Novel PI3Kδ Inhibitors for the Treatment of Inflammatory Diseases

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May 2016

This thesis is the result of the author's original research. It has been composed by the author and has not been previously submitted for examination which has led to the award of a degree.

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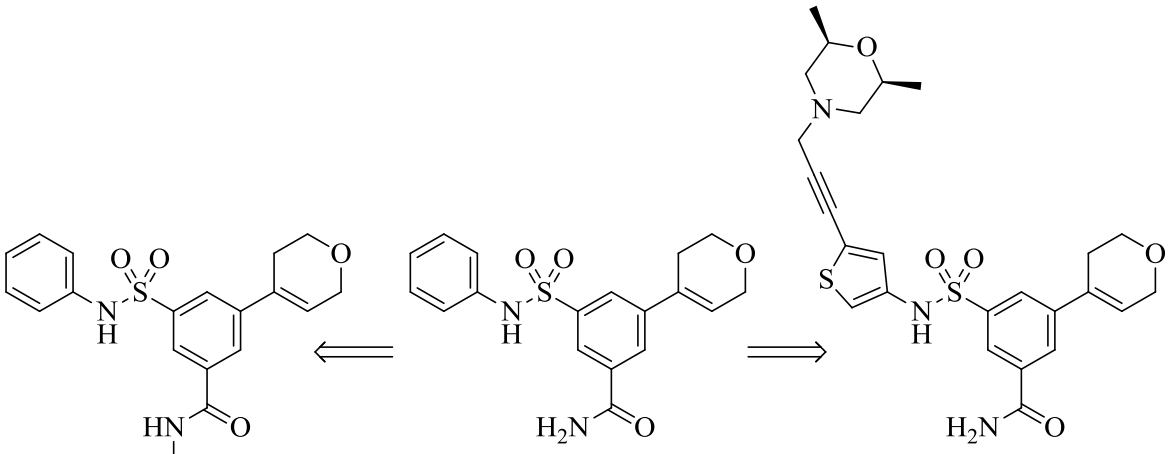
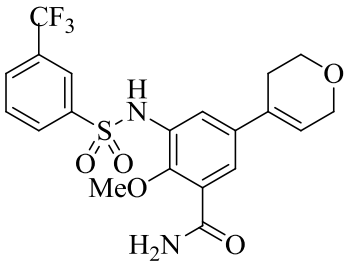
Abstract

This thesis explores the design, synthesis and biological testing of a range of compounds targeting the selective inhibition of phosphoinositide-3-kinase δ (PI3K). The initial aim of this work was to investigate novel replacements for a 2-methoxypyridine moiety, a privileged structure which binds to PI3K in the back pocket region of the protein. Utilising computer aided design, novel groups that bind in the back pocket region were identified to bind to the protein through a conserved water molecule or displace it altogether. From this, a lead compound was selected to further optimise to identify compounds with increased selectivity and potency for PI3Kδ. Investigation of the amide substituent led to an increased understanding of the PI3Kδ back pocket region (e.g. **105**), through the design and synthesis of compounds that open up a region unreported in the literature, whilst enhancing selectivity for PI3Kδ against the other class I PI3K isoforms. Investigation of the sulfonamide substituent led to the identification of potent and selective compounds (e.g. **240**) which accessed an area known to give potency and selectivity from a novel vector unreported in the literature. Although the profiles of the molecules are not optimal, further work in this area could lead to more drug-like compounds with more favourable cellular potency and increased microsomal stability.



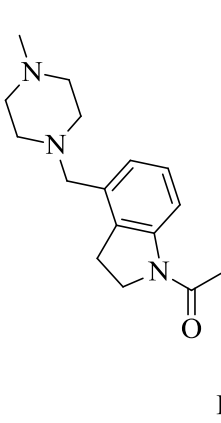
Beyond the work presented in chapters 4 and 5, two further chapters are reported in which two diversified approaches are taken. In chapter 6, utilising a selection of novel back pocket groups discovered in chapter 4, the sulfonamide functionality is reversed in order to ascertain how this would change the binding mode and potency of the compounds. Initial results were

disappointing, however after modification to the structure and crucial co-crystallographic data, compounds of high potency and efficiency have been synthesised (e.g. **284**) which could lead to a promising novel chemical series.

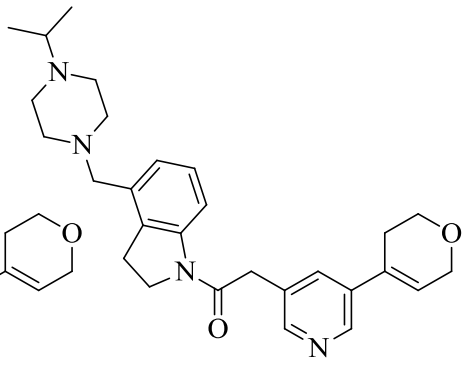


284
PI3Kδ 7.7
Selec. 63-fold

Finally, in chapter 7, initially using a fragment based approach with a compound identified in chapter 4 along with recently reported literature compounds, a combination of computational chemistry and modelling has led to an interesting series of compounds that open an induced pocket. The focus of chapter 7 was to gain potent and selective compounds through the induced pocket, an area of the protein that has proven difficult to access through compounds without a structure similar to Idelalisib, the only marketed PI3Kδ inhibitor. The chapter ends on the establishment of two novel chemical series to be further investigated (e.g. **306** and **328**).



306
PI3Kδ 6.9
Selec. 31-fold



328
PI3Kδ 7.5
Selec. 79-fold

Acknowledgements

I want to thank my supervisors John Murphy and Ken Down for their help and advice throughout my PhD. I would also like to thank Nicole Hamblin and Nick Barton for their support and engaging discussions.

I would like to further thank Nick Barton for BROOD computational chemistry support whilst starting my PhD work and his guidance throughout; I would like to thank Paul Rowland and Máire Convery for protein crystallography data collection, interpretation and discussion; Sean Lynn for NMR support, and the Stevenage collection profiling team and the RRI DPU for their assistance throughout the years. I would also like to acknowledge Dan Thomas, Shenaz Bunally, Chris Edwards and Ken Down for completing data integrity checks on this thesis and Amanda Gladwin for intellectual property checks.

Abbreviations

ADMET	Absorption, distribution, metabolism, excretion, toxicity
AIBN	2,2'-Azobis(2-methylpropionitrile)
AKT	Protein Kinase B
AMP	Artificial membrane permeability
ATP	Adenosine triphosphate
BTK	Bruton tyrosine kinase
Bu	Butyl
^{c}Bu	Cyclobutyl
Cl	Clearance rate
CLND	Chemiluminescent Nitrogen Detection
cLogP	Calculated partition function
COD	1,5-Cyclooctadiene
COPD	Chronic obstructive pulmonary disease
CPME	Cyclopentyl methyl ether
d	Doublet
DABSO	1,4-Diazabicyclo[2.2.2]octane bis(sulfur dioxide) adduct
dba	Dibenzylideneacetone
dd	Doublet of doublets
DCM	Dichloromethane
DHB	Dihydroisobenzofuran
DHP	2-(3,6-Dihydro-2*H*-pyran-4-yl)-

DIPEA	*N*,*N*-Diisopropylethylamine
DMA	*N*,*N*-Dimethylacetamide
DMF	*N*,*N*-Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA-PK	DNA-dependent protein kinase
DPEPhos	(Oxybis(2,1-phenylene))bis(diphenylphosphine)
dppf	1,1'-Bis(diphenylphosphino)ferrocene
DPU	Discovery Performance Unit
dtbpy	4,4'-Di-*tert*-butyl-2,2'-bipyridine
eq	Equivalents
ES	Electrospray
Et	Ethyl
FaSSIF	Fasted state simulated intestinal fluid
GI	Gastrointestinal
GPCR	G-protein coupled receptors
h	Hour(s)
HAC	Heavy atom count
HATU	O-(7-Azabenzotriazol-1-yl)-*N*,*N*,*N*′,*N*′-tetramethyluronium hexafluorophosphate
HBA	Hydrogen bond acceptor
HBD	Hydrogen bond donor
IC_{50}	Half maximal inhibitory concentration
IFNγ	Interferon γ

IgE	Immunoglobulin E
IL8	Interleukin-8
ITK	Interleukin 2-inducible T-cell kinase
LCMS	Liquid chromatography mass spectrometry
LDA	Lithium diisopropylamide
LE	Ligand efficiency
LiDMAE	Lithium dimethylaminoethanol complex
LLE	Lipophilic ligand efficiency
logD	pH dependent partition coefficient
logP	Partition coefficient
m	Multiplet
M.P.	Melting point
MDAP	Mass directed reverse phase automated purification
Me	Methyl
mg	Milligram
min	Minute(s)
mL	Millilitre
mmol	Millimole
MOE™	Molecular Operating Environment™
mol	Mole
MS	Mass spectrometry
mTOR	Mammalian target of rapamycin

n=	Number of tests
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NMR	Nuclear magnetic resonance
NT	Not tested
PH	Pleckstrin homology domain
Ph	Phenyl
PI3K	Phosphoinositide-3-kinase
Pin	Pinacol
PIP_3	Phosphatidylinositol-(3,4,5)-triphosphate
PK	Pharmacokinetics
PPB	Plasma protein binding
ppm	Parts per million
Pr	Propyl
PtdIns	Phosphatidylinositols
*p*TsOH	*para*-Toluenesulfonic acid
RRI	Refractory Respiratory Inflammation
R_t	Retention time
RTK	Receptor Tyrosine Kinases
s	Singlet
Selec.	Selectivity
SHIP	SH2-domain-containing inositol polyphosphate 5'-phosphatase

S_NAr	Aromatic nucleophilic substitution
t	Triplet
$t_{1/2}$	Half-life
T_3P	Propylphosphonic anhydride
TBAB	Tetrabutylammonium bromide
TBME	*tert*-Butyl methyl ether
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
UV	Ultra violet
Vd_{ss}	Volume of distribution at steady state
WB	Whole blood
XantPhos	4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene

Chapter 1 Phosphoinositide-3-kinase

1.1 Kinases

Kinases are a class of intracellular enzymes which catalyse phosphorylation reactions by transferring a phosphate group from adenosine triphosphate (ATP) to a substrate. There are two major types of kinase, protein and lipid. They differ from one another in that protein kinases catalyse the phosphorylation of protein substrates and lipid kinases catalyse the phosphorylation of lipid substrates.[1]

Kinases are comprised of 2 domains, the *N*-and *C*-terminals that are connected by a region of 5-6 residues. This region forms a binding cleft in which ATP binds to the active site through 2 interactions, a HBD from N6 and a HBA from N1 of the adenine ring (Figure 1.1).

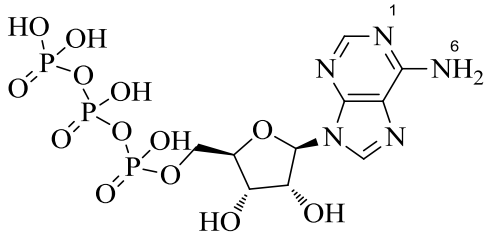


Figure 1.1 Adenosine triphosphate, demonstrating the HBA and HBD interaction points.

This binding cleft is commonly referred to as the hinge as it is the segment that connects the N-and C-terminals of the kinase.[1] The ribose and triphosphate groups are aligned away from the backbone and do not directly interact with the backbone. In PI3Kδ (the relevant target discussed in this thesis), ATP binds to the protein backbone by a HBA interaction from Val828 and a HBD to the backbone carbonyl of Glu826 (Figure 1.2).[2]

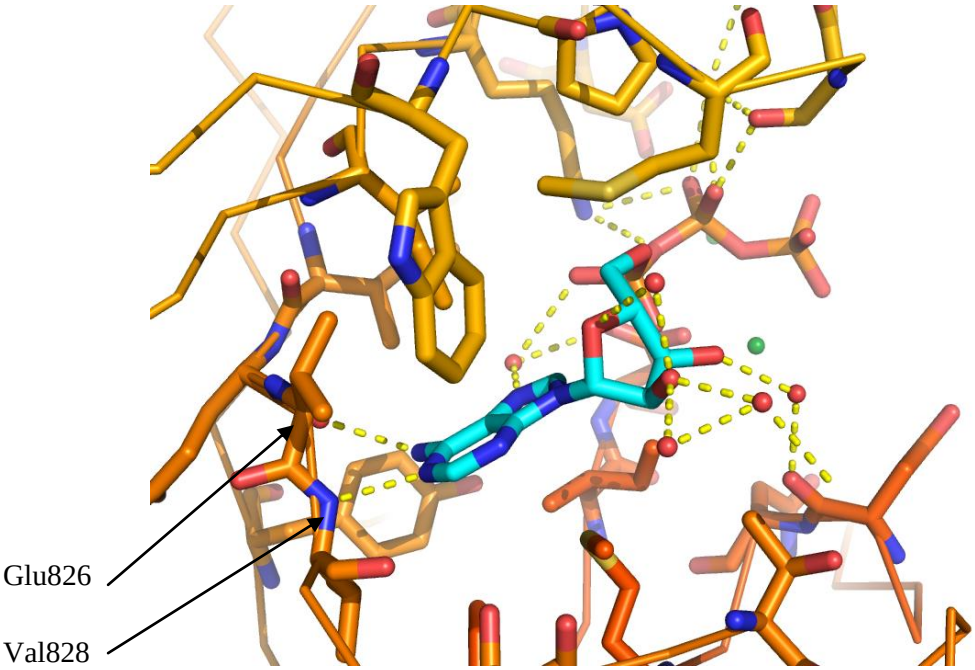


Figure 1.2A Co-crystal structure of ATP in PI3Kδ (Resolution 2.23 Å)[2]

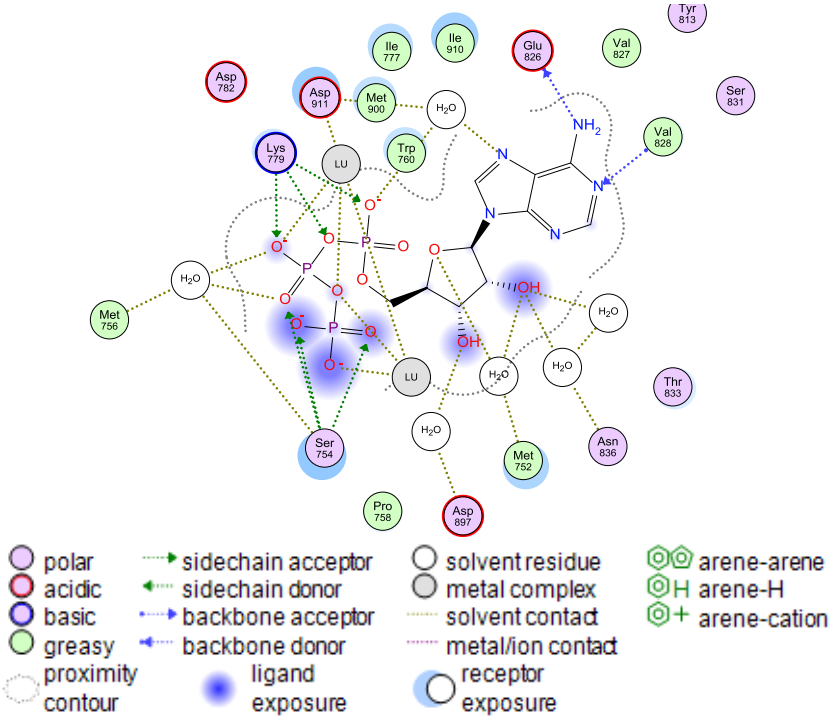


Figure 1.2B 2D representation of ATP binding to PI3Kδ.[2]

The binding strength between ATP and the kinase is weak, but as ATP is abundant in the cell (1-5 mM),[1] the phosphate group is transferred efficiently. A kinase inhibitor has to have high affinity to the active site to compete with this large excess of ATP. A potential kinase inhibitor must utilise the unique differences in residues within the active site of the kinase in order to attain selectivity over the other kinases. Achieving selectivity is not trivial, as each kinase will possess similar, or the same, amino acid sequences in the protein, as shown in the phylogenetic tree (Figure 1.4). In particular, the ATP binding site of the class I PI3K's is highly conserved, so good selectivity can be difficult to achieve.[3]

1.2 Phosphoinositide-3-kinase

The phosphoinositide-3-kinases (PI3K) are a family of intracellular lipid kinases enzymes that are involved in a cascade of cellular processes. First discovered by Lewis Cantley in 1985, PI3Ks have become a popular target for the potential treatment of many diseases including inflammation and cancer.[4-6]

The PI3K-family's primary function is to phosphorylate the 3'-hydroxy group of various phosphatidylinositols (PtdIns) (Figure 1.3). PtdIns are a basic building block for intracellular inositol lipids and consist of a phosphatidic acid that lies within the lipid bilayer and an inositol head group (Figure 1.3).[7,8]

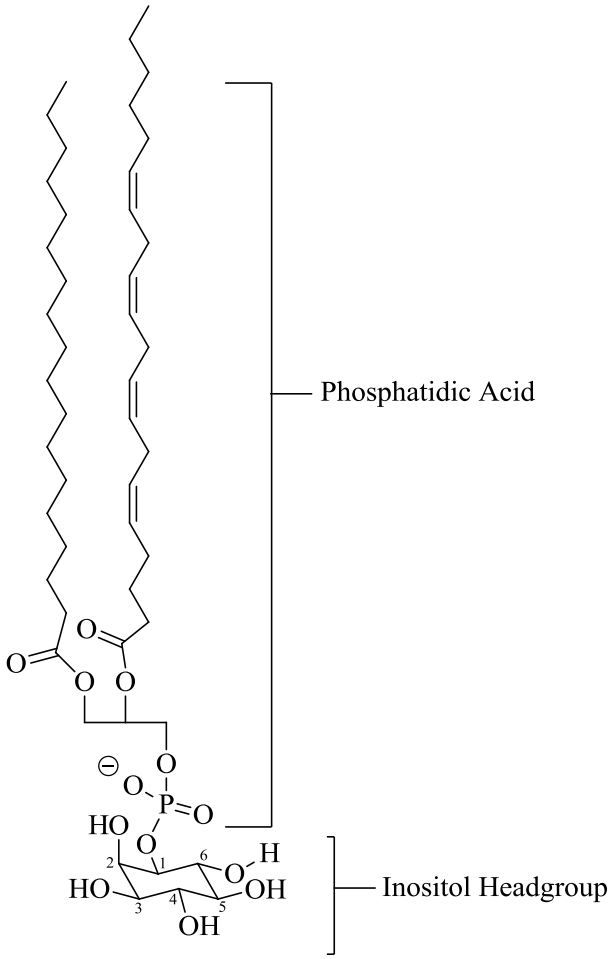


Figure 1.3 The composition of phosphatidylinositols.

Three species of 3-phosphoinositides have been identified; these are PtdInsP3, PtdIns(3,4)P, PtdIns(3,4,5)P_2 and are generated via phosphorylation of the 3-position of PtdInsP, PtdIns(4)P, PtdIns(4,5)P_2. These phospholipids are secondary messengers; they interact with lipid-binding domains of various cellular proteins including serine-threonine kinases, protein kinases and guanosine triphosphate binding proteins (G Proteins). These cellular proteins have specific domains in which to bind the 3'-phosphorylated inositides and this association can cause conformational changes within the proteins' structure, which in turn can activate or deactivate the proteins. For example, the serine-threonine kinase Protein Kinase B (AKT) is activated by phosphatidylinositol-(3,4,5)-triphosphate (PIP_3) associating with the protein. Activation of these proteins can lead to effects downstream such as increased cell growth, cell death, cell differentiation, vesicle trafficking and motility.[7-9]

There are 4 classes of PI3K's, class I, II, III and a separate class related to the PI3K family which includes mammalian target of rapamycin (mTOR) and DNA-dependent protein kinase (DNA-PK) amongst others. The following phylogenetic tree (Figure 1.4), shows the percentage similarity in the amino acid sequence of the catalytic core.[10]

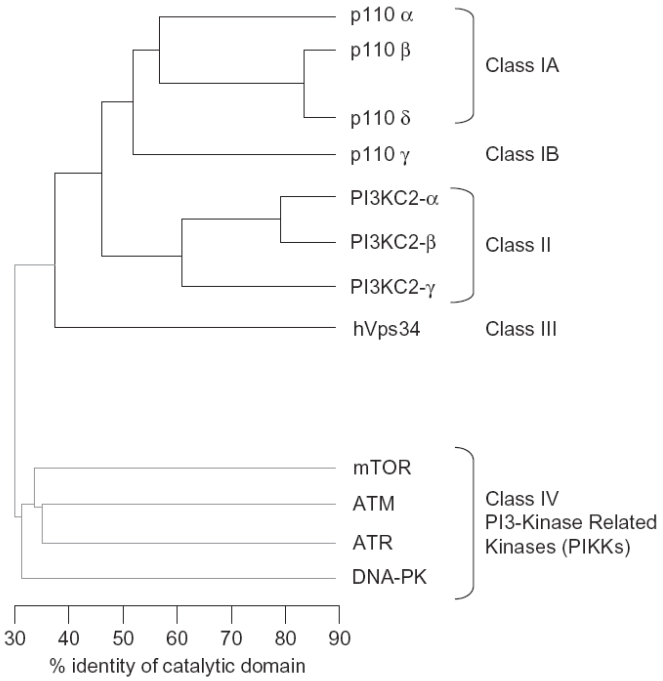


Figure 1.4 Phylogenetic tree showing the percentage similarity of the catalytic cores in the 3 classes of PI3Ks and PIKK's.

Each class of PI3K has a preference for which phosphatidylinositol to phosphorylate at the 3' position. Class I PI3Ks' function is to convert phosphatidylinositol-(4,5)-bisphosphate (PIP_2) into phosphatidylinositol-(3,4,5)-triphosphate (PIP_3) (Figure 1.5).[7,8]

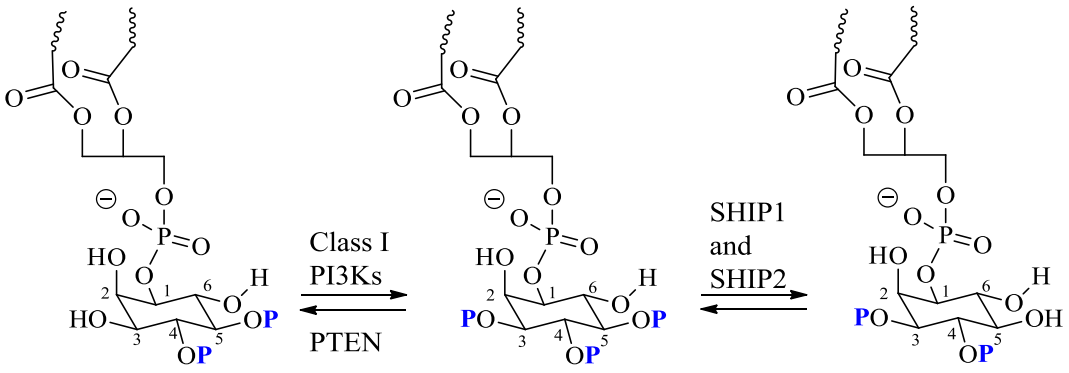


Figure 1.5 The phosphorylation of PIP_2 to PIP_3 using adenosine triphosphate (ATP) by class I PI3K's (**P** = PO_3^{2-})

PIP_3 is an important molecule for cellular function. It is a phosphate transfer agent to many enzymes, particularly for those containing the Pleckstrin-Homology Domain (PH).[4] Examples of kinases containing the PH domains include Protein Kinase B (AKT), Interleukin 2-inducible T-cell kinase (ITK) and Brutons Tyrosine Kinase (BTK).[4] ITK is highly expressed in T-cells and when active, causes extensive T-cell proliferation. Therefore, if PIP_3 production is blocked via PI3K inhibition, then ITK mediated T-cell proliferation will be blocked. This will lead to a decrease in T-cell proliferation which decreases cytokine release in the cell. Brutons Tyrosine Kinase (BTK) is a protein kinase involved in cell growth, especially in B-cells, which fail to mature in the absence of BTK. If BTK is not activated by PIP_3, then B-cells do not mature to form antibodies like immunoglobulin E (IgE) and this can lead to effects such as reduced mast cell degranulation.[7,11]

The modulation of PIP_3 is achieved by the phosphatase and tensin homolog (PTEN) and the SH2-domain-containing inositol polyphosphate-5'-phosphatase (SHIP). PTEN dephosphorylates PIP_3 at the 3' position to form $PI(4,5)P_2$ and SHIP dephosphorylates PIP_3 at the 5'-position to form $PI(3,4)P_2$. Misregulation of PTEN is observed as a potential pathway for cancer.[12] For instance, if the dephosphorylation of PIP_3 by PTEN is stopped, then levels of PIP_3 would increase, leading to overregulation of kinases like AKT, ITK and BTK to detrimental effect.[11]

1.3 Class I PI3K's

Class I PI3K's are hetero-dimers that consist of a regulatory subunit and a 110-kDa catalytic subunit. They are divided into 2 sub-classes; Ia and Ib, and these sub-classes are categorised based on their activation mechanism. There are 3 class Ia PI3Ks; PI3Kα, PI3Kβ and PI3Kδ. They have a p85α/β/δ regulatory sub-unit (PI3Kα can alternatively have p55α and p50α regulatory sub-units) and a p110α/β/δ catalytic core. Class Ia PI3K's are mainly activated by growth factors and cytokines that activate receptor tyrosine kinases (RTK's). Class Ib has a single PI3K isoform, PI3Kγ which exists as a hetero-dimer of a p101, p84 or p87 regulatory subunit and a p110γ catalytic core. PI3Kγ is predominantly activated by G-protein coupled receptors (GPCR's).[7,13-15]

1.4 Class I PI3K's in Disease

A key difference between the Class I PI3K's is the tissue distribution of the individual isoforms throughout the body. This distribution has given guidance to potential diseases that selective PI3K inhibitors could treat. These studies have been supported through the use of knock-out and knock-in mice. Knock-out mice are genetically engineered to have certain genes removed, in order to evaluate the specific gene's effect. Knock-in mice still have the genes required to form the kinase domain, however the enzyme is no longer active so is classed as kinase dead.[6,14] By removing or deactivating the gene associated with each isoform, the biological role of inhibition can be evaluated in order to find potential therapeutic uses for PI3K inhibition.

PI3Kα and PI3Kβ are expressed ubiquitously and when mice have the gene for either PI3Kα or PI3Kβ knocked out they are embryonic lethal, proving that both PI3Kα and PI3Kβ are essential for development. This means that any inhibition of PI3Kα or PI3Kβ could potentially lead to adverse effects. However due to the role of PI3Kα and PI3Kβ in pathways involved in cancer and tissue fibrosis, significant interest in inhibitors for PI3Kα and/or PI3Kβ have been reported. Work by Velculescu and co-workers[16] in 2004 showed that some

tumours from patients with gastric or breast cancer had mutated PI3Kα genes, indicating PI3Kα could have a role in the growth of cancerous cells. Work by Zhao and co-workers[17] in 2008 displayed that PI3Kβ is a key mediator of tumourigenesis, supporting the potential therapeutic effect of PI3Kβ inhibition. However, without further investigation and clinical trials, the exact role of a PI3Kα and/or PI3Kβ inhibitor is relatively unknown.[7,18] In contrast, PI3Kδ and PI3Kγ are not expressed ubiquitously and are primarily expressed in leukocytes. Mice with either PI3Kδ or PI3Kγ deactivated (kinase dead) are viable despite displaying a weakened immune system.[14,15,19,20]

PI3Kγ is highly enriched in leukocytes, but is also present in low levels in the endothelium and heart. Deficiency in PI3Kγ has a key role in the early motility and recruitment of neutrophils and macrophages. Wymann and co-workers[20] showed that upon stimulation of PI3Kγ kinase-dead mice with chemokines such as IL8 and pro-inflammatory proteins, there was a 60 % reduction in the chemotaxis of neutrophils both *in vivo* and *in vitro*. If neutrophils and mast cells are not recruited to the site of inflammation, a reduction in mast cell degranulation and neutrophil oxidative burst could produce a beneficial effect. PI3Kγ kinase dead mice also show significant defects in T-cell activation and differentiation, which could halt T-cell mediated autoimmune responses which are crucial in diseases such as systemic lupus and rheumatoid arthritis.

Similarly to PI3Kγ, PI3Kδ is primarily expressed in leukocytes such as mast cells, neutrophils, B- and T-cells. Studies in PI3Kδ kinase-dead mice have shown a significant role for PI3Kδ in T-cell receptor signalling as well as in B-cell development and the crucial function of T-cells recruitment. Another function of B-cells during an inflammatory response is the recognition of antigens in blood. Upon recognition of antigens in the blood, antibodies such as immunoglobulin E (IgE) are produced which, upon binding to mast cells, cause degranulation of cytokines into the blood. In neutrophils, activation of PI3Kδ has a differential role compared to PI3Kγ, where neutrophil recruitment into inflamed tissue is maintained after the initial response.[11,14,21-24]

Inhibition of PI3Kδ is hypothesised to be a potential therapy for a number of inflammation based diseases and autoimmune diseases. Inhibition of PI3Kδ has also emerged as a

promising target for the treatment of cancers, specifically those which affect white blood cells such as chronic lymphoid leukaemia.[25]

1.5 Inhibition of PI3Kδ for Respiratory Diseases

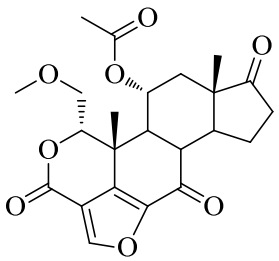
Research into the inhibition of PI3Kδ has shown key roles in both T- and B- cell function which directly affect allergen-IgE-induced mast cell degranulation, neutrophil migration and cytokine release, all important events in respiratory diseases.[15]

Further studies in diseased mouse models have shown that targeting PI3Kδ is a viable and effective strategy for creating a new therapeutic agent. Puri and co-workers[26] demonstrated that upon allergic sensitisation of the airways with ovalbumin, administration of a selective PI3K inhibitor (IC87114 see Table 1.1) inhibited lung tissue eosinophilia and suppressed T-helper-cell dependent inflammation and mucus production in the airway. Further analysis showed a decrease in the expression of IL-4, IL-5, IL-13, IgE, and the downstream phosphorylation of AKT. This was further supported by Marshall and co-workers,[27] who demonstrated that in PI3Kδ kinase inactivated knock-in mice, hyper-responsiveness of the bronchioles using methacholine had a suppressed effect in comparison to the control.

Current treatments for asthma and COPD rely on long-acting muscarinic antagonists (LAMA), long-acting beta agonists (LABA) and corticosteroids. A key problem with corticosteroids is that, over time, patients can develop insensitivity. This can lead to poorly controlled asthma and/or COPD with frequent debilitating exacerbations. Work by Barnes and co-workers in 2010[28] showed that by using a selective PI3Kδ inhibitor (IC87114), steroid insensitivity was reversed in a cigarette-smoking mouse model.

1.6 PI3Kδ Inhibitors

The discovery of the natural product Wortmannin **1** as a pan-PI3K inhibitor in 1993 and then the subsequent discovery of LY294002 **2** in 1994 greatly enhanced the understanding of the basic function of PI3Ks.[29-31]



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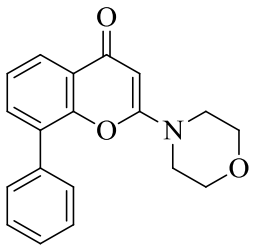
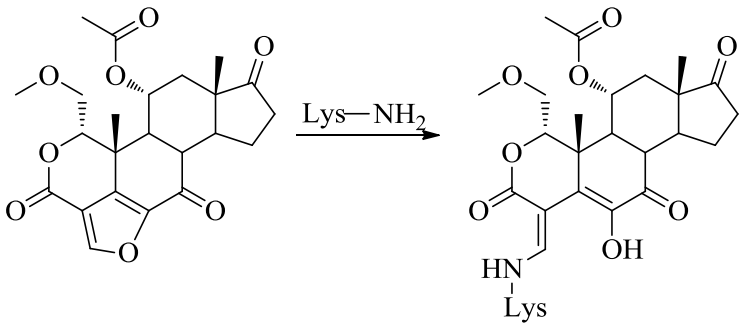


2

Wortmannin is a pan-PI3K inhibitor and also has activity against other lipid kinases such as mTOR, DNA-PK and the mammalian polo like kinase.[29,32] Wortmannin is an irreversible inhibitor of PI3K, forming a covalent bond with the catalytic lysine residue within the active site (Scheme 1.1). Wortmannin is not selective towards transformed cells, exhibiting a level of toxicity rendering it unsuitable as a therapeutic.



Scheme 1.1 Covalent bond formation from the catalytic lysine residue

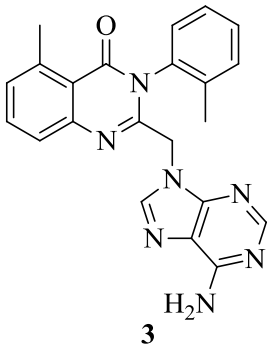
In comparison, LY294002 **2** is the first reported reversible inhibitor of PI3K. Again similarly to wortmannin **1**, LY294002 **2** is not selective for PI3K and displays activity against other lipid kinases DNA-PK, mTOR and more recently against the BET family of bromodomains.[33] These initial compounds gave a platform for the discovery of novel inhibitors with increasing levels of selectivity.

1.7 Structural Elucidation of PI3Kδ

A plethora of small molecule PI3Kδ inhibitors exist in the literature, and the design and synthesis of these have been aided by co-crystallisation of compounds in the PI3Kδ protein construct. Williams and co-workers[34] are pioneers in obtaining crystal structures of compounds co-crystallised in murine PI3K isoforms. Williams and co-workers elucidated the structure of class I PI3Ks binding to adenosine triphosphate (ATP), wortmannin and LY294002. The small molecule co-crystal structures are obtained by 'soaking' the desired compounds dissolved in DMSO into pre-existing PI3K crystals in a defined medium.[34] Glycerol is then added and then the samples frozen. The data of the co-crystallised compounds are collected at a synchrotron facility and refined to elucidate the crystal structure.

The first selective PI3Kδ inhibitor IC87114 **3** was reported in 2002 (Table 1.1).[22,35]

Table 1.1 IC87114 **3** and its inhibition of PI3K class I isoforms.[36]



3

	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
IC87114 3	<4.6 (n=4)	<4.6 (n=4)	5.0 (n=6)	6.9 (n=4)

n= Number of tests.

IC87114 (**3**) shows 79-fold selectivity for PI3Kδ over the closest class I isoform and utilises adenine as its hinge binder. Its binding mode was elucidated by co-crystallography (Figure 1.6).[2]

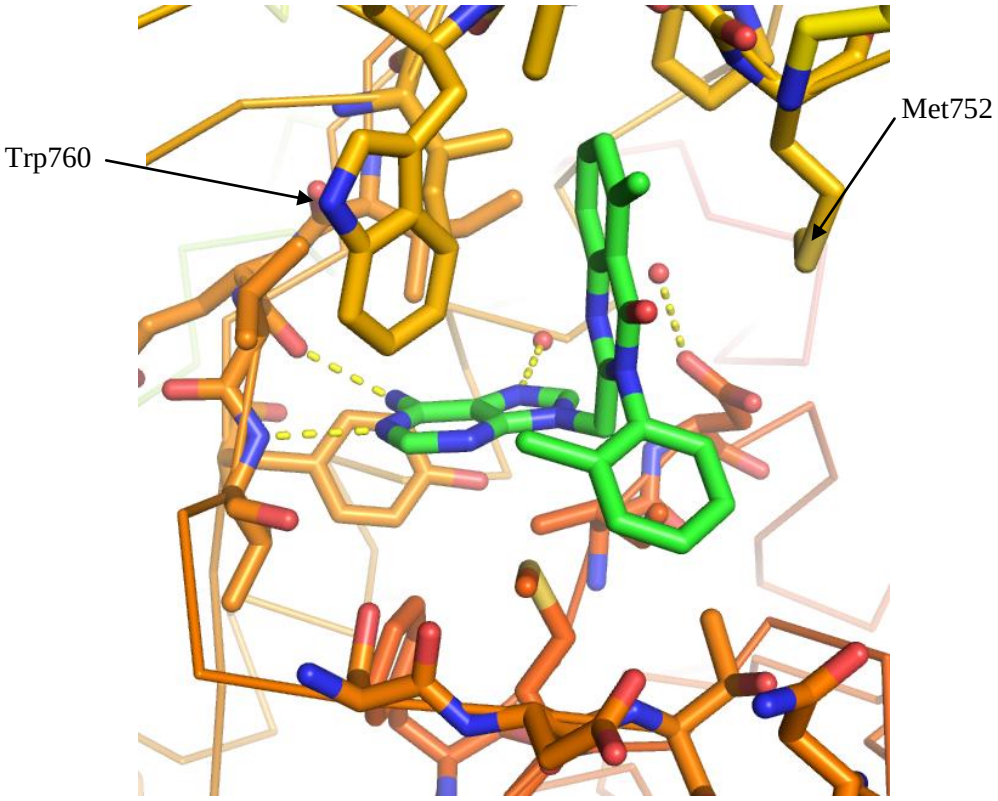


Figure 1.6A Co-crystal structure of **3** in PI3Kδ (Resolution 2.2 Å).[2]

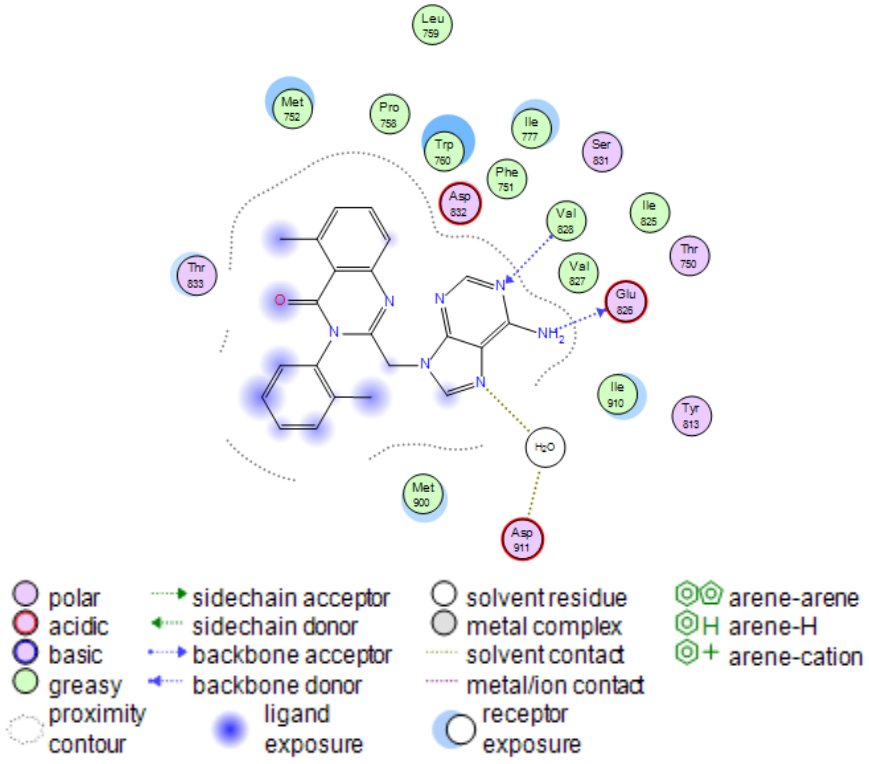
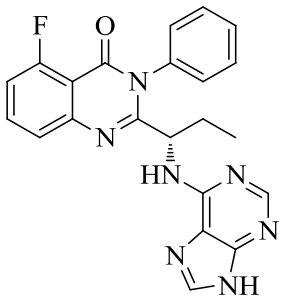


Figure 1.6B 2D representation of **3** binding to PI3Kδ.[2]

The adenine hinge binder in IC87114 forms the same interactions observed in the adenosine ring of ATP binding to PI3Kδ, however no further HBA or HBD interactions are observed. IC87114 gains its potency and selectivity for PI3Kδ by adopting a 'propeller' shape, in which the *N*-(*o*-tolyl)quinazolone induces a conformational change within the protein to occupy a hydrophobic selectivity pocket between Trp760 and Met752. The selectivity gained from this pocket is unique to PI3Kδ, as it has more flexibility than the other PI3K isoforms, possibly due to the RAS binding domain being shifted in comparison to PI3Kα and PI3Kβ.[37,38]

The discovery of this induced selectivity pocket has led to further research into compounds with a similar template. Idelalisib **4** is an oral inhibitor of PI3Kδ and gained approval in 2014 as a first-in-class, third-in-line treatment for chronic lymphocyte leukaemia. Later in 2014, Idelalisib was approved for the treatment of follicular lymphoma and small lymphocytic lymphoma. Preclinical studies have shown Idelalisib to promote cell death in chronic lymphocyte leukaemia cells, but does not promote cell death in T- or natural killer cells.[39,40]

Table 1.2 Idelalisib **4** and its inhibition of PI3K class I isoforms.[36]



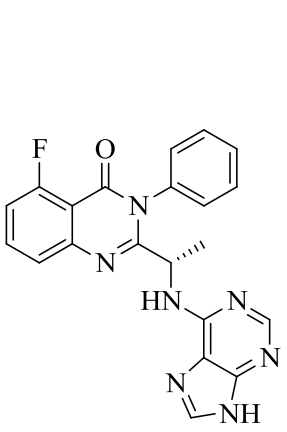
4

	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**WB IFNγ**
Idelalisib 4	5.0 (n=4)	5.8 (n=4)	6.6 (n=4)	8.1 (n=3)	6.7[a] (n=4)

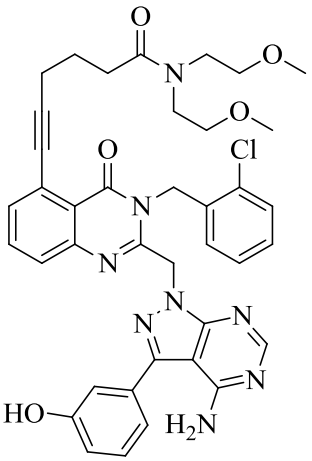
[a]On one occasion, readings of <5.3 were recorded.

Further compounds possessing a similar pharmacophore to IC87114 **3** and Idelalisib **4** are also in development. Examples of these include Duvelisib **5** and an example compound from Respivert's quinazolinone series **6** (Table 1.3).[41,42]

Table 1.3 Reported inhibitions of Duvelisib **5** and RV1729 **6**.[41,42]



5



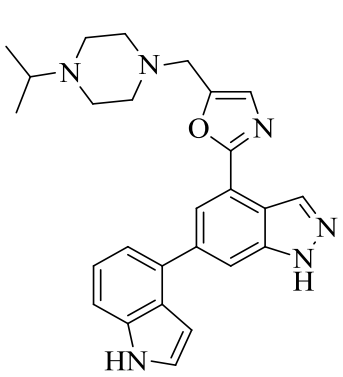
6

	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
5	5.6	7.2	8.1	9.4
6	6.7	Not disclosed	8.6	8.9

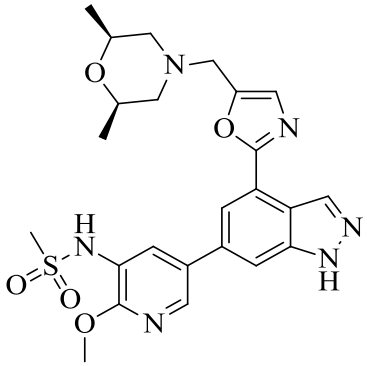
In contrast to Idelalisib **4**, Duvelisib **5** is a dual PI3Kδ/γ oral inhibitor which is currently in phase II trials for the treatment of rheumatoid arthritis and inflammatory diseases. Respivert have also claimed compounds (such as RV1729 **6**) for inhaled use in inflammatory disorders such as COPD and Asthma.[41,42]

Selectivity for PI3Kδ can be achieved by opening the hydrophobic pocket between Trp760 and Met752 as observed in IC87114 **3** and Idelalisib **4**. Other inhibitors of PI3Kδ such as indazoles **7** and **8** do not open the hydrophobic pocket, but attain selectivity for PI3Kδ in an alternative pocket of the protein.[43,44]

Table 1.4 Enzyme inhibition data for compounds **7** and **8** showing excellent potency and selectivity for PI3Kδ.[36,45]



7



8

	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ (pK_i)**
7	5.3[a,b] (n=29)	5.8[a] (n=27)	5.2[c] (n=33)	9.9[d] (n=4)
8	6.3 (n=11)	6.2 (n=10)	6.3 (n=9)	10.1[d] (n=2)

[a]On one occasion, a reading of <4.6 was recorded. [b]On one occasion, readings of <5.3 were recorded. [c]On two occasions, readings of <4.6 was recorded. [d]The standard PI3Kδ assay was run at a 2 mM ATP concentration

The binding mode of **7** and **8** was elucidated by co-crystallography (Figure 1.7).[2]

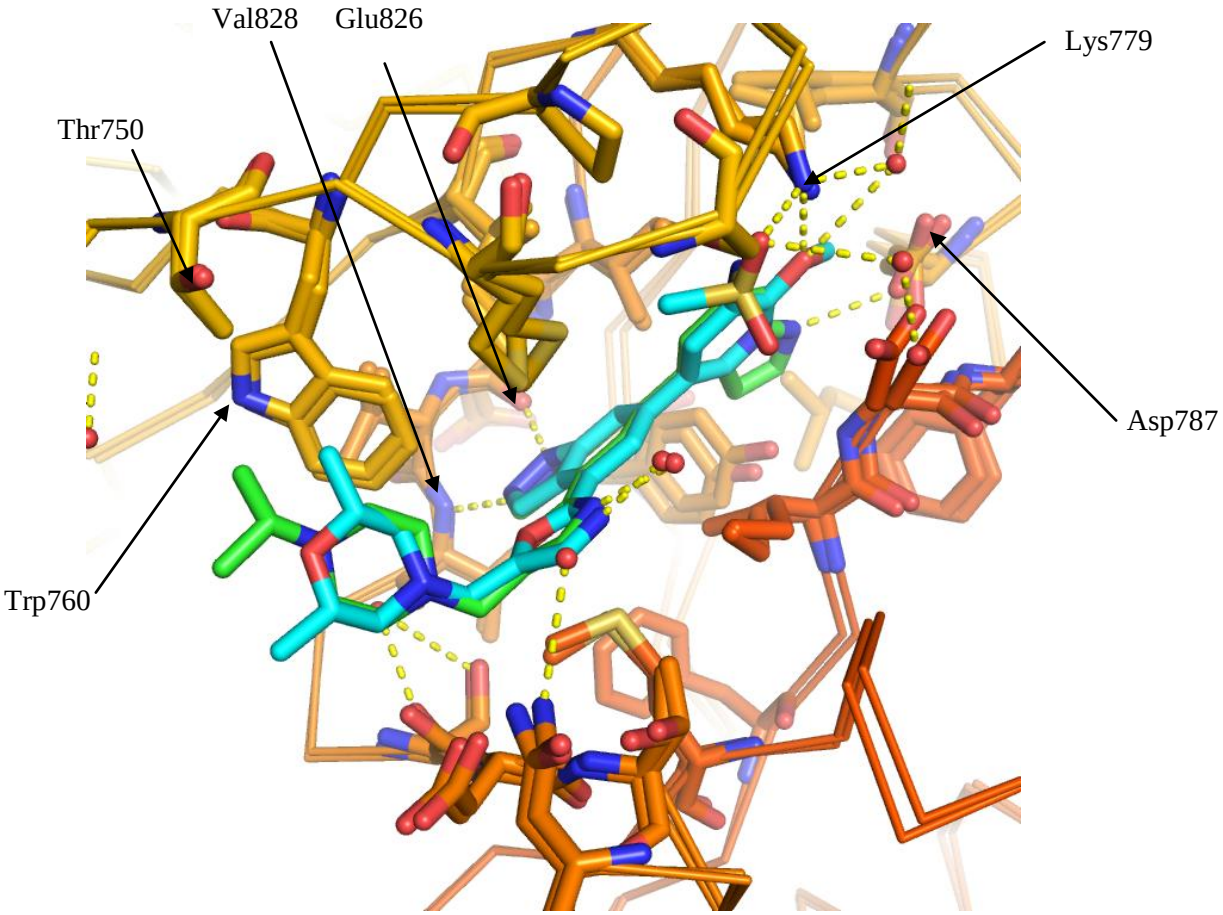
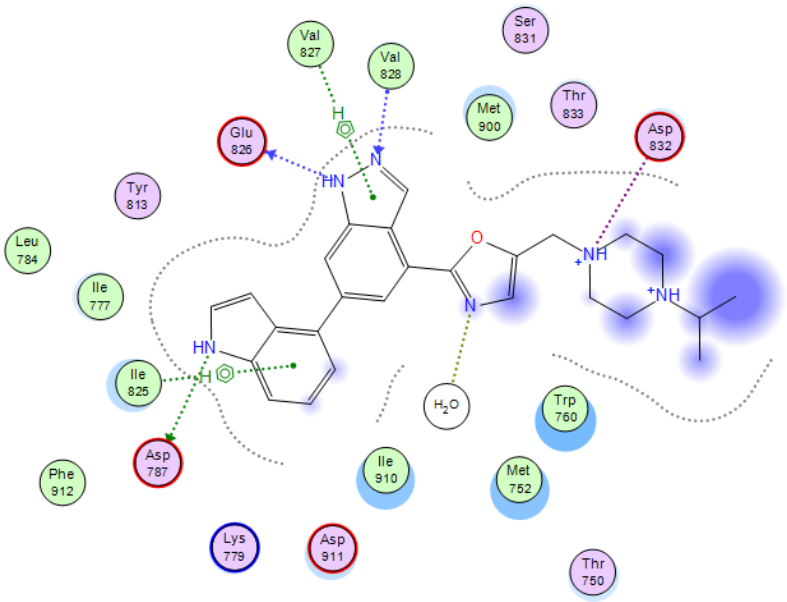


Figure 1.7A Co-crystal structure of **7** (green) and **8** (cyan) in PI3Kδ (Resolution 2.42 Å and 2.63 Å).[2]



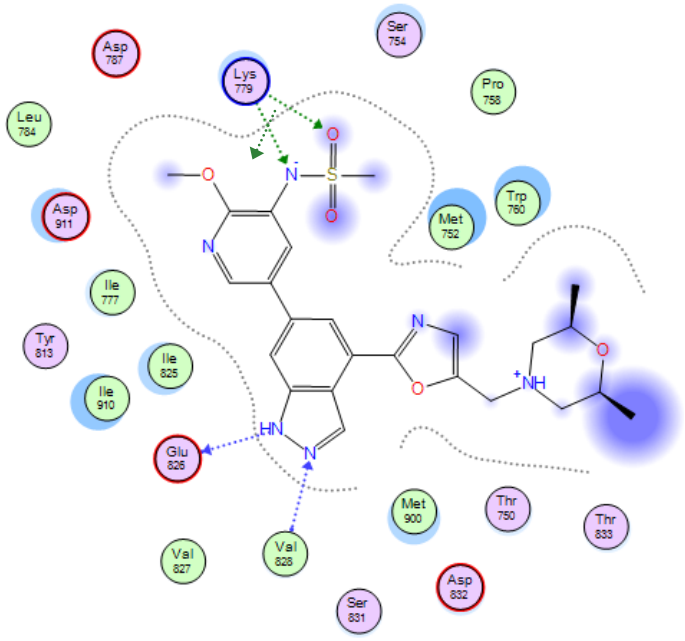


Figure 1.7B 2D Representation of **7** and **8** binding to PI3Kδ.[2]

The crystal structure demonstrates some key interactions. The indazole ring acts as the hinge binder, providing a hydrogen bond acceptor from N2 to the backbone N-H from Val828 and a hydrogen bond donor from N1 to the backbone carbonyl of Glu826. In compound **7**, the indole occupies the area of the kinase known as the back pocket, a lipophilic pocket which is not filled by ATP or by propeller compounds such as IC87114, and is a useful area for gaining potency and selectivity. The indole back pocket group in **7** provides a hydrogen bond donor to the side chain of Asp787, which provides a substantial increase in potency. A decrease of 2 log units of PI3Kδ potency was observed upon methylation of the indole, indicating the detrimental effect of disrupting this interaction. In comparison to this, the 2-methoxypyridine in compound **8** occupies the back pocket and provides different interactions to those observed in the indole back pocket. There is a bifurcated hydrogen bond acceptor from the side-chain of Lys779 to the deprotonated sulfonamide and to the methoxy group. This lysine has a further hydrogen bond to the side-chain of Asp911 through two bridging water molecules. The piperazine (**7**) or morpholine (**8**) moieties make complementary electrostatic interactions near the Trp760 residue and extend towards Thr750. Thr750 is an important residue for achieving selectivity against PI3Kα, PI3Kβ and PI3Kγ, as in the other isoforms, this residue is a larger charged amino acid (Arg in PI3Kα and Lys in PI3Kβ and PI3Kγ. The piperazine (**7**) and morpholine (**8**) occupy space near the Thr750 residue, and

therefore clash with corresponding residues in PI3Kα, PI3Kβ and PI3Kγ, which gives the selectivity observed (Figure 2.8).

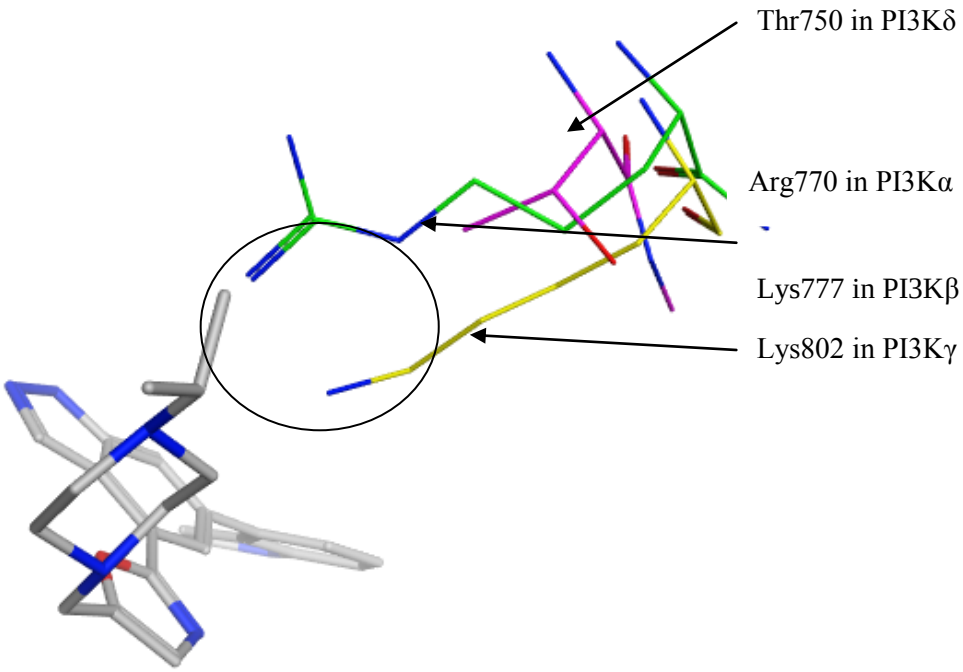
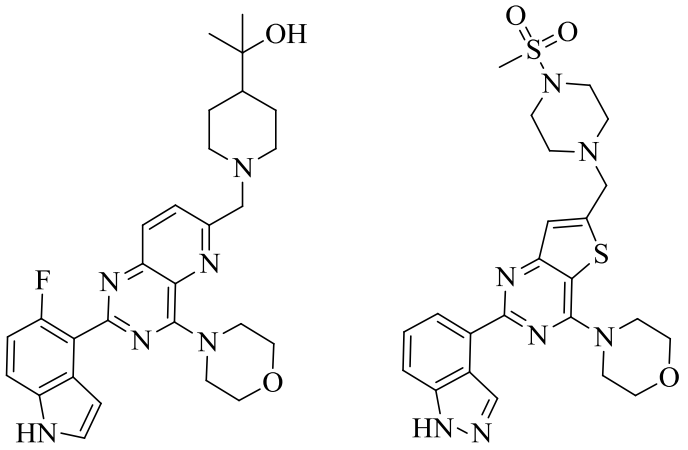


Figure 1.8 Overlay to show PI3Kδ inhibitor **7** clashing with Arg770 in PI3Kα (lime) and Lys777/802 in PI3Kβ and PI3Kγ (yellow).[2]

Indazole **7** is currently in phase II clinical trials[46] for the treatment of COPD and **8** is the pre-clinical backup and both were developed for inhaled delivery. They possess desirable properties such as high potency, selectivity and have a PK profile suitable for inhaled delivery, with a low bioavailability in two animal models. From this starting point, further series have been developed within GSK targeting oral inhibitors of PI3Kδ.

Alongside this within the literature,[47] a number of PI3Kδ inhibitors have been reported that form similar interactions with the Trp760 and Thr750 residues, such as **9**[44] and pictilisib **10**[48] (Figure 1.9).



9 **10**

Figure 1.9 Compounds **9**[44] and pictilisib **10**[48] which interact with T

rp760 and Thr750.

Chapter 2 Properties of Oral Drugs

2.1 Physicochemical Properties of Oral Drugs

The success of oral small molecule drug discovery is directly linked to the physicochemical properties the compounds possess. Compounds have to possess desirable efficacy at their biological target/s, have acceptable toxicological properties and have a suitable dose. In order to achieve this, the physicochemical properties of the molecule such as the lipophilicity and solubility must be tuned in order to achieve good ADMET properties (absorption, distribution, metabolism, excretion and toxicity). In recent years, the number of new drugs produced within the pharmaceutical industry has decreased,[49] and it is widely accepted that a significant cause of this is due to poor physicochemical properties of drugs leading to poor ADMET.

The first collective view on properties required for good oral solubility and permeability are Lipinski's guidelines, first formulated by Christian Lipinski of Pfizer in 1997.[50] Lipinski carried out an analysis of a selection of oral drugs' physical properties and found that if a compound disobeyed more than one of the following guidelines, it would likely have poor permeability and absorption:

- No more than 5 Hydrogen bond donors
- No more than 10 Hydrogen bond acceptors
- LogP lower than or equal to 5
- Molecular weight below 500 g mol^{-1}

Lipinski's guidelines have been used extensively over the past 15 years as a baseline guide of important properties that a potential drug would do well to follow. Since Lipinski's guidelines in 1995, the use of metrics within drug discovery has 'exploded', with numerous guidelines published in order to define and aid the discovery of novel compounds in drug-like space.[51-55]

The ADMET profile of a potential oral drug is important and is governed by the drug's physicochemical properties. These can be modulated by the chemist and these properties include:

- Lipophilicity – logP and logD
- Solubility
- Permeability
- Number of hydrogen bond donors and acceptors
- Polar surface area
- Size

The importance of a drug's physical properties underline many aspects in drug discovery. In particular, the lipophilicity of a compound is recognised as a fundamentally important property that can influence potency, solubility and a variety of pharmacokinetic parameters as well as toxicity. Lipophilicity is measured by the octanol/water partition coefficient, using a shake flask method. The intrinsic lipophilicity (logP) describes the partition of the un-ionised forms of molecules between the octanol and water. Alternatively the effective lipophilicity ($logD_{pH}$) describes the partition between the neutral and ionised species in solution between the two phases at a given pH. Both logP and $logD_{pH}$ can be predicted *in silico* due to the large existing databases which can aid the virtual design of molecules, prior to selection for synthesis.[56] Alternatively, a chromatographic method for $logD_{pH}$ is available ($chromlogD_{pH}$). This is measured by passing compounds through a C_{18} column at various pH levels, and the retention time of the compound gives values of $chromlogD_{pH}$. This can appear as an arbitrary value, however the high throughput nature of the technique allows rapid collection of data.

Another key parameter is the aqueous solubility of molecules. An analysis of over forty-four thousand compounds by Gleeson[57] showed a clear correlation between increasing logP and decreasing aqueous solubility. The ionisation state of a compound at physiological pH has an impact upon solubility; however compounds with high logP still have problems with solubility. Another factor involved in the determination of solubility is the molecular weight. Upon increasing the molecular weight, an inverse correlation with good solubility is observed. The analysis also showed that neutral molecules possessing logP of more than 3

were less soluble than others in any other ionisation state; however upon reducing the logP to less than 3, the solubility was similar to those of ionisable compounds. Young and co-workers[52,55] however proposed that $logD_{pH}$ should be used preferentially alongside the number of aromatic rings to give a guide to the solubility of the molecule. It was concluded that if chrom$logD_{pH=7.4}$ + aromatic ring count is less than or equal to 5, then the compound has a good chance of having good solubility. Young coined this term Property Forecast Index (PFI) and it has become a widely used metric. Gleeson's analysis[57] however displayed a stronger correlation between increasing aromatic ring count and poor solubility than increasing logP and solubility.

Solubility can be predicted *in silico*, however this is only a prediction due to the many variables involved and it is therefore important to measure.[58] Aqueous solubility can be measured using a high throughput chemiluminescence nitrogen detection (CLND) method.[59] This assay is determined by the precipitation of the compound from a solution of DMSO by the addition of a buffered aqueous solution at pH 7.4. This method has a slight caveat in that it measures the amount of the compound left in solution, not the solubility from the solid. A compound is said to have low solubility below 30 μM, moderate from 30-200 μM and high above 200 μM. CLND is a widely used measurement for solubility; however it is not assessing the solubility of molecules in a bio-relevant medium. The solubility in gastrointestinal fluids is important in predicting the *in vivo* behaviour of molecules and the fasted state simulated intestinal fluid (FaSSIF) is used as a good prediction for orally delivered compounds. For inhaled compounds, a good prediction of solubility can be attained using stimulated lung fluid. The majority of drug absorption occurs in the duodenum where the surface area is the largest due to the large concentration of villi and microvilli. The high throughput FaSSIF solubility assay measures solubility from a solid compound in a physiological related medium. A high FaSSIF solubility is classed as greater than 100 μg/mL.

An increase in lipophilicity often results in an increase in cellular solubility due to the hydrophobic effect.[60] The neutral form of an ionisable compound contributes most significantly to the permeability of a compound, as polar (either positively- or negatively-charged) species have reduced affinity towards the phospholipid bilayer. Analysis of over fifty thousand compounds from GSK showed that zwitterions and polar acids were the least

permeable, followed by polar bases, with neutral species the most permeable.[52,55] Despite this, it is not advisable to increase the lipophilicity of compounds above 3 to attain better permeability as it can compromise other properties. Other properties such as the molecular weight, the number of HBA and HBD interactions and the polar surface area can all be tuned in order to achieve permeability, without the negative impact of increased molecular weight. This analysis[52] displayed a correlation between increasing molecular weight and poor permeability. Permeability decreases with the increase in number of HBA and HBD interactions as they are usually polar substituents. This does not always hold true with HBA and HBD interactions that are shielded due to conformation flexibility or ionisation.

The permeation rate of a compound can be measured in a high-throughput assay utilising an artificial membrane.[36] Permeability is classed as low if it is less than 50 nm s^{-1} and high above 200 nm s^{-1}. Other methods for measuring permeability are in cultured cell lines such as the Madin Darby canine kidney cells (MDCK) and Caco-2 cells from human colon carcinoma.[61,62] Both these methods use their respective cells to form a monolayer of cells, upon which the compound is added to the surface. The transport across the monolayer is measured over one hour and is more indicative of the permeability than measuring it through an artificial membrane. However, neither method is high throughput, requiring longer periods of time to form the monolayer upon which the permeability can be measured.

The polar surface area (PSA) is that part of the surface area of a molecule in 3D space that is comprised of polar atoms. It is usually calculated using a two-dimensional additive fragment algorithm which generates a molecular surface and then calculates the polar atoms surface. PSA strongly correlates with the number of heteroatoms. An analysis of over one thousand compounds at GSK analysed the properties required for oral absorption and for the PSA, a value below 140 $Å^2$ resulted in good absorption and below 90 $Å^2$ had good central nervous system penetration.[63]

2.2 Drug Metabolism and Pharmacokinetics

Pharmacokinetics describes the absorption, distribution, metabolism and excretion of a drug that take place following administration. For inhaled delivery, the drug is delivered directly to the target tissue. In comparison oral delivery is not directly delivered to the target tissue, so the drug needs to be absorbed, distributed to the target tissues, be able to pass into the cell and then have effect at the desired target.

Absorption is the transfer of a solubilised compound across the gastrointestinal (GI) tract into the bloodstream. There are many absorption mechanisms which can occur within the body, including passive and active diffusion through the cell and endocytosis. Absorption of a drug relies on many variables including drug stability, rate of efflux and drug solubility. The compound is transported through the hepatic portal vein into the liver (first pass metabolism) and has to avoid renal excretion (second pass metabolism) before entering the systemic blood circulation. A key PK parameter that is calculated by absorption is bioavailability (F), which is the fraction of dose that reaches the systemic blood circulation intact in comparison to an intravenously delivered dose. For an oral project a high bioavailability is desired as this means that most of the drug is reaching the blood.[64,65]

Distribution of a drug takes place via the bloodstream and is mainly based on the drug's lipophilicity, which dictates the passage of the drug through cell membranes. The partition function (logP) and the pH-dependent partition function (logD) are key parameters that influence how much of the drug will pass through the membrane into the cell. This is because increased lipophilicity leads to lower aqueous solubility, so more drug will prefer to distribute into the more lipophilic membrane. Other factors include the drug's solubility and amount of hydrogen bonding. Increasing logP/logD will result in the distribution of the drug into cells, however it can also increase two PK parameters, volume of distribution (Vd_{ss}) and plasma protein binding (PPB). Volume of distribution (Vd_{ss}) is the measure of the amount of drug distributed in the blood plasma and tissue. The larger the volume of distribution, the more distributed the drug is in tissue. However, if the Vd_{ss} is too high then the drug concentration in the blood may be too low (due to its storage in tissue) which can lead to a

poor therapeutic effect if the target is not based in tissue. In contrast, if the Vd_{ss} is too low, then the drug may not reach the desired tissue. The higher the PPB, the more highly bound the drug is to the plasma protein, reducing the amount of drug that is free to diffuse across cell membranes and engage the target.

Metabolism is the chemical modification of "foreign" chemicals by enzymes within the body to make them more readily excreted. Metabolism is a drug clearance process and there are many pathways in which the metabolism of drugs can be studied in order to understand the behaviour of a drug after its administration. Drug metabolising enzymes are mainly located in the liver, but are also present in most tissues, the gastrointestinal tract, blood, lung, kidneys and skin.

There are two phases of metabolism, phase I and II. Phase I metabolism is the chemical alteration of molecules, most commonly by cytochrome P450 enzymes.[66] The cytochrome p450 family are heme-containing proteins, and of the fifty-seven human enzymes, five isoforms are accountable for the majority of metabolism of known drugs; these are 1A4, 2C9, 2C19, 2D6 and 3A4. Cytochrome P450 3A4 is the most abundant and is responsible for the metabolism of approximately 50 % of clinically used drugs; 2D6 is involved in the metabolism of 20 % of clinically used drugs. Other phase I enzymes include monoamine oxidases, aldehyde oxidases and hydrolases.[66]

Phase II metabolism is when the body conjugates drug molecules to polar molecules such as sugars (glucuronidation) and sulfates (sulfation) which increase their water solubility. Key enzymes involved are the aryl sulfatase, the UDP-glucuronyl transferase and the glutathione S-transferase. Both phase I and II metabolism increase the hydrophilic nature of the molecules, leading to more readily excreted products. There are numerous factors that can increase drug metabolism such as high lipophilicity, labile functional groups or just a high affinity for metabolising enzymes such as cytochrome p450. Analysis by Young and co-workers[52] suggest that compounds possessing logP below 3 are less likely to have high affinity to CYP enzymes. One way to measure metabolism of a drug is by observing the clearance of drug *in vivo*.[67]

Excretion of the drug is the removal of the drug from the body. This is either by hepatobiliary elimination through the bile duct or renal excretion.

In vitro studies are useful in gaining an understanding before embarking on *in vivo* models. For the work described in this thesis, if a compound has sufficiently desirable properties which will be outlined later, the *in vitro* clearance (IVC) of the compounds will be measured in microsomes. Two species of microsomes will be utilised; human and rat. Microsomes are composed of differential centrifugation of liver homogenates, which mainly are fragments of endoplasmic reticulum. In order to measure a rate of intrinsic clearance, the disappearance of a compound was monitored over 45 minutes and the elimination rate constant was determined. Compounds can be categorised into low, moderate or high clearance by taking into account the liver blood flow of each species (Table 2.1).

Table 2.1 Liver blood flow and categories for IVC.

	Human	**Rat**
Liver blood flow (ml/min/kg)	18	78
Low IVC (ml/min/kg)	< 0.525	< 1
Moderate IVC (ml/min/kg)	0.525 - 1.6	1 - 4.1
High IVC (ml/min/kg)	> 1.6	> 4.1

The only caveat with *in vitro* clearance is that the clearance of a molecule can be via non-CYP enzymes that are not represented in liver microsomes. Other enzymes that can contribute to drug metabolism include monoamine oxidases (MAO) and aldehyde oxidase (AO). Additionally, microsomes can only conduct phase I metabolism, so any further metabolism in phase II conjugations are not considered. One way this can be overcome is via using hepatocytes as a more sophisticated model which covers all the metabolising enzymes (phase I and II) inside the cell.

The toxicity of a drug is a major cause of late stage drug attrition.[54] Toxicity can be caused by adverse drug reactions, in which compounds engage multiple interactions within the body which can lead to non-therapeutic effects. Much toxicity observed within early phase drug discovery is caused by the promiscuity of compounds. Achieving selectivity for a biological

target is in general not trivial, and therefore any off-target activity observed can result in a toxic effect due to the off-targets' associated pharmacology. One off-target is the human *ether-a-go-go (*hERG) potassium channel, which is essential for normal electrical activity in the heart. Inhibition of the hERG channel is a liability as it can cause cardiac arrhythmia, and is therefore a key target for the identification of cardiovascular liabilities. An analysis by Waring and co-workers[68] saw a strong correlation between increasing lipophilicity and hERG inhibition, further supporting the need to balance the lipophilicity of compounds. Analysis by Young and co-workers displayed a stronger correlation between increasing $\log D_{pH=7.4}$ and hERG inhibition and a correlation between the number of aromatic rings and hERG inhibition. Another key off-target enzyme to avoid the inhibition of is the cytochrome P450 enzymes. Inhibition of cytochrome P450 enzymes is a liability as they are responsible for the metabolism of many active molecules within the body and other drug molecules. If a cytochrome P450 enzyme is inhibited, then it will inhibit its normal function which may lead to a build up of a molecule which at higher concentration may cause toxicity.

Toxicity within the body can also be caused by xenobiotic compounds mutating DNA. Pioneering work by Ames and co-workers[69,70] developed an assay in order to determine compounds that could cause mutations. The Ames test evaluates compounds for potential mutagenicity by measuring mutations in a set of bacteria that lack the ability to make histidine which is necessary for growth. At GSK, a panel of 5 bacteria (four *Salmonella typhimurium* and one *Escherichia coli* strain) is used that all lack the ability to make histidine, an essential amino acid which prevents bacterial growth. The Ames test uses varying concentrations of the compound in a solution of the bacteria. If there is a mutation of the bacteria and bacterial growth is observed above the 2-3 fold background level of mutation, the compound is classed as 'Ames positive'. Ames positivity means development is halted as any toxicity or mutagenicity is impossible to remove from a compound without changing the chemical structure. A common Ames positive functional group is an aromatic amine such as a substituted aniline. Aromatic amines such as substituted anilines are recognised as possible mutagens and are highly linked to cancer.[71] Aniline itself is not mutagenic; however upon phase I oxidative metabolism to 2-aminophenol or 4-aminophenol followed by further oxidative metabolism to form *o*-benzoquinone and *p*-benzoquinone respectively or via *N*-oxidation to form the nitroso-species, aniline can be rapidly metabolised to highly toxic and mutanogenic compounds.[67,72]

2.3 Medicinal Chemistry

The aim of most medicinal chemistry projects is to achieve high enzyme and cellular potency, whilst maintaining selectivity for the desired target and balancing the ADMET properties. For most targets, the biological response to inhibition or activation is a decreased biological response. For the medicinal chemist, the half-maximum inhibitory coefficient (IC_{50}) is commonly reported and measured for antagonist drug potency. This is the concentration of the drug required to inhibit 50 % of the enzyme's activity. The IC_{50} is commonly converted into a negative log scale, i.e. pIC_{50}, to allow easy comparison of data. The pIC_{50} potency can be driven by interactions with the protein, conformational changes, displacement of conserved water molecules and hydrophobic interactions amongst others. One key observation is that increasing the lipophilicity will in general result in an increase in potency due to the hydrophobic effect[73]; however as discussed this increase in lipophilicity will in general lead to poorer ADMET properties.

A way of determining the most efficient groups utilised is through consideration of the ligand efficiency (LE). Kuntz and co-workers[74] first demonstrated that the free energy of ligand binding could be related to the number of 'heavy' atoms, and the term ligand efficiency was then coined by Hopkins.[75] LE can be calculated by dividing the Gibbs free energy (ΔG derived from the interactions between the ligand and protein) by the heavy atom count.

$$\text{Ligand Efficiency (LE)} = \Delta G / \text{HAC}$$

However, as a pIC_{50} value is more readily available, LE can instead be calculated by the following equation.

$$\text{Ligand Efficiency (LE)} = 1.4(pIC_{50}/\text{HAC})$$

Although LE is a useful parameter, it does not take into account the lipophilicity of the molecule, so 'masks' the influence of simply increasing the lipophilicity of the molecule. A modification of LE proposed by Gleeson and Springthorpe, in which the lipophilicity of the molecule would be taken into account, is the lipophilic ligand efficiency (LLE).[76]

$$\text{Lipophilic ligand efficiency (LLE)} = pIC_{50} - \text{cLogP}$$

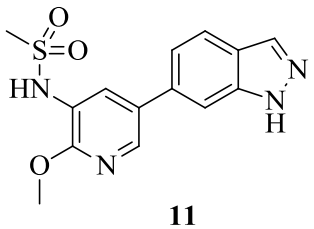
Utilising LE and LLE early within a medicinal chemistry project is important as it can be used to identify the most efficient starting point in order to further optimise the structure of the molecule. In general a higher value of both LE and LLE is favourable, and in general when picking a good lead oral molecule, Reynolds and co-workers[77] suggest that a LE > 0.45 and LLE > 4.4 is optimal. However, Young and co-workers[52,55] report that acceptable values for LE/LLE are highly dependent on the selected biological target. On PI3Kδ projects, a selection of 10 compounds that have good potency and selectivity (pIC_{50} >7.5, selectivity >100 fold) all possessed LE > 0.3 and LLE > 4.5.[78]

Alongside this, other metrics such as PFI can be utilised in order to guide drug discovery. Within GSK, PFI is a highly utilised metric and in general for an oral drug, a value of <6 is desired. However until a scaffold which can be further investigated is discovered, PFI can often be misleading as it can fluctuate depending on the chromlog$D_{pH=7.4}$ of the molecule.

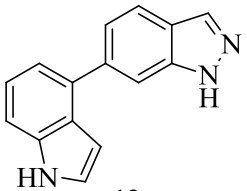
Chapter 3 2-Methoxypyridine replacements

3.1 Introduction

After the discovery of **7** and **8**, work was conducted within GSK which built upon knowledge of the back pocket groups utilised in **7** and **8**. The 2-methoxypyridine fragment **11** is a highly efficient structure with (PI3Kδ pIC_{50} = 6.0 and LE/LLE 0.37/3.7). In comparison, breakdown of **7** to give fragment **12**, was less potent and efficient (PI3Kδ pIC_{50} - = 5.1 and LE/LLE 0.39/1.6) and had four aromatic rings.[36]



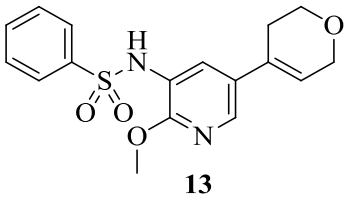
11



12

Using this 2-methoxypyridine back pocket binding motif, an array of new hinge binders was modelled, synthesised and screened in order to find replacements for the indazole.[79] One of the hinge binders found was the dihydropyran **13** (Table 3.1)[36] and its crystal structure is shown in Figure 3.1.[2] Compound **13** has excellent potency for PI3Kδ and possesses some selectivity (10-fold) against the nearest isoform (PI3Kβ).

Table 3.1 Enzyme inhibition data for compound **13**.[36]



13

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**WB IFNγ**
13	5.7 (n=5)	6.4[a] (n=4)	5.9 (n=5)	7.4 (n=4)	5.5 (n=7)

[a]On one occasion, no result was observed.

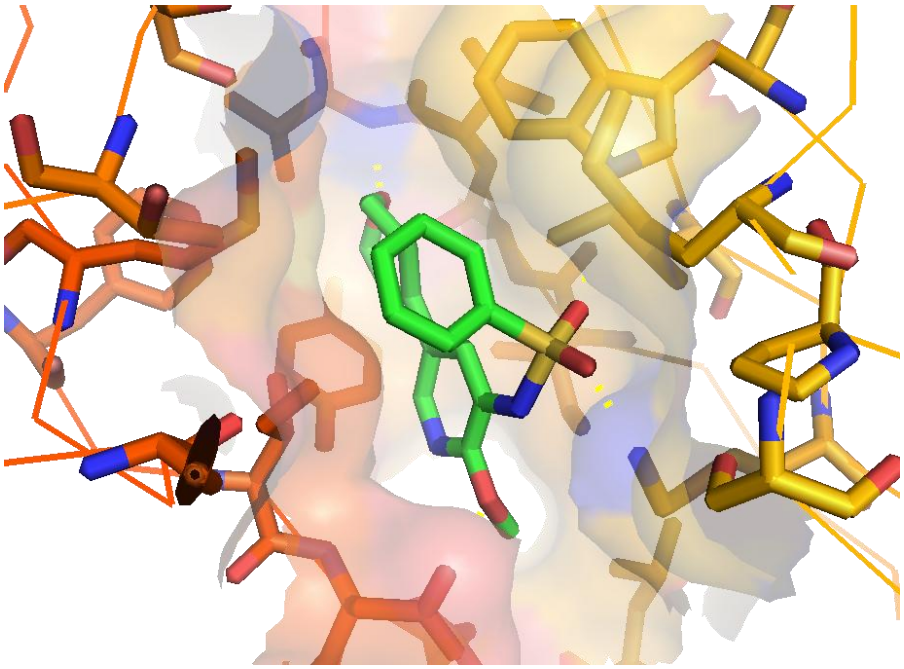


Figure 3.1A. Co-crystal structure of **13** in PI3Kδ (Resolution 2.69 Å).[2]

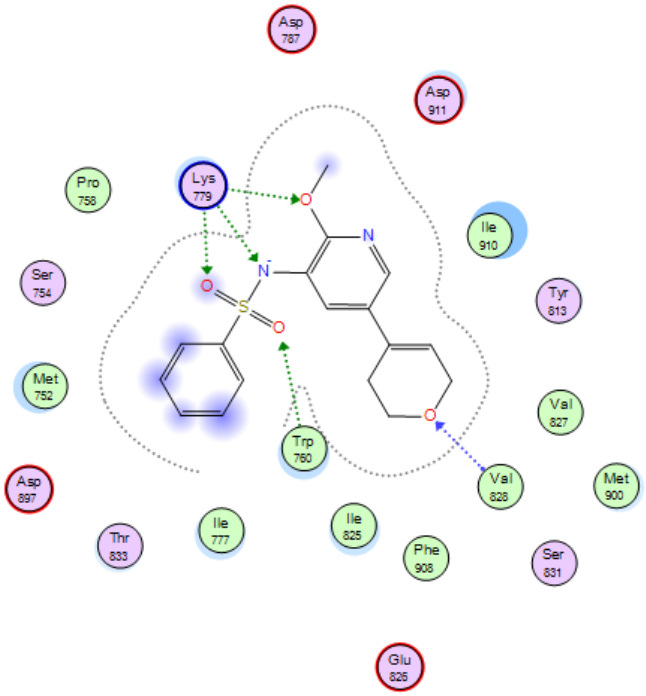


Figure 3.1B 2D representation of **13** binding to PI3Kδ.[2]

From the crystal structure (Figure 3.1A), we observe the dihydropyran binding to the Val828 and the 2-methoxypyridine nitrogen potentially binding to the Asp911 N-H through a

bridging water molecule, however this is not observed via protein crystallography and the 2-methoxypyridine group may not directly interact. However, there are some subtle changes between this and the indazole series. The Lys779 side chain forms a bifurcated hydrogen bond between the sulfonamide S=O and deprotonated sulfonamide nitrogen. The Lys779 residue has moved significantly in comparison to the indazole series. Another interaction observed is between the sulfonamide and the Trp760. In comparison to the indazole compounds **7** and **8**, there is a large twist of the tryptophan residue (Figure 3.2).[2]

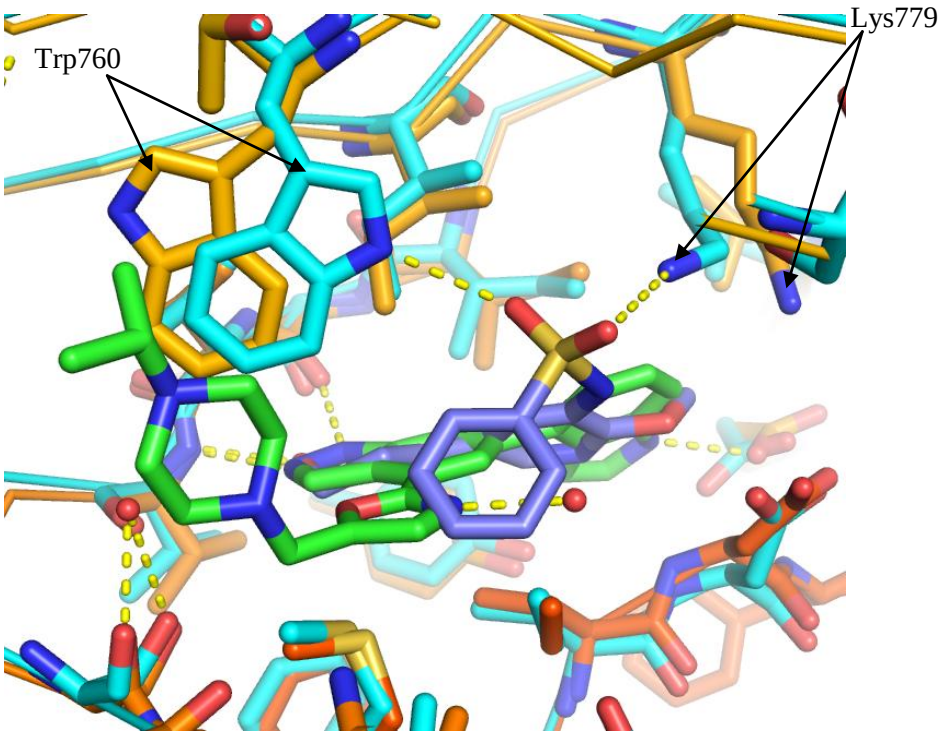
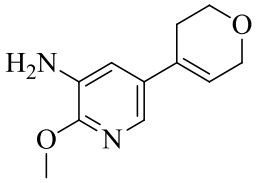


Figure 3.2 The overlay of **13** (blue with cyan protein) and **7** (green with yellow protein) in the PI3Kδ crystal structure showing the twist of Trp760.[2]

This was a positive lead as removal of the indazole was advantageous in relation to the enhanced physical properties such as solubility (31 to 111 μg/mL in **11** to **13**). Removal of the flat hinge binder increases three-dimensional character which can increase selectivity against protein kinases due to this three-dimensional character clashing with the gatekeeper residue. The gatekeeper residue controls access to the hinge region of the protein in which most inhibitors bind. In PI3K's, the gatekeeper residue (Ile777 in PI3Kd) has moved significantly and now resides in the area of the protein commonly known as the back pocket

region. This in turn accommodates more varied hinge binding motifs which can clash against other protein kinases.[80,81] This series had a significant problem in that an intermediate **14** which has the potential to be formed by the cleavage of the sulfonamide bond *in vivo* was Ames positive. The Ames test evaluates compounds for potential mutagenicity by measuring mutations in a set of bacteria that lack the ability to make histidine which is necessary for growth.

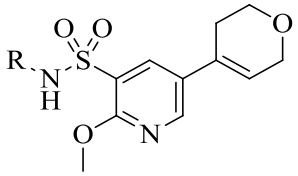


14

At this point, the project team decided to reverse the sulfonamide, therefore keeping a similar structure and hopefully retaining the enzyme potency shown in **13** (Table 3.1).[79] This means that the amines that are added can be known Ames-negative aryl amines.

Initial results were encouraging, as **15** (direct reverse sulfonamide analogue of **13**) displayed similar potency in the enzyme assay in comparison with **13** and against the other PI3K isoforms and similar selectivity to **13**. Incorporation of a *m*-CF_3 aniline **16** resulted in 32-fold selectivity against PI3Kα, PI3Kβ and PI3Kγ (Table 3.2).[36] This *m*-CF_3 group occupies a lipophilic pocket within PI3Kδ which increases the potency and selectivity. Also a *N*-Me sulfonamide **17** retains some potency and selectivity, indicating that removal of the aromatic ring is not completely detrimental to potency. However, further work on this template demonstrated that further elaboration from alkyl sulfonamides such as **17** was not beneficial.

Table 3.2 Enzyme and whole blood inhibition data for compounds **15**, **16** and **17**.[36]



	pIC$_{50}$					
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**WB IFNγ**	**LE/LLE**
15 (R = Ph)	5.4 (n=10)	5.6 (n=9)	5.1[a] (n=7)	6.7 (n=8)	5.4[b] (n=8)	0.38/4.0
16 (R=*m*-CF$_3$C$_6$H$_4$)	6.0 (n=3)	6.2 (n=4)	5.3 (n=3)	7.7 (n=3)	5.0[c,d] (n=1)	0.38/4.1
17 (R = Me)	5.2 (n=7)	5. 5[e] (n=6)	4.7 (n=7)	6.2 (n=7)	5.5 (n=6)	0.45/5.2

[a]On two occasions, a result of <4.5 was received. [b]On four occasions, no result was observed. [c]On one occasion, a result of <4.4 was received. [d]On three occasions, a result of <5.0 was received. [e]On one occasion, no result was observed.

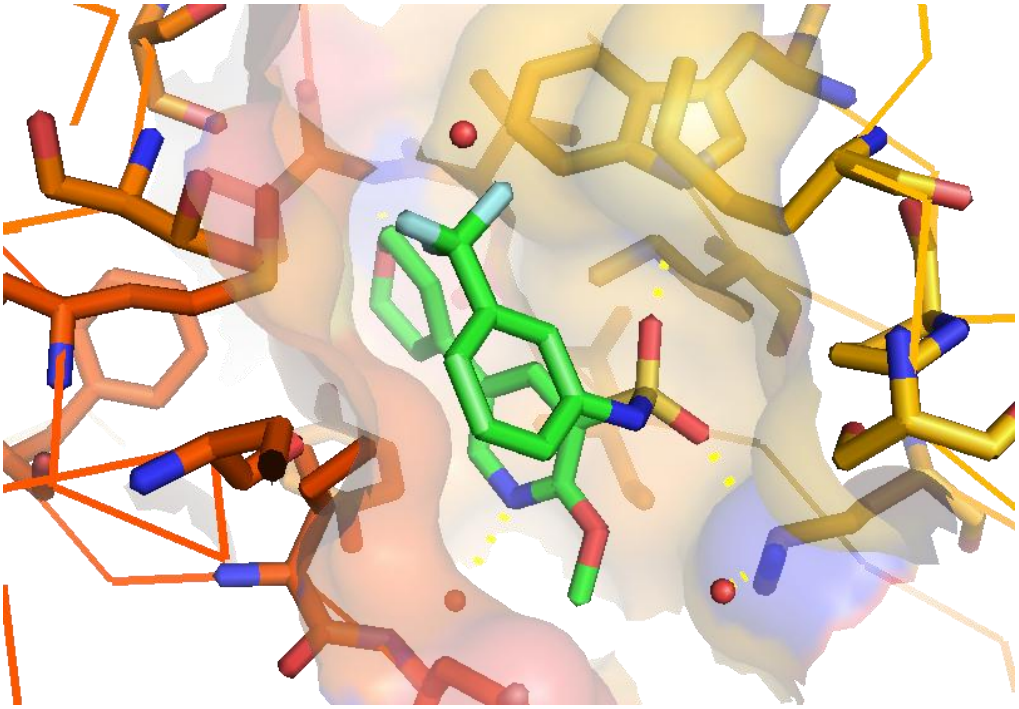


Figure 3.3A. Co-crystal structure of **16** in PI3Kδ (Resolution 2.39 Å).[2]

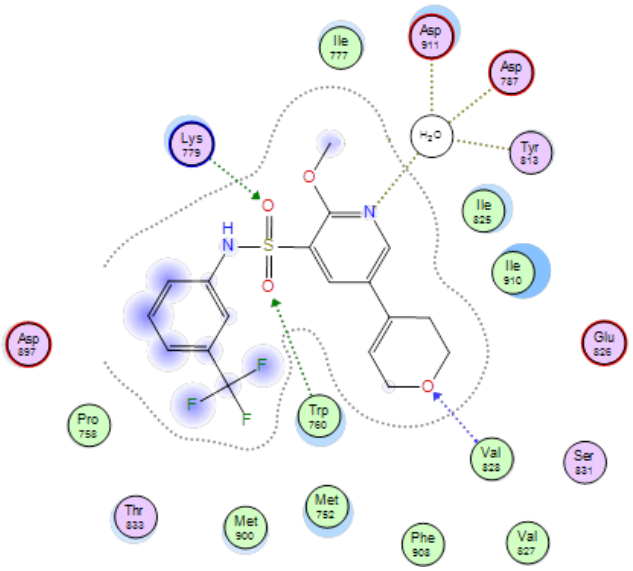


Figure 3.3B 2D representation of **16** binding to PI3Kδ.[2]

From the crystal structure (Figure 3.3) of compound **16**, some similarities with the forward sulfonamide compound **13** (Figure 3.1) can be observed such as the tryptophan twist and the movement of the lysine. However there are some different interactions. The pyridine nitrogen now binds to the protein backbone through a clearly observed bridging water molecule. The sulfonamide S=O bonds now form the key interactions as they accept hydrogen bonds from the Trp760 and Lys779.

The following crystal structure shows the overlap between the forward compound **13** and reverse sulfonamide compound **16** (Figure 3.4).[2]

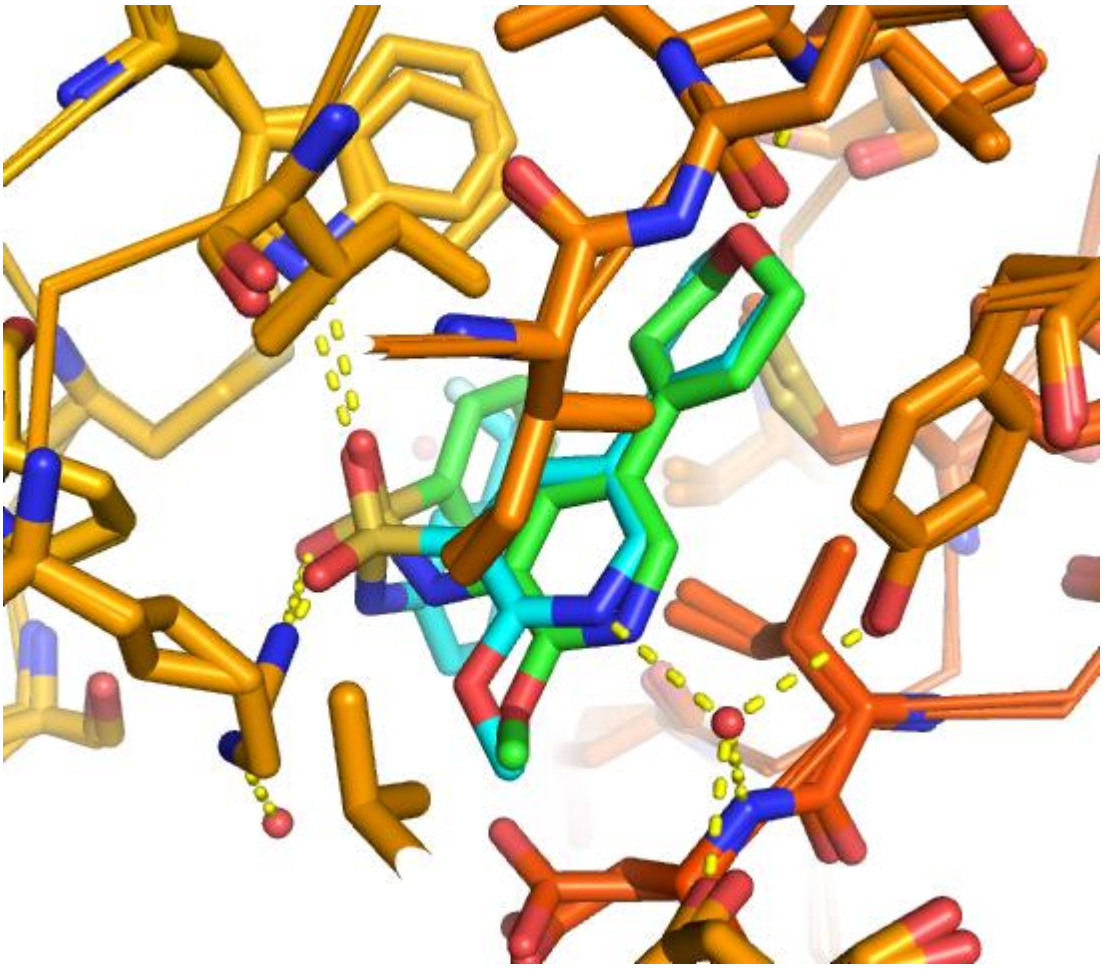


Figure 3.4 The overlay of reverse sulfonamide **16** (cyan) and forward sulfonamide **13** (green), highlighting the water molecule that is observed in the reverse sulfonamide (cyan).

The movement away from the backbone for the reverse sulfonamide **16** compared to the initial forward sulfonamide **13**, is significant in that the reverse sulfonamide **16** binds through an observed water molecule in the active site (this water molecule is conserved in most of the reverse sulfonamide crystal structures). The reverse sulfonamide **16** therefore interacts with the Tyr813 and Asp787 residues through a bridging water molecule.

3.2 Aims

The aim of this work is identify and synthesise novel groups to occupy the back pocket region of the protein in order to replace 2-methoxypyridine moiety in **15**. The novel groups will be predicted using computational experiments in which the shape and electronic requirements of the protein backbone both with the bridging water included (observed in Figure 3.3A) and removed will be investigated. From this the 2-methoxypyridine replacements will be evaluated and selected for synthesis.

The back pocket groups will utilise the reverse pyridine sulfonamide and dihydropyran moieties in order to provide a benchmark upon which the novel back pocket group can be evaluated and compared with **15**. The new back pocket groups could either displace the water molecule or bind through it (Figure 3.5).

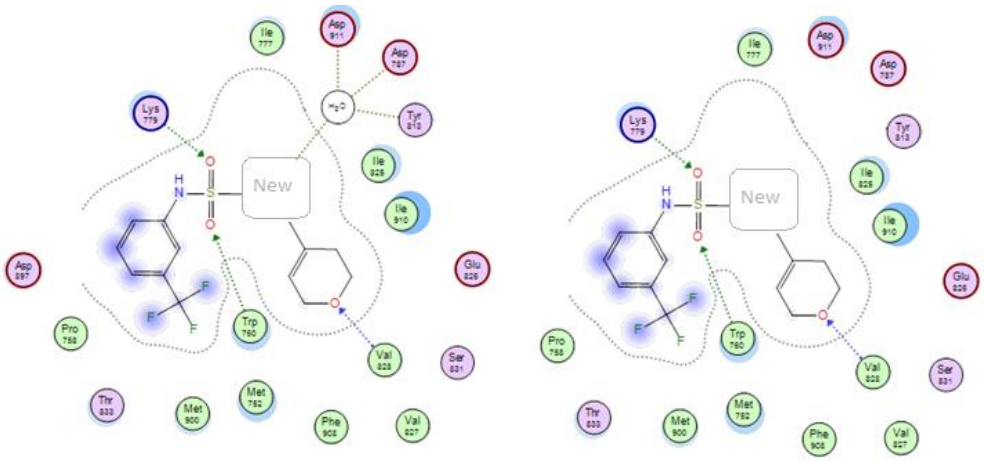


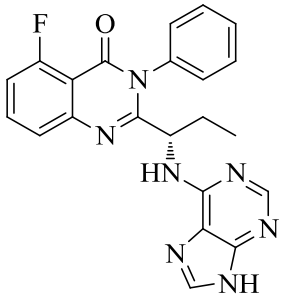
Figure 3.5 A graphical representation of back pocket replacements binding through or displacing the water molecule.

Ideally, a lead compound would meet the following criteria:

- PI3Kδ pIC_{50} > 6
- Good ligand and lipophilic ligand efficiencies LE > 0.3, LLE > 4

- Some evidence of isoform selectivity
- cLogP < 2.5
- Aromatic ring count < 3
- Moderate AMP permeability > 50 nm/s
- Moderate CLND solubility > 30 μg/mL

These criteria would allow the selection of a novel chemical entity to then further investigate, with the aim to boost potency and selectivity whilst maintaining good physicochemical properties to enter preliminary pharmacokinetic analysis *in vitro*. In order to progress any compound further than this, the compound would have to have some advantage over Idelalisib **4**, the current marketed PI3Kδ inhibitor.



4

	Idelalisib 4
PI3Kδ	8.1
PI3Kα/β/γ	5.0/5.8/6.6
LE/LLE	0.36/4.4
WB IFN γ	6.7
Solubility	126 μg/mL
Permeability	410 nm/sec
cLogP	3.6
Number of aromatic rings	5
PFI	8.2

Chapter 4 Replacements for 2-methoxypyridine: Results and Discussion

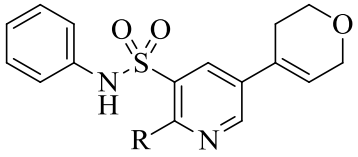
Identification of new back pocket-binding groups for the replacement of the 2-methoxypyridine fragment used a combination of the crystal structure of **16** (R= CF_3) in the PI3Kδ protein (Figure 3.3), BROOD calculations and Molecular Operating Environment (MOE™) in order to generate a variety of pyridine replacements.[82,83]

BROOD is a fragment-based drug design tool which searches a database of fragments to identify similar structures to the query with regards to their shape and electronic properties, in order to develop new leads and/or series (for a review of BROOD see review[84]). For the BROOD calculations the "reverse sulfonamide" and the dihydropyran moieties were not changed, so only replacements of the 2-methoxypyridine were queried. Two calculations were carried out, the first where the 2-methoxypyridine alone was queried for replacement and the second where the bridging water molecule and the 2-methoxypyridine were queried together. MOE™ was utilised to determine the strength and length of the hydrogen bond for this query. A number of different properties including shape and electrostatics were used to scan a database for potential replacements. Then by using the crystal structure of **13** and the PI3Kδ protein (Figure 3.3), the replacement fragments were retained or eliminated based on how they fitted and interacted with the binding pocket. This led to a set of compounds where some would preserve the bridging water molecule and some would displace it.[82-84]

The modelling gave a list of over 50 compounds, which were then ranked using the Admantis Pro add-on in Spotfire. This additional ranking excludes compounds with undesirable chemical properties (such as high cLogP, number of aromatic rings etc) leaving compounds in developable space. Around 25 compounds remained, and a number were prioritised for synthesis.

A key point to start with was a deconstruction of 2-methoxypyridine **15**. Pyridine **18** had previously been synthesised and showed slightly diminished potency and selectivity in comparison with **15 (**Table 4.1**)**.[79]

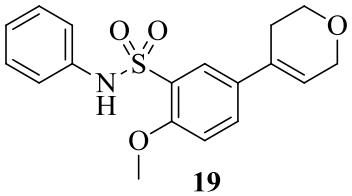
Table 4.1 Enzyme inhibition data for compounds **15** and **18**.[36]



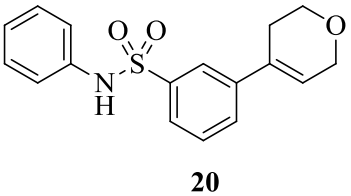
	pIC$_{50}$			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
15 (R= OMe)	5.4 (n=10)	5.6 (n=9)	5.1[a] (n=7)	6.7 (n=8)
18 (R= H)	5.6 (n=4)	5.8 (n=2)	5.5 (n=2)	6.4 (n=2)

[a]On two occasions, a result of <4.5 was received.

From the BROOD screen, methoxybenzene **19** was suggested as a core replacement to probe the requirement for a HBA interaction with the bridging water molecule. Benzene **20** would also be synthesised to have a baseline for the series.

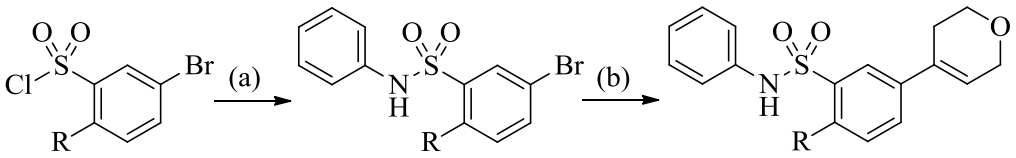


19



20

From sulfonyl chlorides (**21** or **22**) the following scheme was followed (Scheme 4.1).



21 R= OMe
22 R= H

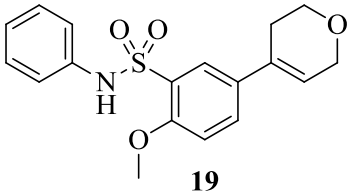
23 (a) R= OMe
24 (a) R= H

19 (c) R= OMe
20 (c) R= H

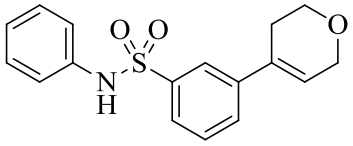
(a) Aniline, pyridine, rt, 3-5 h (**23** = 84 %, **24** = 81 %), (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), Na_2CO_3, 1,4-dioxane and H_2O, μw, 80-120 °C, 30-60 min (**19** = 41 %, **20** = 57 %).

Scheme 4.1 Synthesis of compounds **19** and **20**.

Table 4.2 Enzyme inhibition data for compounds **19** and **20**.[36]



19



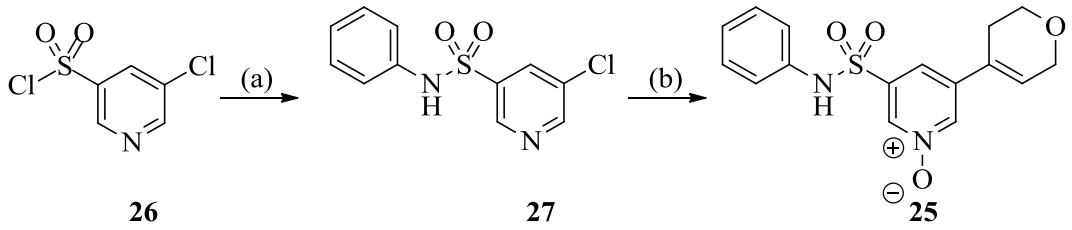
20

	pIC$_{50}$			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
19	<4.5 (n=2)	4.7 (n=2)	<4.5 (n=2)	5.5 (n=2)
20	<4.5[a] (n=2)	5.0[a] (n=2)	4.9[a] (n=2)	5.2 (n=3)

[a]On one occasion, a result of <4.3 was received.

Both compounds showed a decrease in PI3Kδ potency, with **19** 19-fold less active than **15**. This indicates that the HBA interaction at this position with the bridging water molecule makes a significant contribution to the activity (Figure 3.3).

A compound of interest with a similar framework to pyridine **18** is a pyridine *N*-oxide. Pyridine *N*-oxides are common intermediates in the synthesis of compounds due to their increased reactivity with nucleophiles, but are not common drug discovery motifs, other than as metabolites of pyridines.[85] However, there are drugs on the market that possess *N*-oxides (Chlordiazepoxide[86] and Minoxidil[87]). By replacing the pyridine **18** with the pyridine *N*-oxide to give **25**, it could be hypothesised that the oxygen could bind through the water molecule by shifting the water molecule closer to the backbone, or by displacing it entirely. If the water was displaced and some potency maintained, this could indicate the viability of displacing the bridging water molecule. The following scheme was proposed (Scheme 4.2).



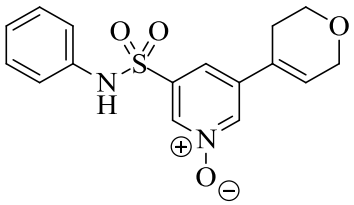
26 **27** **25**

(a) Aniline, pyridine, rt, 1 h (16 %); (b) i) H_2O_2, H_2O, AcOH, 85 °C, 2 h; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 100 °C, 30 min (19 %).

Scheme 4.2 Synthesis of *N*-oxide **25**.

From the commercially available sulfonyl chloride **26**, sulfonamide **27** was formed in poor yield (16 %). Formation of the *N*-oxide was achieved under acidic oxidative conditions and this was used crude in the final Suzuki cross-coupling to give **25** after two purifications in poor yield (19 %).

Table 4.3 Enzyme inhibition data for compound **25**.[36]



	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
25	4.8 (n=4)	5.0 (n=4)	5.1 (n=3)	5.8 (n=4)

In comparison with pyridine **18**, 0.6 log units of activity were lost at PI3Kδ for N-oxide **25**, indicating that its inclusion was not beneficial. The binding mode of **25** was elucidated via protein crystallography (Figure 4.1).

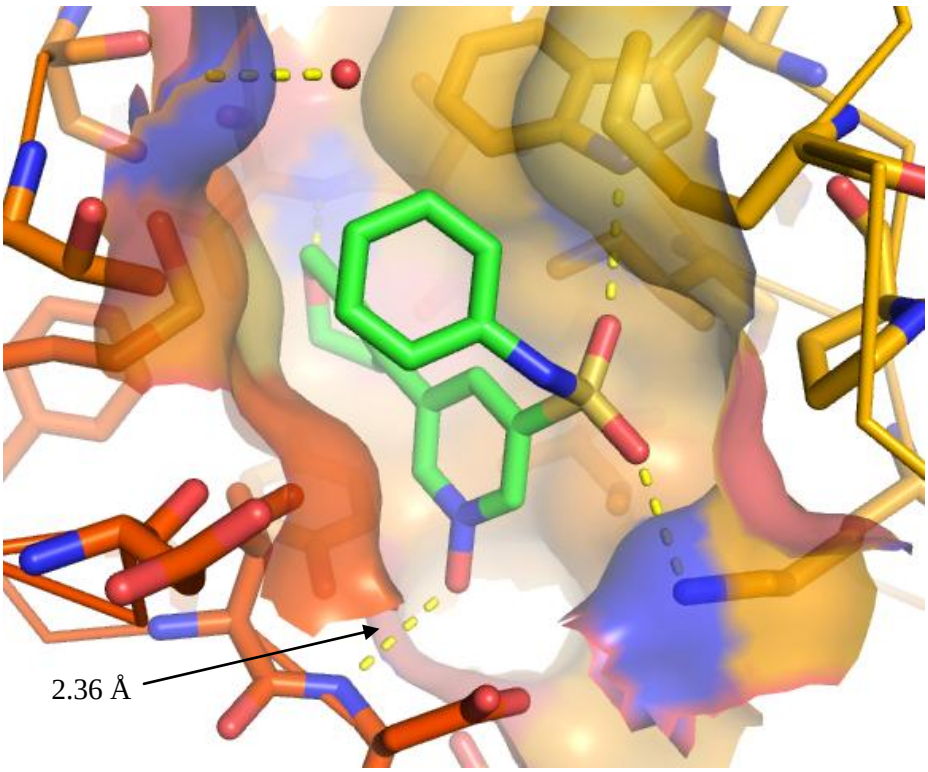


Figure 4.1A Co-crystal structure of **25** in PI3Kδ (Resolution 2.86 Å).[2]

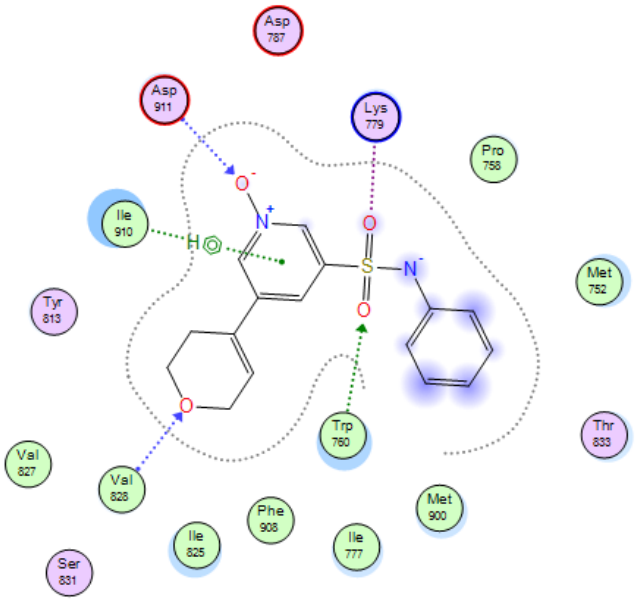


Figure 4.1B 2D Representation of **25** binding to PI3Kδ.

The crystal structure of **25** (Figure 4.1) demonstrated the dihydropyran accepting a hydrogen bond from Val828, the tryptophan twist and lysine movement as observed in **12** and **15**. In the back pocket region, the oxygen of the *N*-oxide acts as a HBA to the backbone N-H of Asp911 and the water molecule is displaced. The *N*-oxide can only make one HBA interaction with the backbone compared to the three interactions that can be made through the water molecule of **18** to Asp911, Asp787 and Tyr813 and this may explain the reduced potency. An overlay of the crystal structure of N-oxide **25** and **18** (Figure 4.2) demonstrates no movement away or towards the backbone, indicating a clear displacement of the bridging water molecule.

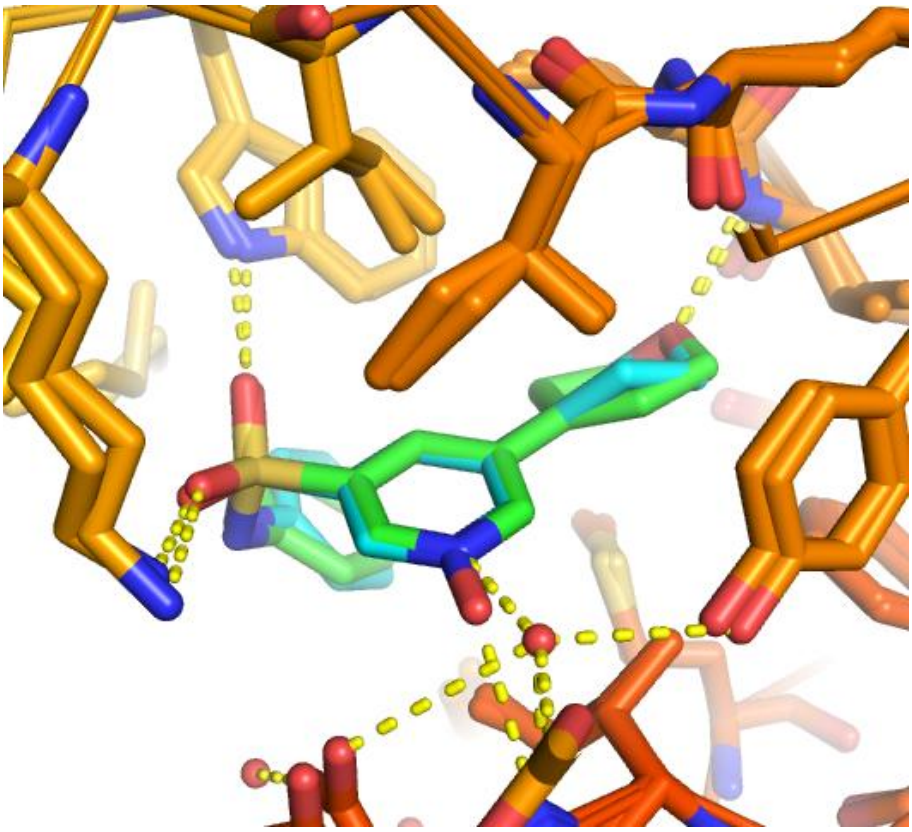
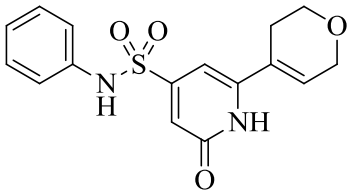
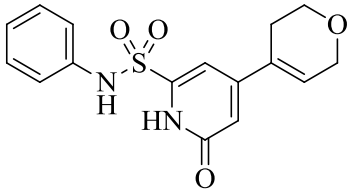


Figure 4.2 An overlay of **25** (cyan) and **18** (green).

Although *N*-oxide **25** was less active than pyridine **18**, this result was positive in that it demonstrated that the bridging water molecule could be displaced whilst maintaining some potency. From the BROOD screen and evaluation of the literature, two compounds possessing pyridones (**28** and **29**) were suggested.



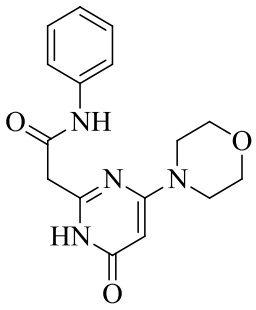
28



29

One of these motifs was utilised in a similar back pocket utilised by Sanofi, who investigated compounds for the selective inhibition of PI3Kβ.[88] Exemplar **30** from the series is shown below and displays excellent potency for PI3Kβ but is not selective against PI3Kδ (Table 4.4).

Table 4.4 Reported inhibition of **30**.[88]



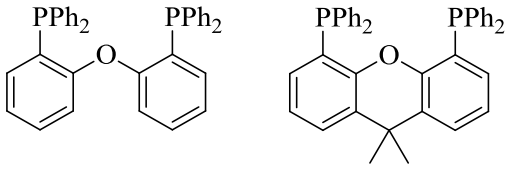
30

	pIC$_{50}$			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
30	5.7	7.4	<5	6.9

Via protein crystallography of the compound in PI3Kγ, the authors demonstrate that the pyrimidone occupies the back pocket region, however no water molecule was observed. Recent literature[89] on this chemical series has elucidated the structure of a pyrimidone bound to the PI3Kβ protein, in which the compound binds through the bridging water molecule to the backbone. Due to the similar homology between PI3Kδ and PI3Kβ, the two pyridones selected for synthesis could bind through the bridging water molecule and retain the beneficial interactions it forms.

For compound **28**, 2,6-dichloro-4-iodopyridine was an ideal starting material as stepwise functionalisation could yield **28**. The first step utilised a palladium-catalysed cross coupling of halides and thiols, which was first reported by Migita and co workers[90] in 1980. Their initial work utilised aryl iodides, thiols, a strong base such as sodium *tert*-butoxide and a palladium(0) source such as $Pd(PPh_3)_4$. The reaction mechanism was studied and is believed to be similar to a Buchwald-Hartwig cross coupling of aryl halides and alkoxides.[90,91]

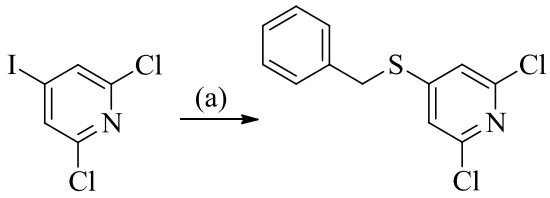
Further work in this area has utilised the bidentate phosphine ligands developed by Buchwald and co-workers such as DPEPhos and XantPhos (Figure 4.3).[92] These have been utilised by Schopfer and co-workers[93] and Itoh and co-workers[94] using a weaker base such as diisopropylethylamine (DIPEA) on more diverse substrates in good yields.



DPEPhos XantPhos

Figure 4.3 Buchwald ligands DPEPhos and XantPhos.[92]

Previous work within GSK has used the procedures by Itoh and co-workers[94] in order to form aryl-benzyl sulfides. The following reaction was carried out (Scheme 4.3).



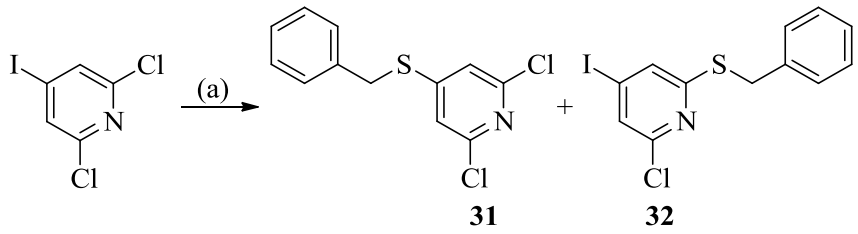
31

(a) Benzyl mercaptan, $Pd_2(dba)_3$, XantPhos, DIPEA, toluene, μw, 100 °C, 1 h (quantitative)

Scheme 4.3 Synthesis of intermediate **31**.

This reaction (Scheme 4.3) was initially selective for the 4-substituted product (using 1.1 eq of benzyl mercaptan), due to the weaker carbon-iodine bond undergoing oxidative addition to the palladium preferentially over the carbon-chlorine bonds. However due to the similarity in lipophilicity between both starting materials and products, isolation via normal phase chromatography was problematic. The poor separation made it vital to push all the starting iodide through to completion, so additional benzyl mercaptan (0.5 eq.) was utilized. However, this led to some formation of the chloro-displaced product possibly through the S_NAr displacement of the C-Cl bond. The reaction proceeded quantitatively (92 % purity, 3 % chloro-displaced product) after 2 purifications. Despite this an alternative route was desired due to the problematic purification and handling issues.

An alternative method to synthesise **31** was the S_NAr displacement of 2,6-dichloro-4-iodopyridine under basic conditions. This approach would hopefully yield both 4- and 2-substituted molecules (**31** and **32** respectively) in order to be used for **28** and for the synthesis of **29**. The following phase transfer catalysed reaction was carried out (Scheme 4.4).[95]



31 **32**

(a) Benzyl mercaptan, NaOH, TBAB, H_2O, toluene, 110 °C, 19 h (**31** 50-66 % and **32** 11-28 %).

Scheme 4.4 Synthesis of intermediates **31** and **32**.

This reaction proceeded with fewer side-products than the palladium-cross coupling and over 6 experiments, an average 4:1 ratio of pure isolated **31** and **32** was obtained after C_{18} reverse phase column chromatography.

For the synthesis of **28**, the next transformation was the oxidative chlorination of the benzyl sulfide **31** to the corresponding sulfonyl chloride **33** (Scheme 4.5). This can be achieved by either chlorine gas in acid, first shown by Dougherty and co-workers in 1940,[96] or via in *situ*

chlorine formation using *N*-chlorosuccinimide in aqueous acid, first published by Kim and co-workers in 1992.[97] Work within GSK[79] has shown that using Kim's method,[97] high yields of the related sulfonyl chloride can be achieved when adding *N*-chlorosuccinimide portion-wise. Although the mechanism has not been fully elucidated, work by Spalding and co-workers[91] has shown that the mechanism proceeds through a series of chlorosulfonium cations which are then quenched by water, whilst the benzyl group is eliminated via an S_N1 mechanism (Figure 4.4).

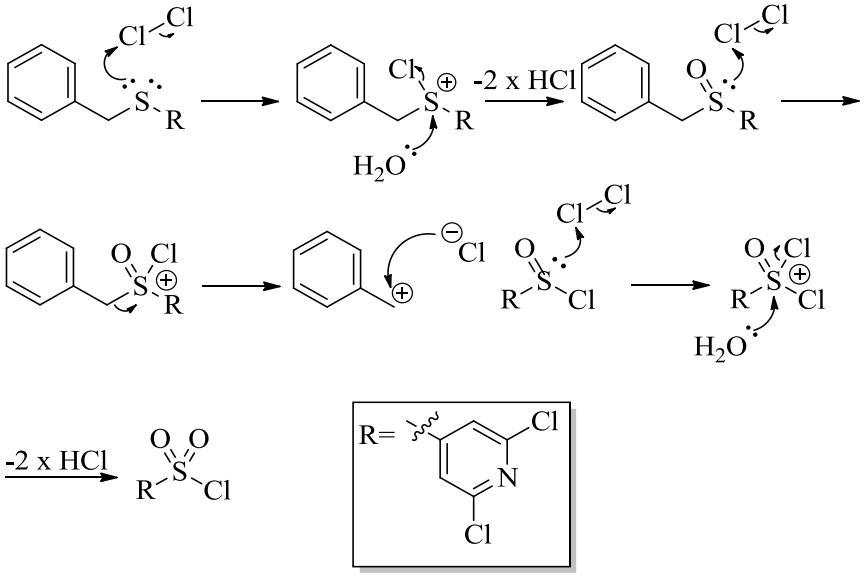
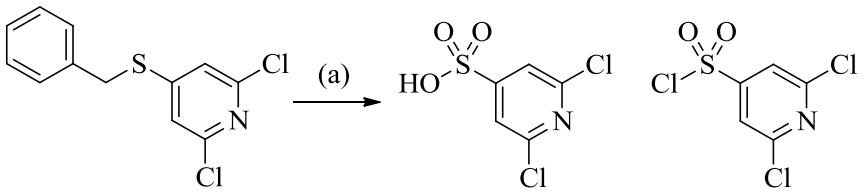


Figure 4.4 Oxidative chlorination mechanism.



31 **33** **34**

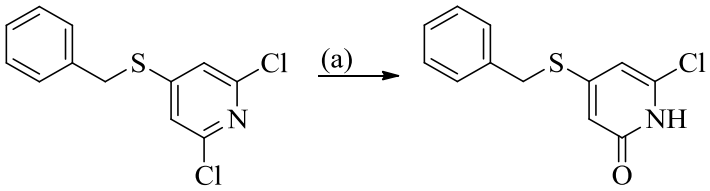
(a) i) $HCl_{(aq)}$, MeCN, rt; ii) NCS, rt.

Scheme 4.5 Oxidative chlorination of **31**.

However on **31**, the reaction yielded the sulfonic acid **33** with no trace of the sulfonyl chloride **34** in 3 experiments, with portion-wise additions of NCS over different times (20, 60 and 120 min). Attempts to purify the acid were unsuccessful due its high aqueous solubility and its lack of separation from the succinimide by-product.[91] The sulfur atom is electron-deficient in **31**, due to the effect of two chlorides and the pyridine ring nitrogen

withdrawing electron density. So upon formation of the sulfonyl chloride, it could be hypothesised that the rapid hydrolysis of the sulfonyl chloride to the sulfonic acid occurs. If the electronics of the ring were changed in order to make the ring more electron-rich, then the sulfonyl chloride would be less likely to hydrolyse upon formation.

Alternatively pyridone **35** was formed (Scheme 4.6) by displacement with potassium t-butoxide, followed by the loss of 2-methylpropene under acidic conditions and this proceeded in good yield (85 %).

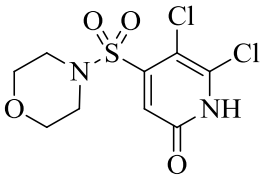


31 **35**

(a) i) ^{t}BuOK, ^{t}BuOH, 100 °C, 17 h; ii) HCl, rt, 20 min (85 %).

Scheme 4.6 Synthesis of pyridone **35**.

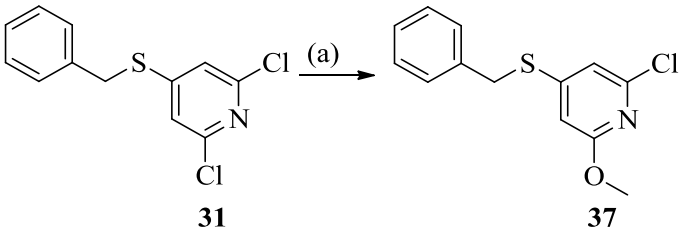
Compound **35** was then subjected to the oxidative chlorination of the benzyl sulfide under the same conditions used previously (Scheme 4.6). No sulfonic acid was observed by crude LCMS (with addition of morpholine to observe sulfonamide), however a new product was formed which ionised as the desired compound but with an additional chlorine. In order to investigate the by-product, a test reaction was utilised in which the oxidative chlorination of the benzyl sulfide was quenched with excess morpholine. This product was then isolated to give **36**.



36

Chlorination occurred in the 3-position of the pyridone ring, observed via NMR, indicating that the pyridone was susceptible under the conditions used. This led to this route being down-prioritised.

An alternative route to increase the electron density of **30** was then undertaken (Scheme 4.7) which involved the displacement of a chloride to give the methoxypyridine **37**.

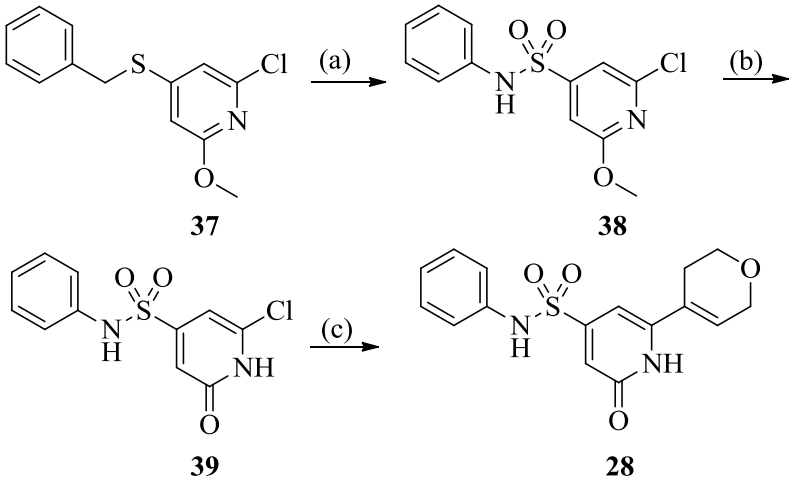


31 **37**

(a) NaOMe, MeOH, 65 °C, 106 h (92 % crude yield).

Scheme 4.7 S_NAr displacement of **31** with methoxide.

The increase in electron density was observed by the ^1H NMR shifts of the heteroaromatic protons (7.08 ppm in **31** to 6.79 and 6.49 ppm in **37**). The following synthetic route was followed to access **28** (Scheme 4.8).



37 **38**

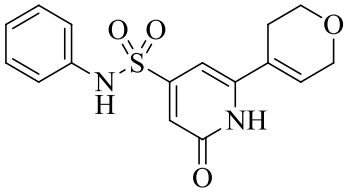
39 **28**

(a) i) $HCl_{(aq)}$, MeCN, rt, 1 h; ii) NCS, 1 h ; iii) aniline, pyridine, rt, 72 h (28 %); (b) TMS-I, MeCN, 65 °C, 1 h (27 %); (c) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 120 °C, 30 min (53 %)

Scheme 4.8 Synthesis of pyridone **28**

This subtle change resulted in the successful formation of the sulfonyl chloride with no evidence of the sulfonic acid or over-chlorination. The sulfonyl chloride formed was quenched with aniline to form **38**. Demethylation of **38** utilising trimethylsilyl iodide (TMS-I)[98,99] proceeded in low yield to give **39** (27 %). Suzuki cross-coupling gave **28** in moderate yield (53 %).

Table 4.5 Enzyme inhibition data for compound **28**.[36]



	pIC$_{50}$			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
28	<4.5 (n=2)	4.6[a] (n=1)	<4.5 (n=2)	5.1 (n=2)

[a]On one occasion, a result of <4.5 was received.

The results for **28** are disappointing; the poor activity at PI3Kδ indicates that the pyridone does not make any complementary interactions with the backbone. By having the pyridone N-H *ortho* to the dihydropyran, there is a potential clash with the Tyr813 residue in the backbone (Figure 4.5). Modelling suggests that the distance is around 3 Å from the π-cloud of the aromatic ring and the pyridone N-H.

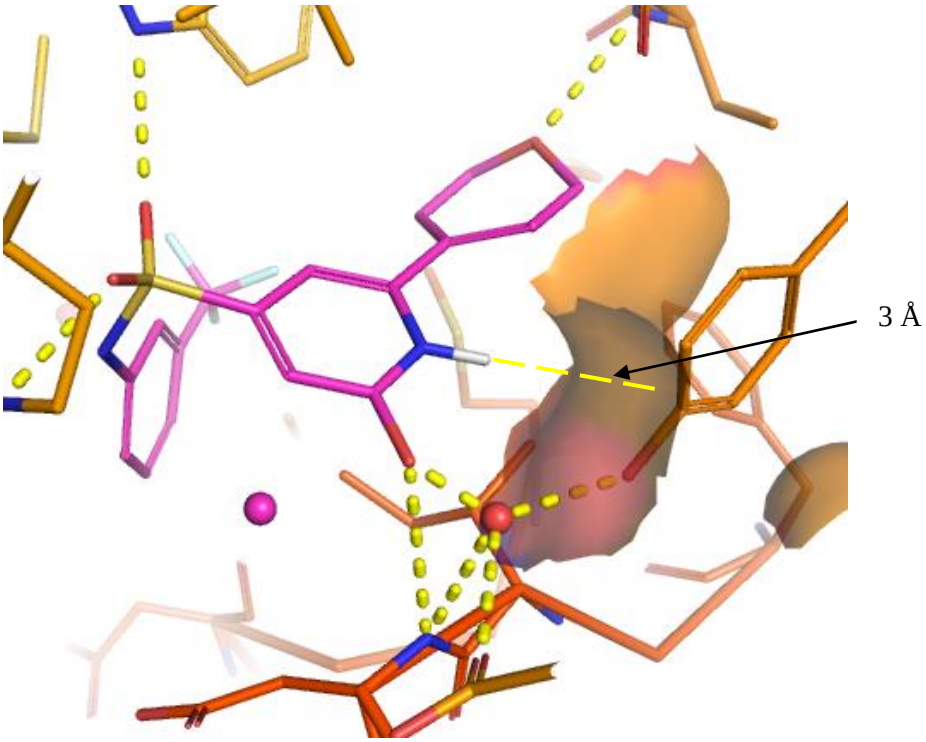
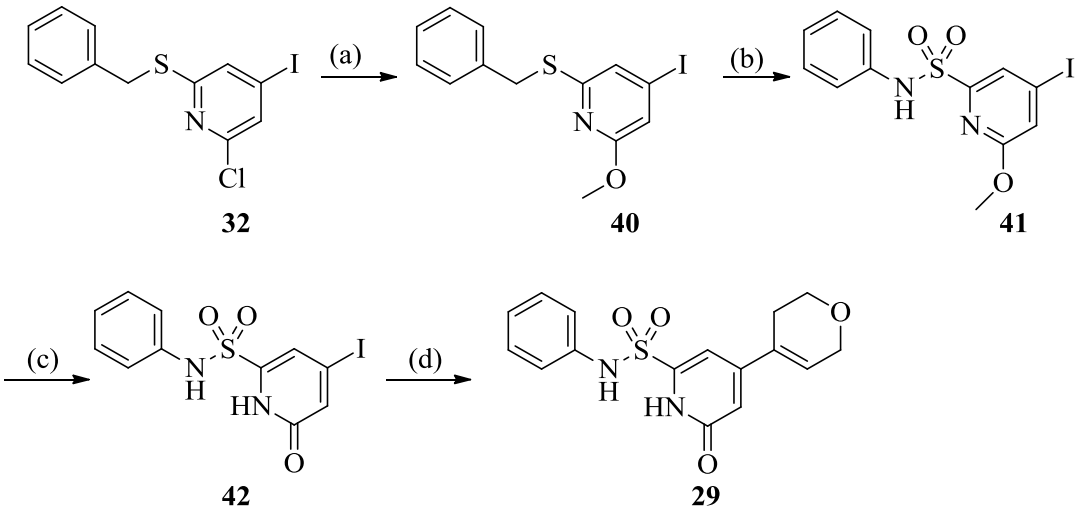


Figure 4.5 Modelling the potential clash of Tyr813 and **28**.

This clash could explain the poor activity at PI3Kδ. No further compounds with N-H *ortho* to the dihydropyran were selected for synthesis.

For the synthesis of **29**, previously synthesised **32** was exposed to oxidative chlorination conditions. On a small scale, initial work suggested that the sulfonyl chloride was formed via LCMS analysis upon quenching with morpholine. However, upon treatment of the crude sulfonyl chloride with anhydrous pyridine and aniline, no reaction occurred giving the sulfonic acid upon workup of the reaction mixture. This result and previously gained knowledge from the synthesis of pyridone **28**, guided the synthesis of **29** via the methoxy substituent **40** (Scheme 4.9).

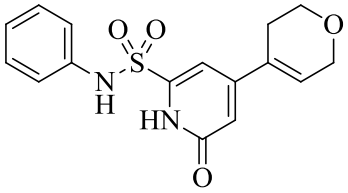


(a) NaOMe, MeOH, 100 °C, 2 h (13 %); (b) i) $HCl_{(aq)}$, MeCN, rt, 1 h; ii) NCS, rt, 1 h ; iii) aniline, pyridine, 1,4-dioxane, rt, 2 h (43-55 %); (c) TMS-I, MeCN, μw, 65 °C, 1 h (53 %); (d) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 120 °C, 1 h (15 %).

Scheme 4.9 Synthesis of pyridone **29**.

The sodium methoxide displacement of **32** in methanol at 65 °C resulted in poor conversion (less than 10 % by LCMS) to the desired methoxypyridine **40**. Alternatively upon increasing the reaction temperature to 100 °C in a sealed tube, moderate conversion (25 % by LCMS) was observed. Careful purification gave the 6-methoxy product **40** in poor yield (13 %). Oxidative chlorination of **40** under standard conditions gave good conversion to the sulfonyl chloride, which was further reacted to give sulfonamide **41** in moderate yield (43-55 %). Cleavage of the methoxy group with TMS-I gave pyridone **42** in moderate yield (53 %). Finally, Suzuki cross-coupling gave **29** in poor yield (15 %).

Table 4.6 Enzyme inhibition data for compound **29**.[36]



	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
29	5.2 (n=3)	6.2 (n=3)	5.3 (n=3)	6.8 (n=3)	0.41/6.0

Pyridone **29** is a potent PI3Kδ inhibitor, with excellent efficiency and selectivity against PI3Kα and PI3Kγ. However it is not selective against PI3Kβ (4- fold). Pyridone **29** is equipotent and of similar efficiency with 2-methoxypyridine **15** and is therefore an interesting motif. The binding mode is as follows (Figure 4.6).

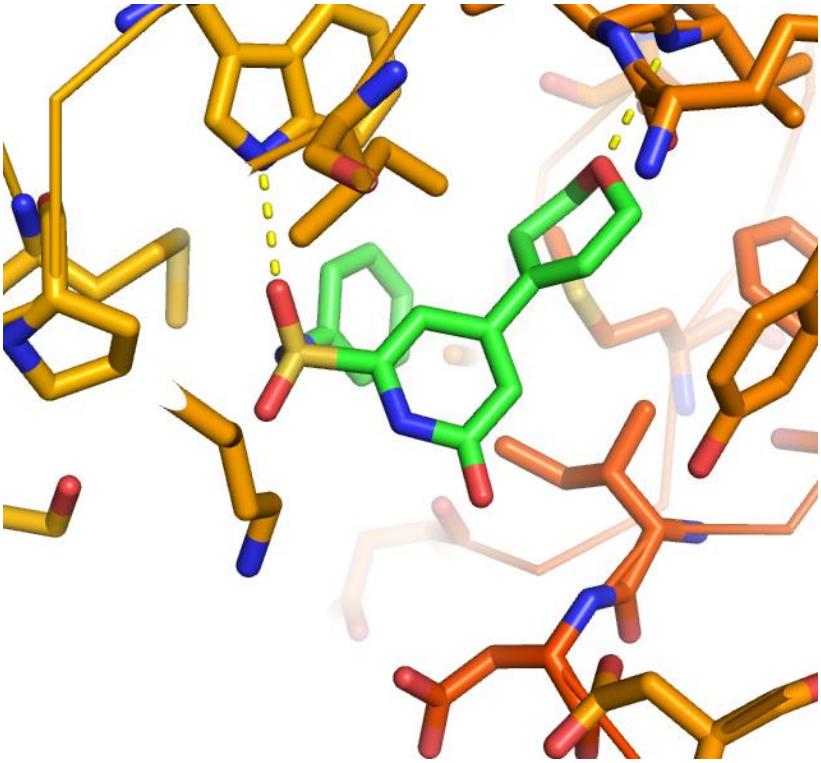


Figure 4.6A Co-crystal structure of **29** in PI3Kδ (Resolution 2.76 Å).[2]

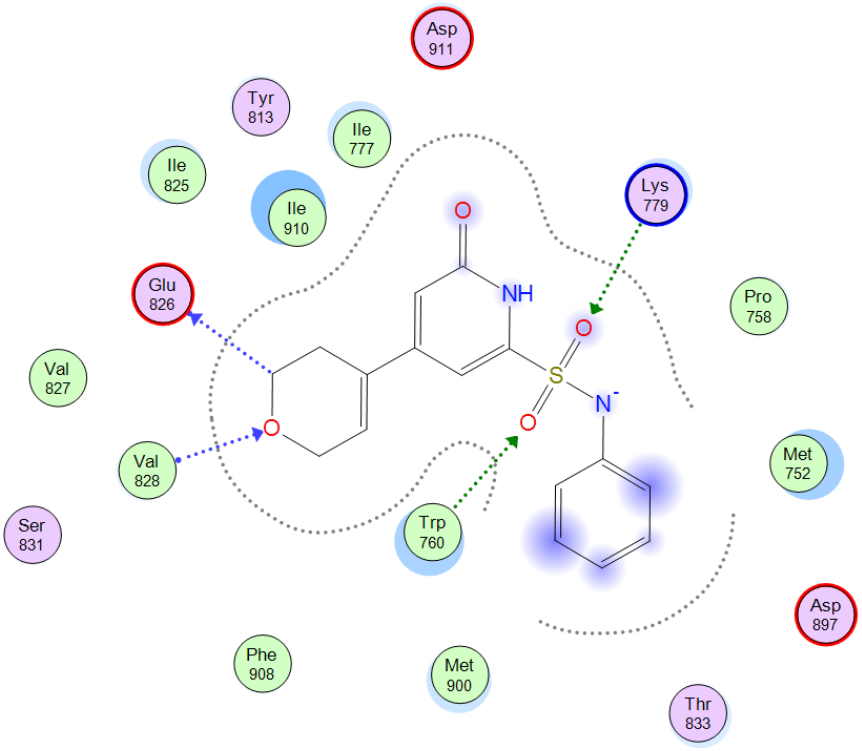


Figure 4.6B 2D Representation of **29** binding to PI3Kδ.

From the crystal structure, the dihydropyran forms the key hinge binding interaction with Val828, the sulfonamide S=O interacting with the 'in' conformation of Trp760 and second S=O interacting with the Lys779. In the back pocket region, no interactions or conserved water molecules were observed. However previously, we observed that compounds that made no interaction in the back pocket region of the protein (such as benzene **20** pIC_{50} 5.2) had poor activity. A plausible explanation of this activity could be down to an unresolved water molecule. For example, an overlay of 2-methoxypyrine **16** and pyridone **29** is shown (Figure 4.7) which clearly shows that a water molecule could be accommodated.

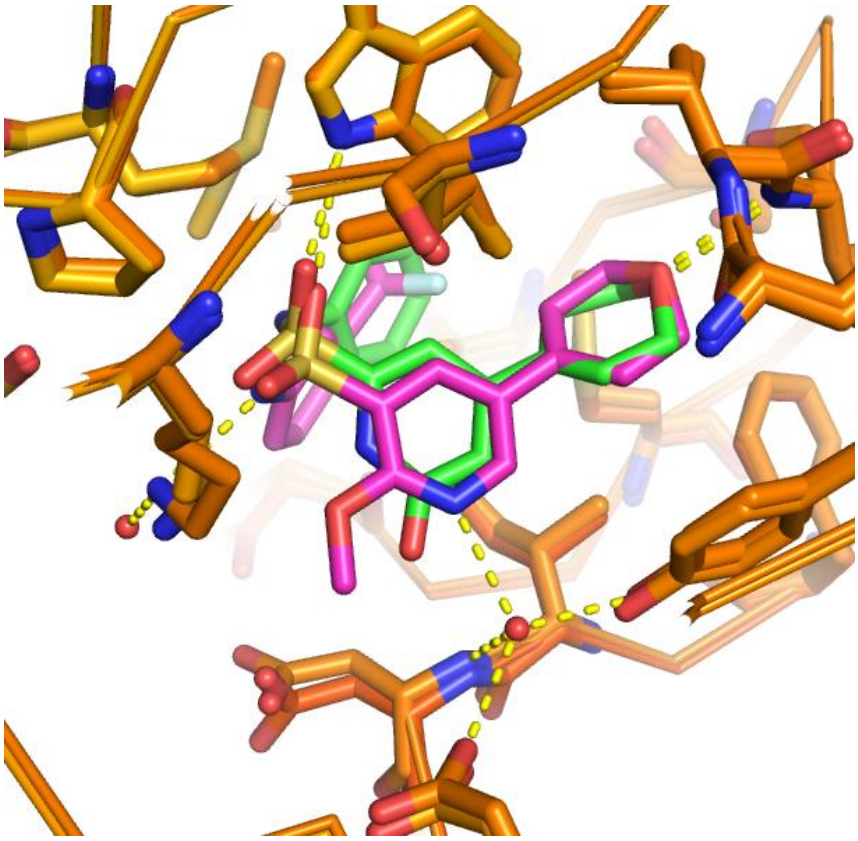
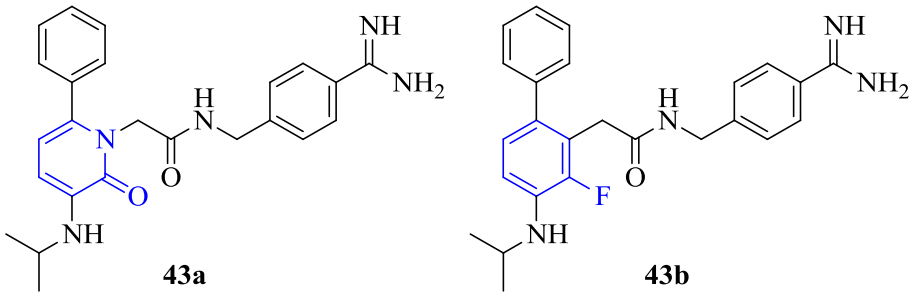


Figure 4.7 Overlay of pyridone **29** (green) and 2-methoxypyridine **16** (magenta).

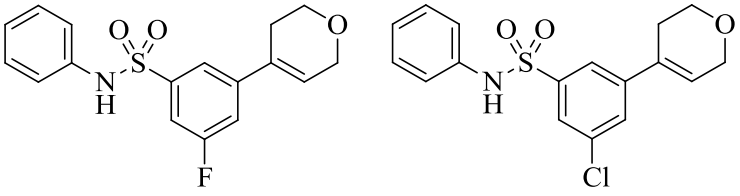
A slight caveat with **29** is a lack of solubility, with the aqueous solubility measured at greater or equal to 72 μg mL^{-1} in CLND and it has low organic solubility in $CDCl_3$, d_4-MeOH or d_6-DMSO.

With this interesting result in mind, there was significant interest in modification of the pyridone in order to investigate the possibility of removing this motif, which although it has significant potency for PI3Kδ, it has poor solubility in a range of solvents possibly due to the preference of 2-pyridones to sit as dimers between the pyridone N-H and C=O bond.[100] Work by Parlow and co-workers[101,102] had explored the use of fluorobenzene as surrogates for 2-pyridone. Parlow and co-workers replaced the pyridone of **43a** with fluorobenzene **43b**.



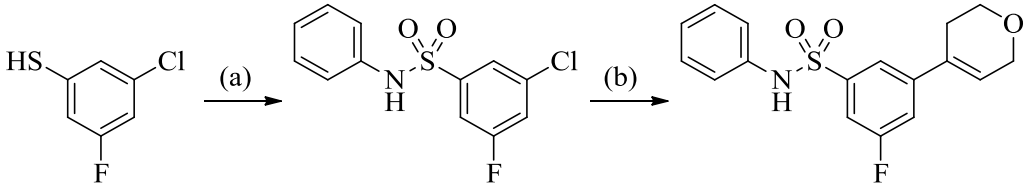
43a **43b**

They observed that this change modestly compromised the potency of the molecule; however they believed that the F atom could form a HBA with N-H bond of a nearby glycine residue, which was observed via X-ray co-crystal structure. In PI3Kδ, although the pyridone does not bind directly to the backbone, the interaction of the fluorine atom would have to be made through a bridging water. Fluorobenzene **44** and chlorobenzene **45** were selected for synthesis, with **45** being a related target that was read-out from the BROOD screen.



44 **45**

For the synthesis of **44**, thiol **46** was subjected to the oxidative chlorination procedure previously used. The crude sulfonyl chloride was reacted after workup with aniline to yield **47** in excellent yield over two steps (77 %). From this, Suzuki cross-coupling yielded fluorobenzene **44** (Scheme 4.10).

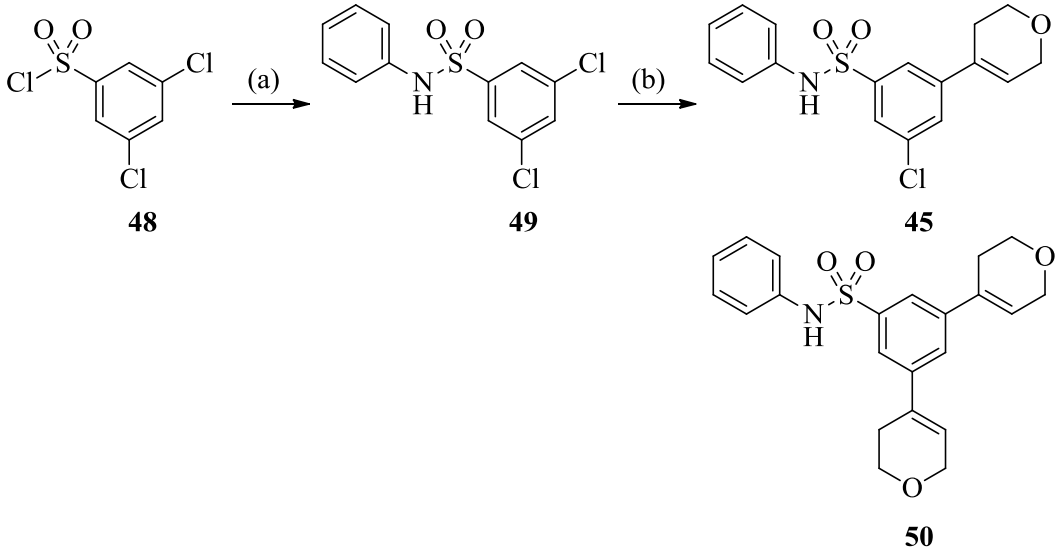


46 **47** **44**

(a) i) $HCl_{(aq)}$, MeCN, 12-16 °C, 1 h; ii) NCS, 1 h; iii) aniline, pyridine, rt, 3h, (77 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (37 %).

Scheme 4.10 Synthesis of fluorobenzene **44**.

For chlorobenzene **45**, the commerically available 3,5-dichlorosulfonylchloride **48** was reacted with aniline in good yield to give **49** (80 %). Suzuki cross-coupling yielded **45** in moderate yield (46 %) due to the unwanted formation of **50** (16 %) the bis-addition product (Scheme 4.11).

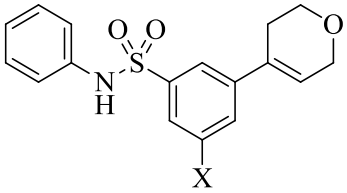


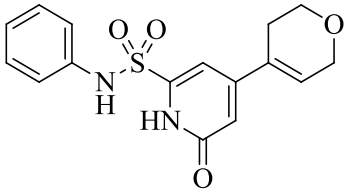
(a) Aniline, pyridine, rt, 2 h, (80 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (46 %).

Scheme 4.11 Synthesis of chlorobenzene **45**.

The results for **45** and **46** are as shown below (Table 4.7).

Table 4.7 Enzyme inhibition data for compounds **44** and **45**.[36]





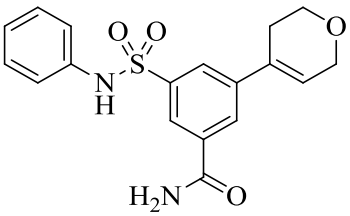
29

	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
44 (X = F)	4.7[a] (n=1)	5.0 (n=2)	4.9 (n=2)	5.7 (n=2)	0.34/2.5
45 (X = Cl)	4.7 (n=2)	5.2 (n=2)	<4.5 (n=2)	5.9 (n=2)	0.35/2.19
20 (X = H)	<4.5[b] (n=2)	4.7[b] (n=2)	<4.5[b] (n=2)	5.2 (n=3)	0.32/2.5
29	5.2 (n=3)	6.2 (n=3)	5.3 (n=3)	6.8 (n=3)	0.41/6.0

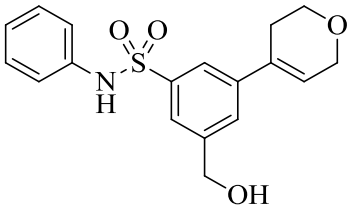
[a]On one occasion, a result of <4.5 was received. [b]On one occasion, a result of <4.3 was received.

The results for **44** were disappointing, as although it was active, it had significantly diminished potency in comparison to pyridone **29**. The compound was more potent than benzene **20** indicating that the fluorine had an effect but was not interacting as favourably. Chlorobenzene **45** was more potent that **20**, but **45** had high lipophilicity, was poorly efficient and therefore the compound was not further pursued.

From the BROOD set, certain compounds were selected that could displace the bridging water molecule and form more than one interaction. Pyridine *N*-oxide **25** displaced the bridging water molecule without replacing the interactions the water molecule made, so it could be hypothesised that amide **51** and alcohol **52** (selected from the BROOD screen) would form more interactions upon water displacement.

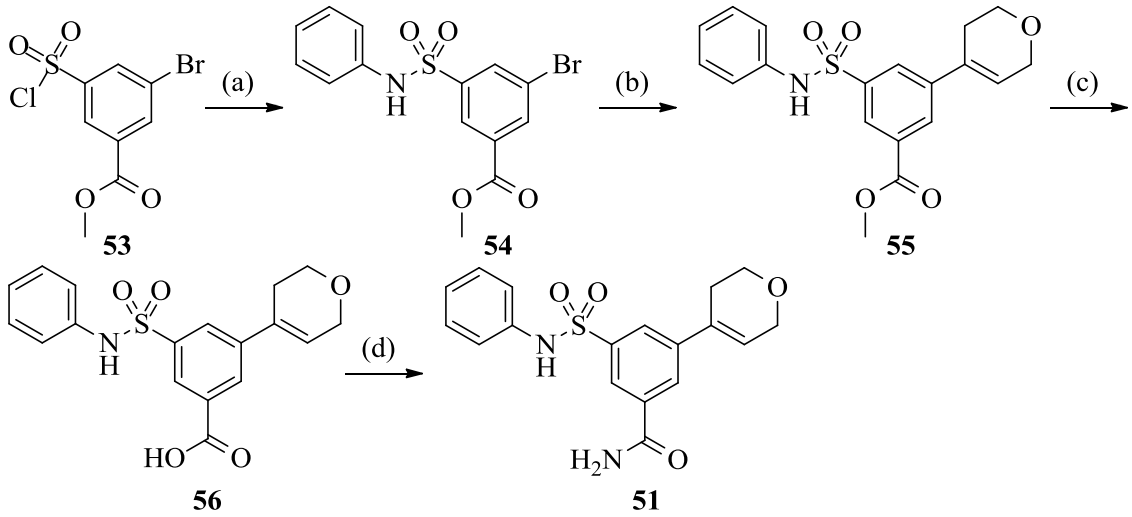


51



52

These targets could be accessed from key intermediate **53**. To access **51**, the following route was chosen (Scheme 4.12).

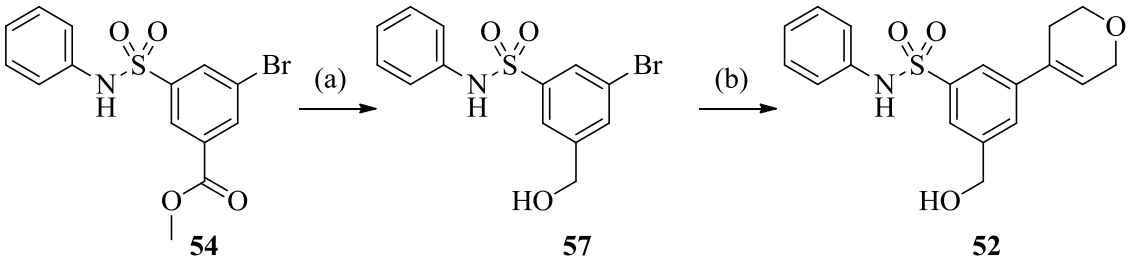


(a) Aniline, pyridine, rt, 3 h (77 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), Na_2CO_3, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (66 %); (c) LiOH, THF, H_2O, rt, 16 h (98 %); (d) i) HATU, DIPEA, DMF, rt, 30 min; ii) NH_3 in 1,4-dioxane, rt, 24 h (41 %).

Scheme 4.12 Synthesis of amide **51**.

Sulfonamide formation with aniline proceeded in good yield (77 %), followed by Suzuki cross-coupling (66 %) and subsequent basic hydrolysis of the methyl ester proceeded in excellent yield (98 %). Activation of the carboxylic acid via HATU and subsequent quenching with ammonia gave **51** in moderate yield (41 %). At this point carboxylic acid **56** was also submitted for testing due to its structural similarity to the amide. It would probe the requirement for a HBD interaction with the backbone as **56** would be ionised at physiological pH.

The primary alcohol **52** was synthesised via the following route (Scheme 4.13).



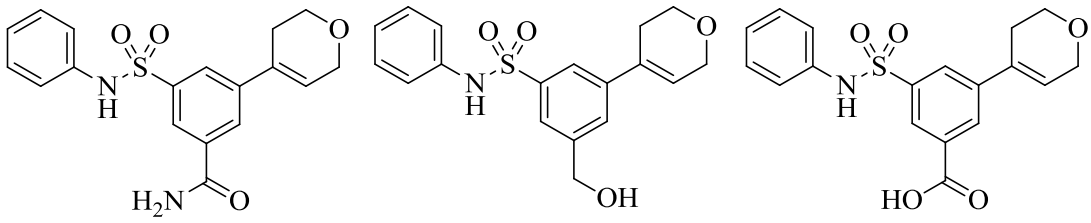
54 **57** **52**

(a) $LiAlH_4$, THF, 0 °C, 1 h (83 %), (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), Na_2CO_3, 1,4-dioxane, H_2O, μw, 80 °C, 20 min (17 %).

Scheme 4.13 Synthesis of alcohol **42**.

Treatment of **53** with lithium aluminium hydride yielded the primary alcohol **57** (83 %) which was used directly in the Suzuki cross-coupling to give **52** (17 %). Enzymatic results for compounds **51**, **52** and **56** are as follows (Table 4.8).

Table 4.8 Enzyme inhibition data for compounds **51**, **52** and **56**.[36]



51 **52** **56**

	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
51	5.2 (n=3)	5.6 (n=3)	5.1[b] (n=1)	6.3 (n=3)	0.35/4.5
52	4.6[a] (n=1)	5.1 (n=2)	4.8 (n=2)	6.0 (n=2)	0.34/4.3
56	5.2 (n=2)	5.4 (n=2)	5.4 (n=2)	6.3 (n=2)	0.35/3.3
15	5.4 (n=10)	5.6 (n=9)	5.1[b] (n=7)	6.7 (n=8)	0.38/4.0

[a]On one occasion, a result of <4.5 was received. [b]On two occasions, a result of <4.5 was received.

For both compounds suggested by the BROOD modelling, initial results were promising as both compounds are active against PI3Kδ and have some selectivity over the other PI3K isoforms. Primary carboxamide **51** and alcohol **52** were less active (0.4 and 0.7 log units respectively) and less selective (5-fold and 8-fold respectively) in comparison to 2-methoxy pyridine **15**. Both amide **51** and alcohol **52** are of similar efficiencies to 2-methoxypyridine **15**. Both compounds were submitted for X-ray crystallography to observe the interactions made. Results for amide **51** are shown below (Figure 4.8A).

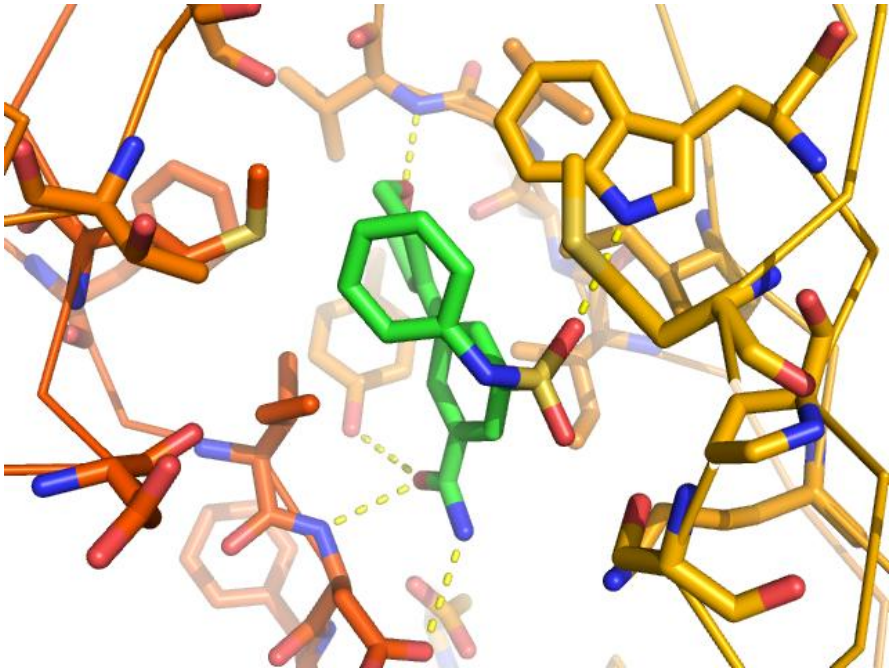


Figure 4.8A Co-crystal structure of **51** in PI3Kδ (Resolution 2.79 Å).[2]

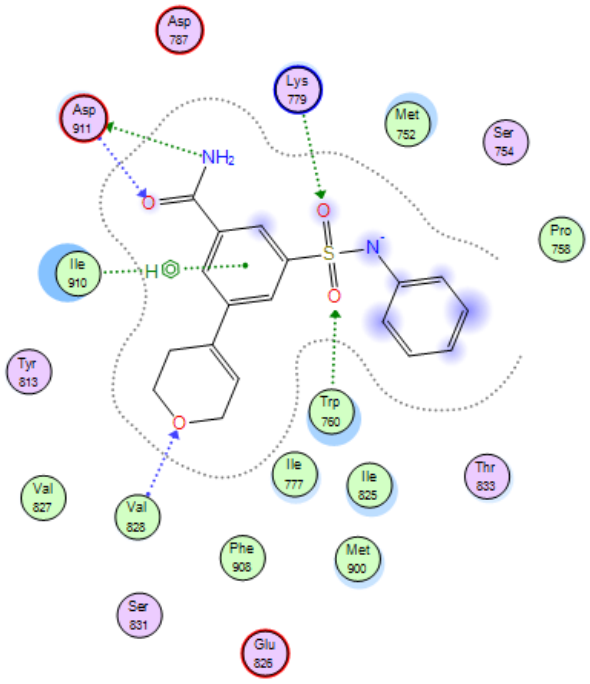


Figure 4.8B 2D Representation of **51** to PI3Kδ.

The crystal structure of amide **51** in the protein demonstrates the dihydropyran accepting a hydrogen bond from Val828, the Trp760 twist and Lys779 movement as observed in **16** (Figure 3.3). The following crystal structure shows the overlay between the amide **51** and 2-methoxypyridine **16** (Figure 3.11).[2] The primary carboxamide clearly displaces the bridging water molecule that is observed in the crystal structure of **51**, with the carboxamide forming HBA interactions with the phenolic proton of Tyr813 and the backbone N-H of Asp911 and one HBD interaction with the Asp911 side chain (Figure 4.9). Clearly observed (Figure 4.9) is the benzamide core moving away from the backbone to accommodate the amide moiety. This could be a factor relating to the drop in potency of **51** in comparison with **15**.

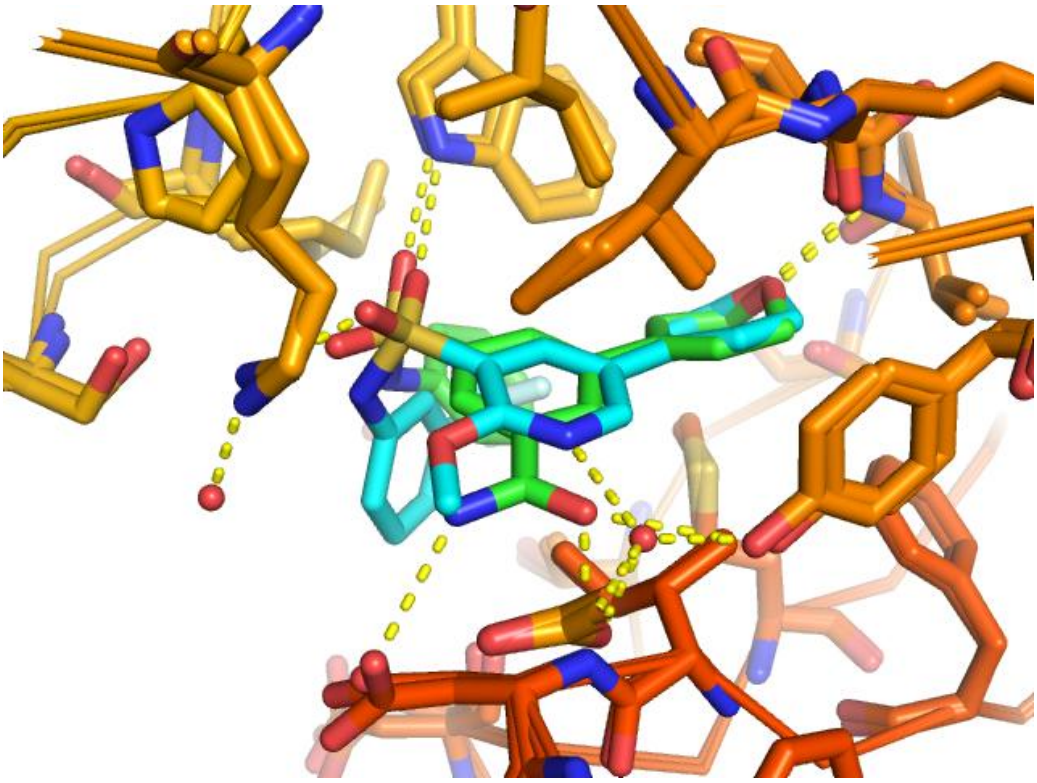


Figure 4.9 The overlay of compounds **16** (cyan) and **51** (green).[2]

Alcohol **52** is shown below (Figure 4.10).[2]

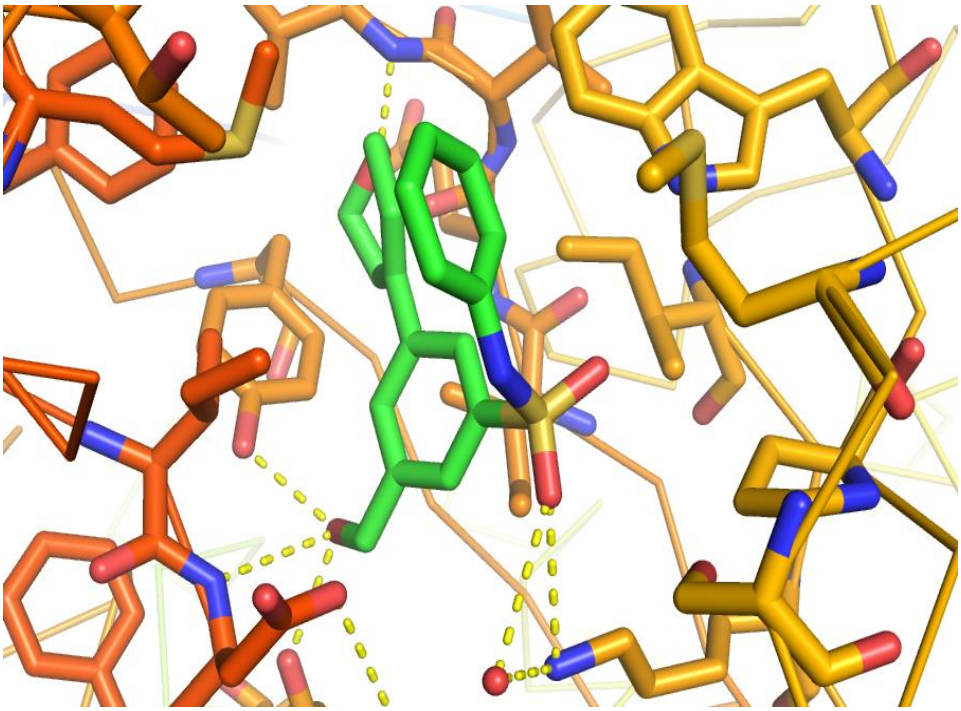


Figure 4.10A Co-crystal structure of **52** in PI3Kδ (Resolution 2.99 Å).[2]

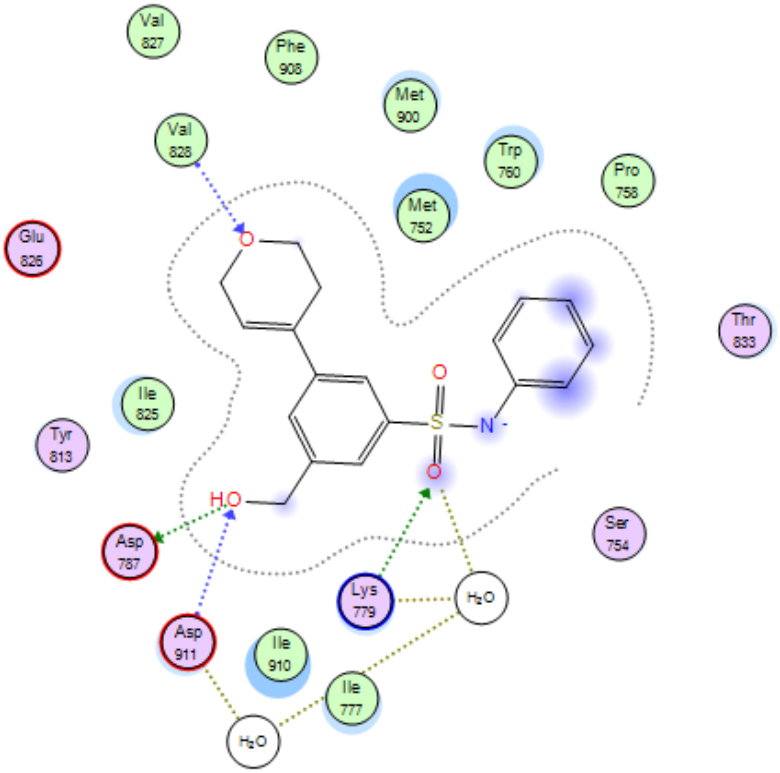


Figure 4.10B 2D Representation of **52** binding to PI3Kδ.

The crystal structure of **52** (Figure 4.10) demonstrates the dihydropyran accepting a hydrogen bond from Val828, the Trp760 twist and Lys779 movement as observed in **16** (Figure 3.3A). The primary alcohol displaces the conserved water molecule, with the alcohol forming a HBA interaction with the backbone N-H of Asp911 and one HBD interaction with the Asp911 side chain. An overlay of pyridine **18** and 2-methoxypyridine **16** with alcohol **52** displays a near perfect overlay, proving that displacement of the water molecule is viable (Figure 4.11).

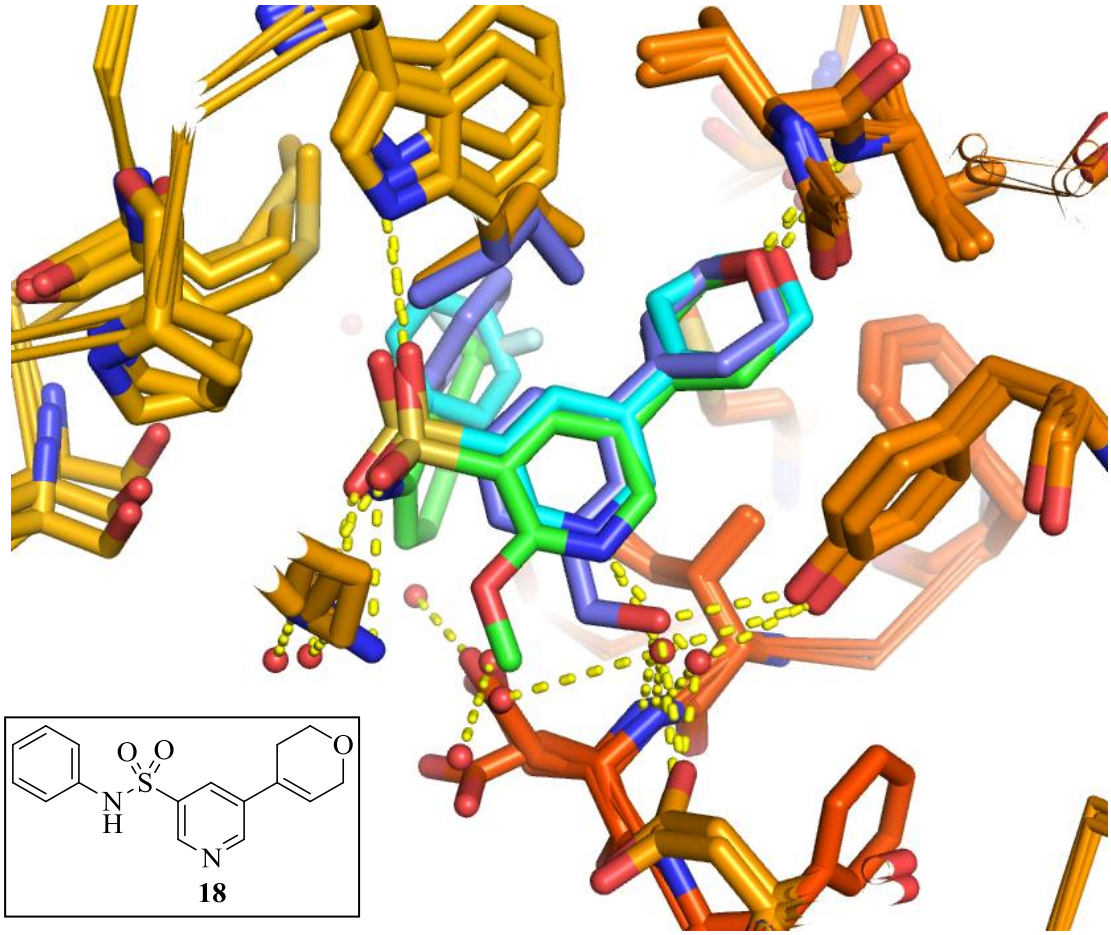


Figure 4.11 The overlay of compounds **16** (green), **18** (cyan) and **52** (blue).

Although the primary alcohol **52** is 5-fold less active than 2-methoxypyridine **15**, in comparison with the bioisostere pyridine **18**, primary alcohol **52** has similar if not slightly diminished activity (pIC_{50} 6.4 and 6.0 respectively), and similar selectivity against the other PI3K isoforms (4-fold and 9-fold respectively against the next nearest isoform). This suggests that further modifications to alcohol **52** could yield a compound with similar activity to the 2-methoxypyridine.

Carboxylic acid **56** showed good inhibition and selectivity for PI3Kδ with a similar profile to amide **51**. This suggests that it could make similar interactions as the amide with the protein, as the acid carbonyl can form a HBA interaction with the N-H of Asp911. The binding mode of **56** may not be the same as amide **51** as at physiological pH the carboxylic acid would be

ionised (calculated p*K*a 3.6), which would lead to a clash with Asp911. Protein crystallography greatly aided the understanding at this position (Figure 4.12).

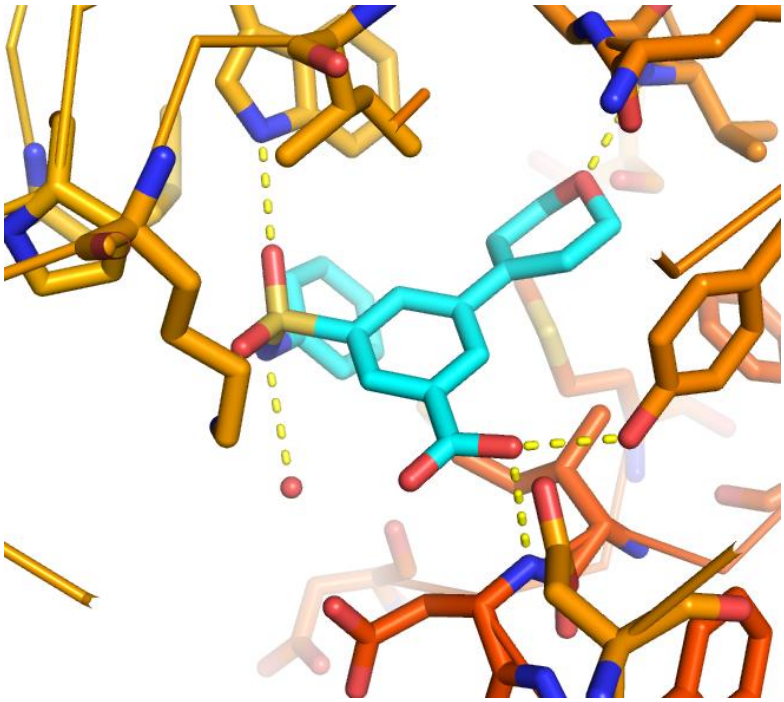


Figure 4.12 Co-crystal structure of **56** in PI3Kδ (Resolution 2.28 Å).[2]

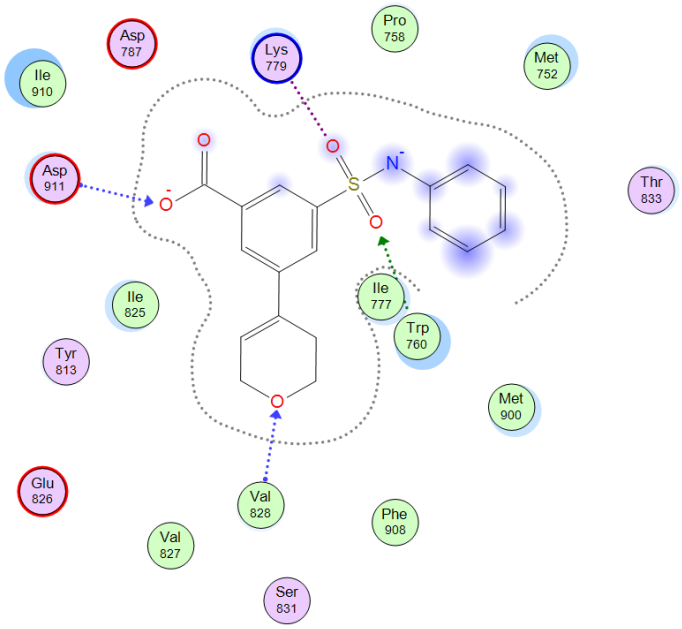
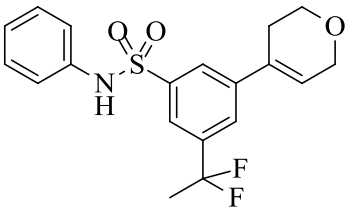


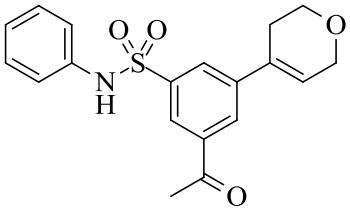
Figure 4.12 2D Representation of **56** binding to PI3Kδ.

From the crystallography, we can clearly observe that the acid **56** is bound in a similar way to amide **51**, in that the carbonyl motif forms a HBA interaction with the Asp911. However, in contrast to amide **51**, acid **56** enforces minor twists in the carboxylic acid side-chains of Asp911 and Asp787 within the back pocket region.

In order to further understand displacing the water with substitutions at this position, further compounds were selected in order to probe certain interactions observed in **51**, **52** and **56**. From the original BROOD set, difluoroethyl **58** was suggested as a target, however as this is a bioisostere of ketone **59**, ketone **59** was selected for synthesis.[103] Ketone **59** would probe the requirement for a hydrogen bond donor interaction with the backbone whilst maintaining the carbonyl interactions observed in amide **51**.

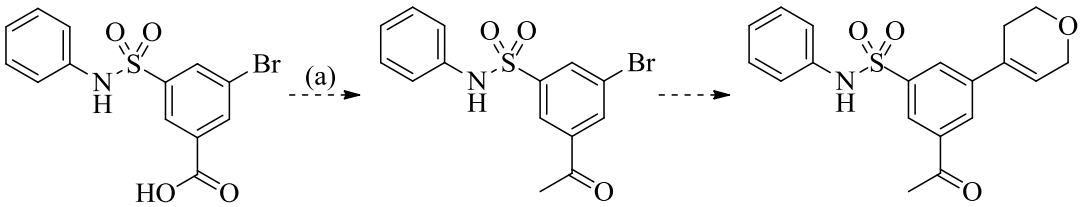


58



59

To access **59**, ketone **61** was proposed to be synthesised from intermediate **60** (Scheme 4.14).

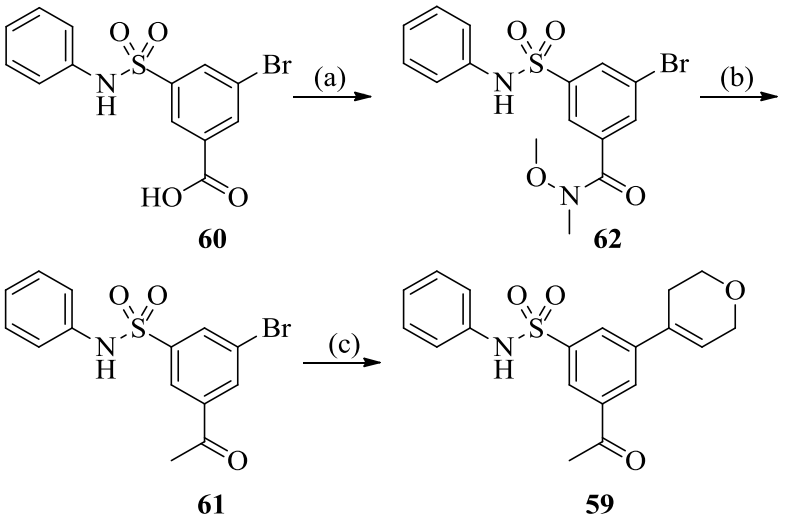


60 **61** **59**

(a) Methyllithium (3 eq.), THF, 0 °C, 18 h.

Scheme 4.14 Proposed synthesis of compound **61**.

Initially, ketone **61** was envisaged to be formed by the reaction of **60** with methyllithium. However during the reaction, bis-anion formation upon deprotonation of the carboxylic acid and sulfonamide resulted in the starting material precipitating without any desired reaction occurring. Alternatively, formation of the Weinreb amide **62** and then treatment with methylmagnesium bromide was attempted (Scheme 4.15)

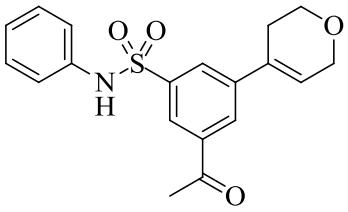


(a) i) 1*H*-Benzo[*d*][1,2,3]triazol-1-ol hydrate, *N*-((ethylimino)methylene)-*N*,*N*-dimethylpropane-1,3-diamine hydrochloride, DIPEA, DMF, rt, 5 min; ii) *N*,*O*-dimethylhydroxylamine hydrochloride, DMF, rt, 48 h (14 %); (b) MeMgBr, THF, 0 °C, 30 min (64 %); (c) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 100 °C, 30 min (49 %).

Scheme 4.15 Synthesis of ketone **59**

Formation of the Weinreb amide **62** under standard conditions proceeded in poor yield (14 %) and it was used immediately. Treatment of **62** with methyl magnesium bromide (64 %) and subsequent Suzuki cross-coupling gave ketone **59** (49 %). The results are as follows (Table 4.9).

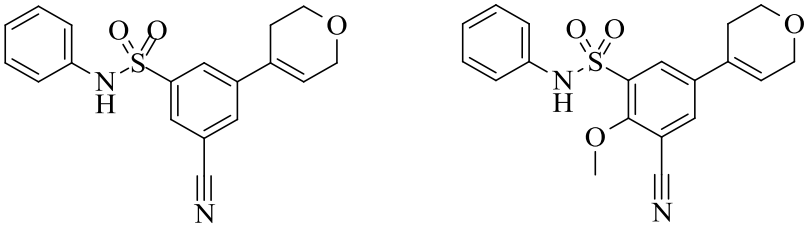
Table 4.9 Enzyme inhibition data for compound **59**.[36]



	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
59	4.8 (n=4)	5.0 (n=4)	4.7 (n=4)	5.6 (n=4)

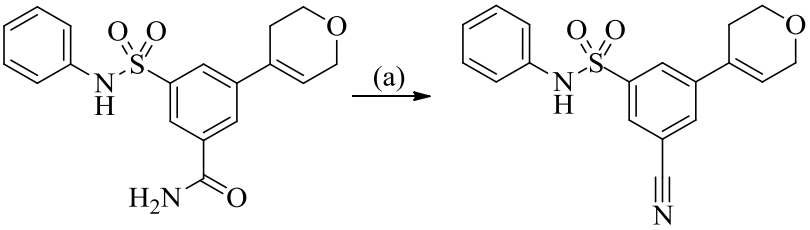
Ketone **59** showed moderate inhibition and selectivity for PI3Kδ, but was 5-fold less active than amide **51**. This indicated that removing a HBD interaction with Asp911 and the introduction of the lipophilic methyl group impacts on the potency. The drop in potency observed in **59** resulted in difluoroethyl **58** being down-prioritised.

One further 1,3,5-substituted product proposed for synthesis was nitrile **63**. BROOD calculations gave methoxy-nitrile **64** as a potential target, however to understand the effect of the nitrile alone, **63** was selected for synthesis. Nitrile groups are sometimes utilised in medicinal chemistry to displace conserved water molecules.[104]



63 **64**

To access **63**, dehydration of amide **51** was trialled (Scheme 4.16).



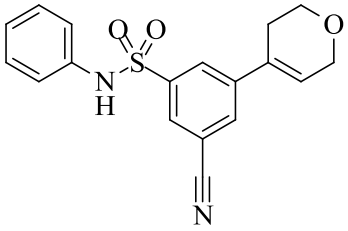
51 **63**

(a) Triflic anhydride, DIPEA, DCM, rt, 16 h (52 %).

Scheme 4.16 Dehydration of amide **51** to access nitrile **63**.

To prevent side-reactions with the dihydropyran, mild conditions utilising triflic anhydride and DIPEA were used to give **63** in moderate yield (52 %). The results are as follows (Table 4.10).

Table 4.10 Enzyme inhibition data for compound **63**.[36]

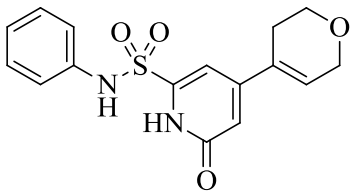


	pIC$_{50}$			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
63	<4.5 (n=2)	4.6 (n=2)	5.0 (n=2)	5.0 (n=2)

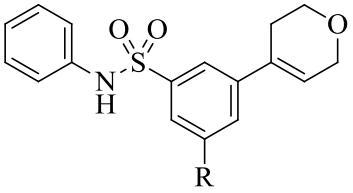
Nitrile **63** showed poor potency for PI3Kδ, which indicates that the cyano group may not form any beneficial interactions with the protein backbone to compensate the displacement of the conserved water molecule if it binds in the same region to amide **41**. In comparison with benzene **20**, nitrile **63** was 2-fold less active indicating the detrimental effect of disrupting the conserved water molecules interactions with the backbone without replacing them.

Having discovered that pyridone **29**, amide **51**, alcohol **52** and acid **56** possessed moderate potency and selectivity for PI3Kδ, the wider properties are considered below (Table 4.11)

Table 4.11 Enzyme inhibition data for compounds **29**, **51**, **52** and **56**.[36]



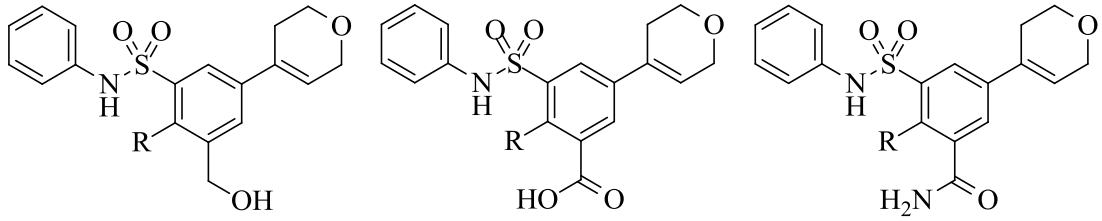
29



		R		
	29	**51 $CONH_2$**	**52 CH_2OH**	**56 COOH**
PI3Kδ pIC_{50}	6.8	6.3	6.0	6.3
Selectivity (nearest isoform)	4-fold	5-fold	8- fold	8- fold
AMP (nm/sec)	230	175	470	<3
CLND Solubility (µg/mL)	72	88	9	106
cLogP	0.8	1.8	1.7	2.9
LE/LLE	0.41/6.0	0.35/4.5	0.34/4.3	0.35/3.3

Pyridone **29** was an exciting compound, with excellent activity and efficiency for PI3Kδ. It had good permeability and aqueous solubility; however its poor organic solubility discouraged further work on the template. The wider properties of **51**, **52** and **56** demonstrate that only amide **51** meets the criteria outlined within the aims of this work. Alcohol **52** is a highly permeable compound with acceptable cLogP for a fragment of this size, however it possesses poor solubility, a key property for any drug molecule. Acid **56** is poorly permeable, with an un-measurable value observed via AMP likely due to the ionisable carboxylic acid at physiological pH. Amide **51** demonstrated moderate solubility and permeability, with good cLogP and ligand efficiencies.

Before selecting a compound to further elaborate, we wanted to investigate whether the potency, selectivity or physical properties of amide **51**, alcohol **52** and acid **56** could be enhanced by the incorporation of a methoxy or methyl group *ortho* to the sulfonamide (Figure 4.13).



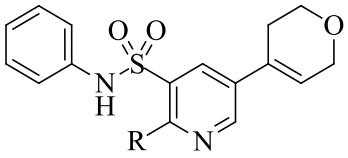
R = Me **65** **66** **67**

R = OMe **68** **69** **70**

Figure 4.13 Methyl- and methoxy- compounds **65-70**.

This work is interesting in that incorporation of the methoxy and methyl groups in the 2-position of the pyridine derivative **18** results in increased potency and, with the methoxy substituent, significant improvements in the selectivity of the compounds (Table 4.12).

Table 4.12 Enzyme inhibition data for compounds **18**, **71** and **15**.[36]



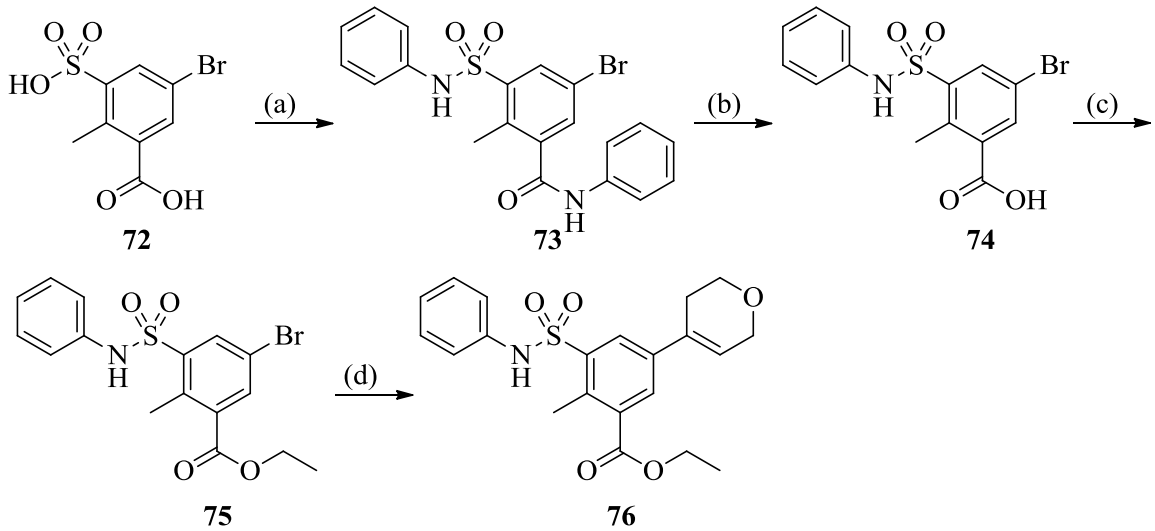
	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
18 (R= H)	5.6 (n=4)	5.8 (n=2)	5.5 (n=2)	6.4 (n=2)
71 (R = Me)	5.6 (n=3)	6.1[a] (n=2)	5.1 (n=3)	6.5 (n=3)
15 (R= OMe)	5.4 (n=10)	5.6 (n=9)	5.1[b] (n=7)	6.7 (n=9)

[a]On one occasion, a result of <4.5 was received. [b]On two occasions, a result of <4.5 was received.

By incorporation of a methoxy or methyl group in the alcohol/amide/acid series (Figure 4.13), it could be hypothesised that the potency and or selectivity of the compounds might increase due to the similarity of the binding mode (Figure 4.9 and Figure 4.11). The methyl substituent in **71** fills a lipophilic space within the protein and in the alcohol/amide/acid series, the methyl group again would fill a lipophilic space within the protein due to similarities in the binding mode. However for alcohol **65**, the lowest energy conformation of the alcohol could pre-organise the molecule with the alcohol moiety adopting a similar

conformation to that observed in the crystal structure of alcohol **52** (Figure 4.10), which may enhance the potency observed. The methoxy substituent in pyridine **15** fills a lipophilic space within the protein, accepts a hydrogen bond from Lys779 and increases the electron density of the aromatic ring. This increase in electron density could enhance the nitrogen's HBA interaction with the water molecule through which it binds to the protein backbone. For methoxy alcohol **68** and methoxy amide **70**, their lowest energy conformations could pre-organise either the alcohol moiety or the amide moiety to their desired conformations as observed through the crystal structures of **51** and **52** respectively (Figure 4.8 for **51** and Figure 4.10 for **52**). This hypothesis is further supported by the BROOD fragment screen, which identified the methyl and methoxy primary alcohols **65** and **68** as potential targets (Figure 4.13).

For the synthesis of **65**, **66** and **67**; the following route was utilised to access common intermediate **76** (Scheme 4.17).

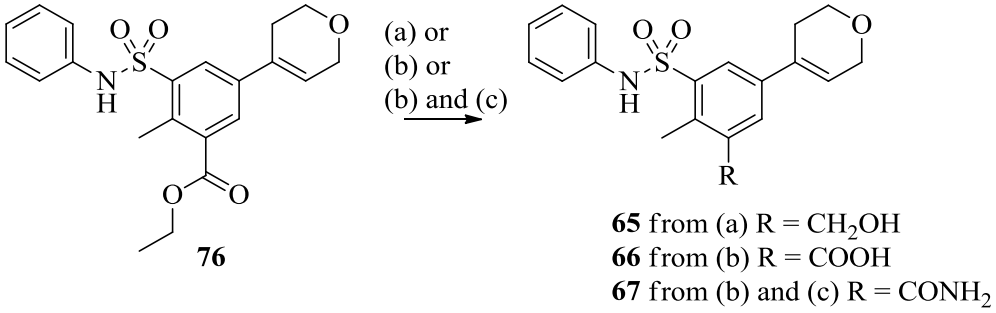


(a) i) $SOCl_2$, 0 °C, 48 h; ii) aniline, pyridine, 1,4-dioxane, rt, 18 h (37 %); (b) LiOH, MeOH, H_2O, rt, 96 h; (c) $SOCl_2$, EtOH, rt, 96 h (62 % over 2 steps); (d) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 1 h (56 %).

Scheme 4.17 Synthesis of common intermediate **76**.

From the commercially available diacid **72**, *in situ* formation of the sulfonyl chloride-acid chloride and subsequent reaction with aniline gave **73** in moderate yield (37 % over 2 steps). From the literature,[105] basic conditions selectively cleaved the carboxamide to give acid **74**,

which was used crude, due to the inability to separate the water soluble acid from the inorganic salts formed during the reaction. Subsequent esterification **75** (62 % yield) and Suzuki cross-coupling (56 % yield) gave common intermediate **76**. Reduction of **76** via *in situ* formed calcium borohydride gave alcohol **65** (52 % yield). Hydrolysis of the ethyl ester gave acid **66** (94 % yield), which was further reacted to give the primary carboxamide **67** (40 % yield) (Scheme 4.18).

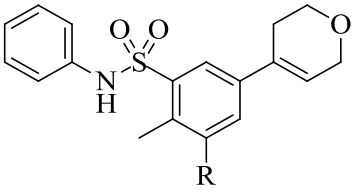


(a) $CaCl_2$, $NaBH_4$, 0 °C, 120 h (52 %); (b) LiOH, THF, H_2O, rt, 16 h (94 %); (c) i) HATU, DIPEA, DMF, rt, 30 min; ii) NH_3, 72 h (40 %).

Scheme 4.18 Synthesis of compound **65**-**67** from common intermediate **76**.

The enzymatic results for compounds **65**-**67** are shown below (Table 4.13).

Table 4.13 Enzyme inhibition data for compounds **65**-**67**.[36]



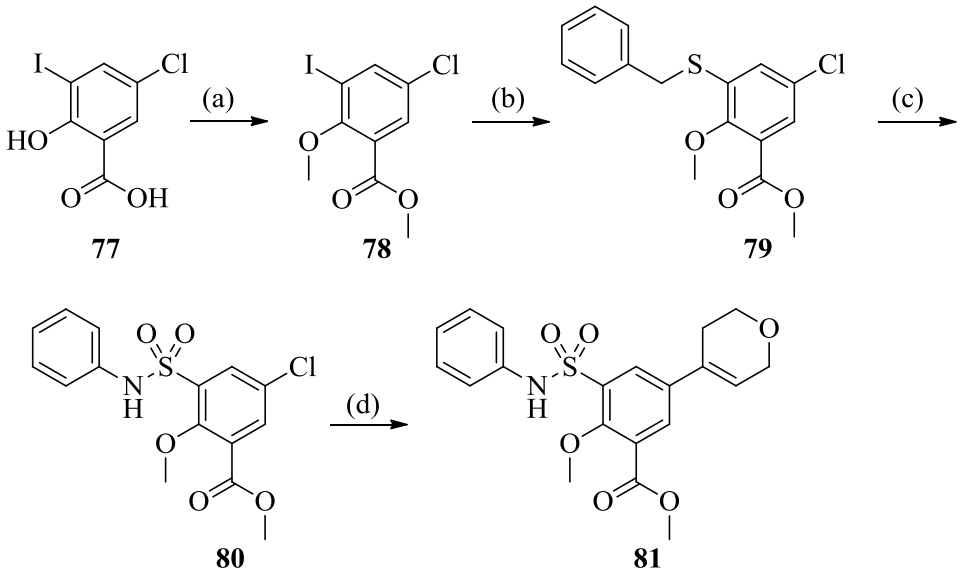
	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
65 R=CH_2OH	<4.5 (n=3)	<4.5 (n=3)	<4.5 (n=3)	5.1 (n=3)
66 R=COOH	<4.5 (n=2)	4.6 (n=2)	<4.5 (n=2)	<4.5 (n=2)
67 R=$CONH_2$	5.0[a] (n=4)	4.9[b] (n=1)	4.9[c] (n=5)	5.4 (n=5)

[a]On two occasions, a result of <4.5 was received. [b]On five occasions, a result of <4.5 was received. [c]On one occasion, a result of <4.5 was received.

All three compounds drop in potency in comparison with the non-methyl versions **51**, **52** and **56**. For alcohol **65**, proposed from the initial BROOD screen, PI3Kδ potency has dropped 10-fold in comparison with **52**. From the crystal structure of **52** (Figure 3.12A and 3.12B) and by docking compound **65** in the crystal structure, the methyl group clashes with Lys779 and a water molecule that form interactions with the sulfonamide. However, this water molecule is not usually observed in crystallography of 2-methoxypyridine compounds (data not shown) and the lysine residue has significant flexibility within the region, so this clash was difficult to predict before synthesising the compound.

PI3Kδ potency drops for amide **67** in comparison with amide **51**. This loss in activity is probably due to the methyl substituent clashing with the carboxamide/acid moiety. This clash may force the amide and acid moieties out of their preferred planar conformation and therefore the interactions they make with Asp911 are no longer ideal. Acid **66** is completely inactive, as upon its movement out of the plane, it would clash with the Asp787 side chain and the HBA interaction with the N-H of Asp911 would become weaker due to increased length. Amide **67** retains some potency as its movement out of the plane would lose the HBD interaction with the carboxylate of Asp911; however it may pick up an alternative interaction with Asp787.

For the synthesis of **68**, **69** and **70**, the following route was utilised to access a common intermediate **81** which could subsequently be diversified (Scheme 4.19).



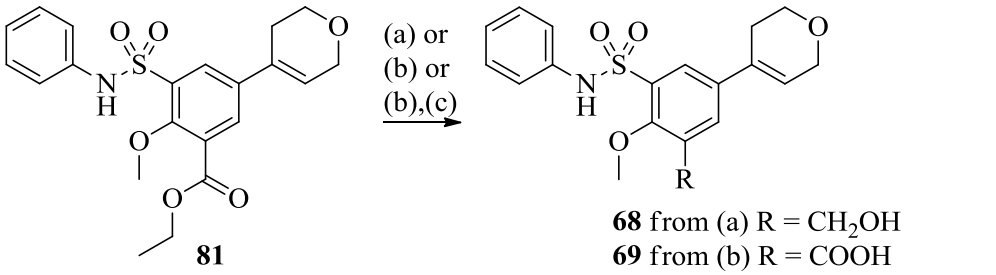
77 **78** **79** **80** **81**

(a) MeI, K_2CO_3, acetone, 60 °C, 6 h (57 %); (b) BnSH, $Pd_2(dba)_3$, XantPhos, DIPEA, toluene, 100 °C, 1 h (77 %); (c) i) $HCl_{(aq)}$, MeCN, rt; ii) NCS, 1 h; iii) pyridine, aniline, rt, 21 h (38 %); (d) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 100 °C, 90 min (98 %).

Scheme 4.19 Synthesis of intermediate **81**.

From the commercially available **77**, dimethylation with methyl iodide gave **78** in moderate yield (57 %). Palladium-catalysed cross-coupling of **78** and benzyl mercaptan gave impure **79** (77 % yield). Subsequent oxidative chlorination of **79** and sulfonamide formation with aniline gave **80** (38 % yield) which underwent Suzuki cross-coupling in excellent yield (98 %) to give versatile **81**.

Reduction of ester **81** via *in situ* formation of calcium borohydride gave alcohol **68** (18 % yield). Hydrolysis of the methyl ester gave carboxylic acid **69** (75 % yield), which was further reacted to give the primary carboxamide **70** (46 % yield) (Scheme 4.20).

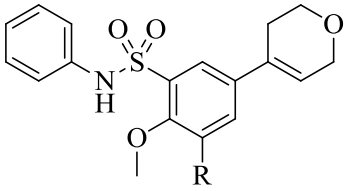


(a) $CaCl_2$, $NaBH_4$, 0 °C, 21 h (18 %); (b) LiOH, THF, H_2O, rt, 16 h (75 %); (c) i) HATU, DIPEA, DMF, rt, 10 min; ii) NH_3, 16 h (46 %).

Scheme 4.20 Synthesis of **68**-**70** from common intermediate **81**.

The enzymatic results are shown below (Table 4.14).

Table 4.14 Enzyme inhibition data for compounds **68**-**70**.[36]



	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
68 R = CH_2OH	4.6[a] (n=2)	4.9 (n=4)	<4.5 (n=4)	5.8 (n=4)	0.31/4.3
69 R = COOH	4.6[b] (n=1)	4.7[b] (n=1)	<4.5 (n=2)	5.4 (n=2)	0.27/3.3
70 R =$CONH_2$	4.8[b] (n=3)	5.1 (n=4)	4.6 (n=4)	6.2 (n=4)	0.31/4.9

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.5 was received

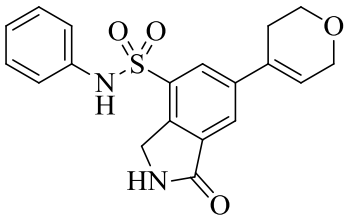
Compounds **68**, **69** and **70** all show some activity and selectivity for PI3Kδ. Alcohol **68** is similar to alcohol **52** in both activity and selectivity, indicating that the methoxy substituent does not contribute towards or diminish the potency. Its inclusion boosted both its solubility (from 9 to 43μg/mL in **52** and **68**) and maintained good permeability (470 and 520 nm/sec in **52** and **68**), but this compound was not of sufficient activity for further work.

For **70**, the potency is very similar to amide **51**, again indicating that the methoxy substituent has little to no effect. The selectivity of **70** for PI3Kδ has not significantly changed in comparison with **51**, again showing that its inclusion does not impair or improve selectivity. The methoxy moiety may pre-organise the amide moiety in the desired conformation in the protein by the formation of a hydrogen bond between the amide N-H and the lone pair of the methoxy group, that would lead to enhanced potency. However no increase in potency is observed, indicating that this has little or no effect.

Compound **69** showed diminished potency in comparison with acid **56**, again indicating that substitution at this position is detrimental for potency. This may again be caused by the acid moiety clashing with the oxygen of the methoxy group, which causes the carboxylic acid moiety to move out of plane that is not tolerated upon binding to the protein.

At this stage a decision was made to look into modifying the amide to enhance the interactions made. One way in which the binding mode could be enhanced would be cyclization of the amide to lock its conformation. Locking a conformation can be beneficial, as it stops rotation around the bond, so the substituents are conformationally pre-organised in the preferred binding conformation prior to binding with the protein backbone. This results in lowering the entropy of the system which, if beneficial to the interactions formed, can lead to enhanced binding potency. By lowering the entropy, the Gibbs free energy of the system will become more negative ($\Delta G = \Delta H - T\Delta S$) from which the K_i (the inhibition constant) can be calculated (because $\Delta G = -RT\ln K_i$).[106,107]

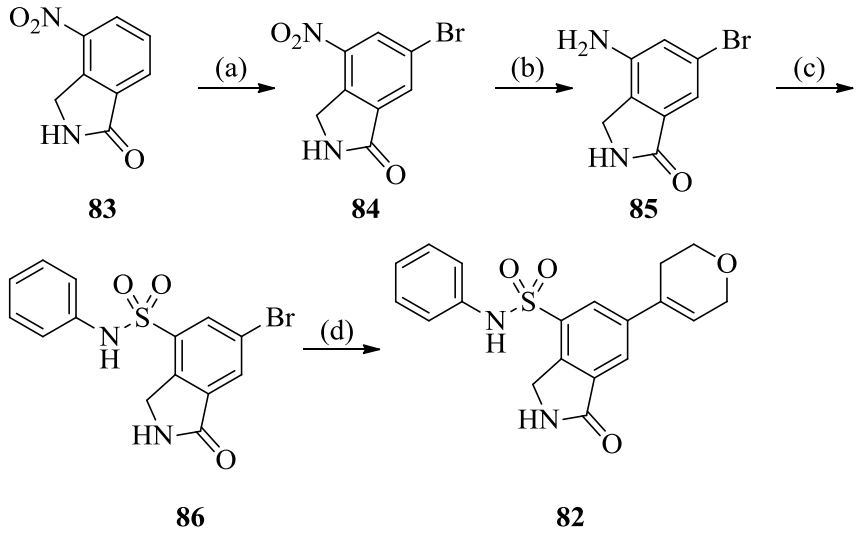
Isoindolin-1-one **82** was selected in order to probe cyclization of the ring.



82

This would change the amides bond angle from 120° to 108°, but would give a more rigid and therefore more entropically negative system, which could cause an increase in potency.

Isoindolin-1-one **83** was observed as a good starting material and led to the following route being investigated (Scheme 4.21).



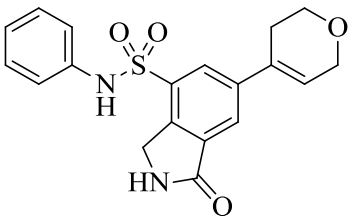
(a) NBS, H_2SO_4, 70 °C, 2 h, (97 %); (b) Fe, NH_4Cl, EtOH, H_2O, 80 °C, 17 h, (59 %); (c) i) $HCl_{(aq)}$, 10-15 °C; ii) $NaNO_2$, -5 °C, 1 h; iii) $SOCl_2$, H_2O, CuCl, -5 °C, 2 h; iii) aniline, pyridine, rt, 2 h (62 %); (d) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, (63 %).

Scheme 4.21 Synthesis of isoindolin-1-one **82**.

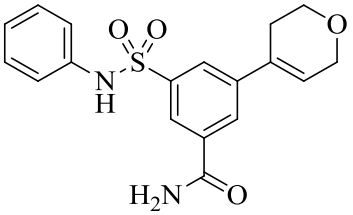
Bromination of **83** with NBS in sulfuric acid yielded **84** in near-quantitative yield and was regioselective for the 6-position of the indolin-1-one ring. From this, reduction of the nitro to the aniline proceeded smoothly, however, upon workup and column chromatography, only a moderate yield (59 %) was obtained. From this, diazotisation of the aniline yielded the sulfonyl chloride upon addition to the pre-generated sulfur dioxide/hydrochloric acid mixture,[108] which was used without purification due to instability on silica and reverse phase chromatography. Sulfonamide formation under standard conditions gave **86** (62 %) which

was then used in the final step to afford **82** in moderate yield. The results for **82** are shown below (Table 4.15).

Table 4.15 Enzyme inhibition data for compounds **82** and **51**.[36]



82



51

	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
82	5.0 (n=4)	5.1 (n=4)	5.2 (n=4)	6.1 (n=4)	0.32/4.1
51	5.2 (n=3)	5.6 (n=3)	5.1[a] (n=3)	6.3 (n=3)	0.35/4.5

[a]On two occasions, a result of <4.5 was received.

Isoindolin-1-one **82** is a moderately potent PI3Kδ inhibitor, with some selectivity against the other PI3K class-I isoforms. However, in comparison to amide **51**, cyclization of the amide has little effect on the potency or selectivity against PI3Kα and PI3Kγ, indicating that although it is tolerated, it is not beneficial to the compound resulting in a drop in both LE and LLE. The only major change is that incorporation of this group appears to improve PI3Kβ selectivity in comparison to amide **51**. Incorporation of the isoindol-1-one increases the permeability from 175 nm/sec in **51** to 390 nm/sec in **82**, but due to the more rigid system, poorer solubility in CLND is observed (88 μg/mL in **51** to 33 μg/mL in **82**). To confirm the binding mode, co-crystallography of **82** in the PI3Kδ protein was attained (Figure 4.14).

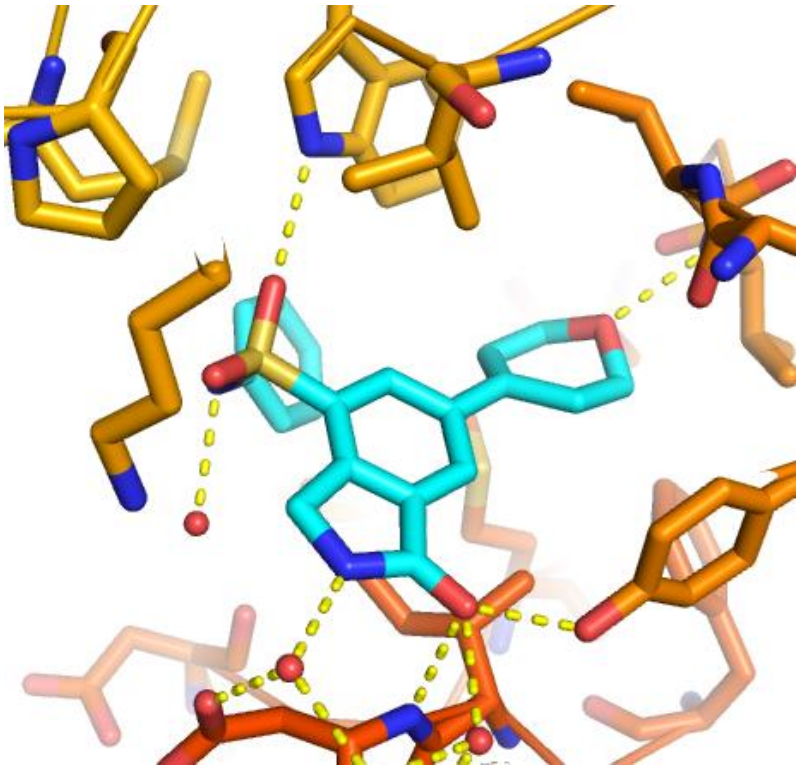


Figure 4.14A Co-crystal structure of **82** in PI3Kδ (Resolution 2.08 Å).[2]

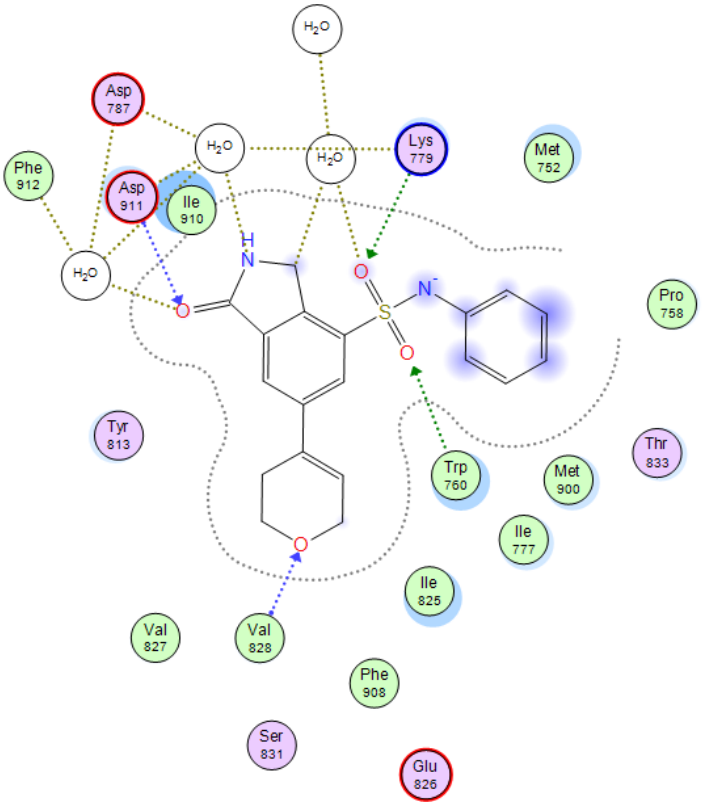


Figure 4.14B 2D Representation of **82** binding to PI3Kδ

Compound **82** forms the hinge interaction with Val828, a HBA interaction with Trp760 and two HBA interactions through the carbonyl moiety to Tyr813 and Asp911 (Figure 4.14). The isoindoline N-H interacts through a water molecule to the Asp911 carboxylic acid side chain, which could be caused by the change in vector of the N-H isoindoline. In comparison to primary amide **51** (Figure 4.15), **82** forms the same interactions with the protein backbone except the through water isoindoline N-H interaction, however due to the similarities in potency, this is not significant.

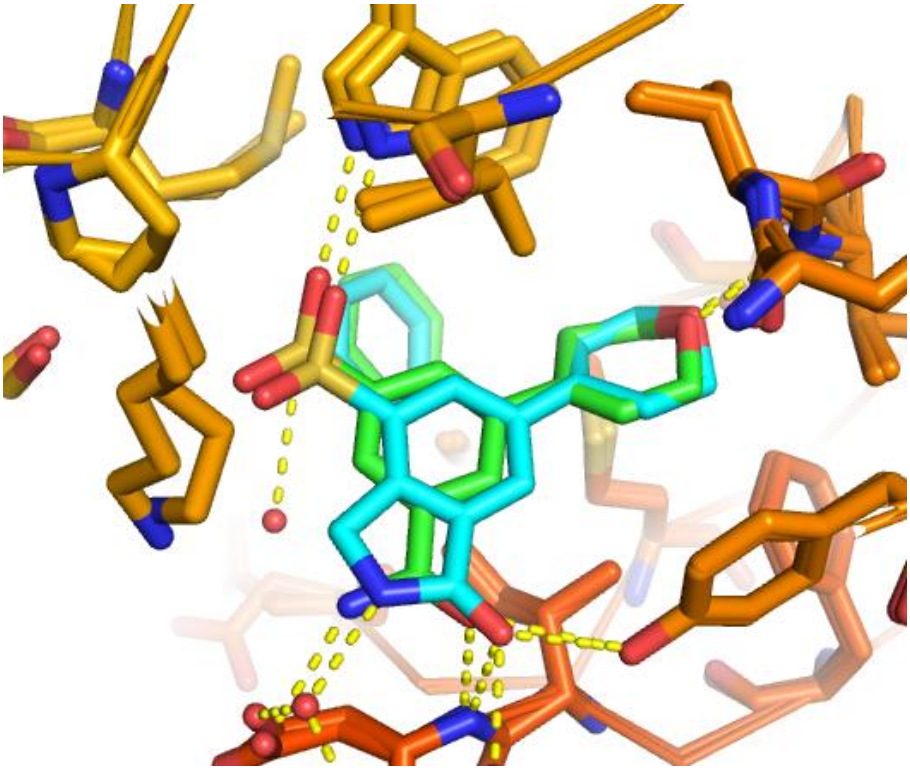
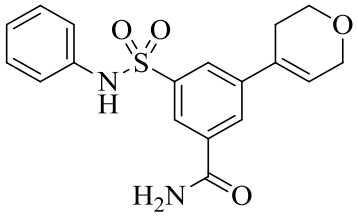


Figure 4.15 Overlay of compounds **51** (green) and **82** (teal).

Unfortunately, changes at the 2-position highlighted within the BROOD screen did not enhance the compounds significantly in comparison to the unsubstituted compounds **51**, **52** and **56**. This showed that amide **51** was the best novel lead chemical entity, fulfilling all the initially described criteria (Table 4.16) and so was selected for further optimisation.

Table 4.16 Aims set for a lead compound and compound **51**.[36]



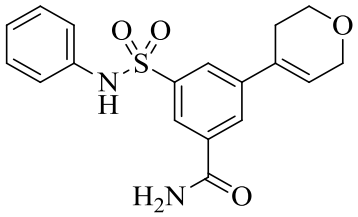
51

Target Profile	**51**
PI3Kδ pIC_{50} > 6	6.3
Good ligand and lipophilic ligand efficiencies (LE > 0.3, LLE > 4)	0.35/4.5
Some evidence of isoform selectivity	5-fold
cLogP (< 2.5)	1.8
Aromatic ring count (< 3)	2
Moderate AMP permeability (> 50 nm/s)	175 nm/s
Moderate CLND solubility (> 30 µg/mL)	88 µg/mL

Chapter 5 Exploration of lead **51**

From chapter four, work to replace the 2-methoxypyridine group with an alternate group led to the discovery of some potent and selective compounds. Amide **51** was of particular interest, as it was an efficient, small molecule with good physical properties outlined previously (Table 5.1) and met all the lead criteria set out in the initial aims.

Table 5.1 Enzyme inhibition and physical data for compound **51**.[36]

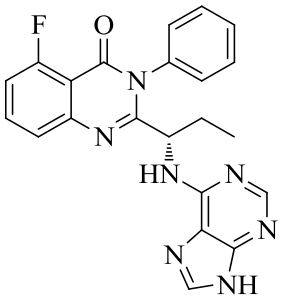


51

	pIC$_{50}$				
	PI3Kδ	**PI3Kα/β/γ**	**LE/LLE**	**Permeability (nm/sec)**	**Solubility; (µg/mL)**
51 Amide	6.3	5.2/5.6/5.1	0.35/4.5	175	88

Previously in chapter four, modifications to the 2-position of the benzamide ring offered few benefits in regard to potency or selectivity; however modulations of physical chemical properties were possible at this position.

Further changes in chemical structure to boost potency or selectivity were not investigated, which will entail the work explored in this chapter. In order to progress a molecule further, the compound would have to have some advantage over Idelalisib **4**, the current marketed PI3Kδ inhibitor. Primary aims would be to boost PI3Kδ potency to pIC$_{50}$ 8 whilst possessing 100-fold selectivity against the other class I PI3K isoforms.



4

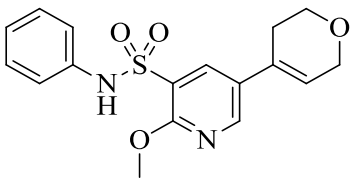
Idelalisib

PI3Kδ pIC_{50}	8.1
PI3Kα/β/γ pIC_{50}	5.0/5.8/6.6
LE/LLE	0.36/4.4
WB IFN γ pIC_{50}	6.7
Solubility	126 μg/mL
Permeability	410 nm/sec
cLogP	3.6
Number of aromatic rings	5
PFI	8.2

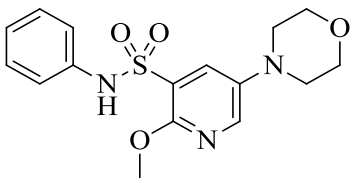
5.1 Hinge binder modifications

One structural change that could be effected was to replace the dihydropyran with morpholine. In the 2-methoxypyridine series, this resulted in a drop in potency.

Table 5.2 Enzyme inhibition data for compounds **15** and **87**.[36]



15

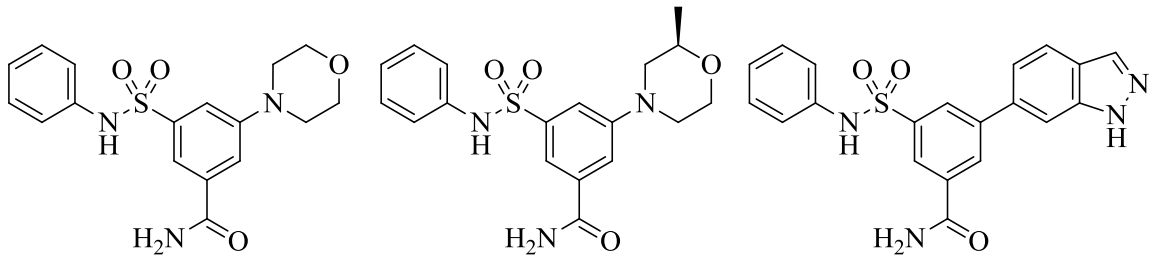


87

	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
15	5.4 (n=10)	5.6 (n=9)	5.1[a] (n=7)	6.7 (n=8)
87	4.8 (n=2)	5.2 (n=2)	4.8 (n=2)	6.1 (n=2)

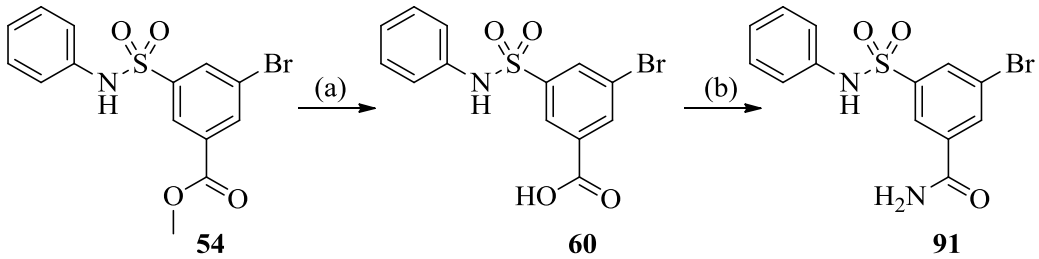
[a]On two occasions, a result of <4.5 was received.

The morpholine oxygen acts as the hinge binding motif, and it could be hypothesised that the drop in potency is due to the increased sp^3 character in the hinge region. As the hinge binder is required to sit 'flat' near the back pocket group, the morpholino-compound **87** would pay an energy penalty to adopt this conformation. One further change that can enhance the potency of the morpholine is the inclusion of a (*R*)-methyl group at the 2-position of the morpholine which is believed to occupy a small lipophilic pocket within the protein.[78] With this in mind, **88** and **89** were proposed for synthesis as potential alternatives for dihydropyran. Another hinge binding motif which was of interest was 6-indazole **90**, a 'longer' hinge binding motif, which would provide an insight into whether the 'short' hinge was a crucial component.



88 **89** **90**

The synthesis of **88** and **89** would be via Buchwald-cross-couplings and indazole **90** via a Suzuki cross-coupling. Common intermediate **91** was synthesised via the following route (Scheme 5.1) in excellent yields (96 % and 79 %).

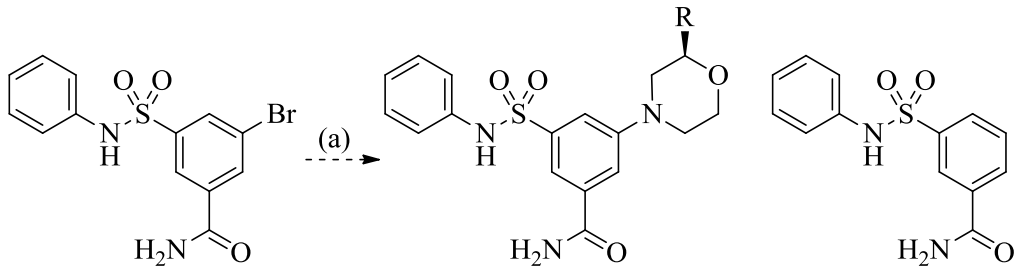


54 **60** **91**

(a) LiOH, THF, H_2O, rt, 16 h (96 %); (b) i) $SOCl_2$, 70 °C, 3 h; ii) NH_4OH, THF 0 °C (79 %).

Scheme 5.1 Synthesis of intermediate **91**.

With this intermediate, Buchwald-cross-coupling on this substrate proved challenging with both morpholine and (*R*)-2-methylmorpholine (Scheme 5.2).

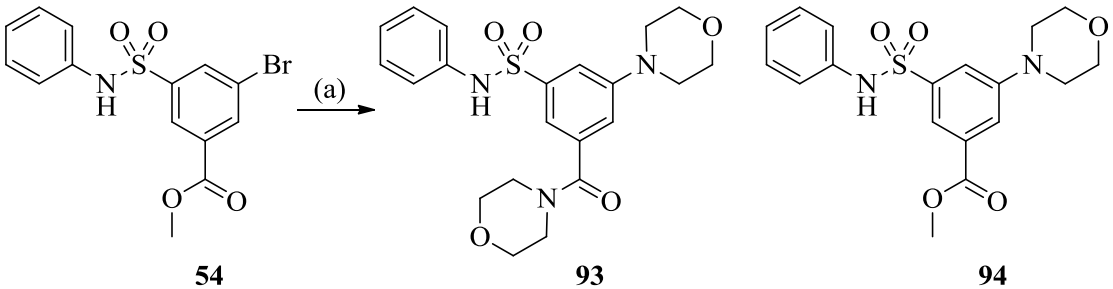


91 **88** R= H **89** R= CH_3 **92**

(a) RuPhos precatalyst, RuPhos, morpholine, NaO^tBu or Cs_2CO_3, 1,4-dioxane, 120 °C, 1 h.

Scheme 5.2 Proposed Buchwald cross-coupling of **88** and **89**

Initially, the reactions were trialled under microwave irradiation, utilising RuPhos precatalyst, RuPhos and sodium *tert*-butoxide (Scheme 5.2**)** however, only minimal traces of **88** and **89** were observed. The major product observed was the proto-debrominated product **92** by LCMS, indicating upon oxidative addition of the arylbromide, displacement of the palladium bromide bond with the morpholine was not occurring efficiently. Changing the base to caesium carbonate or increasing catalyst loading offered no improvement in conversion or reaction profile. At this stage, we envisaged that by changing the starting material to methyl ester **54**, the potentially problematic primary amide would be removed from the system.[109] Again under similar conditions, the following reaction was trialled with morpholine (Scheme 5.3).

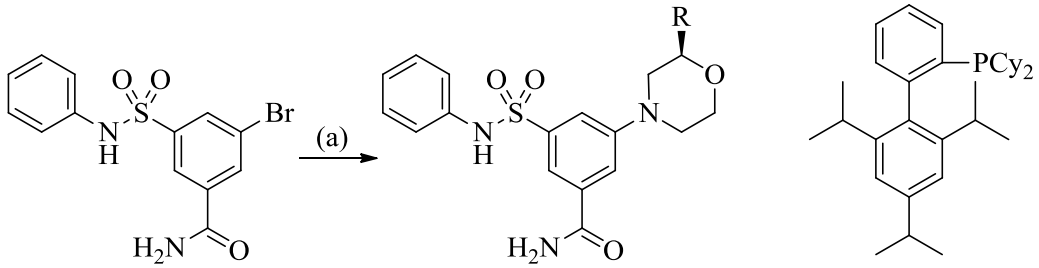


54 **93** **94**

(a) RuPhos precatalyst, RuPhos, morpholine, NaO^tBu or Cs_2CO_3, 1,4-dioxane, 120 °C, 1 h.

Scheme 5.3 Proposed synthesis of compound **94**.

Again under microwave irradiation, no desired product **94** was observed, however a new product was formed which was found to be **93**. With this observation, a change of base was proposed as a good option to remove the potential of amide formation. Cesium carbonate was chosen, and again under the same conditions, poor conversion was observed in three separate reactions. On one occasion after 1 h heating the major component of the reaction mixture was the carboxylic acid **60**. However, upon re-heating, the vial's pressure increased significantly leading to an explosion in the microwave cavity, which could suggest hydrolysis and subsequent decarboxylation under these conditions. At this stage, an alternative set of conditions from Buchwald and co-workers,[109] in which they describe how a mono-dentate ligand such as XPhos is efficient at forming a highly active Pd-catalyst for the Buchwald-cross coupling of challenging substrates. The paper featured compounds containing primary amides such as 3-chlorobenzamide, so the conditions were applied to **91** (Scheme 5.4).

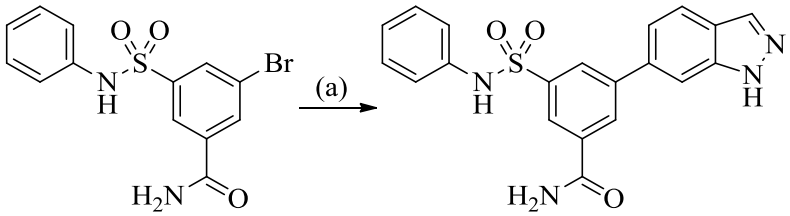


91 **88** R= H **89** R= CH_3

(a) $Pd_2(dba)_3$, XPhos, morpholine or (*R*)-2-methylmorpholine, LiHMDS, 65 °C, 17 h (23-36 %).

Scheme 5.4 Synthesis of compounds **88** and **89**.

Thankfully, this methodology gave acceptable levels of conversion to the desired products **88** and **89** after heating overnight, giving the products in poor yield (**88** 23 %, **89** 36 %). For indazole **90**, Suzuki cross-coupling with the corresponding boronic ester gave **90** in poor yield (34 %) (Scheme 5.5).



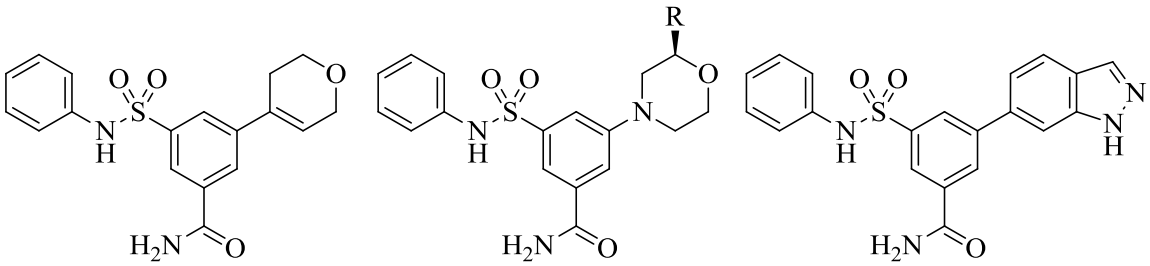
91 **90**

(a) 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 120 °C, 1 h (34 %).

Scheme 5.5 Synthesis of indazole **90**.

The results for compounds **88**, **89** and **90** are as followed (Table 5.3).

Table 5.3 Enzyme inhibition data for compounds **51** and **88**-**90**.[36]



51 **88** R= H **89** R= CH_3 **90**

	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
51	5.2 (n=3)	5.6 (n=3)	5.1[a] (n=1)	6.3 (n=3)	0.35/4.5
88	4.8 (n=7)	5.0[b] (n=6)	4.7[b] (n=6)	5.6 (n=7)	0.31/4.4
89	5.6 (n=4)	5.4 (n=4)	5.8 (n=4)	6.5 (n=4)	0.34/4.7
90	4.6 (n=2)	4.6 (n=2)	5.7 (n=2)	4.8 (n=2)	0.23/1.7

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.5 was received.

Results for morpholine **88** were as expected, in which a 5-fold drop in potency was observed, which is consistent with the 2-methoxypyridine series of compounds. Methylmorpholine **89**

was as similar in potency and selectivity to dihydropyran **51**, whilst maintaining similar ligand efficiencies and other physicochemical properties such as moderate solubility (128 μg/mL) and permeability (210 nm/s). In contrast, **90** was found to be at the lower borderline of the PI3Kδ assay, demonstrating that the longer hinge binding motif is not tolerated with the benzamide back pocket group in PI3Kδ. Indazole **90** now possessed some PI3Kγ activity, which indicates that the longer hinge binding motif positions the back pocket group in a position to interact better with PI3Kγ.

5.2 Amide modifications

The second area of the molecule we wanted to investigate was substitution from the amide. This was a novel area of the protein, as within the literature and GSK, no compounds had entered into this region in PI3Kδ. If substitution from this position was tolerated, it could provide a novel area in which some physical properties of the compound could be tuned. In chapter 3, we had previously observed that removal of a hydrogen bond donor of amide **51** with isoindolin-1-one **82** had improved permeability (175 to 390 nm/sec) however other properties such as solubility were compromised. The partial double-bond nature of the amide bond means it is not completely rigid and therefore its dihedral angle can adopt both *cis* and *trans* values. The *cis* configuration, however, is less frequently observed due to the much higher stability of the *trans* isomer (Figure 5.1). This is a desired vector from which substitution could be investigated.[110]

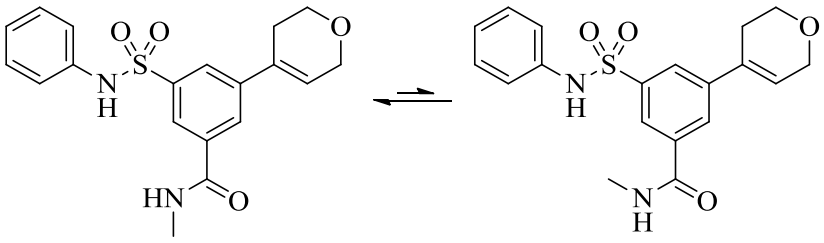


Figure 5.1 The preference of *cis* and *trans* amide conformation.

With this key observation noted, initially substitution from the amide was investigated computationally. Building from the *trans*-position of the amide with a methyl group, we

initially minimised the structure and then the resulting compound was docked into PI3Kδ. No poses of the docked compound were collected. This was because in this region of the protein, an interaction between Lys779 and Asp911 closed the pocket, which suggested that there was no space to extend from this position (Figure 5.2).

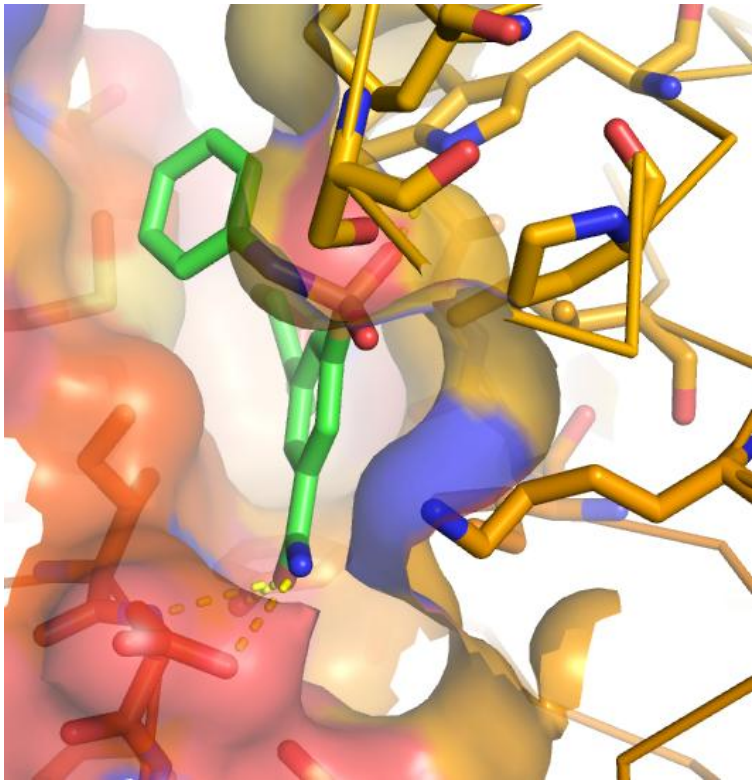
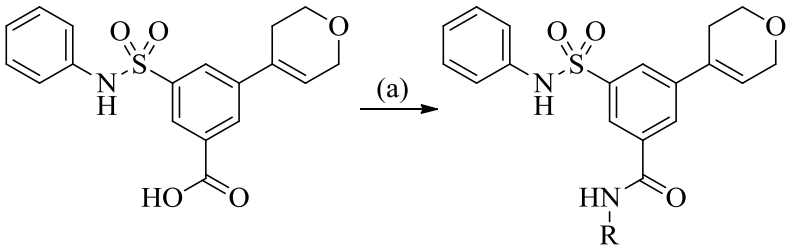


Figure 5.2 Co-crystal structure of **51** in PI3Kδ highlighting the tight binding pocket.[2]

However, within GSK, a number of compounds break the HBD/HBA interaction between the two residues and open the pocket as observed by crystallography. Unfortunately there are significant differences in the crystal structure of **51** such as the displaced water molecule and residue side-chain movement that using an alternative crystal structure with significant protein chain changes would not be relevant. With this in mind, we envisaged that methyl amide **95** and ethyl amide **96** would be critical to observe whether any substitution is tolerated. Using a previously described intermediate **56**, amides **95** and **96** (Scheme 5.6) were synthesised in moderate yield (**95** 54 %, **96** 47 %)



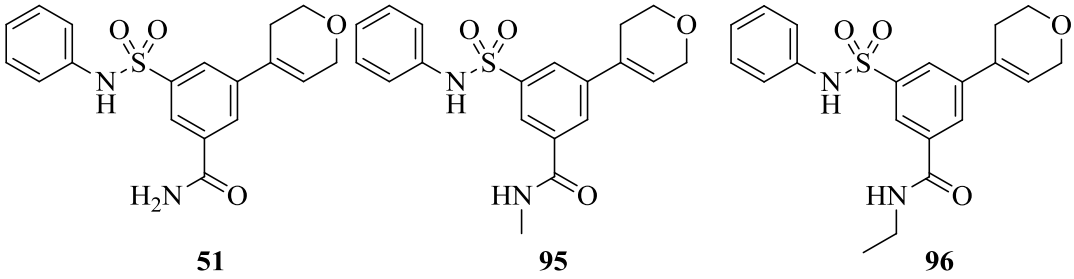
56 **95** R = Me
96 R = Et

(a) i) HATU, DIPEA, DMF, rt, 10-30 min; ii) R-NH_2 16 h (47-54 %)

Scheme 5.6 Synthesis of amides **95** and **96**.

The results for compounds **51**, **95** and **96** are as followed (Table 5.4).

Table 5.4 Enzyme inhibition data for compounds **51**, **95** and **96**.[36]



51 **95** **96**

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
51	5.2 (n=3)	5.6 (n=3)	5.1[a] (n=1)	6.3 (n=3)	0.35/4.5
95	<4.5 (n=4)	<4.5 (n=4)	4.9 (n=4)	5.7 (n=4)	0.3/3.6
96	<4.5 (n=4)	4.5[a] (n=2)	4.6[a] (n=2)	5.8 (n=4)	0.29/3.2

[a]On two occasions, a result of <4.5 was received.

Compounds methyl **95** and ethyl **96** showed very similar inhibition profiles in which, although a 4-fold decrease in potency against PI3Kδ was observed in comparison to **51**, both compounds showed very similar potency, indicating that substitution was not completely detrimental to potency. Despite this decrease at PI3Kδ, a significant decrease at the three

other PI3K class I isoforms is observed in which both methyl **95** and ethyl amides **96** show minimal activity. These initial results were encouraging as they suggested that substitution from this position was tolerated with the potential to enhance selectivity.

At this stage, we wanted to explore the scope and functionality that could be tolerated in this region in order to boost the potency of the compounds. In this region of the protein, if the interaction between Lys779 and Asp911 was disrupted, there would be two Asp residues side chains (Asp911 and Asp787) and the Lys779 side chain to interact with. With this in mind, a small selection of monomers was selected to probe this pocket (Figure 5.3).

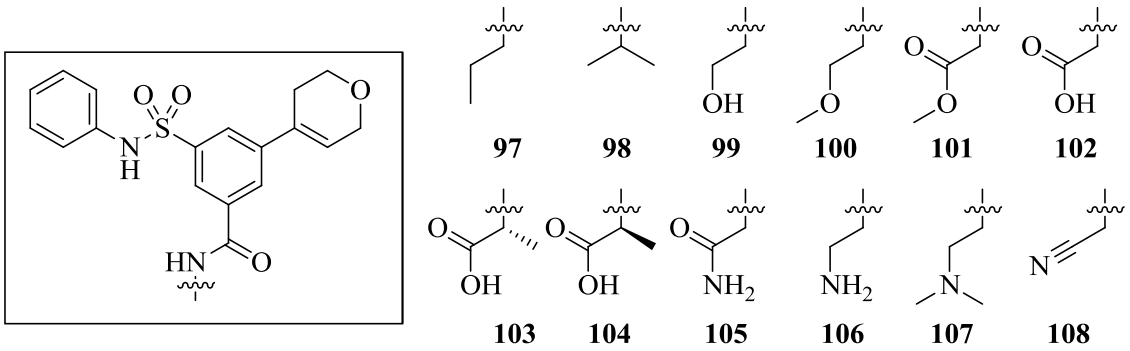
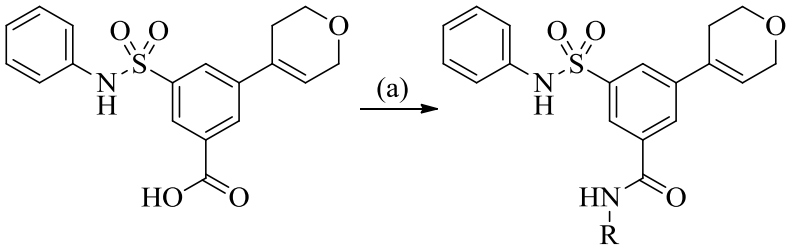


Figure 5.3 Amides **97**-**108** selected for synthesis.

Compounds **97** and **98** would probe the amount of space within the protein, the effect of increased lipophilicity and **98** would probe the effect of branching. Alcohol **99** would then test how a HBD would interact with the two acidic Asp side chains residues, and how removal of this HBD interaction with ether **100** would effect this change. Ester **101** would introduce a HBA into the region, which would not clash with the two Asp side chains, but potentially interact with Lys779 through the carbonyl. Carboxylic acids **102**, **103** and **104** were selected as compounds to interact with Lys779. Enantiomers **103** and **104** were selected as to see the effect on having some 3D character in this region with acid **102** as the reference. Amide **105** was selected to combine a HBA and HBD group in the same molecule. Introduction of basic groups **106** and **107** were chosen to probe the interaction with either Asp side chain. Nitrile **108** was selected to observe how a decrease in sp^3 character and the inclusion of a nitrogen atom would affect the compound in comparison to ethyl **96** and propyl **97**. The compounds were synthesised using either HATU or T_3P (Scheme 5.7).

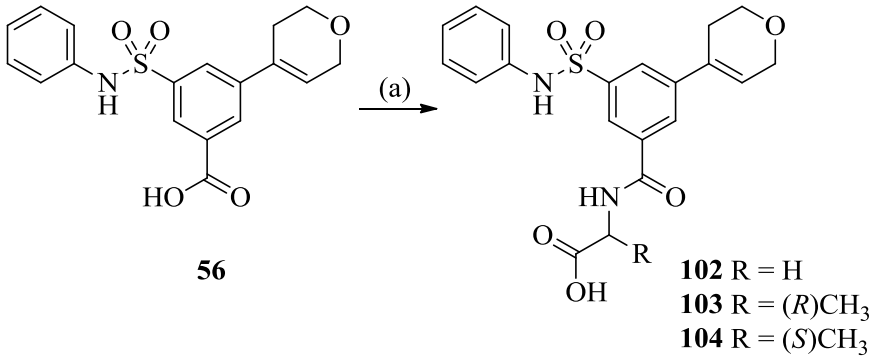


56

(a) i) HATU, DIPEA, DMF, rt, 10-30 min; ii) R-NH_2 16 h (30-89 %); (b) T_3P, DIPEA, R-NH_2, DMF, rt, 16 h (41-89 %).

Scheme 5.7 Synthesis of amides **97**-**101** and **105**-**108**.

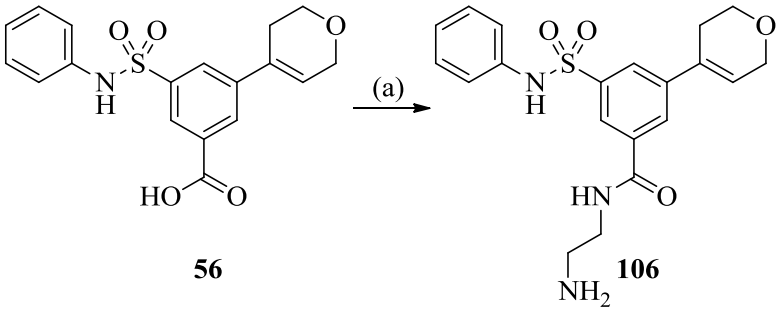
For **102**, **103** and **104** after the initial amide coupling with the corresponding glycine or alanine-esters, ester hydrolysis with lithium hydroxide furnished the desired carboxylic acids **102**, **103** and **104** (Scheme 5.8).



(a) i) HATU, DIPEA, DMF, rt, 10-30 min; ii) R-NH_2 16 h; iii) LiOH, THF, H_2O, rt, 16 h (17-31 %); (b) i) T_3P, DIPEA, R-NH_2, DMF, rt, 16 h; ii) LiOH, THF, H_2O, rt, 16 h (39 %).

Scheme 5.8 Synthesis of amides **102**-**104**.

For amine **106**, amide formation with the commercially available *N*-Boc-ethylenediamine was followed by deprotection with TFA to yield **106** (Scheme 5.9).



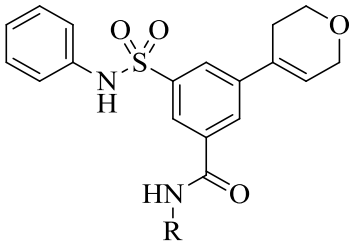
56 **106**

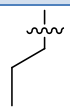
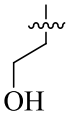
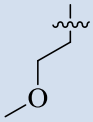
(a) T_3P, DIPEA, R-NH_2, DMF, rt, 16 h; ii) TFA, DCM, rt, 1 h (62 %).

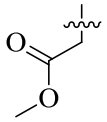
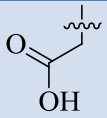
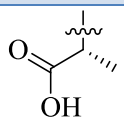
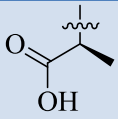
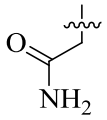
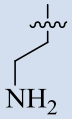
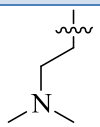
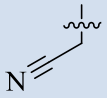
Scheme 5.9 Synthesis of amide **106**.

The results are as follows (Table 5.5).

Table 5.5 Enzyme inhibition data for compounds **97**-**108**.[36]



		pIC_{50}				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
97		<4.5 (n=2)	4.8[a] (n=1)	4.6[a] (n=1)	5.4 (n=2)	0.25/2.5
98		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.2 (n=2)	0.25/2.3
99		<4.5 (n=3)	4.7[a] (n=2)	4.5[a] (n=2)	6.0 (n=3)	0.29/4.5
100		<4.5 (n=2)	4.6 (n=2)	<4.5 (n=2)	5.1 (n=2)	0.24/2.9

101		<4.5 (n=3)	<4.5 (n=3)	<4.5 (n=3)	5.7 (n=3)	0.26/3.6
102		<4.5 (n=2)	<4.5 (n=2)	4.6[a] (n=1)	4.7 (n=2)	0.22/2.9
103		<4.5 (n=3)	<4.5 (n=3)	<4.5 (n=3)	5.5 (n=3)	0.25/3.4
104		<4.5 (n=3)	<4.5 (n=3)	<4.5 (n=3)	5.3 (n=3)	0.24/3.2
105		4.9[b] (n=2)	4.9 (n=4)	4.6[b] (n=2)	6.5 (n=4)	0.31/5.6
106		<4.5 (n=2)	4.7[a] (n=1)	4.6[a] (n=1)	5.6 (n=2)	0.27/4.1
107		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.0 (n=2)	0.23/2.6
108		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.1 (n=2)	0.25/3.7

[a]On one occasion, a result of <4.5 was received. [b]On two occasion, a result of <4.5 was received.

Analysis of the resulting data in comparison to methyl **95** and ethyl **96** indicated that extension by an additional carbon atom to give **97** or branching to an isopropyl group to give **98** further loses potency, indicating that increased lipophilicity in this region is not tolerated. This is consistent with the potential interactions that could form with the ionised lysine or aspartic acid residues at physiological pH. With an ethanolamine side-chain **99**, potency against PI3Kδ increased in comparison to methyl **95**, indicating that the inclusion of the polar hydroxyl group was beneficial to potency. This compound possessed greater than 20-fold selectivity against the closest PI3K isoform, but was still less potent than the initial starting amide **51**. In contrast, removal of the HBD interaction with ether **100** proved

significantly detrimental to potency. To probe the interactions formed, co-crystallography of **99** was obtained (Figure 5.4).

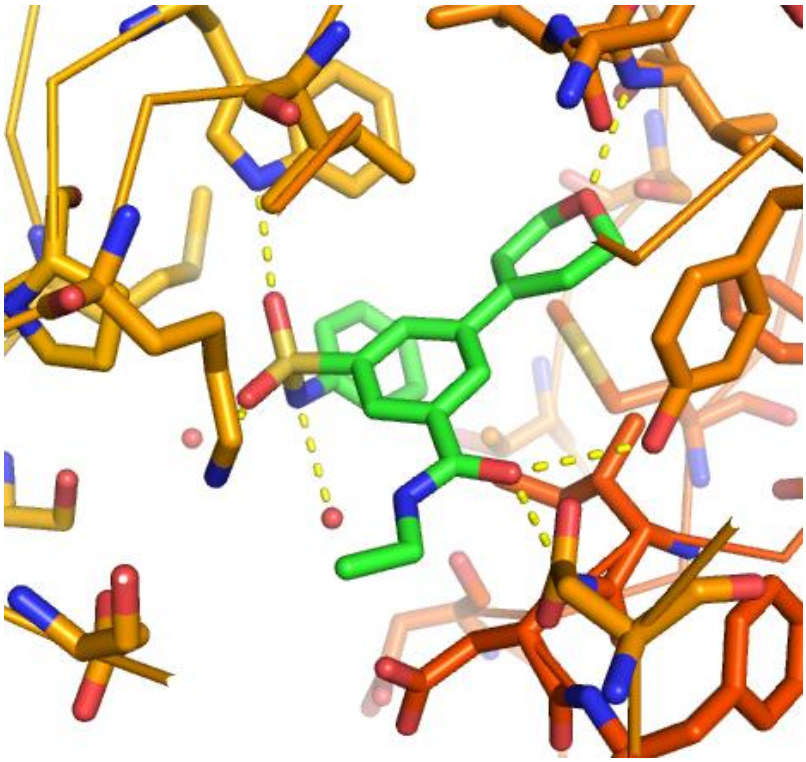


Figure 5.4A Co-crystal structure of **99** in PI3Kδ (Resolution 2.52 Å)[2]

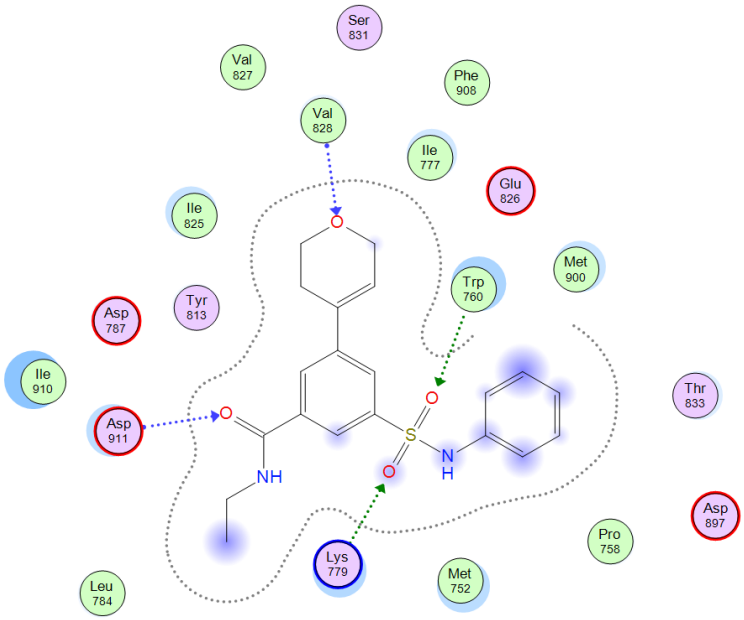


Figure 5.4B 2D Representation of **99** in PI3Kδ.

Good density and resolution (2.52 Å) was observed for the majority of the ligand leaving the binding mode unambiguous; however no density was observed for the hydroxyl group. In concordance with previous structures, the dihydropyran forms a HBD interaction with Val828, the amide carbonyl formed interactions with both Tyr813 and Asp911, and Trp760 forms an interaction with a S=O bond. However in this crystal structure a very well defined Lys779 interaction with the second S=O bond was observed. This suggests that disruption of the salt bridge formation between Lys779 and Asp911 results in a clear interaction with the sulfonamide.

The lack of density observed for the hydroxyl group could be due to it adopting more than 1-conformation within the protein. One such conformation (Figure 5.5) would move Lys779 towards the S=O bond and this conformation is the most energetically favoured within the protein when modelled computationally. By fixing of the protein and compound, computational calculations around the CH_2-CH_2OH bond were conducted and a clear minimum is observed (Figure 5.5). However there are other minima's around 2-4 kcal/mol higher in energy that are not significantly unfavourable, which could give an insight into the lack of density around the hydroxyl group.

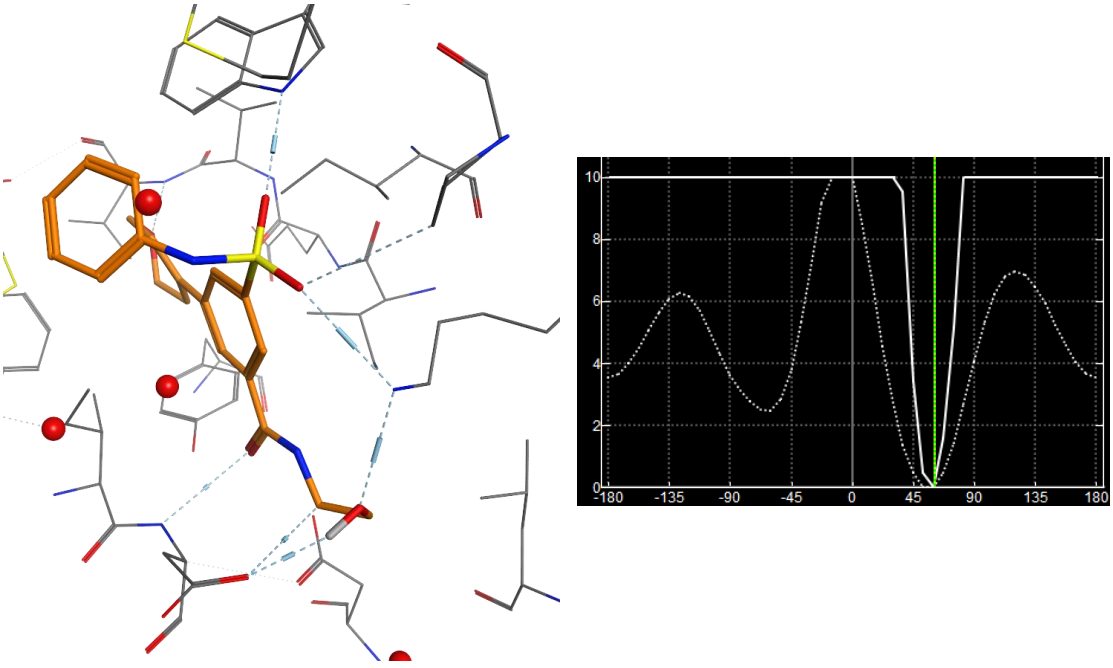


Figure 5.5 Hypothesised binding mode of **99** with the energy profile of the CH_2-CH_2OH bond (x-axis degree rotation (°), y-axis energy (kcal/mol)).

Ester **101** was found to be similarly potent and selective for PI3Kδ as ethyl **96**, suggesting that the ester functionality had little to no effect on the compound. However, in comparison acid **102** was at the borderline of our assay for potency against PI3Kδ, and was 39-fold less active than the primary amide **51**, indicating that inclusion of a negatively ionised group was severely detrimental to potency. Enantiomers **103** and **104** were significantly less potent than primary amide **51**, although they were more potent than acid **102**. This could indicate the methyl group occupies a small lipophilic pocket, or simply that the carboxylic acid was moved to no longer clash as significantly with the ionised aspartic acid groups in the region.

Amide **105** was the standout compound from the initial twelve compounds synthesised. The compound was of similar potency to primary amide **51**, with good efficiencies and superb selectivity (40-fold against the nearest isoform). This indicated that the combination of a HBA and HBD was important in this region of the protein. Structural data resulting from co-crystallography of **105** with PI3Kδ was collected (Figure 5.6).

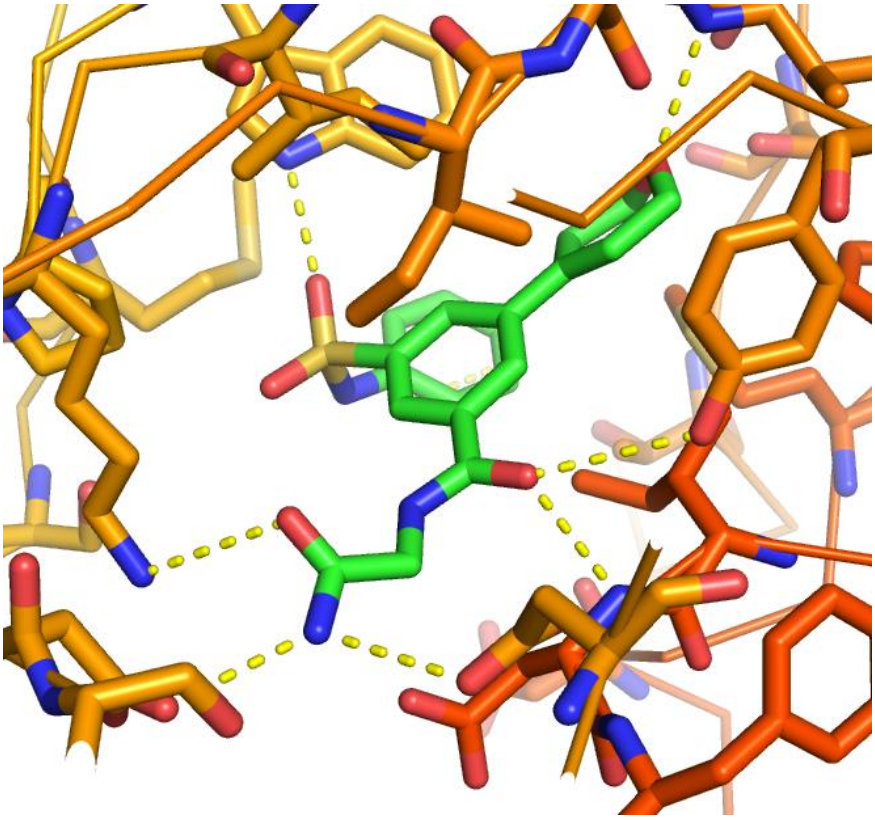


Figure 5.6A Co-crystal structure of **105** in PI3Kδ (Resolution 2.53 Å)[2]

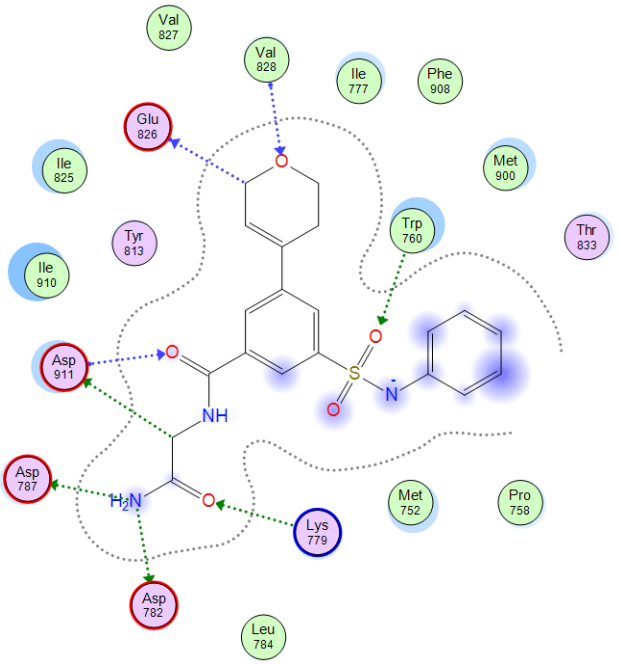


Figure 5.6B 2D Representation of **105** binding to PI3Kδ.

Compound **105** forms the hinge interaction with Val828, a HBA interaction with Trp760 and two HBA interactions through the carbonyl moiety to Tyr813 and Asp911 (Figure 5.6B). The major difference in this crystal structure in comparison to **51** is the clear opening of this pocket in the protein, in which the terminal amide forms a number of HBD interactions with the side chains of Asp787 and Asp782, and a HBA interaction with Lys779, which is now orientated towards the carbonyl, rather than the S=O bond as previously observed in **99**. This however does not explain how **105** is 40-fold selective for PI3Kδ over the other class I isoforms. The back pocket region of the PI3K protein isoforms is highly conserved, with all four isoforms having the same amino acid homology in this region, so an explanation for the selectivity obtained is challenging. An overlay of the co-crystal structure of **105** with PI3Kα, PI3Kβ and PI3Kγ displayed no clear explanation for the selectivity gained due to the high similarity in protein folding of PI3Kα and PI3Kβ, and although PI3Kγ has a 1-residue smaller loop within the protein, this does not appear to be close enough to cause this selectivity. A potential issue with this compound is its low permeability, of < 3 nm/sec. However as this amide side chain lowers the cLogP by 0.9 log units and permeability is partially dependent on the lipophilicity of the compound, this may be overcome by further modifications. Permeability is also influenced by HBD and PSA, and for compound **105** the

addition of a further HBD contributes to a higher PSA (**105** 127 $Å^2$ compared to phenyl **51** 98 $Å^2$) and this could cause the low permeability.

Removal of the carbonyl moiety from amide **105** to give amine **106** was significantly detrimental to potency. Primary amine **106** was found to be of similar potency to ethyl **96**, indicating that the amino group had had little to no effect. Increasing the steric bulk, lipophilicity and basicity of the amine in **107** was further detrimental to potency. Finally, nitrile **108** had poor activity for PI3Kδ, indicating that it was unable to inhibit in a similar fashion to amide **51**.

To follow on from the positive result of **105** we wanted to explore further around the primary amide moiety. The compounds shown below were the second wave of compounds designed to enhance, mimic or replace the terminal amide (Figure 5.7).

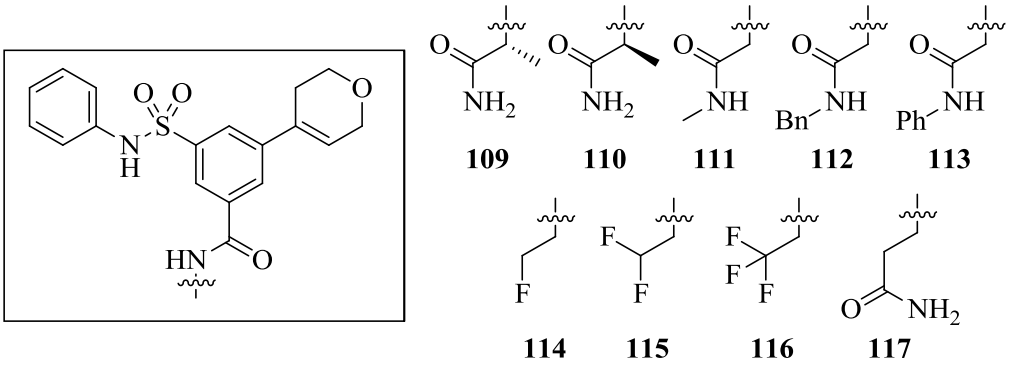
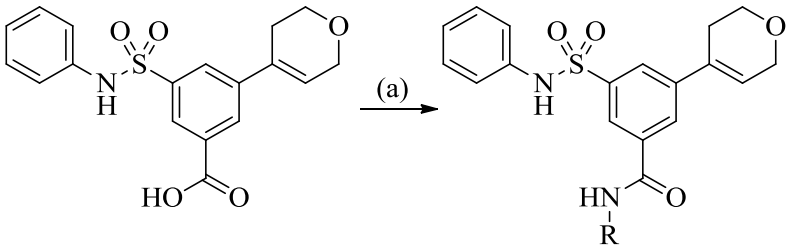


Figure 5.7 Amides **109**-**117** selected for synthesis.

Enantiomers **109** and **110** were proposed to investigate whether a synergistic effect could be replicated from acid **102**, in which a potency boost was observed upon incorporation of the chiral methyl groups (**103** and **104)**. If this led to a boost, different amino acids side chains could be incorporated to further test the requirements of the region. Further substitution from the amide would also be investigated, as via the crystal structure of **105**, we can observe that past the primary amide the protein opens out. Initially **111**, **112** and **113** were proposed as compounds that would investigate the amount of space available. We also wanted to probe whether we could mimic the amide without removal of the hydrogen bond donor properties

of the amide. We proposed to do this via **114** and **115**, in which the fluorine atoms would hopefully mimic the carbonyl and the polarised C-H bond(s) would behave as acidic proton(s), allowing them to interact with the Asp residues. In order to observe the effects of removing the hydrogen bond donor properties, trifluoro **116** would also be synthesised.[111] Finally, homologation of amide **116** to the beta-amino acid **117** would be investigated.

The majority of the monomers required were commercially available, so amide formation using HATU gave the desired compounds **109-117** (Scheme 5.10).

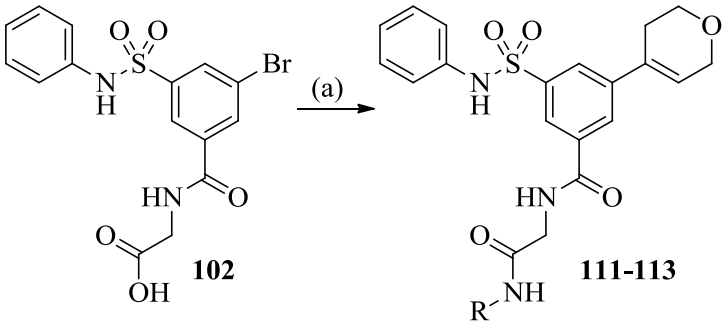


56 **109-117**

(a) i) HATU, DIPEA, DMF, rt, 30 min; ii) R-NH_2 16 h (41-72 %);

Scheme 5.10 Synthesis of amides **109-117**.

For **111-113**, synthesis of acid **102**, followed by amide couplings utilising T_3P, gave **111-113** (Scheme 5.11).



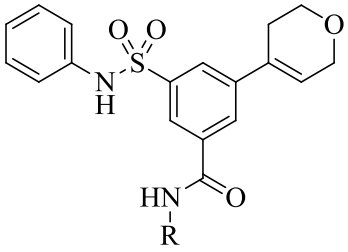
102 **111-113**

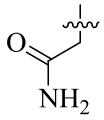
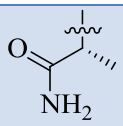
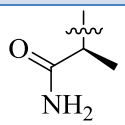
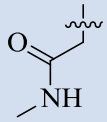
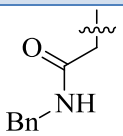
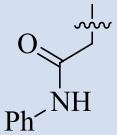
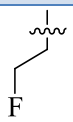
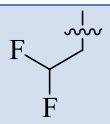
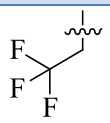
(a) T_3P, DIPEA, R-NH_2, DMF, rt, 16 h (34-44 %).

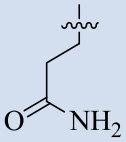
Scheme 5.11 Synthesis of amides **111-113**.

The results were as follows (Table 5.6).

Table 5.6 Enzyme inhibition data for compounds **105** and **109**-**117**.[36]



		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
105		4.9[a] (n=2)	4.9 (n=4)	4.6[a] (n=2)	6.5 (n=4)	0.31/5.6
109		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.2 (n=2)	0.24/4.0
110		<4.5 (n=2)	4.5[b] (n=1)	4.7[b] (n=1)	5.8 (n=2)	0.26/4.6
111		4.6 (n=3)	5.0 (n=3)	<4.5 (n=3)	6.4 (n=3)	0.29/5.2
112		4.5 (n=2)	4.7 (n=2)	5.4 (n=2)	5.6 (n=2)	0.21/2.5
113		4.7 (n=2)	4.6 (n=2)	5.4 (n=2)	5.7 (n=2)	0.22/2.6
114		5.1 (n=5)	5.0 (n=5)	4.8[b] (n=4)	6.4 (n=5)	0.31/4.1
115		5.0 (n=5)	5.0 (n=5)	4.9 (n=5)	6.5 (n=5)	0.31/3.9
116		4.7[a] (n=1)	4.9[b] (n=2)	4.8 (n=3)	5.9 (n=3)	0.27/3.0

117		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.6 (n=2)	0.26/4.4

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.5 was received.

Both enantiomers (**109** and **110**) resulted in a drop in potency in comparison to **105**, suggesting that branching from the α-position with the primary amide **105** is not tolerated. This is in accordance with isopropyl **98**, in which branching is observed to be detrimental to potency. This also indicates that acids **103** and **104** have an alternative binding mode to accommodate the ionised carboxylic acid. Unfortunately, branching from the amide N-H with methyl, benzyl or phenyl groups (**111-113**) provided no further boost in potency, with both **112** and **113** losing potency. Compound **111** was comparable to **105** in both potency and selectivity profile. Importantly **111** was found to improve permeability and even though this value was low (27 nm/sec) it did offer a benefit in comparison to **105** (< 3 nm/sec). Compounds **114** and **115** showed comparable potency and selectivity to **105** whilst improving the permeability (350 and 410 nm/sec), further demonstrating that from this position, multiple physical properties can be modulated. A crystal structure of **115** was obtained (Figure 5.8).

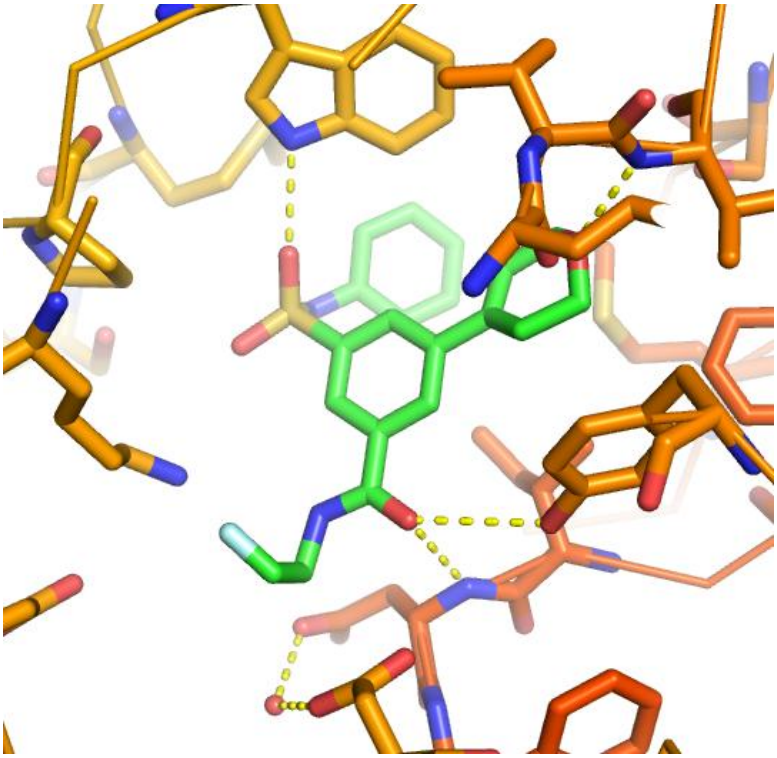


Figure 5.8A Co-crystal structure of **115** in PI3Kδ (Resolution 2.20 Å)[2]

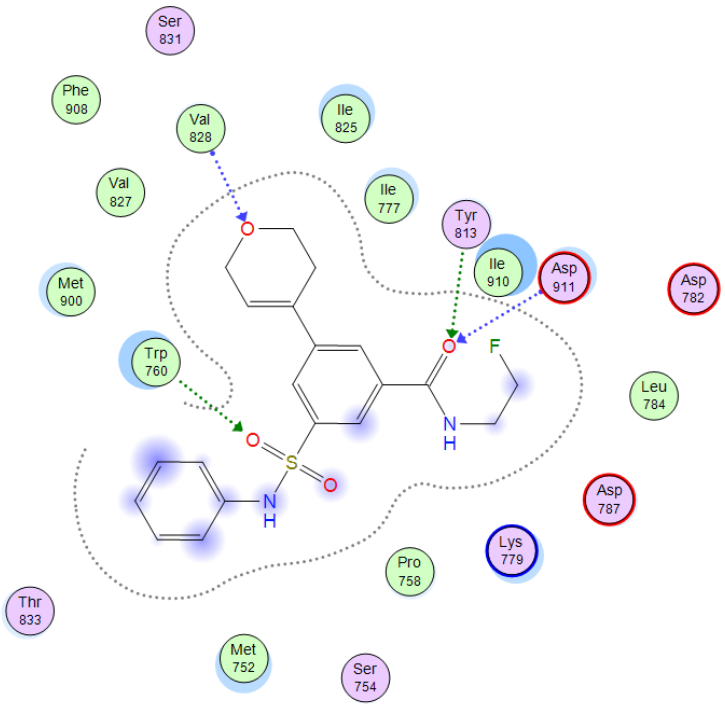


Figure 5.8B 2D Representation of **115** binding to PI3Kδ.

The crystal structure demonstrated that the compound bound in a similar fashion to amide **105**. Only one fluorine atom was observed in the crystal structure and this appears to mimic the carbonyl of the primary amide of **105**, maintaining a similar vector pointing towards Lys779 which is situated 2.8 Å away (Figure 5.8A). This could indicate the formation of a HBA from the ionised lysine residue.[111]

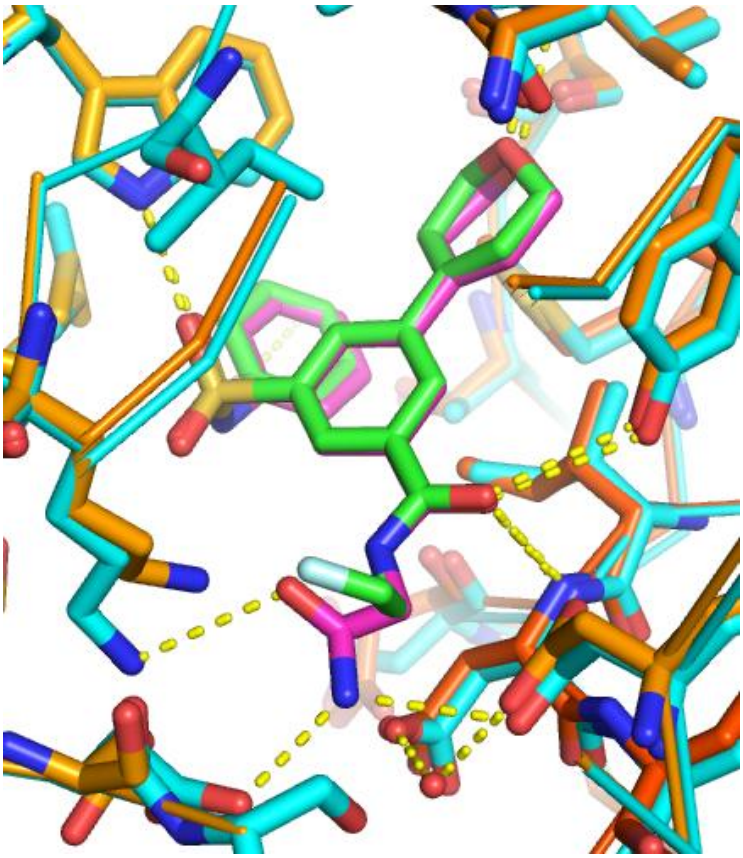


Figure 5.9 Overlay of compounds **105** (magenta) and **115** (green).

Trifluoroethyl **116** demonstrated a 4-fold drop in potency in comparison to **114** and **115**, highlighting the importance of a HBD in this area of the protein. Finally, **117** was found to decrease in potency, indicating homologation of the chain by 1-carbon atom orientated the primary amide into a region unfavourable to make positive interactions.

The final changes initiated in this area were to replace the amide with small 5-membered ring heterocycles. We made the conscious decision to avoid pyrrole due its metabolic liabilities,[112] and instead focussed on heterocycles that would have at least one HBD and HBA and not have an acidic centre at physiological pH (Figure 5.10).

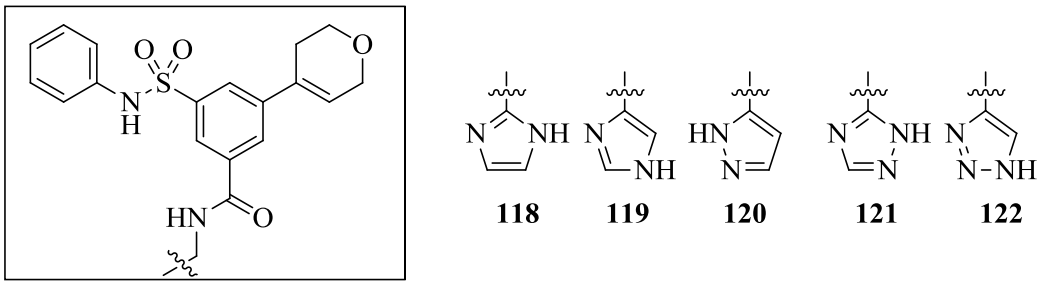
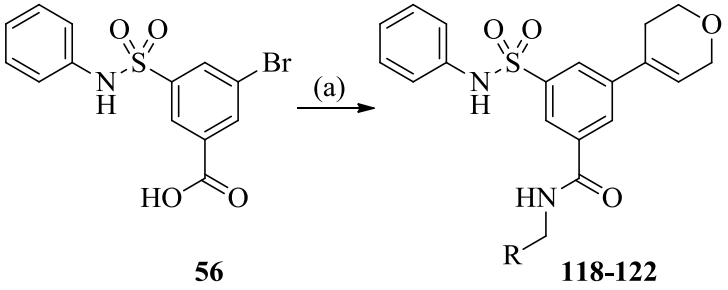


Figure 5.10 Compounds **118-122** selected for synthesis.

The compounds were synthesised using HATU in poor to moderate yields (19-57 %) (Scheme 5.12).

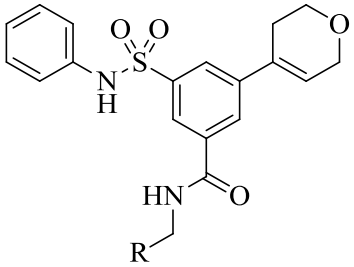


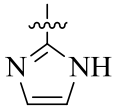
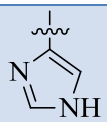
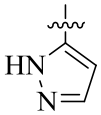
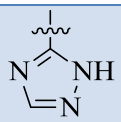
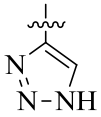
(a) i) HATU, DIPEA, DMF, rt, 30 min; ii) R-NH_2, rt, 16 h (19-57 %);

Scheme 5.12 Synthesis of amides **118-122**.

The results were as follows (Table 5.7).

Table 5.7 Enzyme inhibition data for compounds **118**-**122**.[36]



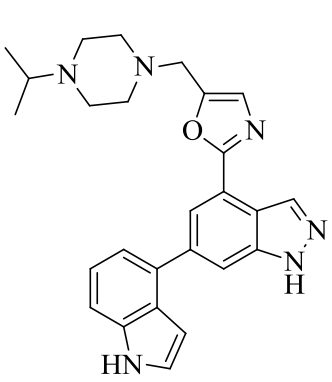
		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
118		<4.5 (n=2)	4.7[a] (n=1)	<4.5 (n=2)	5.7 (n=2)	0.25/3.8
119		<4.5 (n=3)	<4.5 (n=3)	<4.5 (n=3)	5.7 (n=3)	0.25/3.8
120		<4.5 (n=2)	<4.5 (n=2)	4.7[a] (n=1)	4.8 (n=2)	0.21/2.7
121		5.2[a] (n=1)	<4.5 (n=2)	<4.5 (n=2)	5.3 (n=2)	0.23/3.7
122		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	4.9 (n=2)	0.22/3.2

[a]On one occasion, a result of <4.5 was received.

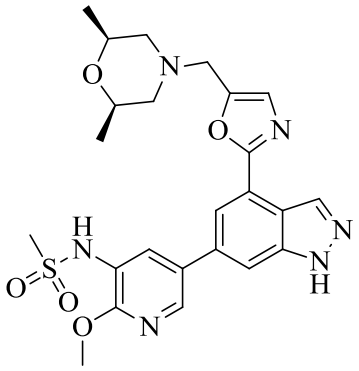
Both imidazoles **118** and **119** were moderately active, however both compounds had diminished potency in relation to the standout amides synthesised previously. Pyrazole **120**, and triazoles **121** and **122** were weakly active, indicating that their inclusion was detrimental to potency. No further interest was invested into heterocyclic replacements.

5.3 Sulfonamide modifications

The final stage to modify the benzamide **51** was by modification to the sulfonamide group. Within historic GSK series, access to the selectivity pocket was by substitution from the hinge binding motif. For example indazole **7** accesses the selectivity pocket by substitution of an oxazole ring with an isopropyl-piperazine, whereas indazole **8** accesses the same region with a dimethylmorpholine, both of which possess some steric bulk in order to clash with Arg770 in PI3Kα and Lys777/802 in PI3Kβ and PI3Kγ. These groups also contribute significantly to the potency of the compound, in which it was found that a basic amine was crucial for whole blood potency.[45]



7



8

However within the benzamide template, building from the dihydropyran hinge binder was not as attractive as an option compared to building from the sulfonamide due to the distance required to build into this region from the hinge. An overlay of the indazole **8** and benzamide **51** demonstrates the distance between the dimethylmorpholine nitrogen **8** and the benzamide **51** phenyl ring *meta*-position is 3.2 Å and the *para*-position 3.5 Å (Figure 5.11). This approach has been successfully utilised on the 2-methoxypyridine series, however due to the differences in binding of amide **51** and 2-methoxypyridine **15**, especially in the sulfonamide region, there is no overlap between the series.

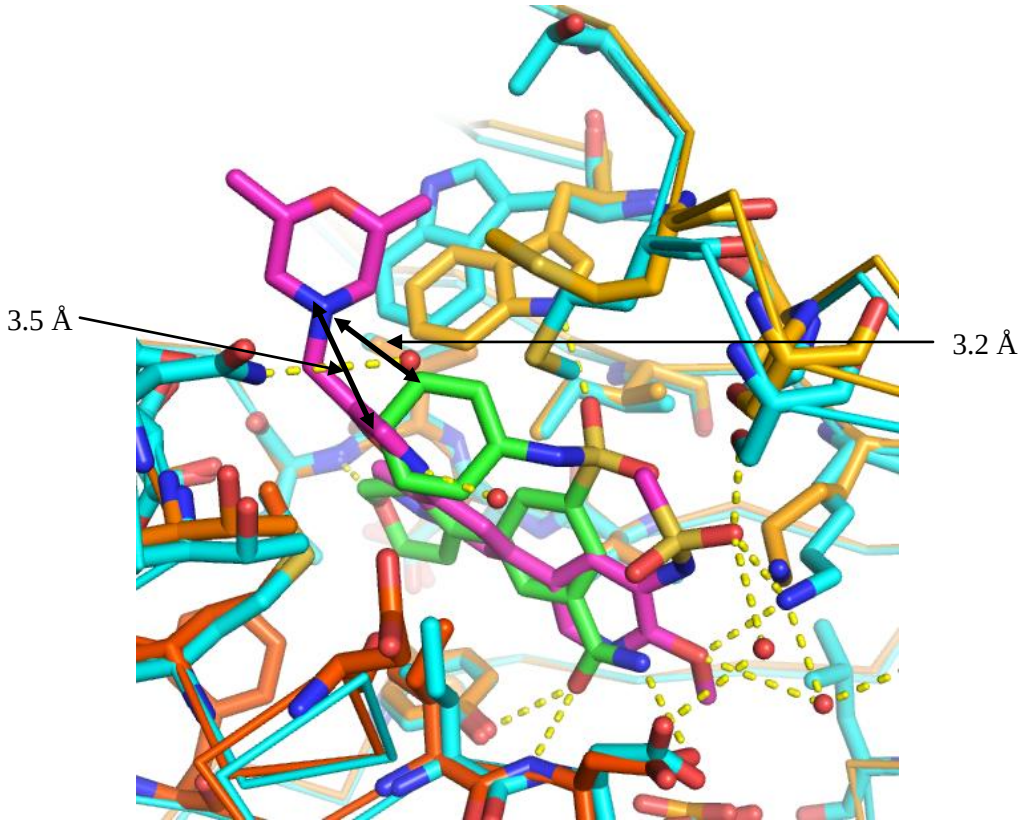
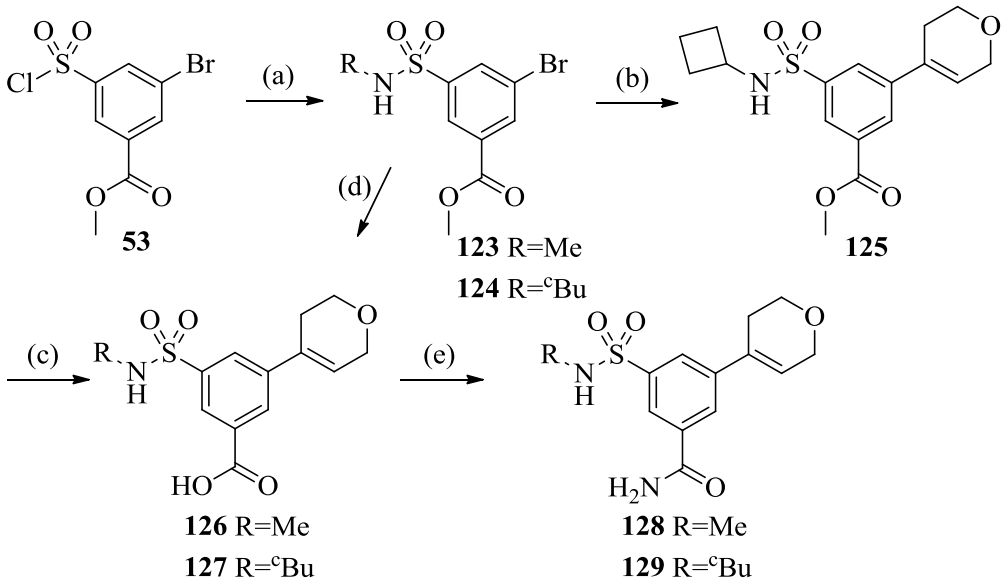


Figure 5.11 Overlay of **8** and **51** demonstrating the distance between the *meta*- and *para*-positions of the phenyl ring and the morpholino nitrogen.

This demonstrated a novel vector to access the selectivity pocket. Initially to probe the requirement of a phenyl ring, two alkyl moieties **128** and **129** were selected. Methyl **128** would test whether removal of the phenyl group would lead to a compound of similar efficiency. Cyclobutyl **129** would probe how an increase in lipophilicity in comparison to methyl **128** would affect potency (Scheme 5.13).

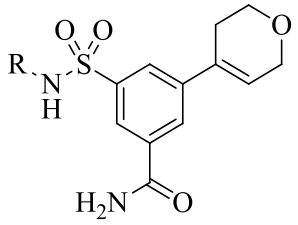


(a) R-NH_2, pyridine, rt, 3 h (52-70 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (82 %); (c) LiOH, THF, Water, rt, 16 h (83 %); (d) i) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min; ii) LiOH, THF, Water, rt, 5 h (98 %); (e) i) HATU, DIPEA, DMF, rt, 30 min; ii) NH_3 in 1,4-dioxane, rt, 24 h (7-42 %).

Scheme 5.13 Synthesis of sulfonamides **128** and **129**.

Compounds **128** and **129** were synthesised in accordance with the previously utilised route. Methyl **126** was a problematic compound as during the Suzuki cross-coupling (Scheme 5.13 Step (d)) partial hydrolysis of the methyl ester occurred. To overcome this, the partially hydrolysed mixture was used without purification in the ester hydrolysis step to give **126**. Amide formation with **126** led to a challenging final purification of **128**, due to close running impurities which gave a poor yield.

Table 5.8 Enzyme inhibition data for compounds **128** and **129**.[36]

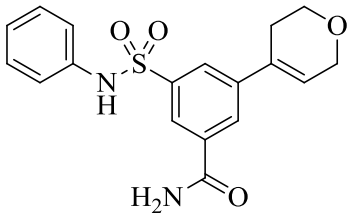


		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
128	Me	<4.5 (n=2)	4.6[a] (n=1)	4.5[a] (n=1)	5.0 (n=2)	0.34/4.9
129		<4.5 (n=2)	4.9[a] (n=1)	<4.5 (n=2)	4.9 (n=2)	0.29/3.9

[a]On one occasion, a reading of <4.5 were recorded.

The methyl sulfonamide **128** demonstrated low potency, but remained an efficient compound. This potency drop however was more detrimental in comparison with 2-methoxypyridine **15** (pIC$_{50}$ 6.8, LE 0.38) and its corresponding methyl version **17** (pIC$_{50}$ 6.2, LE 0.45). Increasing the lipophilicity of the alkyl group with a cyclobutyl sulfonamide **129** had no effect, symbolising how simply increasing lipophilicity was not beneficial.

In order to conduct this work, a computational approach was utilised in order to investigate growth from a compound such as benzamide **51**.



51

Initially, focusing on '*meta*' substituents, mapping the nitrogen atom of the morpholine ring into the same position as in indazole **8** was stipulated. Due to limitations within the software,

'*meta*' substitution is specified as the vector from which substitution occurs, not specifically *meta*-substitution from a phenyl ring. This therefore includes 5- and 6- membered rings with substitution from this vector. By using a custom pharmacophore, in which the benzamide core, hinge binder and sulfonamide moieties are fixed and the only requirements were the nitrogen atom and an aromatic linker, a 'scaffold' replacement search was conducted (Figure 5.12). A scaffold replacement involves selecting the portion of the molecule to be replaced, in this case from the sulfonamide nitrogen to the morpholino- nitrogen, whilst the pharmacophore defines the requirements of the fragment.

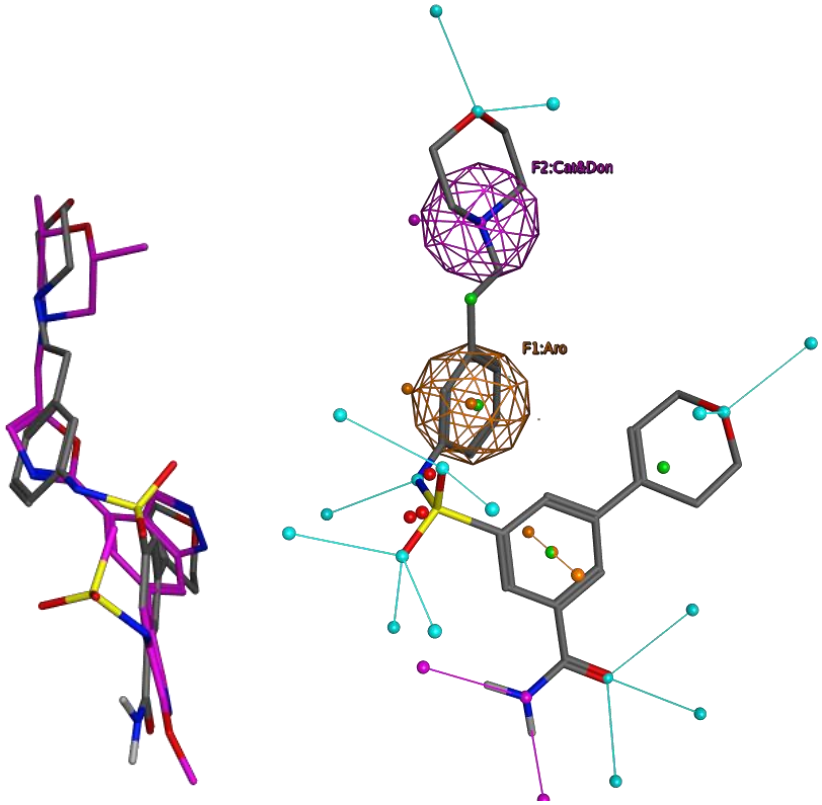


Figure 5.12 Pharmacophore utilised in the scaffold replacement search.

Using both internal and external databases, the scaffold replacement gave 435 hypothetical compounds. However it quickly became evident that a lot of the compounds were simple changes within the composition of the 5- or 6-membered sulfonamide ring. For example Figure 5.13 demonstrates a selection of different 5-membered rings which maintain a propylmorpholine-side chain

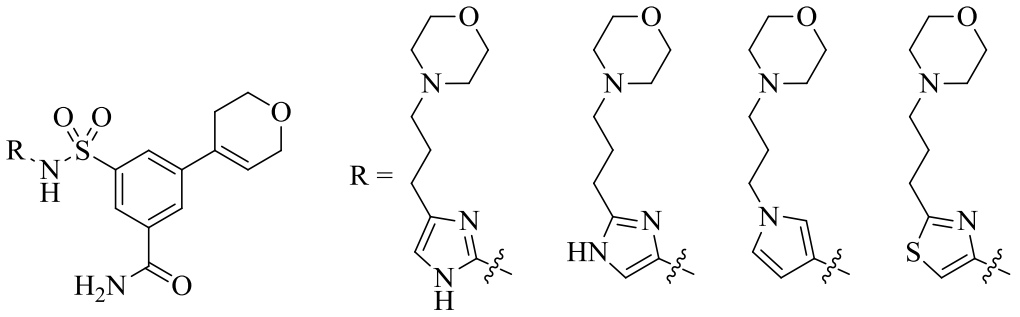


Figure 5.13 Example compounds from the scaffold replacement.

These results suggested that from a 5-membered heterocycle, a 3-atom linked chain was required. From a 6-membered ring the results were more varied and found to be dependent on the linking atoms which varied the geometry/vector of the linking chain (Figure 5.14). On a significant number of the 6-membered rings substitution of methyls, methoxy or fluorine groups at various positions around the ring were found within the results with some examples shown below.

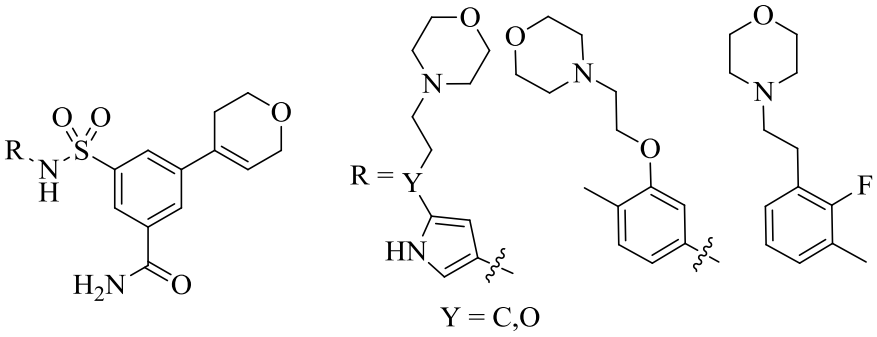


Figure 5.14 Example compounds from the scaffold replacement.

This strategy was then further applied to the '*para*' substituent of **51**. Again due to limitations in the software, '*para*' substituents are specified as the vector from which substituents occur, not specifically *para*-substitution from a phenyl ring. This scaffold replacement gave 65 theoretical compounds. These followed a very similar pattern in which the hypothetical compounds read-out were 3-atom linked morpholine rings from a 6-membered aromatic or heteroaromatic ring (Figure 5.15).

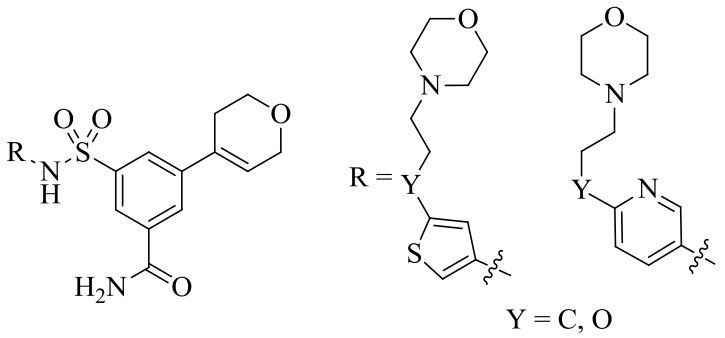
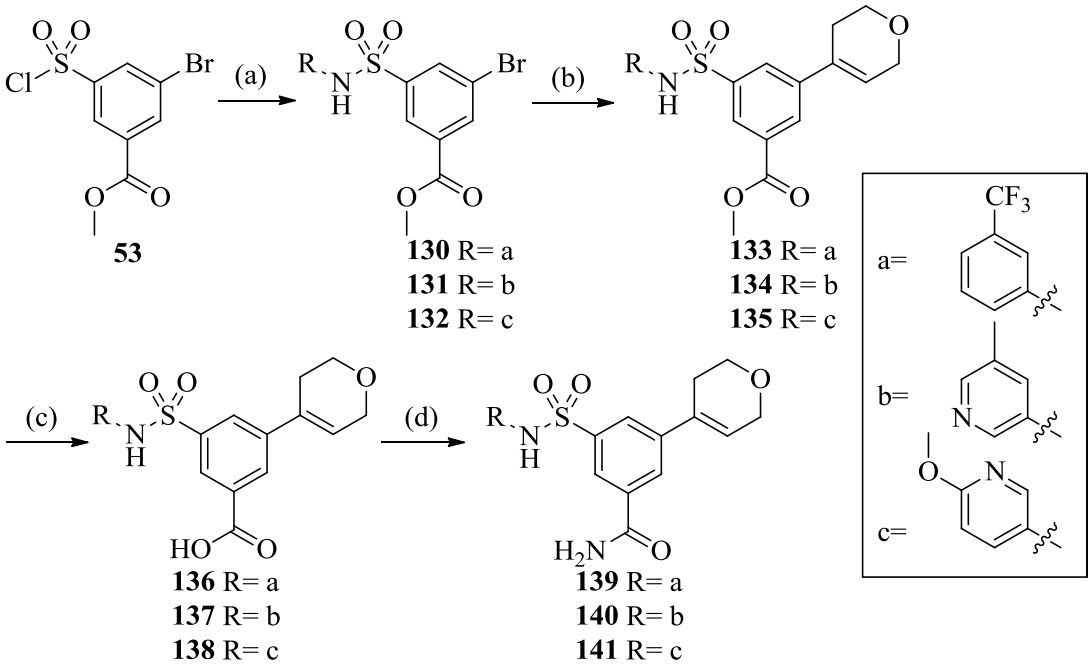


Figure 5.15 Example compounds from the scaffold replacement.

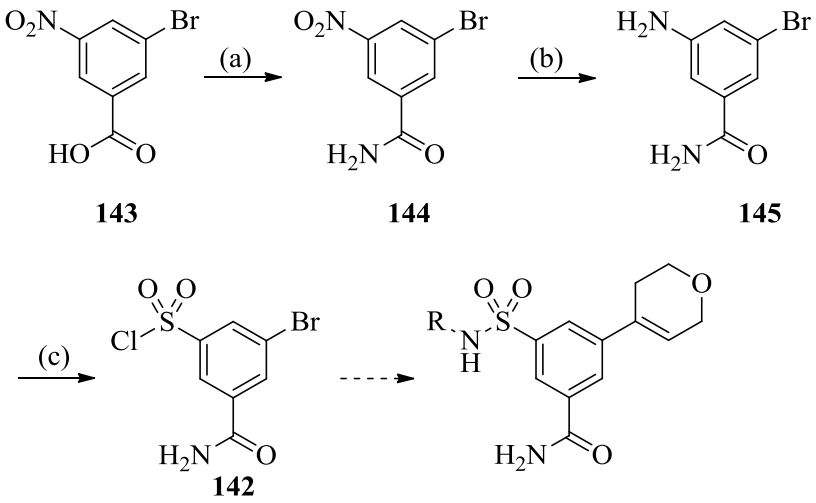
Given that the computational chemistry successfully identified potential compounds to investigate from both 5- and 6- membered aromatic/heteroaromatic rings with a variety of substituents and linkers we first decided to investigate small substituents around different aromatic and heteroaromatic rings before synthesising fully elaborated compounds. The first compounds chosen were **139-141** (Scheme 5.14)



(a) R-NH_2, pyridine, rt, 3 h (52-72 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, $PdCl_2$(dppf), Na_2CO_3, 1,4-dioxane, H_2O, μw, 100 °C, 30 min (46-93 %); (c) LiOH, THF, Water, rt, 16 h (89-100 %); (d) i) HATU, DIPEA, DMF, rt, 20 min; ii) NH_3 in 1,4-dioxane, rt, 24 h (27-46 %).

Scheme 5.14 Synthesis of sulfonamides **139-141**.

All 3 compounds were synthesised without incident, however whilst synthesising **139-141**, it quickly became apparent that in order to explore SAR of the sulfonamide group, a change in route was required. As each compound required a four-step synthesis due to the sulfonamide formation as the initial step, we wanted to investigate whether a convergent synthesis was possible. As we would have to generate a sulfonyl chloride **142** on gram scale quantities with the ability to store the compound to make multiple iterations of compounds, we initially ruled out 'in situ' based sulfonyl chloride syntheses such as palladium-catalysed sulfonyl chloride synthesis with DABSO pioneered by Willis and co-workers[113,114] due to poor compatibility with aniline nucleophiles or quenching of organo-metallic reagents with aryl sulfochloridates[115] or sulfuryl chloride[116] due to the intrinsic primary amide. Instead, we focused on the following route (Scheme 5.15) as we had previously used the diazotisation based sulfonyl chloride synthesis on scale, the reaction was found to be robust and the desired sulfonyl chloride **142** could be collected easily by filtration.[108]



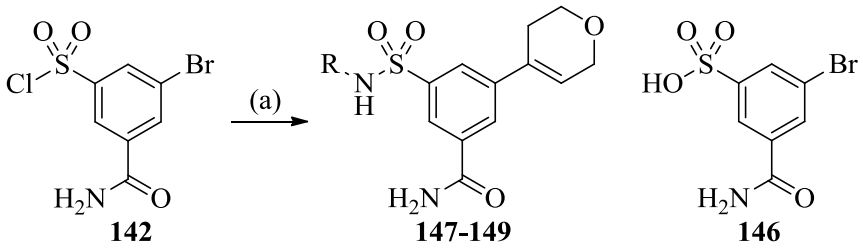
(a) i) $SOCl_2$, 70 °C, 3 h; ii) NH_4OH, THF, 0 °C (94-99 %); (b) Fe, NH_4Cl, EtOH, H_2O, 80 °C, 17 h (87-99 %); (c) i) $HCl_{(aq)}$, 20-0 °C; ii) $NaNO_2$, -5 °C, 1 h; iii) $SOCl_2$, H_2O, CuCl, -5 °C, 1 h (43-67 %).

Scheme 5.15 Synthesis of sulfonylchloride **142**.

The starting material for this synthesis **143** was relatively inexpensive (£6 per g) and was readily converted into **144** via *in situ* acyl chloride formation and subsequent quenching with ammonia to give **144** in high purity (94-99 % yield). Reduction of the nitro-substituent

proceeded well under mild-iron-catalysed conditions and gave good yields (87-99 %) without the need for column chromatography. At this stage, sulfonyl chloride formation under the previously utilised conditions in chapter 3 yielded **142** in moderate purity (44 %) and yield (56 %) on a small scale. The reaction was scaled up two-fold and on two occasions, via very close monitoring and cooling of the reaction temperature, a significantly purer (64 - 71 % by LCMS) product was isolated in moderate yield (43-44 %). These batches were subsequently used for the chemistry outlined below; however replication of these conditions proved troublesome. The issue was tracked down to the transfer of the diazotised aniline **145** to the sulfur dioxide/hydrochloric acid, where significant 'foaming' of the reaction mixture was observed. This led to an exothermic degradation of the reaction mixture. We attributed this to the poor solubility of the aniline hydrochloride salt, which we believed was slow in formation and led to poor diazotisation upon addition of the sodium nitrite. To overcome this, the aniline **145** and the hydrochloric acid were heated for 1 h before the flask was cooled and the sodium nitrite added. This led to high purity (84 %) product in moderate yield (67 %).[108]

Three reactive amines were used to test the two-step (sulfonamide formation and Suzuki cross-coupling) procedure involved. A small silica plug would be used to remove any hydrolysed sulfonic acid **146** after sulfonamide formation (Scheme 5.16). Compound **147** would probe how homologation of the phenyl ring by a CH_2 would affect potency. As the sulfonamide substituent was no longer aromatic, this would increase the pK_a of the sulfonamide N-H (calculated pK_a 9.8) in comparison to phenyl **51** (calculated pK_a 7.7, measured pK_a 8). Methyl **148** and ethyl **149** would probe whether removal of the acidic sulfonamide N-H was tolerated. As the sulfonamide nitrogen did not make an observable interaction via crystallography (Figure 4.8), it could be hypothesised that unless the sulfonamide nitrogen was having a subtle effect, **148** and **149** would not affect the potency significantly.

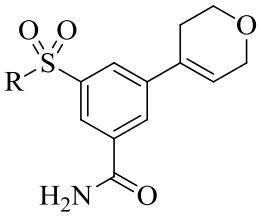


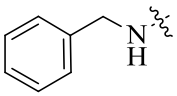
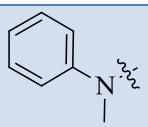
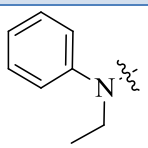
(a) i) R-NH_2, pyridine, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (11-24 %);

Scheme 5.16 Synthesis of sulfonamides **147-149**

Under the 2-step procedure, **147 - 149** were synthesised in poor yields (11-24 %) due to the low purity of the starting material and difficult purification. Possibly contributing towards this was the reagent's low solubility in the reaction solvent pyridine.

Table 5.9 Enzyme inhibition data for compounds **147-149**.[36]



		pIC_{50}				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
147		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.0 (n=2)	0.26/3.1
148		5.0 (n=3)	5.4 (n=3)	5.1 (n=3)	6.2 (n=3)	0.33/4.3
149		5.2 (n=3)	5.0 (n=3)	5.0 (n=3)	6.1 (n=3)	0.31/3.8

Homologation of the phenyl ring by one carbon is significantly detrimental to potency, with **147** equipotent with methyl sulfonamide analogue **128**, further supporting the need for an aromatic sulfonamide substituent. Methyl **148** and ethyl **149** maintained similar potency to phenyl **51** (pIC_{50} 6.3). This suggests that the sulfonamide N-H in **51** is not fundamentally important and by incorporation of a methyl group, other properties such as permeability can be increased (175 nm/sec in phenyl **51** to 500 nm/sec in **148**).

The following set of compounds (Figure 5.16) were chosen to evaluate the ring and potential ring SAR. Multiple compounds were chosen to probe whether a nitrogen atom could be incorporated on the phenyl ring in order to lower lipophilicity, whilst maintaining a vector to investigate interacting with Trp760. The corresponding phenyl versions were also selected to provide a direct comparison. We also wanted to probe whether fluorine could be incorporated on this ring, as fluorines can be used to block sites of metabolism or modulate pK_a.

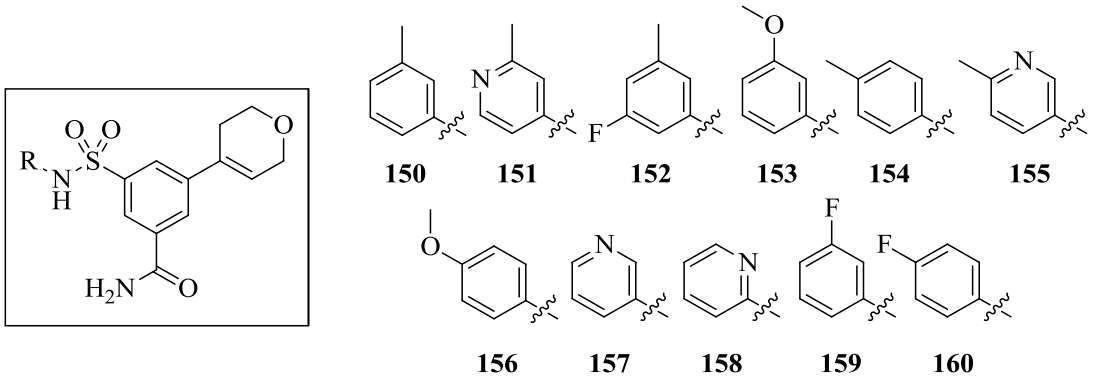
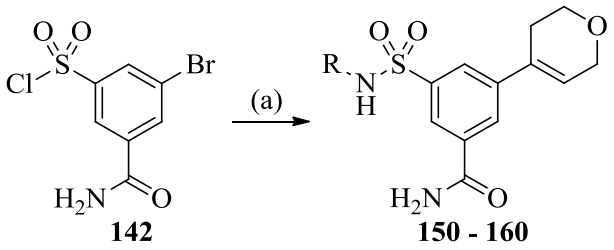


Figure 5.16 Sulfonamides **150**-**160** selected for synthesis.

As **142** had previously shown poor solubility in pyridine, we first investigated the solubility of **142** in various solvents. Sulfonyl chloride **142** was not soluble in a range of solvents such as DCM, chloroform, DMF and acetonitrile. In 1,4-dioxane the compound was minimally soluble and in THF the compound was readily soluble. Using triethylamine as the base, this change in solvent was successfully used on a range of compounds (Scheme 5.17).

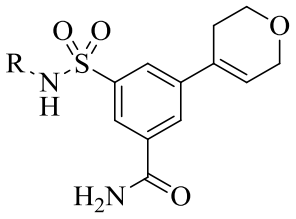


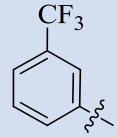
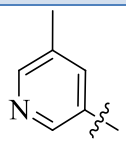
(a) i) R-NH_2, triethylamine, 1,4-dioxane or THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (3-39 %);

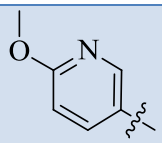
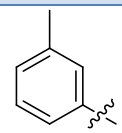
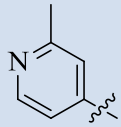
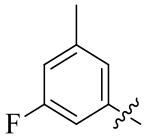
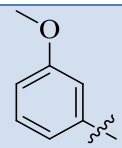
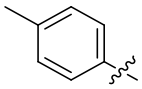
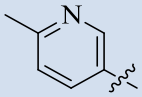
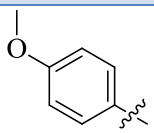
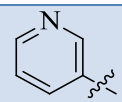
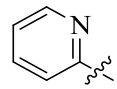
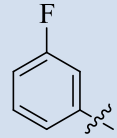
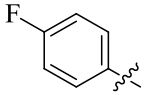
Scheme 5.17 Synthesis of sulfonamides **150-160**.

Poor yields were observed for a number of compounds (such as **151** and **158**) due to the poor nucleophilicity of the corresponding anilines. The results for **139**, **140** and **141** are included (Table 5.10).

Table 5.10 Enzyme inhibition data for compounds **51**, **139-141** and **150-160**.[36]



		pIC_{50}				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
51	Ph	5.2 (n=3)	5.6 (n=3)	5.1[a] (n=3)	6.3 (n=3)	0.35/4.5
139		5.0 (n=3)	5.1 (n=3)	5.2 (n=3)	5.9 (n=3)	0.28/3.13
140		<4.5 (n=2)	4.6 (n=2)	<4.5 (n=2)	5.4 (n=2)	0.28/4.23

141		4.9[a] (n=1)	4.9 (n=2)	<4.5 (n=2)	5.8 (n=2)	0.29/4.33
150		5.2[a] (n=3)	5.2 (n=4)	5.2 (n=4)	6.1 (n=4)	0.32/3.8
151		<4.5 (n=4)	<4.5 (n=4)	<4.5 (n=4)	5.3 (n=4)	0.28/4.1
152		5.0 (n=4)	4.7 (n=4)	5.1 (n=4)	5.9 (n=4)	0.29/3.2
153		5.1 (n=2)	5.0 (n=2)	4.9 (n=2)	6.1 (n=2)	0.31/4.3
154		5.1 (n=3)	5.2 (n=3)	5.1[b] (n=2)	6.4 (n=3)	0.34/4.1
155		<4.5 (n=5)	4.7[c] (n=1)	<4.5 (n=5)	5.5 (n=5)	0.29/4.3
156		5.3 (n=3)	5.1 (n=3)	4.9 (n=3)	6.6 (n=3)	0.33/4.8
157		4.6[b] (n=2)	4.6[b] (n=2)	<4.5 (n=3)	5.1 (n=3)	0.27/4.2
158		<4.5 (n=2)	4.8[b] (n=1)	<4.5 (n=2)	5.5 (n=2)	0.30/4.8
159		4.9 (n=3)	5.0 (n=3)	4.9[b] (n=2)	5.9 (n=3)	0.31/3.9
160		5.0 (n=2)	4.8[b] (n=2)	5.0 (n=2)	5.8 (n=2)	0.31/3.8

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.5 was received. [c]On four occasions, a result of <4.5 was received.

Meta-substitution with a methyl group to give **150** gave comparable potency to phenyl **51**. This suggested that the methyl group was tolerated and could lead to a vector to further investigate. In comparison, **139** demonstrated diminished potency in comparison to phenyl **51** and, due to the increase in lipophilicity, was significantly less efficient than phenyl **51**. In the 2-methoxypyridine series, the inclusion of the trifluoromethyl group had increased potency 6-fold (pIC_{50} 6.8 to 7.6) where it is located in a lipophilic region of the protein. This suggests that the phenyl of benzamide **51** is already occupying the lipophilic pocket and that further lipophilicity outside this pocket is not beneficial. In comparison to **150**, pyridyl compounds **140** and **151** were less potent, suggesting a clear distinction between phenyl (**150)** and pyridyl cores (**140** and **151**). Inspection of the crystal structure of **51** indicated no clear clash with any protein residues or observed water molecules, suggesting that the effect was more subtle. Fluoromethyl **152** was comparable in activity compared to methyl **150**. Methoxy analogue **153** was equipotent with **150** which provided an alternative linking group to explore substitution.

Para-substitution with a methyl group **154** is of similar potency to phenyl **51**. However, as observed with *meta*-substituted compounds **140** and **151**, inclusion of a pyridyl group to give **155** is 8-fold less potent. This suggests that the inclusion of a basic nitrogen atom is significantly detrimental to potency. Methoxy **156** was found to boost potency 2.5-fold compared to phenyl **51**, indicating it is a linker from which to grow with enhanced potency. In comparison **141** lost 8-fold potency for PI3Kδ in comparison to **156**. This again may be a simple case of a basic nitrogen in this area, or could be due to a disfavoured conformation due to the preference of 2-methoxypyridines for the *anti*-conformer (Figure 5.17).[117]

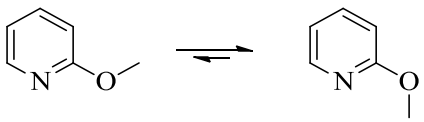


Figure 5.17 Conformational preference of 2-methoxypyridine.

To identify how the methoxy-group would orientate, co-crystallography of **156** was sought, however on two occasions; no conclusive density was obtained and on both occasions poor occupancy within the active site was observed. Pyridyl compounds **157** and **158** both showed a decrease in potency, with *ortho*-pyridyl **158** 6-fold and *para*-pyridyl **157** 15-fold less

potent than phenyl **51**. Both fluoro compounds **159** and **160** demonstrated similar potency, indicating inclusion of the group was tolerated.

As **156** was an interesting motif from which to build, we first wanted to investigate whether additional functionalities could enhance potency (Figure 5.18).

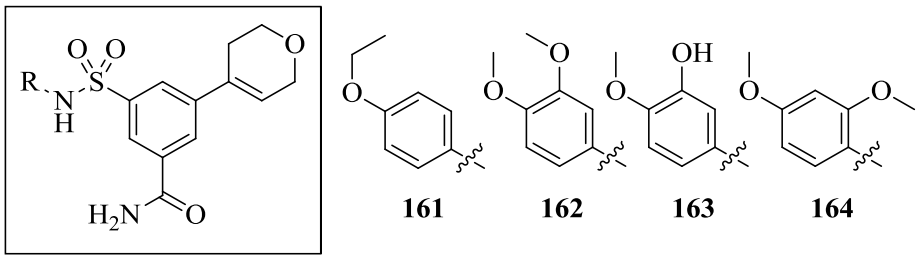
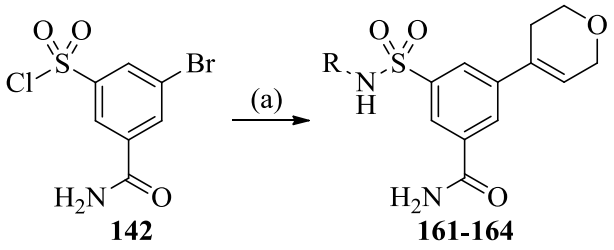


Figure 5.18 Compounds **161**-**164** selected for synthesis.

Ethoxy derivative **161** would probe whether additional growth in this region would be tolerated. Dimethoxy analogues **162** and **164** would investigate how additional electron density on the phenyl ring would affect potency, whereas phenol **163** would investigate how a HBD would interact within this area of the protein. Compounds were successfully prepared using the standard procedure (Scheme 5.18).

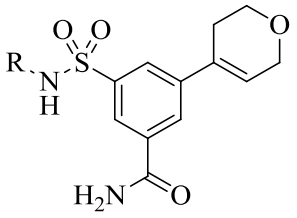


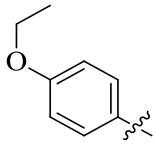
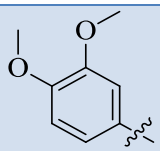
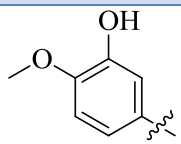
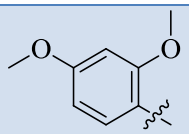
(a) i) R-NH_2, triethylamine, 1,4-dioxane or THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (5-41 %);

Scheme 5.18 Synthesis of sulfonamides **161**-**164**.

The results for **161**-**164** are as follows (Table 5.11).

Table 5.11 Enzyme inhibition data for compounds **161**-**164**.[36]



		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
161		5.3 (n=3)	4.9 (n=3)	5.1[a] (n=2)	6.8 (n=3)	0.33/4.5
162		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.0 (n=2)	0.24/3.6
163		4.9 (n=2)	4.5[a] (n=1)	<4.5 (n=2)	5.1 (n=2)	0.25/4.1
164		4.5[a] (n=1)	4.5[a] (n=1)	<4.5 (n=2)	5.3 (n=2)	0.25/3.5

[a]On one occasion, a result of <4.5 was received.

Ethoxy compound **161** demonstrated similar potency to methoxy derivative **156**, indicating that although the additional CH_2 does not increase potency it is well tolerated. Compounds **162**-**164** demonstrated diminished potency in comparison to methoxy **156**, indicating that introduction of a second methoxy group either *ortho*- or *para*- is detrimental to potency, whilst introduction of phenol **163** is not tolerated.

Whilst selecting 5-membered heterocycles, there were some limitations on the availability, stability and reactivity of the corresponding amines. We wanted to probe both the effects of

methyl-substituents and of heterocycles in the region. However compounds such as 2- and 3-aminothiophene,[118] 2- and 3-aminofuran and 2-aminopyrole[119] are not bench stable, so it somewhat limited the range of amines available. With this in mind, we proposed the following targets (Figure 5.19).

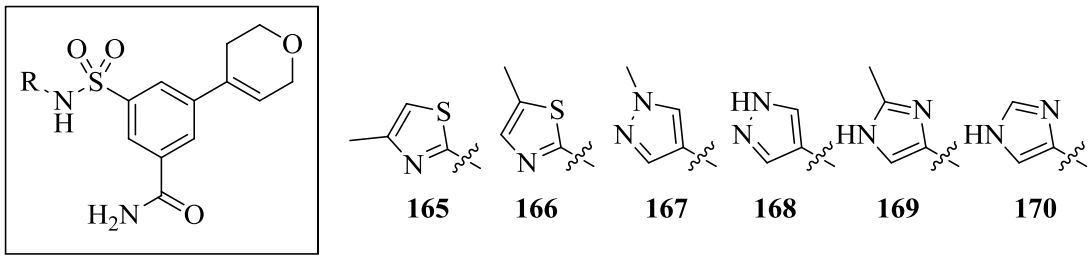
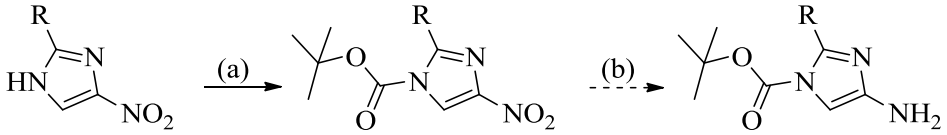


Figure 5.19 Compounds **165-170** selected for synthesis.

Aromatic amines for final compounds **165-168** were commercially available; however aromatic amines for **169** and **170** required synthesis (Scheme 5.19).



171 R = H
172 R = CH_3

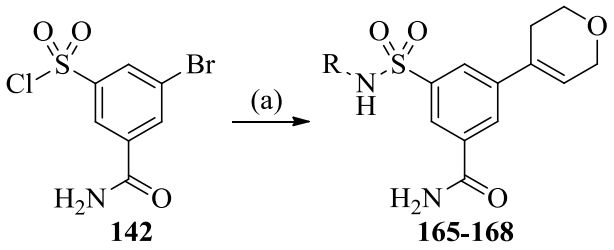
173 R = H
174 R = CH_3

175 R = H
176 R = CH_3

(a) Boc anhydride, NEt_3, DCM (91 %); (b) H_2, Pd/C, EtOH or Fe, NH_4Cl, EtOH, H_2O.

Scheme 5.19 Proposed synthesis of amines **175** and **176**.

Boc-protection of imidazoles **171** and **172** proceeded well under standard conditions (Scheme 5.19). Using either palladium on carbon or iron-catalysed reduction conditions, decomposition of **173** and **174** was observed, indicating that the desired anilines **175** and **176** were unstable. We chose not to further pursue these targets and instead focussed on **165-168** (Scheme 5.20).

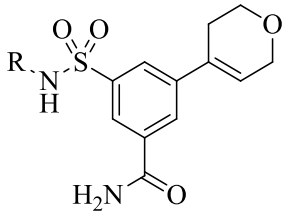


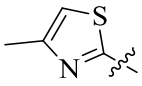
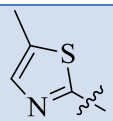
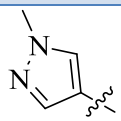
(a) i) R-NH_2, triethylamine, 1,4-dioxane or THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (5-16 %);

Scheme 5.20 Synthesis of sulfonamides **165-168**.

Under the standard reaction conditions **165-167** were synthesised in poor yields (5-16 %), however for **168**, we found the corresponding amine to be unreactive under the conditions used.

Table 5.12 Enzyme inhibition data for compounds **165-167**.[36]



		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
165		<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.5 (n=2)	0.30/4.4
166		4.6 (n=2)	4.6 (n=2)	<4.5 (n=2)	5.6 (n=2)	0.31/4.5
167		<4.5 (n=4)	<4.5 (n=4)	4.6[a] (n=2)	5.4 (n=4)	0.3/5.4

[a]On two occasions, a result of <4.5 was received

Potency for the three heterocycles synthesised was lower than for **51**, indicating once more that some heterocycles in this region are not tolerated.

From this work it was clear that the best aryl group to substitute from was a phenyl and both *meta*- and *para*- substitution could be tolerated. The previous computational modelling suggested either a 2- or 3- atom linker to the morpholine ring would best fit the pharmacophore. The following structures were proposed for synthesis (Figure 5.20).

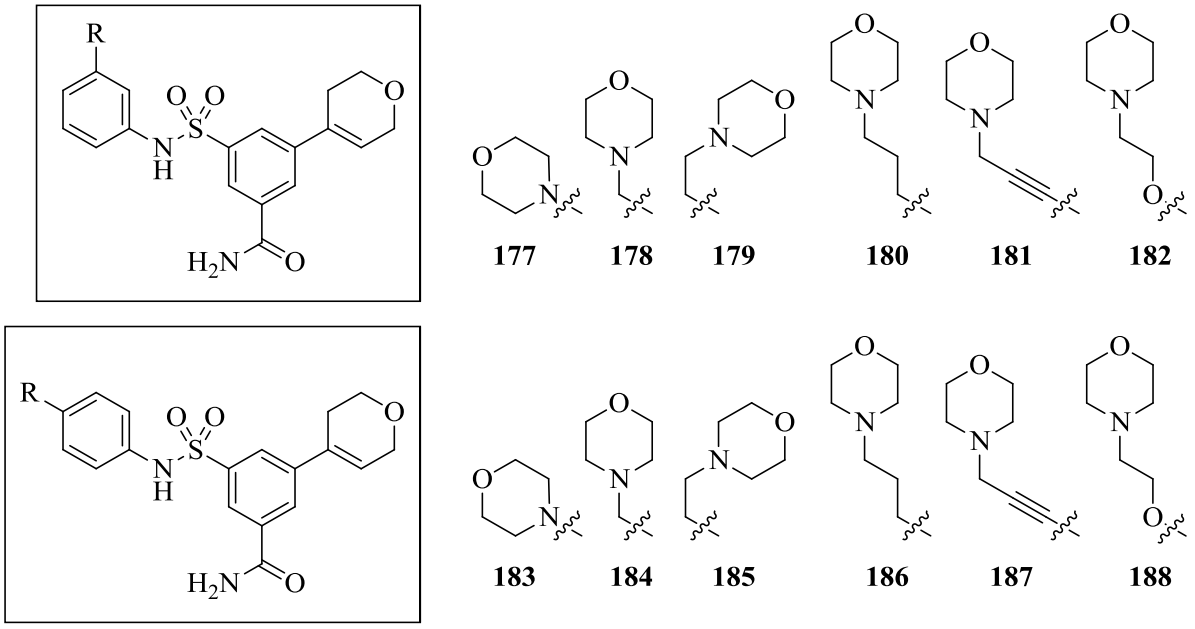
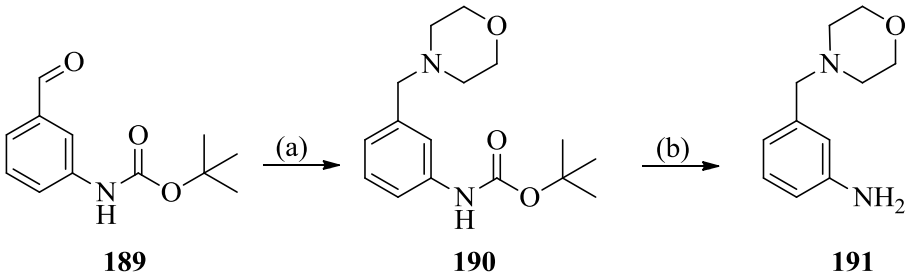


Figure 5.20 Compounds **177**-**188** selected for synthesis.

In order to really probe this region, we selected both ether linkers (**182** and **188**) and alkyl chains (**179**, **180**, **185** and **186**). With the carbon atom linkers, we decided to evaluate not only the desired 2- or 3- atom linkers, but also directly attached (**177** and **183**) and one-atom (**178** and **184**) linkers to fully probe the chain length required. Alkyne compounds **181** and **187** were selected to observe the effect, if any, of giving the alkyl chain a more rigid and defined structure.

With the targets selected, the monomers that could not be obtained from commercial suppliers were synthesised. For **191**, the following route was followed (Scheme 5.21).



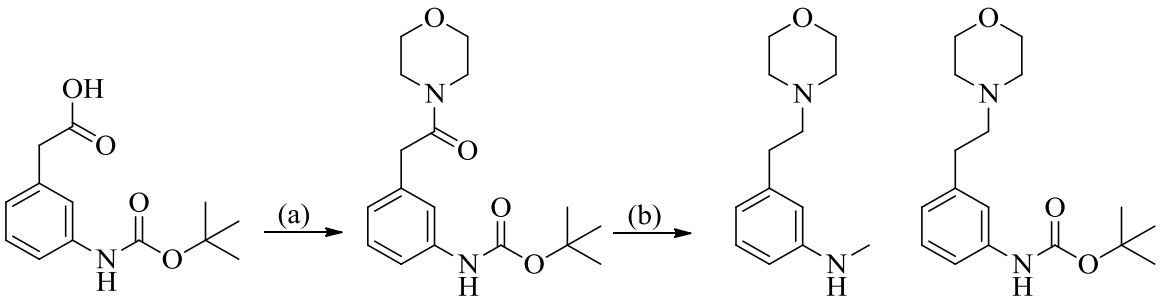
189 **190** **191**

(a) i) Morpholine, THF, rt, 5 min; ii) $NaBH(OAc)_3$, rt, 16 h, (79 %); (b) HCl, rt, 16 h (89 %).

Scheme 5.21 Synthesis of aniline **191**.

Reaction of aldehyde **189** with morpholine under typical reductive amination conditions gave **190** in good yield (79 %) which was subsequently deprotected with hydrochloric acid to give **191**, again in high yield (89 %).

For aniline **194**, we envisaged the following route (Scheme 5.22).



192 **193** **195** **194**

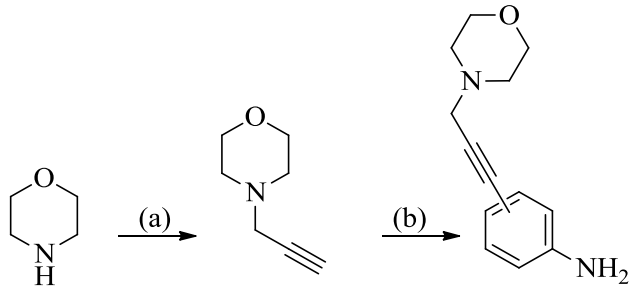
(a) Morpholine, T_3P, DIPEA, DMF, rt, (67 %); (b) BH_3, THF, 0 °C, 16 h; then 60 °C, 4 h, (97 %).

Scheme 5.22 Synthesis of aniline **195**.

Amide coupling of **192** and morpholine with T_3P proceeded in moderate yield (67 %). This was followed by reduction of the amide **193** with borane in THF. At low temperature, no reduction product **194** was observed via LCMS. However, upon heating the reaction for four hours and workup, analysis of the reaction mixture via NMR revealed no desired product.

The product observed was methyl aniline **195** in high yield and moderate purity (97 % and 78 % purity via LCMS). As we had previously observed that the methyl tertiary sulfonamide **148** (page 119) had little to no effect upon the potency of the compound, we used **195** as a surrogate monomer. If the compound was found to be an exciting target, we would re-visit the NH_2 monomers synthesis.

As propyl anilines **203** and **204** could be accessed through reduction of the alkyne anilines **197** and **198**, we observed that both could be synthesised through a similar route. With this in mind, we first trialled the synthesis of **197** and **198** (Scheme 5.23) under standard Sonogashira-cross-coupling conditions with 4-(prop-2-yn-1-yl)morpholine **196**, prepared in accordance with the literature.[120]

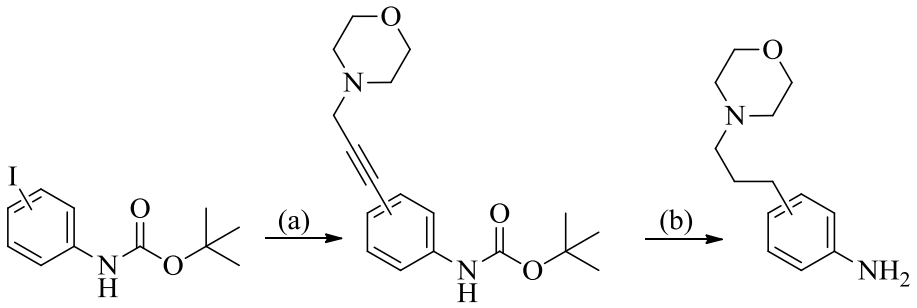


196 **197** *meta* **198** *para*

(a) 3-Bromoprop-1-yne, Cs_2CO_3, acetone, rt, 16 h (87 %); (b) 3-iodoaniline or 4-iodoaniline, triethylamine, $Pd_2Cl_2(PPh_3)_2$, CuI, THF, rt, 72h (34-44 %).

Scheme 5.23 Synthesis of anilines **197** and **198**.

On a small scale, this reaction yielded the desired monomers **197** and **198** in poor yields (**197** 34 % and **198** 44 % (with 10 % by weight triphenylphosphine oxide) in moderate purity. To improve the yield to allow subsequent reactions to form propyl derivatives **203** and **204**, *tert*-butyl (3-iodophenyl)carbamate **199** or *tert*-butyl (4-iodophenyl)carbamate **200** were used to give the desired products **201** and **202** in good yield (71 % and 96 %). Hydrogenation of the triple bond over palladium on carbon followed by Boc-deprotection gave **203** and **204** in variable yield (58 % and 83 %) (Scheme 5.24).

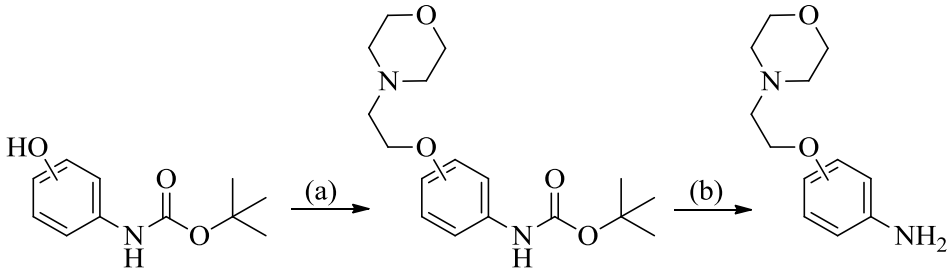


199 *meta* **201** *meta* **203** *meta*
200 *para* **202** *para* **204** *para*

(a) 4-(Prop-2-yn-1-yl)morpholine, triethylamine, $Pd_2Cl_2(PPh_3)_2$, CuI, THF, rt, 72h (71-96 %); (b) i) Pd/C, H_2, 16 h; ii) HCl, rt,1 h (58-83 %).

Scheme 5.24 Synthesis of anilines **203** and **204**.

For ether anilines **209** and **210**, the following route was undertaken (Scheme 5.25).



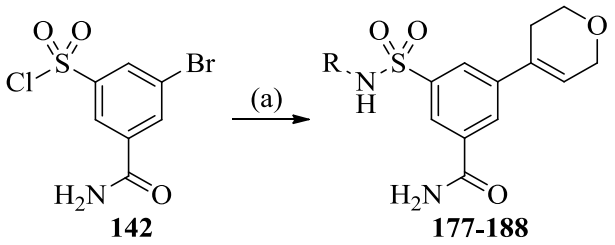
205 *meta* **207** *meta* **209** *meta*
206 *para* **208** *para* **210** *para*

(a) 4-(2- chloroethyl)morpholine hydrochloride, K_2CO_3, MeCN, 80 °C, 6 h (91-94 %); (b) HCl, rt, 1 h (90-95 %).

Scheme 5.25 Synthesis of anilines **209** and **210**.

From the Boc-protected aminophenols **205** and **206**, alkylation with commercially available 4-(2-chloroethyl)morpholine hydrochloride proceeded without incident giving intermediates **207** and **208** in high yield (91 and 94 %) which were subsequently deprotected with hydrochloric acid to give anilines **209** and **210** (95 % and 90 %).

With the anilines in hand, under the same conditions used previously, all the desired compounds were synthesised in variable yield (9 – 57 %) (Scheme 5.26).

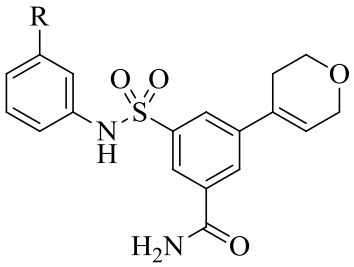


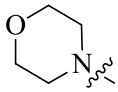
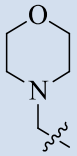
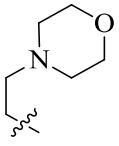
(a) i) R-NH_2, triethylamine, THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (9-57 %);

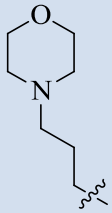
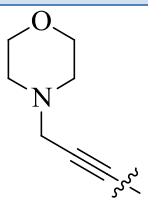
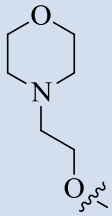
Scheme 5.26 Synthesis of sulfonamides **177-188**.

Enzymatic results for *meta*-substituted compounds **177-182** are shown below (Table 5.13).

Table 5.13 Enzyme inhibition data for compounds **177-182**.[36]



		pIC_{50}				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
177		4.9 (n=3)	4.8 (n=3)	4.9[a,b] (n=1)	6.3 (n=3)	0.28/5.0
178		4.8 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.4 (n=2)	0.23/3.9
179	 NMe	5.2 (n=4)	4.9 (n=4)	4.6[a,c] (n=1)	6.6 (n=4)	0.27/4.7

180		5.0 (n=3)	5.1 (n=3)	4.6[a,b] (n=1)	6.5 (n=3)	0.26/4.2
181		5.6 (n=4)	4.9 (n=4)	5.3 (n=3)	7.1 (n=4)	0.29/5.5
182		5.2 (n=3)	4.9 (n=3)	4.6[d] (n=3)	6.5 (n=3)	0.26/4.6

[a]On one occasion, a result of <4.3 was received. [b]On three occasions, a result of <4.5 was received. [c]On four occasions, a result of <4.5 was received. [d]On two occasions, a result of <4.5 was received.

Directly attached morpholine compound **177** maintained the potency observed in phenyl **51**, however the compound showed an increase in selectivity for PI3Kδ against PI3Kβ. Homologation to a one-carbon linker **178** resulted in a drop in potency, indicating there was no advantage to its inclusion. Both two- and three- carbon linkers **179** and **180** showed comparable potency, and showed an increase in potency in comparison to *meta*-toluidine **150**, however neither compound gained enough potency or selectivity to suggest interacting with the tryptophan residue.[45] Alkyne **181** was the standout compound from the *meta*-position, in which good potency and selectivity (31-fold against the nearest isoform PI3Kα) was observed, indicating that this alkyne was the ideal linker to enter the desired region. Ether linker **182** was comparable to **180**, however was less active against PI3Kδ in comparison to **181**.

Co-crystallography of alkyne **181** was obtained (Figure 5.21)

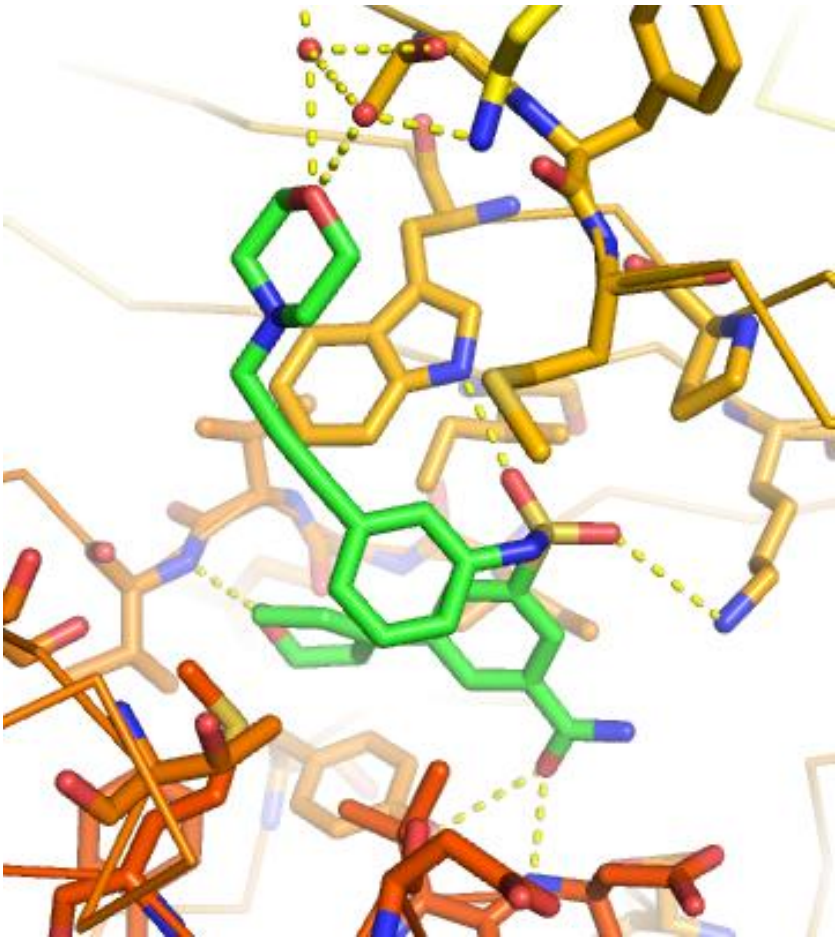


Figure 5.21A Co-crystal structure of **181** in PI3Kδ (Resolution 2.17 Å)[2]

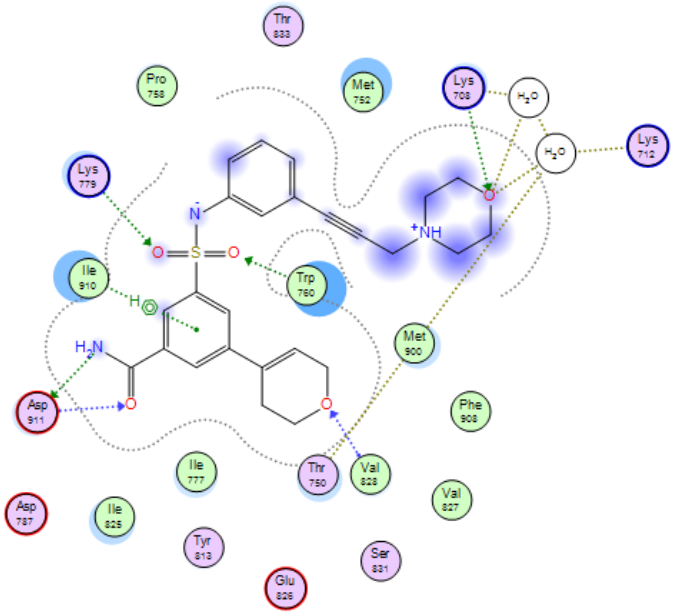
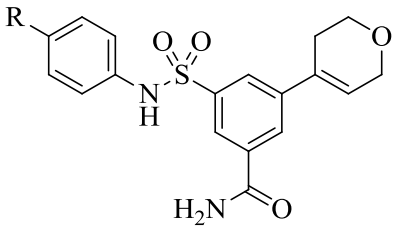


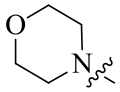
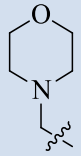
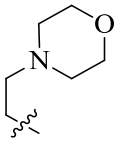
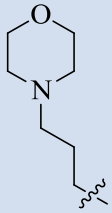
Figure 5.21B 2D Representation of **181** binding to PI3Kδ.

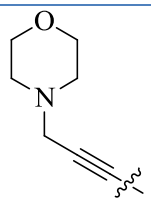
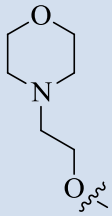
The crystal structure (Figure 5.21) demonstrated that inclusion of the alkyne moiety maintained the same interactions observed in the phenyl compound **51**, such as the hinge binding interaction with Val828, sulfonamide HBA interactions with Trp760 and Lys777 and interactions with the primary amide through Asp911 and Tyr813. The alkyne moiety provides a planar linker to the morpholine, where the nitrogen atom of the morpholine is 4.6 Å away from the π-cloud of Trp760, indicating that it is within distance to make a π-cation interaction. The oxygen atom of the morpholine is 4.2 Å away from Thr750.

Para-substituted compounds are shown below (Table 5.14).

Table 5.14 Enzyme inhibition data for compounds **183**-**188**.[36]



		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
183		5.0 (n=2)	<4.5 (n=2)	4.7[a] (n=1)	6.2 (n=2)	0.27/4.9
184		<4.5 (n=3)	<4.5 (n=3)	4.6[b] (n=1)	5.9 (n=3)	0.25/4.3
185		4.6[b] (n=2)	4.6[b] (n=2)	4.7[c] (n=1)	6.2 (n=4)	0.26/4.3
186		5.1 (n=5)	5.1 (n=5)	4.6[a] (n=6)	7.1 (n=5)	0.29/4.8

187		5.6 (n=5)	4.9 (n=5)	5.5 (n=5)	7.2 (n=5)	0.29/5.6
188		5.0[a] (n=4)	4.6[b] (n=3)	4.7[b] (n=5)	6.7 (n=5)	0.27/4.8

[a]On one occasion, a result of <4.5 was received. [b]On two occasions, a result of <4.5 was received. [c]On three occasions, a result of <4.5 was received.

From the *para*-position, we observe that a directly attached morpholine **183**, one- and two-carbon linkers (**184** and **185**) all show similar potency against PI3Kδ and have moderate selectivity. Propyl linker **186** possesses good potency and 100-fold selectivity for PI3Kδ against the other class I isoforms unlike the equivalent *meta*-substituted propyl **180**. Alkyne **187** was similar in potency to propyl linker **186**, however alkyne **187** was not as selective (40-fold against the nearest isoform). Ether linker **188** demonstrated similar potency to ethoxy **161**; however it had increased selectivity against PI3Kα and PI3Kβ.

Co-crystallography of alkyne **187** was obtained (Figure 5.22).

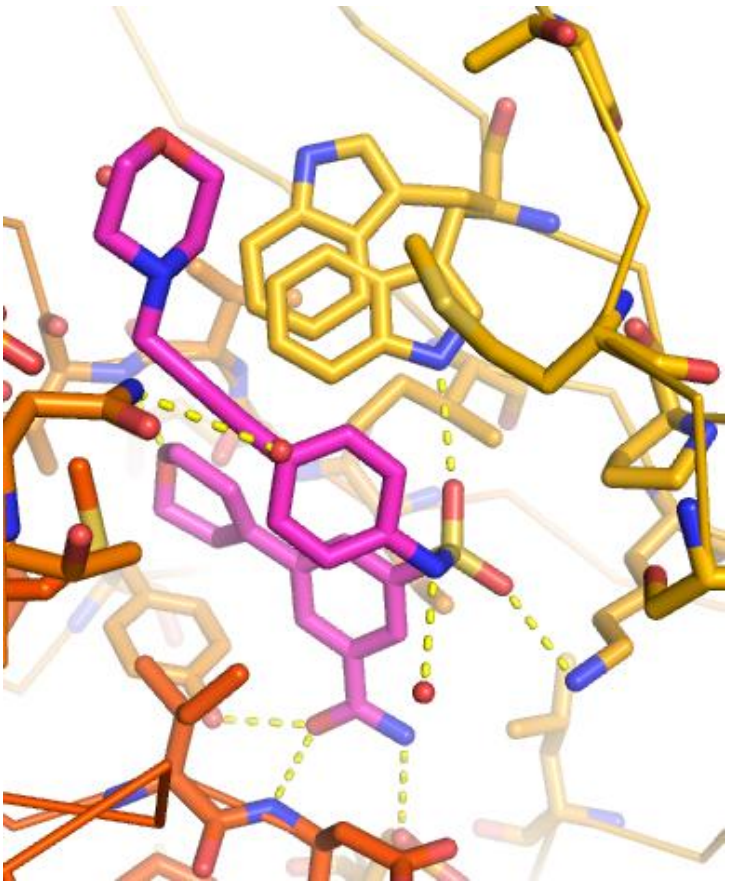


Figure 5.22A Co-crystal structure of **187** in PI3Kδ (Resolution 2.22 Å)[2]

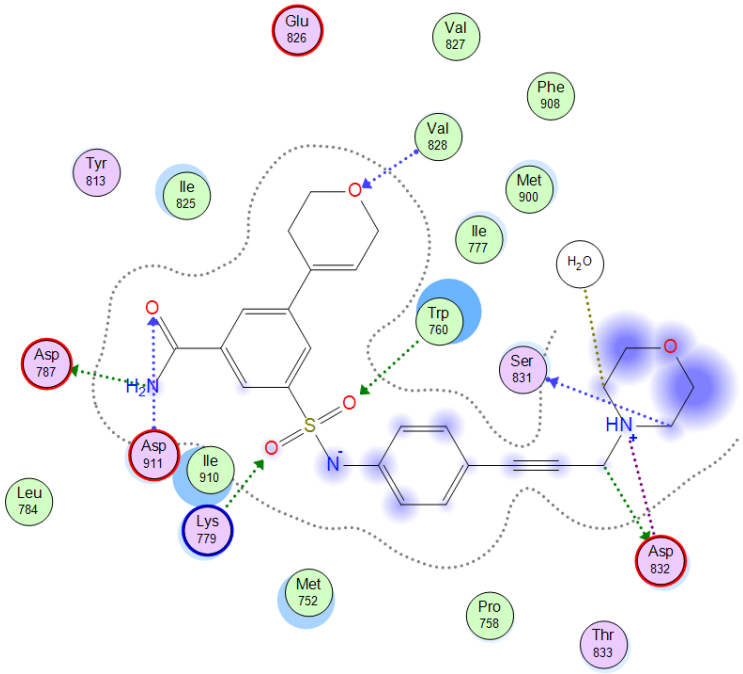


Figure 5.22B 2D Representation of **187** binding to PI3Kδ.

The crystal structure of **187** (Figure 5.22) demonstrated that inclusion of the *para*-alkyne moiety maintained the same interactions observed in the phenyl compound **51**, such as the hinge binding interaction with Val828, a sulfonamide HBA interaction with Lys777 and interactions with the primary amide through Asp911 and Tyr813. The crystal structure revealed dual binding conformations for Trp760 with both the 'in' (forming a hydrogen bond interaction with sulfonamide S=O bond) and 'out' (forming no interaction) conformers observed in equal density. The alkyne moiety was clearly observed in the density; however the group does not extend itself toward the Trp760/Thr750 residues and instead moves into a solvent-exposed region of the protein. The morpholine nitrogen forms an interaction with the ionised carboxylic acid side chain of Asp832. However despite the similarities in binding interactions to *meta*-alkyne **181**, the orientation of the entire molecule changes, with the benzamide core fragment binding closer to the protein backbone than observed previously. The overlays of alkynes **181** and **187** are shown below to highlight the difference in how the core binding fragment changes and how the sulfonamide changes affect potency (Figure 5.23).

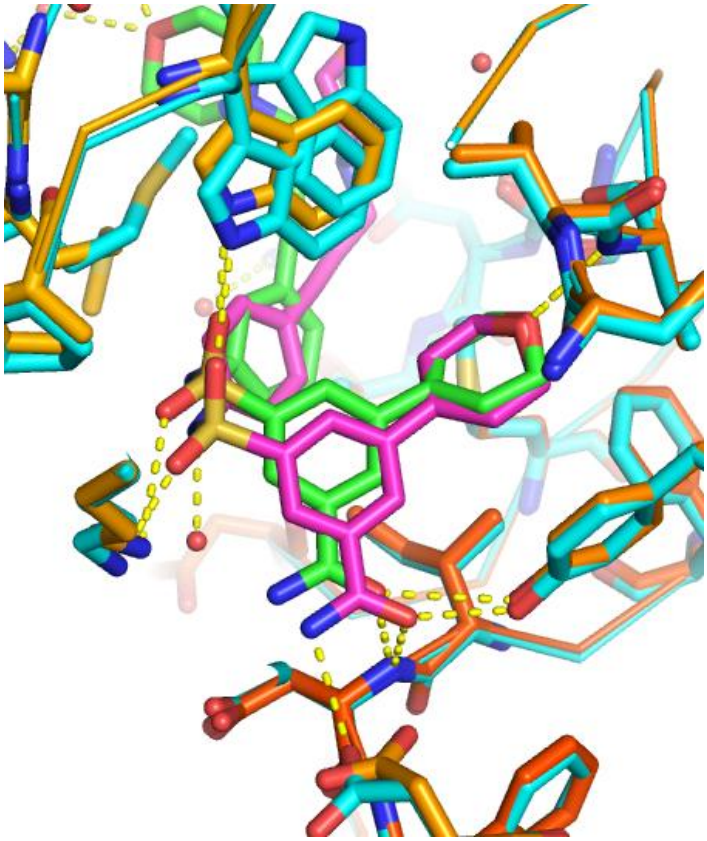


Figure 5.23A Overlay of compounds **181** (green) and **187** (magenta).

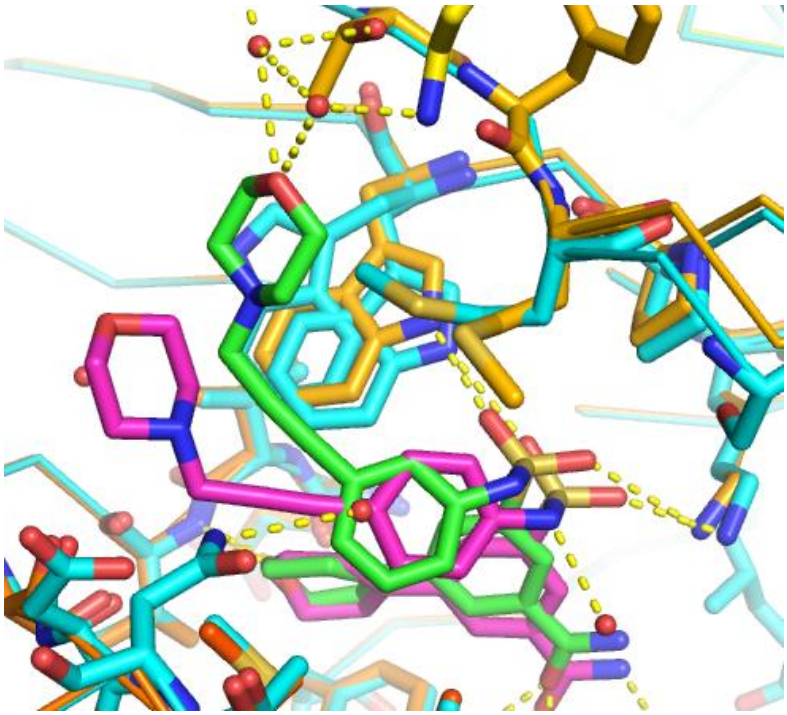


Figure 5.23B Alternative overlay of compounds **181** (green) and **187** (magenta).

These results were a real insight into how growth from the sulfonamide into the area of the protein that is associated with high levels of potency and selectivity for PI3Kδ can be achieved. Despite this, the most active compounds were not all achieving the desired level of selectivity (>100-fold) or potency (pIC_{50} ~8). One way in which potency and selectivity in this region can be increased is by additional bulking around the pendant morpholine. One such pendant morpholine is cis-2,6-dimethylmorpholine. Although this change increases the lipophilicity by 0.9 log units, as active compounds **186**, **187** and **188** are all below a cLogP of 2.3, this was an acceptable increase. The following compounds were selected for synthesis (Figure 5.24).

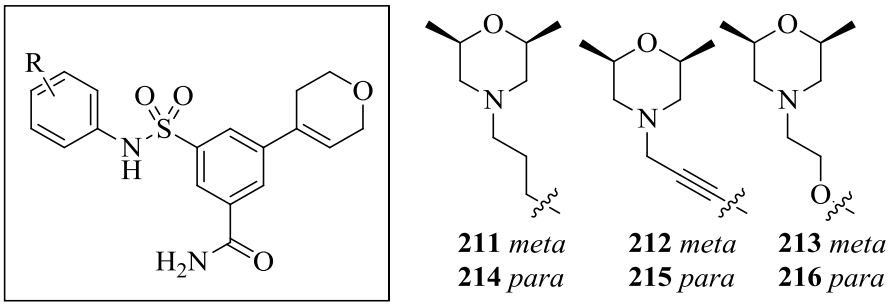
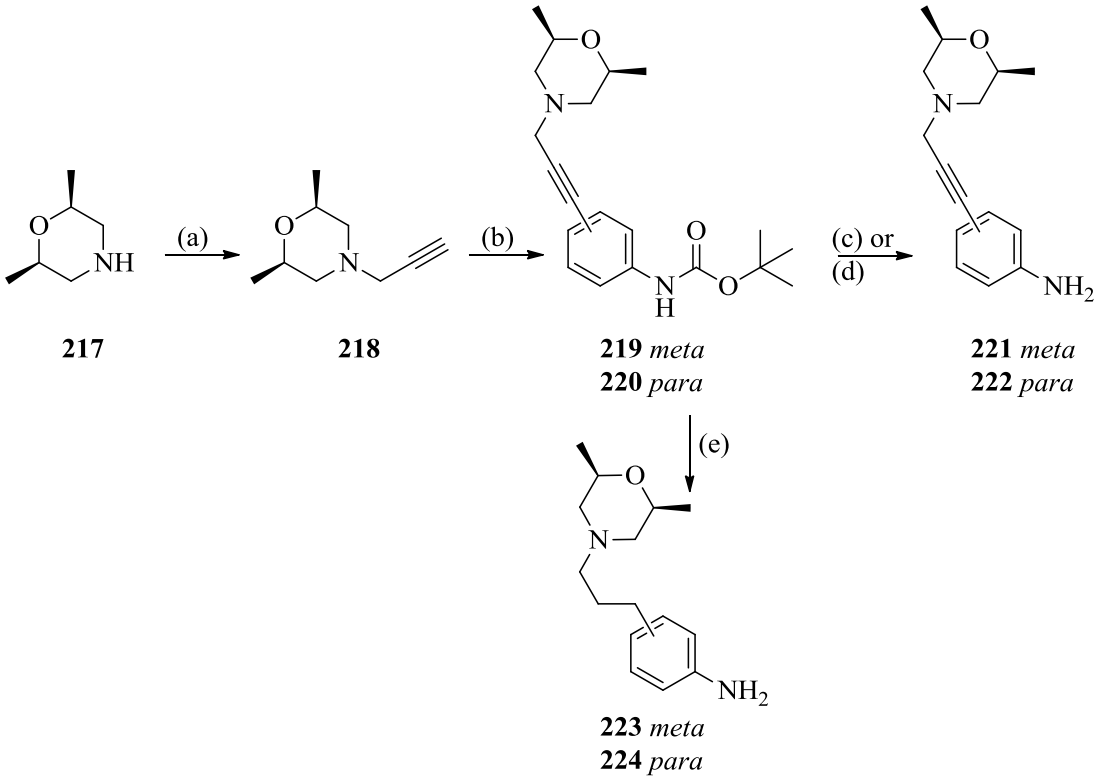


Figure 5.24 Compounds **211-216** selected for synthesis.

All six anilines for **211-216** required synthesis. For anilines of **211**, **212**, **214** and **215** the following route was investigated (Scheme 5.27).

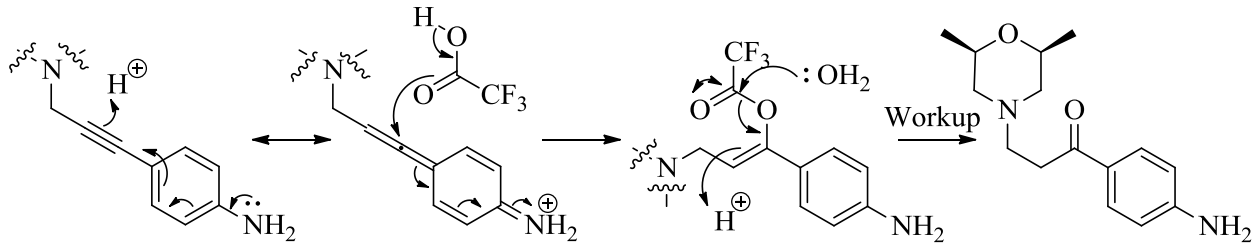


(a) Cs_2CO_3, 3-bromoprop-1-yne, acetone, rt, 16 h (92 %); (b) *tert*-butyl (3-iodophenyl)carbamate or *tert*-butyl (4-iodophenyl)carbamate, triethylamine, $Pd_2Cl_2(PPh_3)_2$, CuI, THF, rt, 72h (63-71 %); (c)TFA, DCM, rt, 1h, (79 %); (d) TBAF, THF, 70 °C, 24 h; (e) i) Pd/C, H_2, 16 h; ii) HCl, rt, 1 h (59-83 %).

Scheme 5.27 Synthesis of anilines **221-224**.

Alkylation of **217** proceeded in high yield (92 %) and subsequent Sonogashira cross-coupling gave **219** and **220** under previously used conditions in good yield (63 % and 71 % respectively). To access propyl monomers **223** and **224**, hydrogenation over palladium on carbon followed by Boc-deprotection yielded **223** and **224** (59 % and 83 % respectively). To access alkyne monomer **221**, deprotection with hydrochloric acid was not successful due to hydrogen chloride addition across the triple bond which was observed via LCMS. However, switching to TFA proceeded in good yield (79 %).

For *para*-alkyne monomer **222**, we again observed HCl addition across the triple bond during the deprotection step. Using TFA, a different side-reaction was observed, which was believed to be hydrolysis of the triple-bond to give ketone **225**. This could possibly proceed via the following mechanism (Scheme 5.28).

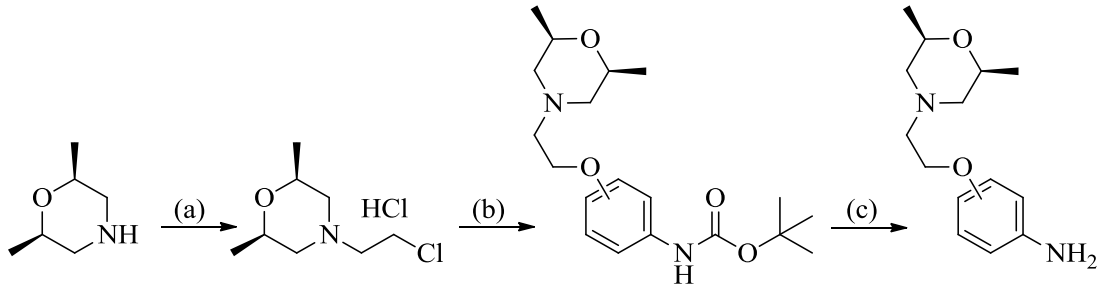


225

Scheme 5.28 Side reaction observed upon treating **222** with TFA to give **225**.

To overcome this side reaction Boc-deprotection was carried out using TBAF, a mild deprotection reagent and this proceeded in near quantitative yield (99 %).

For ethers **213** and **216**, the following route was used (Scheme 5.29).



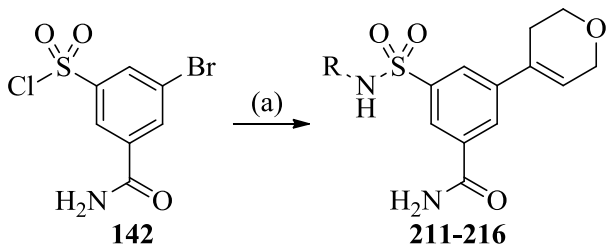
217 **226** **227** *meta* **228** *para* **229** *meta* **230** *para*

(a) i) 2-Bromoethanol, K_2CO_3, MeCN, 80 °C, 6 h; ii) $SOCl_2$, 70 °C, 9 h, (84 %); (b) *tert*-butyl (3-hydroxyphenyl)carbamate or *tert*-butyl (4-hydroxyphenyl)carbamate, K_2CO_3, MeCN, 80 °C, 6 h (57-94 %); (c) HCl, rt, 1 h (79-89 %).

Scheme 5.29 Synthesis of anilines **229** and **230**.

Alkylation of **217** with 2-bromoethanol followed by chlorination with thionyl chloride gave **226**, which was then used to alkylate the corresponding phenol derivative to give **227** and **228** in variable yield (57 % and 94 %). Deprotection with hydrochloric acid gave **229** and **230** (79 % and 89 %).

The desired compounds **211-216** were synthesised in variable yield (8 – 34 %) (Scheme 5.26).



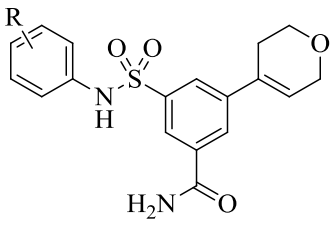
142 **211-216**

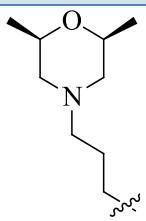
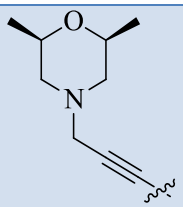
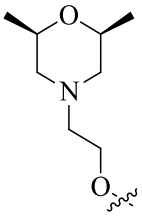
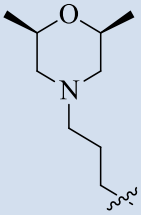
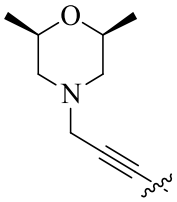
(a) i) R-NH_2, triethylamine, THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (8-34 %);

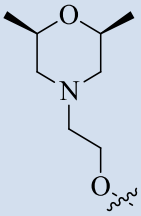
Scheme 5.30 Synthesis of sulfonamides **211-216**.

The results are shown below (Table 5.15).

Table 5.15 Enzyme inhibition data for compounds **211-216**.[36]



		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
211 *meta*		5.2 (n=3)	4.9 (n=3)	4.5[a] (n=3)	6.6 (n=3)	0.25/3.4
212 *meta*		5.8 (n=4)	5.1 (n=4)	5.3 (n=5)	7.4 (n=4)	0.28/4.9
213 *meta*		5.0 (n=3)	4.8 (n=3)	4.5[a,b] (n=2)	6.6 (n=3)	0.25/3.8
214 *para*		4.9 (n=3)	5.1 (n=3)	4.6 (n=5)	7.5 (n=3)	0.29/4.3
215 *para*		5.2 (n=3)	4.7[c] (n=2)	5.2 (n=5)	7.7 (n=3)	0.29/5.2

216 *para*		4.8 (n=4)	4.8[c] (n=3)	4.7 (n=6)	7.3 (n=4)	0.28/4.5

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.3 was received. [c]On one occasion, a result of <4.5 was received.

From the *meta*-position bulking out the terminal morpholine group with either the propyl **211** or ethoxy **213** linker has no effect on potency or selectivity. This in turn lowers the efficiency of the molecule. This indicates that this group is not reaching the desired area of the protein. *Meta*-alkyne **212** showed a small increase in potency for all four PI3K isoforms, This pan-increase is unusual as via crystallography of morpholine **181** (Scheme 5.21) we can see the compound binding close to Thr750 and this is the region where the selectivity is usually attained.

The bulking of the morpholine at the *para*-position was more successful, as all three compounds **214-216** demonstrated above 100-fold selectivity for PI3Kδ against the other class I isoforms and enhanced potency. By incorporation of the dimethylmorpholine in propyl linker **214**, the compound was two-and-a-half times more potent at PI3Kδ, whilst maintaining the same level of potency at PI3Kα and PI3Kβ. This resulted in an excellent 250-fold selectivity against the nearest isoform. Alkyne **215** was three times more potent than the corresponding morpholine alkyne **187**, with excellent 316- fold selectivity against PI3Kα and 1000-fold selectivity against PI3Kβ. Ethoxy **216** PI3K potency increased 4-fold and was 316-fold selective against both PI3Kα and PI3Kβ and 500-fold selective against PI3Kγ.

The co-crystal structure for alkyne **215** was collected (Figure 5.25).

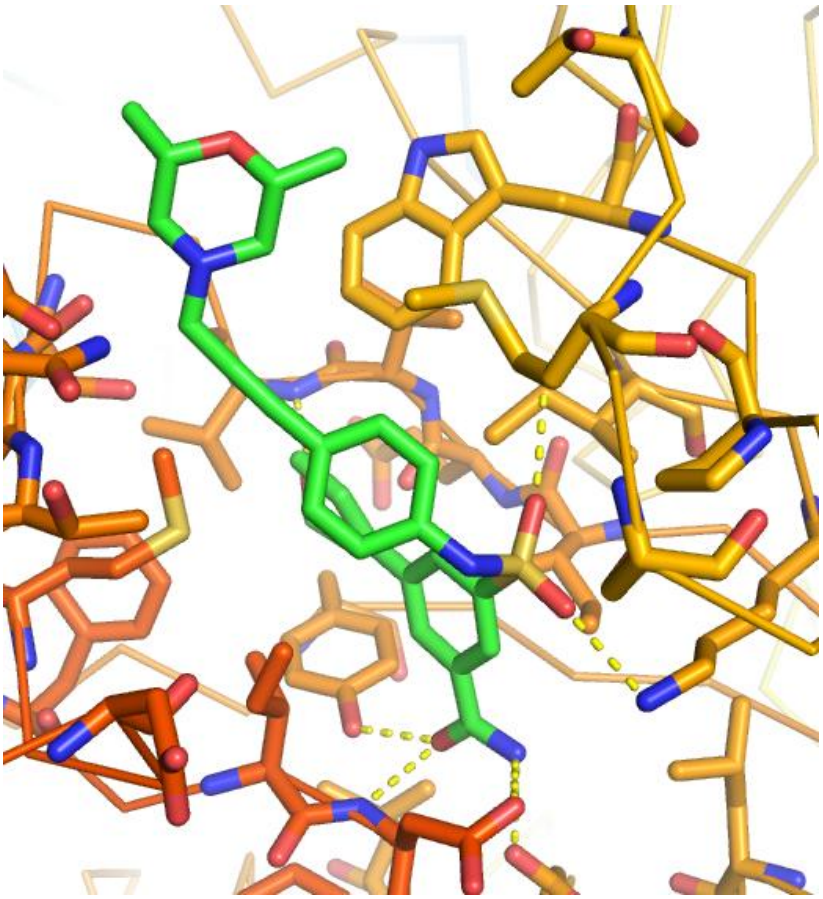


Figure 5.25A Co-crystal structure of **215** in PI3Kδ (Resolution 2.14 Å)[2]

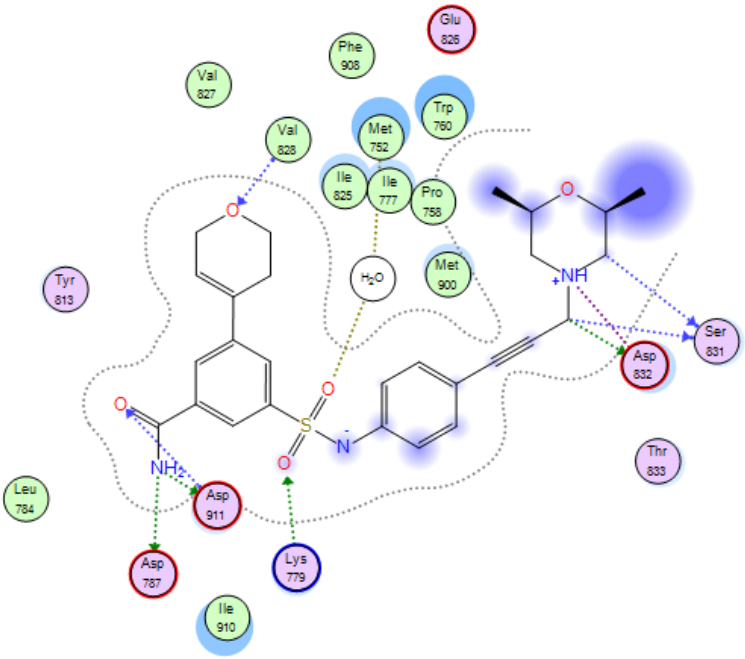
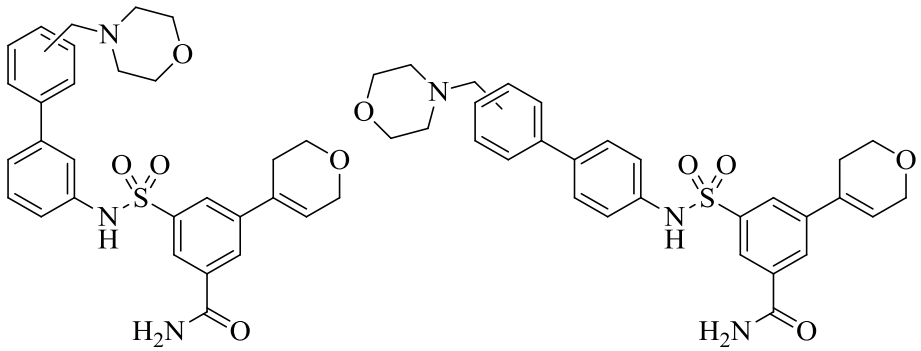


Figure 5.25A 2D Representation of **215** to PI3Kδ.

From the crystal structure, we observed very similar binding to alkyne **215**, signifying that incorporation of the dimethylmorpholine has no effect on binding mode. In comparison to the crystal structure of alkyne **187**, dimethyl alkyne **215** shows a dominant conformer for the Trp760 residue as 'out', and no longer making the interaction with the sulfonamide S=O bond. The enhanced selectivity may be accounted for by a methyl group extending towards the Thr750 residue, therefore clashing in PI3Kα, PI3Kβ and PI3Kγ.

At this stage, we wanted to observe whether the alkyne could be replaced by a phenyl ring. Exchange of the alkyne with a phenyl ring would maintain a similar distance (4.1 Å and 5.7 Å) and vector. We chose to initially make two *meta*-biphenyls **231** and **232** as in the crystal structure (Figure 5.25A) we believed that incorporation of the *para*-biphenyls **234** and **235** would clash with the protein (Figure 5.26).

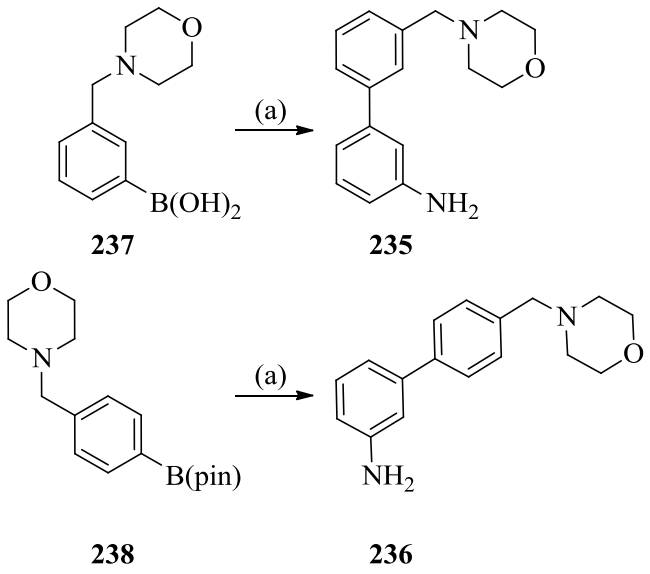


231 *meta*
232 *para*

233 *meta*
234 *para*

Figure 5.26 Compounds **231**-**234** from which **231** and **232** were selected for synthesis.

With these in-mind, Suzuki cross-coupling yielded **235** and **236** from the commercially available boronic acid **237** and boronic ester **238** (Scheme 5.31).



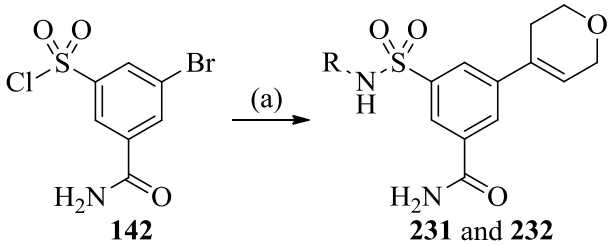
237 **235**

238 **236**

(a) 3-Iodoaniline, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (76-87 %);

Scheme 5.31 Synthesis of anilines **235** and **236**.

The anilines **235** and **236** were then successfully coupled with sulfonyl chloride **142** in moderate yields (30 % and 35 % respectively) (Scheme 5.32).



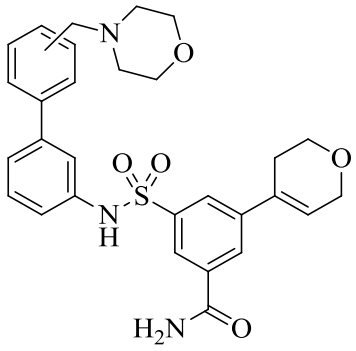
142 **231** and **232**

(a) i) R-NH_2, triethylamine, THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (30-35 %);

Scheme 5.32 Synthesis of sulfonamides **231** and **232**.

The results are shown below (Table 5.16).

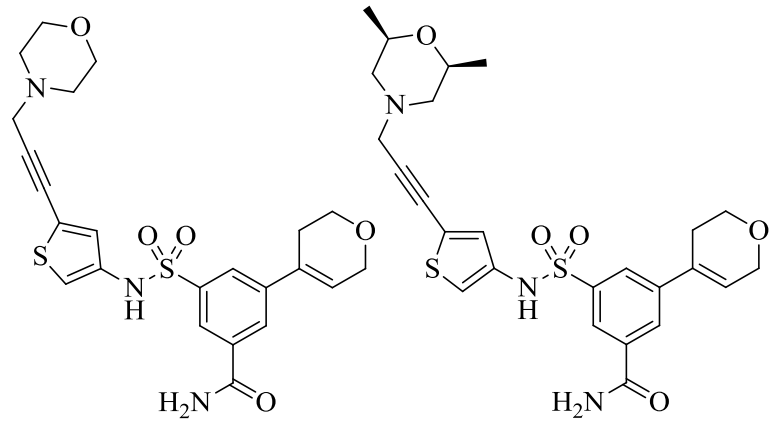
Table 5.16 Enzyme inhibition data for compounds **231** and **232**.[36]



	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
231 *meta*	6.0 (n=3)	5.5 (n=3)	5.1 (n=5)	7.2 (n=3)	0.26/3.8
232 *para*	6.1 (n=3)	5.7 (n=3)	5.5 (n=5)	7.7 (n=3)	0.28/4.3

Both compounds were active and possessed some selectivity; however we can clearly observe that the *para*-substitution was the preferred position for the morpholinomethyl group, with excellent potency at PI3Kδ. Unfortunately, these compounds possessed moderate lipophilicity (cLogP = 3.4), so incorporation of a dimethylmorpholine would drive the compound into undesired chemical space (cLogP 4.3) so at this time were not synthesised.

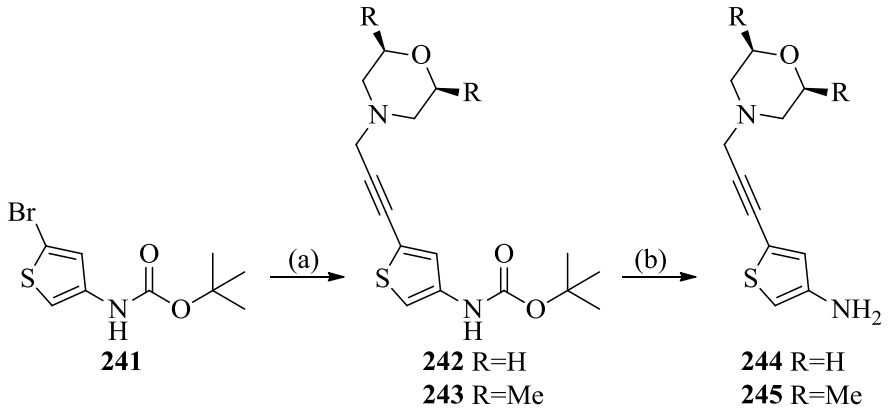
One further change we were previously unable to investigate was the incorporation of a 5-membered ring. Unsubstituted 5-membered aromatic amines such as aminothiophene and aminofuran are chemically unstable, however substitution upon these rings can impart enhanced stability. With this in mind, we first wanted to incorporate thiophene, a group that would direct a vector similar to **212**, whilst lowering the logP by 0.3. The following compounds were selected for synthesis (Figure 5.27).



239 **240**

Figure 5.27 Compounds **239** and **240** selected for synthesis.

The following route was trialled to access monomers **244** and **245** (Scheme 5.33).



241 **242** R=H **243** R=Me **244** R=H **245** R=Me

(a) see Table 5.17; (b) TFA, DCM, rt, 1 h, (80-84 %)

Scheme 5.33 Synthesis of aromatic amines **244** and **245**.

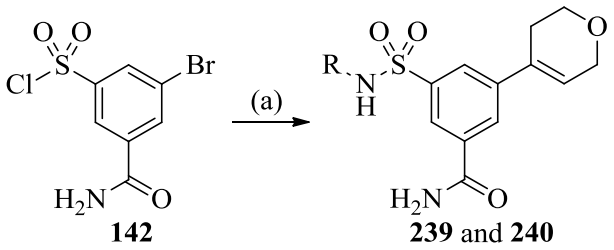
For the Sonogashira cross-coupling, the following reaction conditions were trialled (Table 5.17).

Table 5.17 Sonogashira conditions trialled for the synthesis of **242** and **243**.

Conditions (a)	**Conversion % (Yield %)**
$PdCl_2(PPh_3)_2$ (0.1 eq), CuI (0.1 eq), NEt_3 (3 eq), DMF, rt - 70 °C	0 (0)
$Pd_2(dba)_3$ (0.05 eq), BINAP (0.1 eq), CuI (0.1 eq), NEt_3 (3 eq), DMF, rt - 70 °C	0 (0)
$PdCl_2(PPh_3)_2$ (0.1 eq), (*t*-Bu)$_3$PHBF$_4$ (0.1 eq), DBU (0.2 eq), Cs_2CO_3 (2 eq), 100 °C μw	100 (50 - 79)

Under standard conditions with either $PdCl_2(PPh_3)_2$ or $Pd_2(dba)_3$/BINAP, no conversion or degradation of the starting material was observed. Alternatively copper-free Buchwald cross-coupling utilising DBU proved successful, giving **242** and **243** in moderate to high yield (50-79 %). Subsequent deprotection in TFA yielded **244** and **245** which, although not 100 % pure, were of sufficient purity.

The amines **244** and **245** were then successfully reacted in the two-step process previously used in poor yields (16 % and 7 % respectively) (Scheme 5.34).

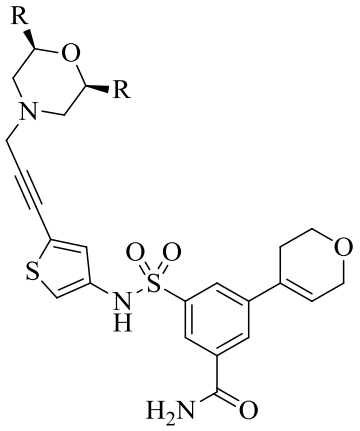


(a) i) R-NH_2, triethylamine, THF, rt, 30 min; ii) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (7-16 %);

Scheme 5.34 Synthesis of sulfonamides **239** and **240**.

The results are shown below (Table 5.18).

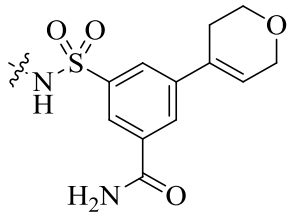
Table 5.18 Enzyme inhibition data for compounds **239** and **240**.[36]

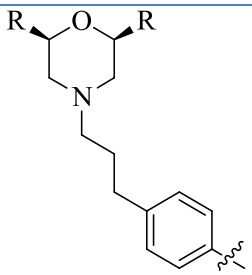
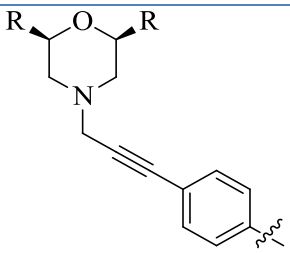
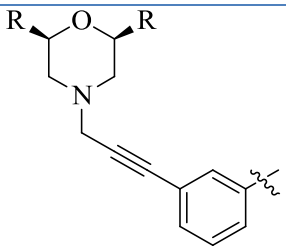
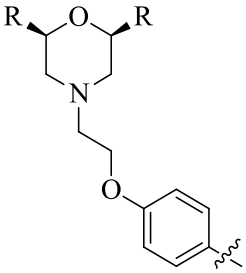


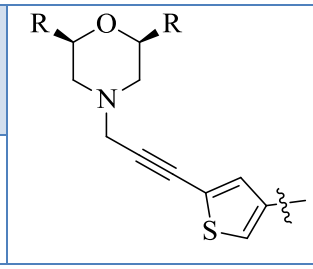
		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
239	H	5.4 (n=2)	5.0 (n=2)	5.2 (n=4)	7.2 (n=2)	0.30/6.0
240	Me	5.3 (n=2)	5.1 (n=2)	5.0 (n=4)	7.8 (n=2)	0.31/5.6

Both compounds showed comparable activity to *para*-phenyls **239** and **240**, indicating that the thiophene was tolerated and mimicked a similar vector. The results were exciting so we decided to further investigate the standout compounds (**212**, **214**, **215**, **216** and **240**) and five comparative morpholino compounds (**181**, **186**, **187**, **188** and **239**) for their cellular activity and physicochemical profile (Table 5.19).

Table 5.19 Wider properties of compounds **181**, **186-188**, **212-216**, **239** and **240**.[36]



		PI3Kδ (pIC_{50})	WB IFNγ (pIC_{50})	Permeability AMP/MDCK (nm/sec)	Solubility (µg/mL)
186 R=H		7.1 (n=5)	5.5[a] (n=3)	11	> 287
214 R=Me		7.5 (n=3)	6.5 (n=3)	78	> 195
187 R=H		7.2 (n=5)	4.6[b,c] (n=1)	7.4	> 249
215 R=Me		7.7 (n=3)	5.2 (n=3)	NT/47.5	108
181 R=H		7.1 (n=4)	5.6[d] (n=3)	28	> 201
212 R=Me		7.4 (n=4)	4.9 (n=1)	94/48.1	>262
188 R=H		6.7 (n=5)	4.9[d] (n=2)	< 3	> 162
216 R=Me		7.3 (n=4)	5.9 (n=3)	66/47.1	> 258

239 R=H		7.2 (n=2)	NR[e]	< 3	>174
240 R=Me		7.8 (n=2)	5.5 (n=2)	38/47.8	>194

[a]On three occassions, no result was observed. [b]On two occassions, a result of <4.5 was observed. [c]On five occassions, no result was observed. [d]On two occassions, no result was observed. [e]On four occassions, no result was observed.

For all eight compounds a drop in potency is observed at PI3Kδ from the isolated enzyme to the cellular assay. In general, the dimethylmorpholino- compounds (**212**, **214**, **215**, **216** and **240**) possessed greater activity in the cellular assay than the morpholino- matched pairs (**181**, **186**, **187**, **188** and **239**). This is likely due to the increase in lipophilicity which increases the permeability of the dimethylmorpholino- compounds. All compounds possessed moderate to high aqueous solubility indicating that the increase in lipophilicity does not impair this series of compounds in relation to aqueous solubility

Alongside this, we also investigated the *in vitro* clearance of four compounds (**215**, **212**, **216** and **240**) in order to gain an initial insight into the hepatic metabolic clearance of this series of compounds (Table 5.20).

Table 5.20 *In vitro* clearance data for **212**, **215**, **216** and **240**.[36]

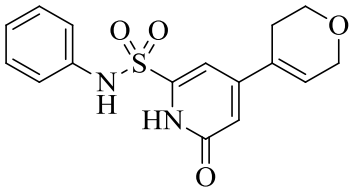
			Liver Microsomal *in vitro* clearance (mL/min/g)	
	PI3Kδ (pIC_{50})	**cLogP**	**Rat**	**Human**
215	7.7	2.4	2.17	13.82
212	7.4	2.4	12.60	8.73
216	7.3	2.8	4.01	12.86
240	7.8	2.1	2.40	7.84

The data shown demonstrates that all four compounds are highly cleared in human microsomes. When scaled using the 'well-stirred model' and a non-restrictive approach, the compounds are all cleared above two thirds of liver blood flow (human LBF = 18 mL/min/g). A larger value in human IVC correlates with increasing lipophilicity, indicating that lowering this could provide some benefit. In rat, the data is more encouraging as three compounds are moderately cleared and one highly cleared. Again, some correlation can be observed between increased lipophilicity and IVC for the three moderately cleared compounds. Interestingly, *meta*-**212** is cleared six-times faster than *para*-**215**, in rat, indicating a potential structural liability.

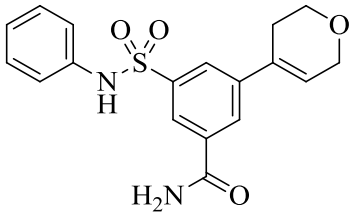
Despite observing moderate to high IVC, this work has highlighted a novel back pocket group that can be utilised in order to access highly selective and potent PI3Kδ inhibitors from a vector not utilised or explored within the literature.

Chapter 6 Re-reversal of the sulfonamide

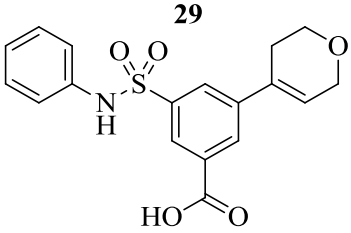
In chapter 4, we identified four interesting and diverse compounds with reverse sulfonamides to replace the 2-methoxypyridine back pocket group.



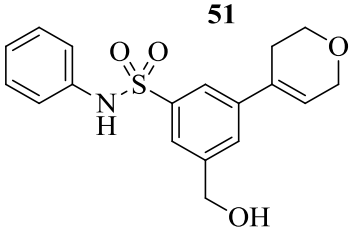
29



51



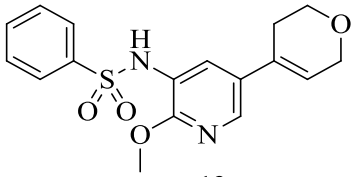
56



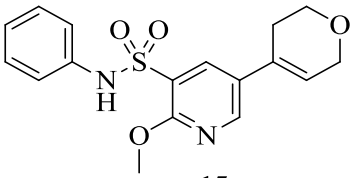
52

This chapter of the thesis will focus on the 're-reversal' of the sulfonamide. In the 2-methoxypyridine series, it was found that reversal of the forward sulfonamide **13** to **15** led to a small drop in potency (4-fold) but in general was well tolerated (Table 6.1).

Table 6.1 Enzyme inhibition data for compounds **13** and **15**.[36]



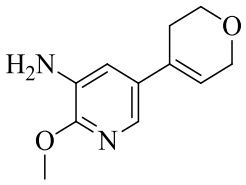
13



15

	pIC$_{50}$					
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**WB IFNγ**	**LE/LLE**
13	5.7 (n=5)	6.4 (n=4)	5.9 (n=5)	7.4 (n=4)	5.5 (n=7)	0.42/4.7
15	5.4 (n=10)	5.6 (n=9)	5.1[a] (n=7)	6.7 (n=8)	5.4 (n=8)	0.38/4.0

Although **13** was a more potent PI3Kδ inhibitor, reversal of the sulfonamide removed the Ames positive aniline **14** which led to a developable series of compounds.[121]



14

Both **13** and **15** bound to the protein backbone through a bridging water, although this was not observed in the crystal structure of **13**. As observed in the overlay in crystal structure of **13** and **16** (Figure 6.1) the forward sulfonamide **13** binds closer to the backbone due to the alternative binding mode of the sulfonamide moiety in which Lys779 interacts with the ionised nitrogen and S=O bond of the sulfonamide.

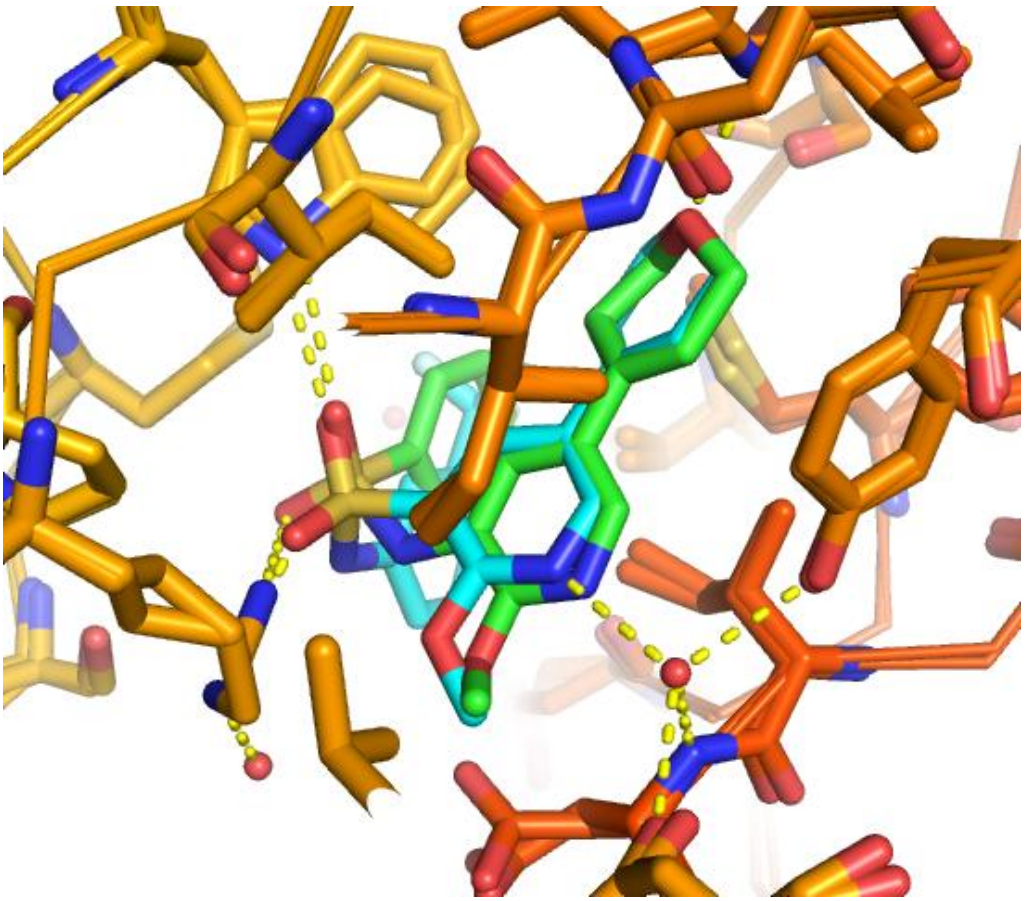
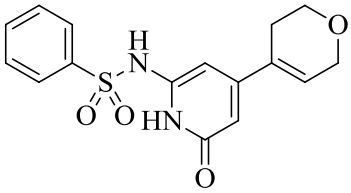
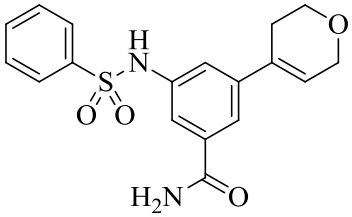


Figure 6.1 The overlay of reverse sulfonamide **16** (cyan) and forward sulfonamide **13** (green), highlighting the water molecule that is observed in the reverse sulfonamide (cyan).

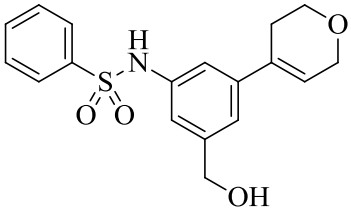
If the sulfonamides of pyridone **29**, amide **51**, alcohol **52** and acid **56** were 're-reversed' to give the forward sulfonamides, it could be hypothesized that the binding mode of the crucial sulfonamide would change to interact with the ionized nitrogen and a sulfonamide oxygen.



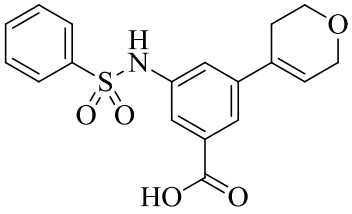
246



247



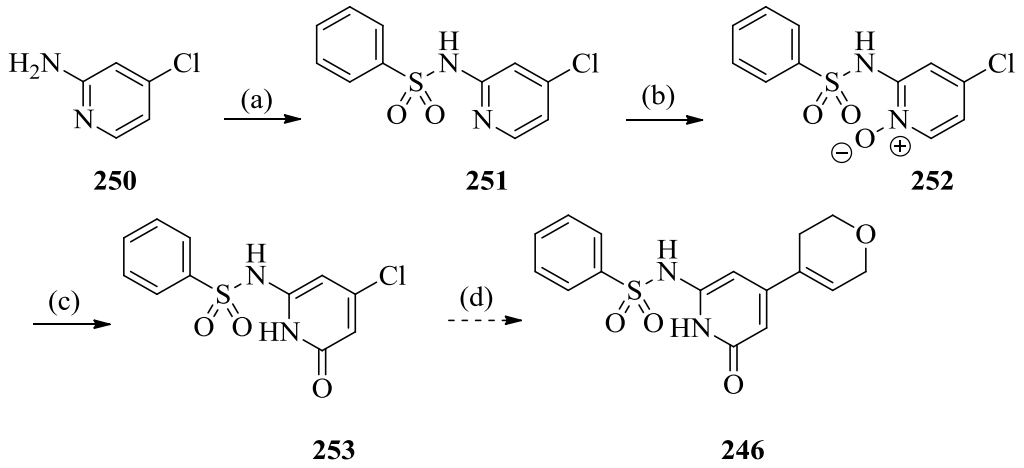
248



249

By directly changing the back pocket binding group, the potential toxicity of the aromatic ring would be different due to the change in ring electronics and its substituents, which may lead to an Ames negative aniline.[122,123] If a potent forward sulfonamide was discovered during this work, synthesis of the corresponding aniline would be a critical endeavour in order to rule out potential Ames liabilities.

For pyridone **246**, it was envisaged that the compound could be synthesised via a re-arrangement of *N*-oxide **252** using acetic anhydride to give the desired chloro-pyridone **253** which could be further derivatized to desired pyridone **246** (Scheme 6.1).



(a) Benzenesulfonyl chloride, pyridine, rt, 5 h (71 %); (b) see Table 6.2; (c) acetic anhydride 120 °C, 3 h.

Scheme 6.1 Proposed synthesis of pyridone **246**.

Sulfonamide formation under standard conditions proceeded smoothly after recrystallization from ethanol to give **251** (71 %). The following conditions were trialled for the *N*-oxidation of **251** (Table 6.2)

Table 6.2 *N*-Oxidation conditions trialled.

Conditions	**% Product with MH=284 via LCMS**
*m*CPBA, $CHCl_3$, 40 °C, 4 h	82 %
30 % H_2O_2 in H_2O, AcOH, 80 °C, 16 h	0 %
30 % H_2O_2 in H_2O, AcOH, 80 °C, 2 h	73 %

Heating of pyridine **251** with *m*CPBA showed promising conversion to a product with the desired mass via LCMS. Despite this, the workup of the reaction mixture was challenging due to the high aqueous solubility of the product formed and the *m*CPBA by-product 3-chlorobenzoic acid. After repeated extraction of the aqueous layer, the desired product was observed via LCMS. However upon column chromatography, only 3-chlorobenzoic acid was eluted, indicating the desired product had decomposed on silica.

Alternative conditions utilising hydrogen peroxide in acetic acid were chosen in order to limit organic by-products and again, initial results looked positive however upon prolonged heating overnight, significant decomposition to unknown products was observed via LCMS. The reaction was repeated and by quenching the reaction at 2 h, minimal decomposition was observed. Workup of the reaction was again problematic, however by reducing the amount of aqueous sodium sulfite used in the quench of the excess hydrogen peroxide, the compound extracted into ethyl acetate. To avoid purification on silica the compound was dissolved neat in acetic anhydride and heated to 120 °C for 3 h, upon which a new peak was observed with the desired mass. Purification by HPLC gave 76 mg (28 %) of a compound that was not the desired pyridone **253**, but in fact the N-hydroxy sulfonamide **254**. A proposed mechanism for its formation is highlighted below (Figure 6.2).

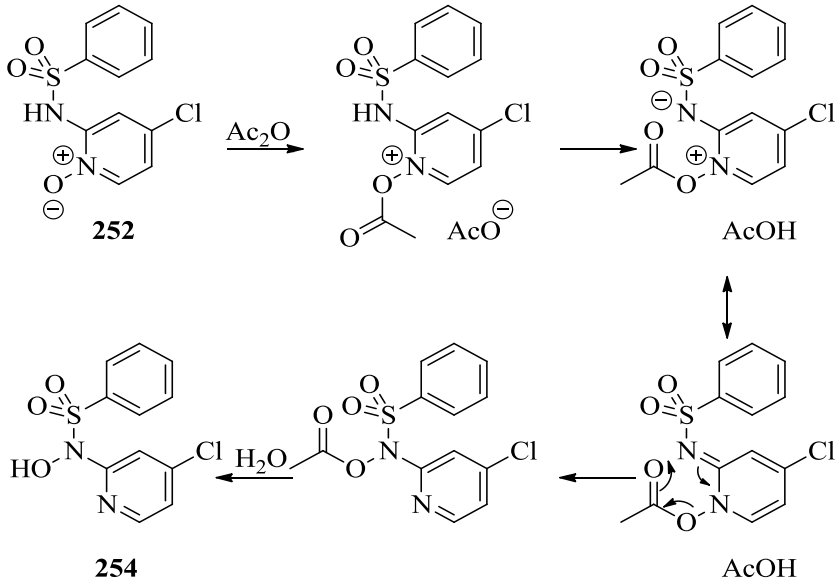
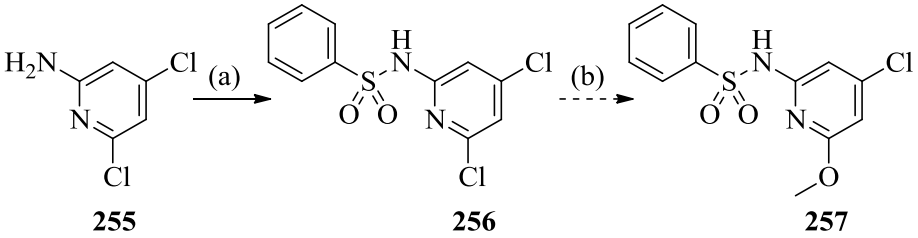


Figure 6.2 Proposed formation of N-hydroxy sulfonamide **254**.

NMR spectroscopy of the product revealed 8 aromatic protons, which was inconsistent with the desired 2-pyridone **253**. The peaks were not well resolved in the NMR spectrum, however in comparison to the starting material, there was not significant downfield shift observed of the proton or carbon at the 6-position of the pyridine ring to suggest the pyridine N-oxide. 2-Pyridyl-*N*-hydroxysulfonamides are known in the literature however no ^{1}H NMR chemical shifts were reported.[124]

An alternate route was initiated by starting from pyridine **255** (Scheme 6.2).



255 **256** **257**

(a) Benzenesulfonyl chloride, pyridine, rt, 5 h, (83 %); (b) NaOMe, MeOH, 135 °C, 2 h.

Scheme 6.2 Proposed synthesis of compound **257**.

Sulfonamide formation under standard conditions gave **256** (83 %). Intermediate **256** was unreactive with sodium methoxide at 135 °C under microwave irradiation. This is probably due to the deprotonated nitrogen of the sulfonamide delocalizing into the pyridine ring, deactivating the ring towards a S_NAr reaction (Figure 6.3).

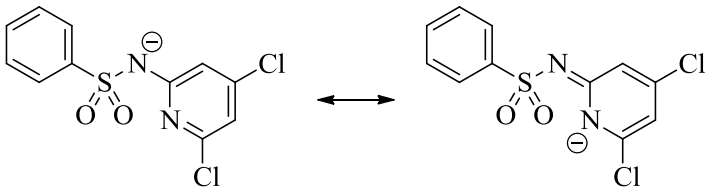
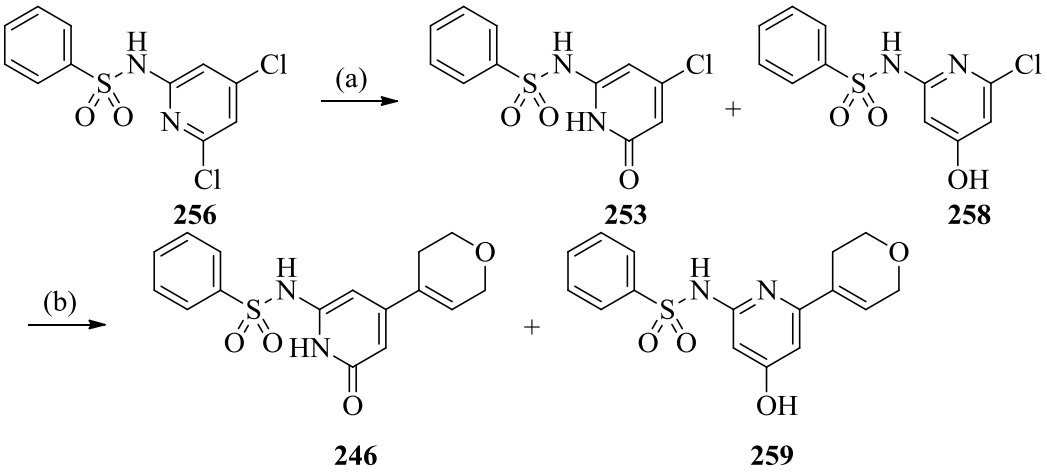


Figure 6.3 Delocalization of the ionised sulfonamide.

At this point, to avoid protection of the sulfonamide N-H, it was decided to trial a Buchwald-cross-coupling with alkoxides (Scheme 6.3).



(a) XPhos, $Pd_2(dba)_3$, NaO^tBu, THF, H_2O, 100 °C, 16 h; (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min (11-24 %);

Scheme 6.3 Synthesis of pyridone **246** and hydroxypyridine **259**.

Clean conversion was observed to a single peak via LCMS of the desired pyridone **253**. However, upon purification, it was found that this was a mixture of **253** and **258**. Analysis via NMR spectroscopy was challenging due to the similarity in chemical shift of the two protons of 2-pyridone and 4-hydroxypyridine respectively. In an attempt to access the final compound, the mixture was used in a Suzuki cross-coupling and it was found that after this stage, the compounds could be separated in poor yields (**246** = 20 %, **259** = 19 %). The compounds were differentiated from one another using ROESY spectroscopy, in which correlations were observed between the two C-H bonds of the pyridone **246** to the C-C*H*$_2$ and C=C*H* bonds of the dihydropyran. This was in contrast to **259**, in ROESY spectroscopy of which only 1 correlation was observed (Figure 6.4).

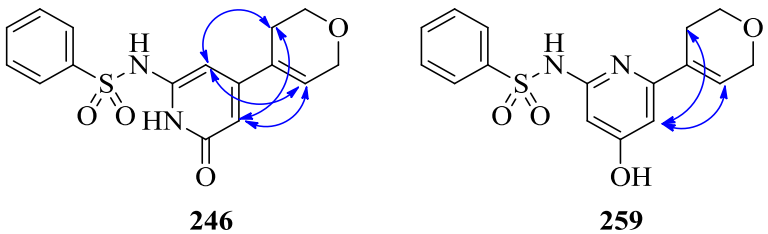
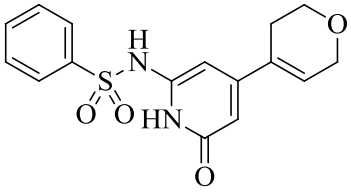


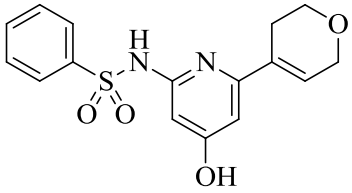
Figure 6.4 ROESY correlations observed.

The results are as follows (Table 6.3).

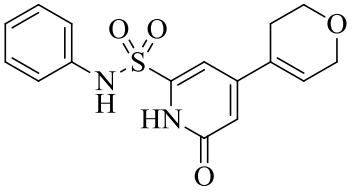
Table 6.3 Enzyme inhibition data for compounds **29**, **246** and **259**.[36]



246



259



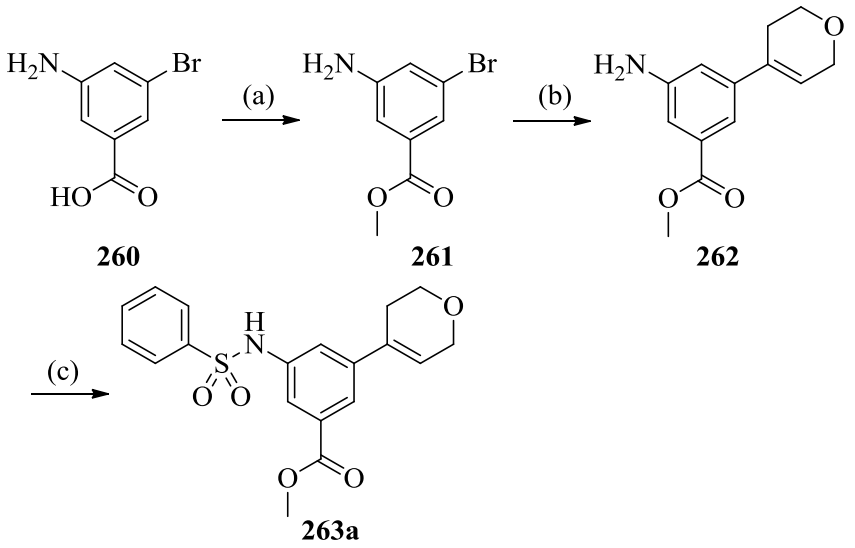
29

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
246	5.1 (n=3)	5.3 (n=3)	5.0 (n=5)	6.3 (n=3)	0.38/5.9
259	4.6[a] (n=1)	4.6[a] (n=1)	4.4[b,c] (n=1)	5.2 (n=2)	0.31/3.0
29	5.2 (n=3)	6.3 (n=3)	5.3 (n=3)	6.8 (n=3)	0.41/6.1

[a]On one occasion, a result of <4.5 was received. [b]On one occasion, a result of <4.3 was received. [c]On two occasions, a result of <4.5 was received.

Pyridone **246** was an active PI3Kδ inhibitor, displaying moderate potency and some selectivity for PI3Kδ against the other class I isoforms. This indicated that the forward sulfonamide was tolerated, however in comparison to the reverse sulfonamide **29**, the compound was 4-fold less potent against PI3Kδ. Pyridone **246** had no measurable permeability (< 3 nm/sec) and poor organic solubility. 4-Hydroxypyridyl **259** was also tested and was found to be a weakly active PI3Kδ inhibitor.

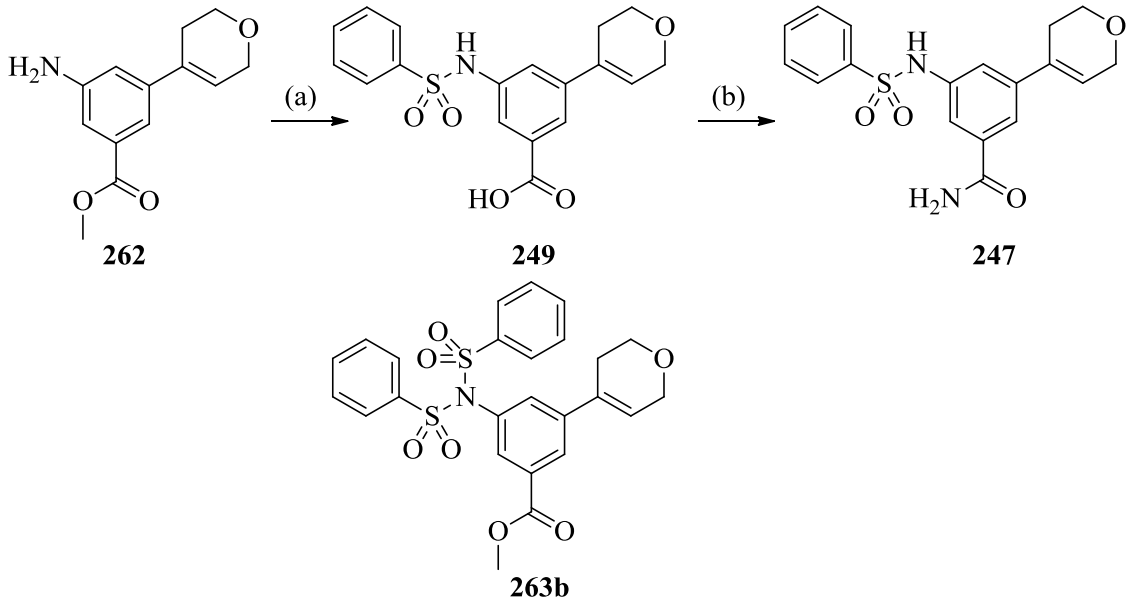
For amide **247**, alcohol **248** and acid **249**, the following route was initiated to access the key intermediate **263a** which could be then further substituted (Scheme 6.4).



(a) H_2SO_4, MeOH, rt, 24 h (90-93 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 60 min (98 %); (c) benzenesulfonyl chloride, pyridine, rt, 3 h (86 %).

Scheme 6.4 Synthesis of common intermediate **263a**

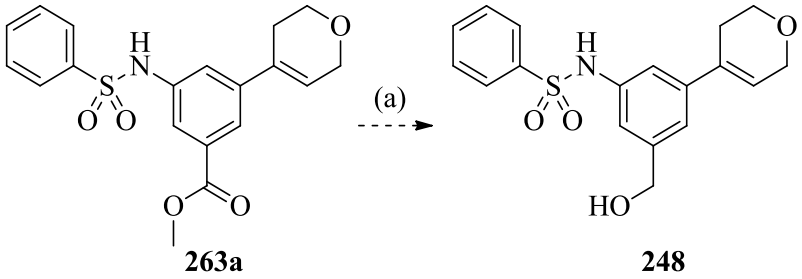
Fisher esterification of **260** and subsequent Suzuki cross-coupling proceeded in excellent yields (90 - 93 % and 98 %). An initial sulfonamide formation test reaction was performed which proceeded in good yield (86 %). On a larger scale during the sulfonamide formation to form **263a**, a small proportion of the bis-sulfonamide **263b** was observed by LCMS analysis (Scheme 6.5, step (a)). In order to directly access the acid, the bis-sulfonamide **264** and methyl ester were cleaved *in situ* utilizing sodium hydroxide in methanol to give acid **249** in moderate yield (71 % over 2 steps). Subsequent amide coupling under standard conditions gave amide **247** (41 % yield).



(a) i) Benzenesulfonyl chloride, pyridine, rt, 3 h; ii) NaOH, rt, 30 min, (71 %); (b) i) HATU, DIPEA, DMF, rt, 30 min; ii) NH_3, rt, 72 h, (41 %).

Scheme 6.5 Synthesis of acid **249** and amide **247**.

At this stage, the reduction to form alcohol **248** was trialled (Scheme 6.6).

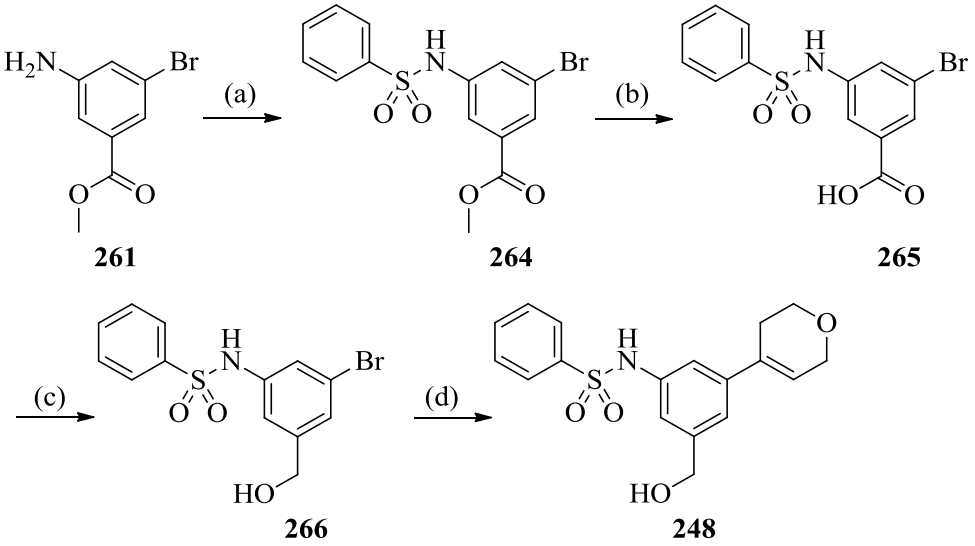


(a) $LiAlH_4$, THF or $CaCl_2$, $NaBH_4$, THF

Scheme 6.6 Proposed synthesis of **248**.

The reaction formed the desired product, as observed by LCMS. Despite this, purification of **248** proved challenging due to an unknown close running impurity that formed during the

reaction with either lithium aluminium hydride or *in situ* generated calcium borohydride. To access alcohol **248**, the following scheme was envisaged (Scheme 6.7).



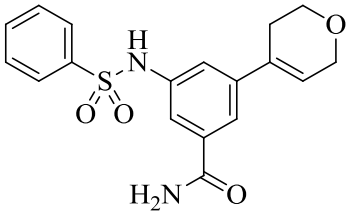
(a) Benzenesulfonyl chloride, pyridine, rt, 3 h, (70 %); (b) LiOH, THF, H_2O, rt, 16 h, (99 %); (c) BH_3, THF, 0 °C, 16 h, (81 %); (d) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (49 %)

Scheme 6.7 Synthesis of compound **248**.

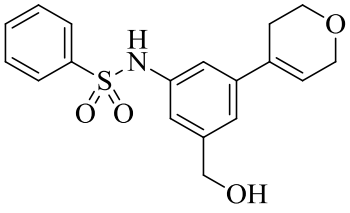
From methyl ester **261**, sulfonamide formation proceeded in good yield after recrystallization from boiling ethanol (70 %) to give **264**, which was subsequently hydrolysed in excellent yield (99 %). Reduction with borane yielded **266** in moderate yield (81 %). Suzuki cross-coupling proceeded in moderate yield (49 %) to give **248**.

The results for compounds **247**, **248** and **249** are as follows (Table 6.4).

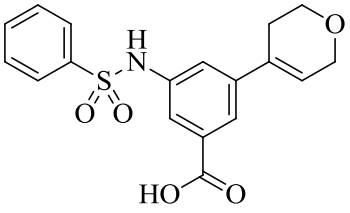
Table 6.4 Enzyme inhibition data for compounds **247**-**249**.[36]



247



248



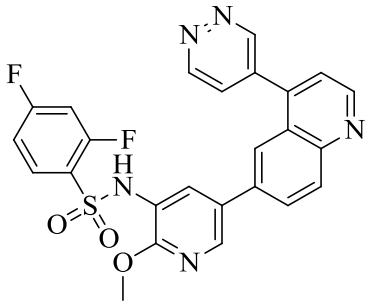
249

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
247	<4.5 (n=2)	<4.5 (n=2)	4.8[a] (n=1)	4.9 (n=2)	0.27/3.4
248	<4.5 (n=1)	4.7 (n=1)	<4.5 (n=2)	4.7 (n=2)	0.27/3.0
249	<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	5.0 (n=2)	0.27/2.3

[a]On one occasion, a result of <4.5 was received.

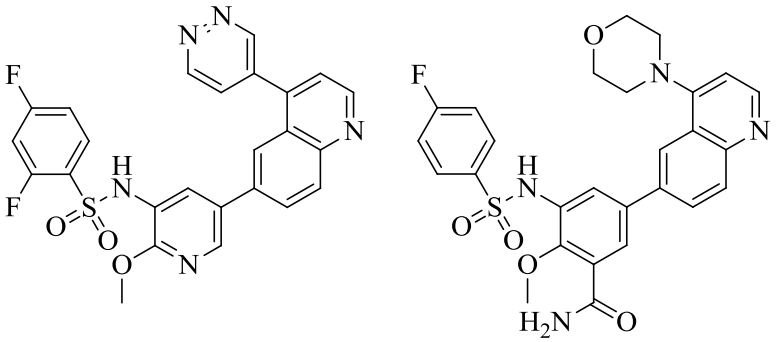
All three compounds showed weak potency for PI3Kδ, indicating that re-reversal of the sulfonamide was significantly detrimental to potency. This suggests that in order for the amide/acid/alcohol moieties to be able to bind in the back pocket region, the key sulfonamide interactions observed in **13** are lost, leading to a drop in potency.

Whilst conducting this work, a paper was published by Zhang and co-workers[125] which described the synthesis of some benzamide back pocket binding inhibitors of the PI3K/MTOR pathway, which possessed a similar benzamide core utilised in **248**. They built upon work by Knight and co-workers[126] in which the discovery of **267**, a potent pan-PI3K/MTOR inhibitor in human clinical trials for the treatment of cancer, is described.



267

The binding mode of **267** in the crystal structure of PI3Kγ was described in which the 2-methoxypyridine acts as the back pocket group binding to the protein through a bridging water molecule and the quinoline nitrogen forms the key hinge-binding interaction to Val828. The sulfonamide nitrogen and a single S=O bond of the sulfonamide interact with Lys779. Zhang and co-workers then describe the novel replacement of the 2-methoxypyridine and bridging water molecule with the benzamide core. Zhang and co-workers published a second paper[127] which again utilised this back pocket in further pan-PI3K inhibitors. Some exemplars are given below (Figure 6.5).



267 **268**

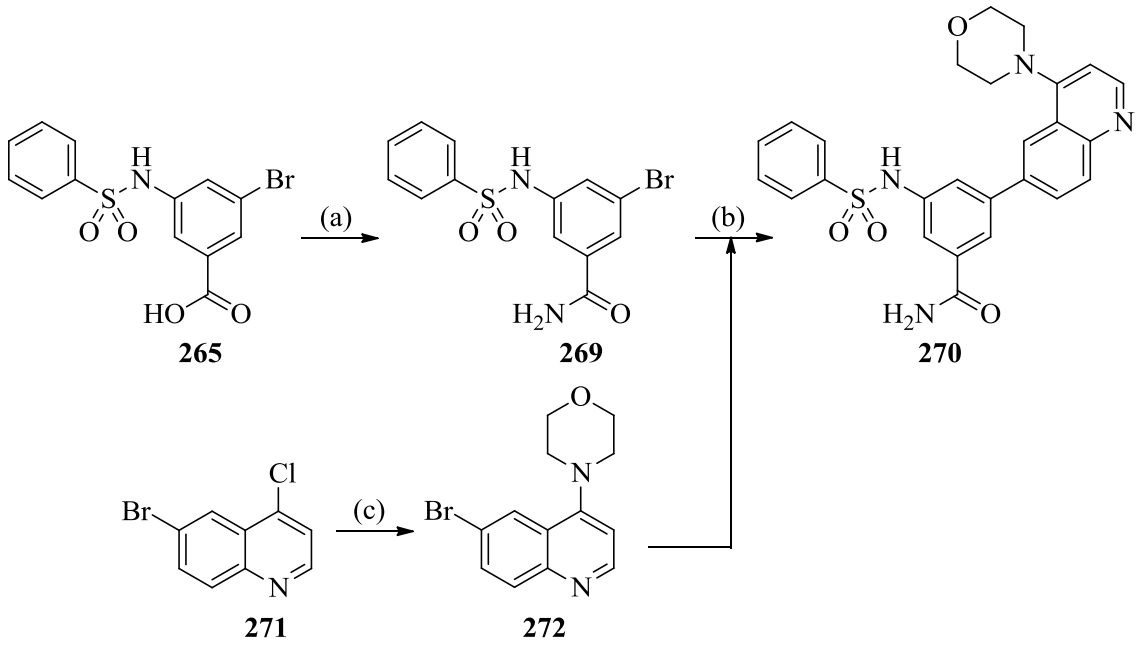
Figure 6.5 Replacement of the 2-methoxypyridine with 2-methoxycarboxamide.

The reported inhibition of **267** and **268** are shown below (Table 6.5).[126,128]

Table 6.5 Reported enzyme inhibition data for compounds **267** and **268**.[36,125,126]

	pIC$_{50}$			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
267	10.7	9.8	10.2	10.6
268	7.8	6.7	7.2	7.1

An interesting observation was that removal of the methoxy group in **268** was severely detrimental to potency in a variety of cancer cell lines. However as the PI3K pIC$_{50}$ values were not reported, it was synthesised to determine the change in potency. From common intermediate **266**, the following route was utilised to access **270** (Scheme 6.8).



(a) i) $SOCl_2$, 3 h, 70 °C; ii) NH_4OH, THF, rt, (99 %); (b) i) 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane), KOAc, $PdCl_2$(dppf), 1,4-dioxane, 6 h, 80 °C; ii) **272**, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 60 min, (56 %); (c) morpholine, IPA, 2h, 85 °C, (95 %).

Scheme 6.8 Synthesis of **270**.

In situ acyl chloride formation with thionyl chloride and subsequent quenching with ammonia gave primary carboxamide **269** in excellent yield (99 %). The morpholino-quinoline **272** cross-coupling partner was synthesised via a S_NAr reaction under literature conditions and again proceeded in excellent yield (95 %).[129] A one-pot Miyaura borylation Suzuki cross-coupling reaction yielded **270** in moderate yield over 2 steps (56 %).

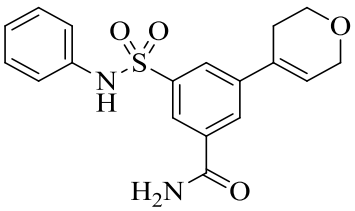
Table 6.6 Enzyme inhibition data for compound **270**.[36]

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
270	5.0 (n=2)	4.7[a] (n=1)	5.9 (n=2)	6.1 (n=2)	0.24/3.0

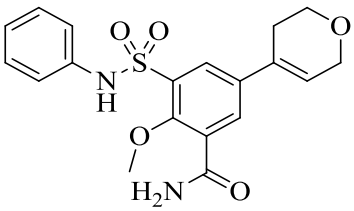
[a]On one occasion, a result of <4.5 was received.

Compound **270** has moderate potency, albeit poor efficiency, against PI3Kδ and PI3Kγ, and in comparison to the reported data for **268**, **270** is significantly less potent against all the PI3K class I isoforms. This comparison of the 2-methoxy group was interesting as in the previous reverse sulfonamide series, the inclusion of the methoxy group had no impact in potency or selectivity (Table 6.7).

Table 6.7. Enzyme inhibition data for compounds **51** and **70**.[36]



51

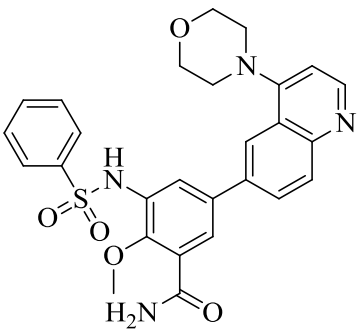


70

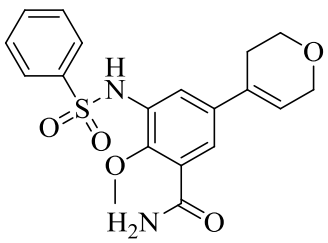
	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
51	5.2 (n=3)	5.6 (n=3)	5.1[a] (n=1)	6.3 (n=3)	0.35/4.5
70	4.8[b] (n=3)	5.1 (n=4)	4.6 (n=4)	6.2 (n=4)	0.31/4.9

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.5 was received.

This highlighted a disconnect between the SAR of forward and reverse sulfonamide, therefore, **273** and **274** were proposed for synthesis (Figure 6.6).



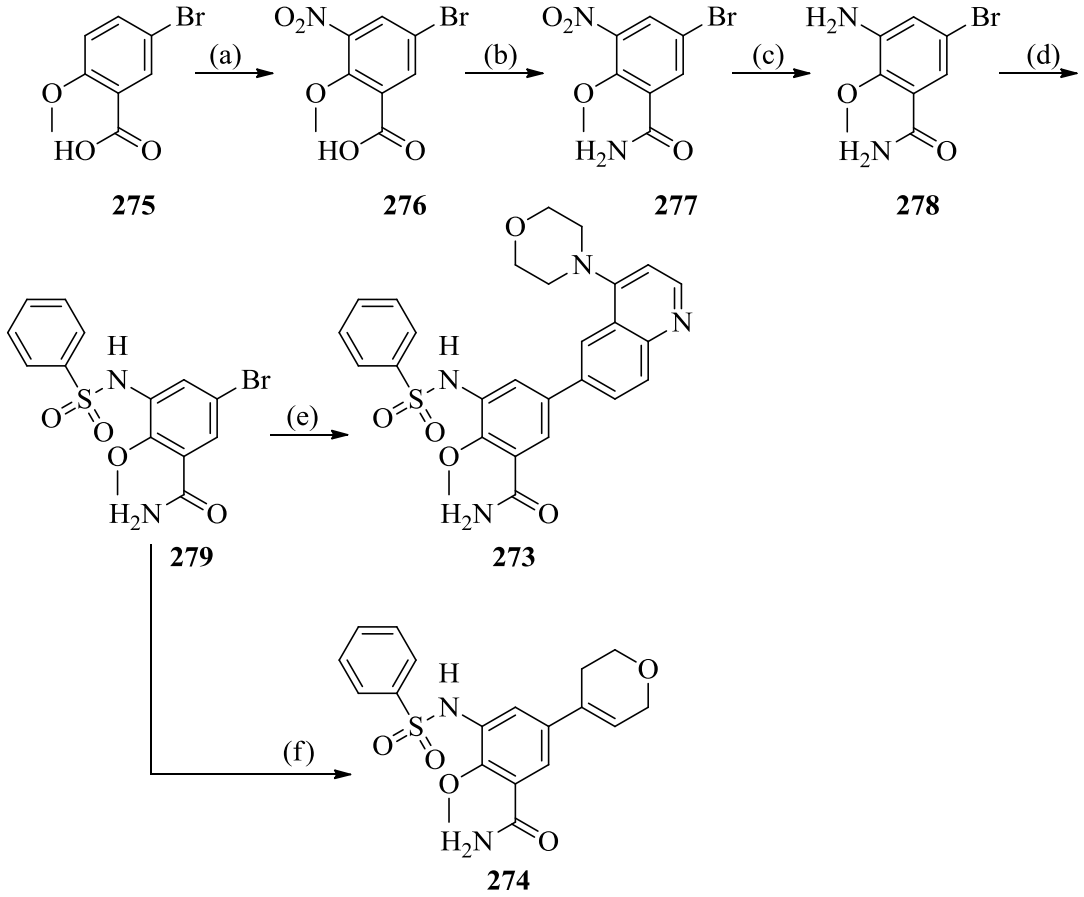
273



274

Figure 6.6 Compounds **273** and **274** selected for synthesis.

To access this methoxy template, the following route was initiated (Scheme 6.9).



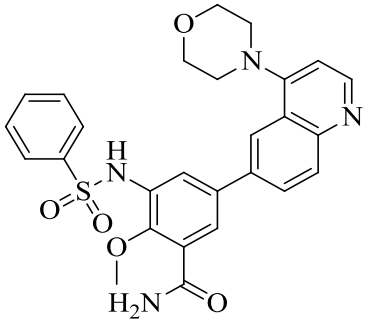
(a) H_2SO_4, HNO_3, 0 °C, 30 min, (61 - 74 %); (b) $SOCl_2$, 70 °C, 3 h; ii) NH_4OH, THF, 0 °C, (88 - 97 %); (c) Fe, NH_4Cl, EtOH, H_2O, 80 °C, 17 h, (82 - 97 %); (d) benzenesulfonyl chloride, pyridine, rt, 5 h, (41 %); (e) i) **272**, 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane), KOAc, $PdCl_2$(dppf), 1,4-dioxane, 6 h, 80 °C; ii) **279**, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 60 min, (72 %); (f) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (51 %).

Scheme 6.9 Synthesis of compounds **273** and **274**.

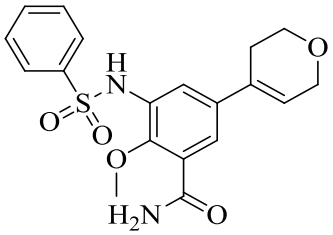
From salicylic acid derivative **275**, *ortho*-directed nitration by the methoxy group under standard conditions gave **276** in good yield (61-74 %). *In situ* acyl chloride formation and subsequent quench with aqueous ammonia gave the primary carboxamide **277**. Reduction of the nitro-substituent to the aniline **278** proceeded in good yield (82-97 %). Subsequent

sulfonamide formation displayed a clean reaction profile; however the isolation of the product from the pyridinium salts formed proved problematic. To overcome this, recrystallization from boiling ethanol gave **279** in poor yield (41 %). A one-pot Miyaura borylation Suzuki cross-coupling reaction yielded **273** in good yield over two steps (72 %) and Suzuki cross-coupling yielded **274** (51 %). The results are shown below (Table 6.8).

Table 6.8 Reported and in-house enzyme inhibition data for compounds **273** and **274**.[36]



273



274

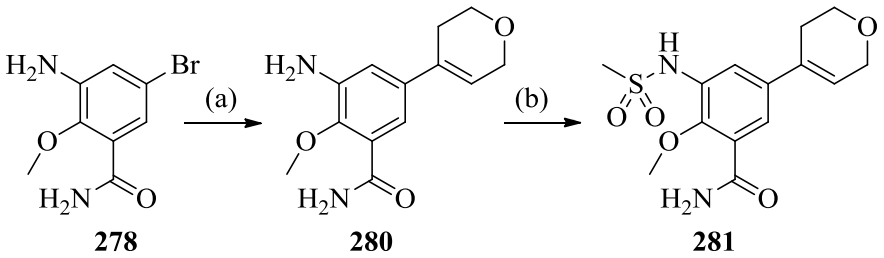
	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
273	6.2 (n=3)	5.5 (n=3)	6.3 (n=3)	7.0 (n=3)	0.26/3.9
273 Reported	7.8	6.7	7.2	7.1	0.26/4.0
274	5.0[a] (n=3)	4.9 (n=4)	4.6[a] (n=3)	6.3 (n=4)	0.32/4.9

[a]On one occasion, a result of <4.5 was received.

Compound **273** demonstrated high potency for PI3Kδ, moderate potency for PI3Kα and PI3Kγ, but was not an efficient compound. Inclusion of the methoxy group increased potency against all the isoforms in comparison to **270**, signifying that it has a profound effect. Compound **273** was significantly less potent at the PI3K isoforms in the assay run within GSK than reported within the literature.[125,128] The assay format utilised within GSK (as highlighted in chapter 2) is a homogeneous time resolved fluorescence, whereas Zhang and co-workers utilised an ATP depletion assay. The different concentrations of PI3K enzymes and ATP utilised in both sets of work can lead to different results.

Methoxy analogue **274** showed moderate potency against PI3Kδ and good selectivity for PI3Kδ against all three class I isoforms with good LE and LLE. In fact methoxy analogue **274** was 25- fold more active at PI3Kδ compared to **247**, signifying that the methoxy was important for potency with the forward phenyl sulfonamide. The increase in potency observed is difficult to rationalise and in this case attempts to crystallise the compound in the PI3Kδ protein failed to give interpretable X-ray density on three occasions. The compound was moderately soluble (> 172 μg/mL) in CLND and in variety of organic solvents (deuterochloroform and d^6-dimethylsulfoxide) so the reason for the poor density is unknown.

To explore this, methylsulfonamide analogue **281** was proposed for synthesis as it was hoped that it would have a similar binding mode, due to the similarity observed previously within GSK between methyl and phenyl sulfonamides.[78] To achieve this, intermediate **278** was first used in a Suzuki cross-coupling to yield **280** and was used crude to form sulfonamide in moderate yield (31 % over 2 steps) (Scheme 6.10).



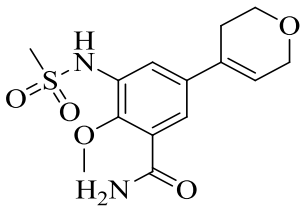
278 **280** **281**

(a) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (98 - 99 %); (b) methanesulfonyl chloride, pyridine, rt, 3 h, (31 %).

Scheme 6.10 Synthesis of **281**.

The results are as follows (Table 6.9).

Table 6.9 Enzyme inhibition data for compound **281**.[36]



	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
281	4.6 (n=3)	4.6[a] (n=2)	4.7 (n=3)	5.1 (n=3)	0.32/5.3

[a]On one occasion, a result of <4.5 was received.

Compound **281** still maintained some potency for PI3Kδ and remained equally efficient in comparison to phenyl sulfonamide **274**. Co-crystallography of **281** in the protein construct gave good density with good resolution around the active site (2.09 Å) (Figure 6.7).

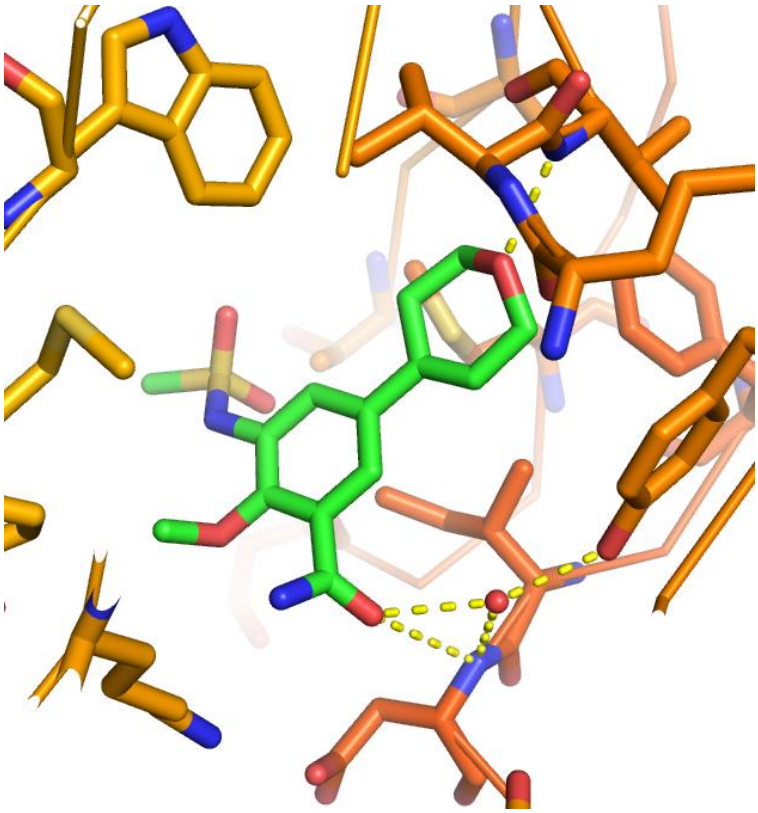


Figure 6.7A Co-crystal structure of **281** in PI3Kδ (Resolution 2.09 Å)[2]

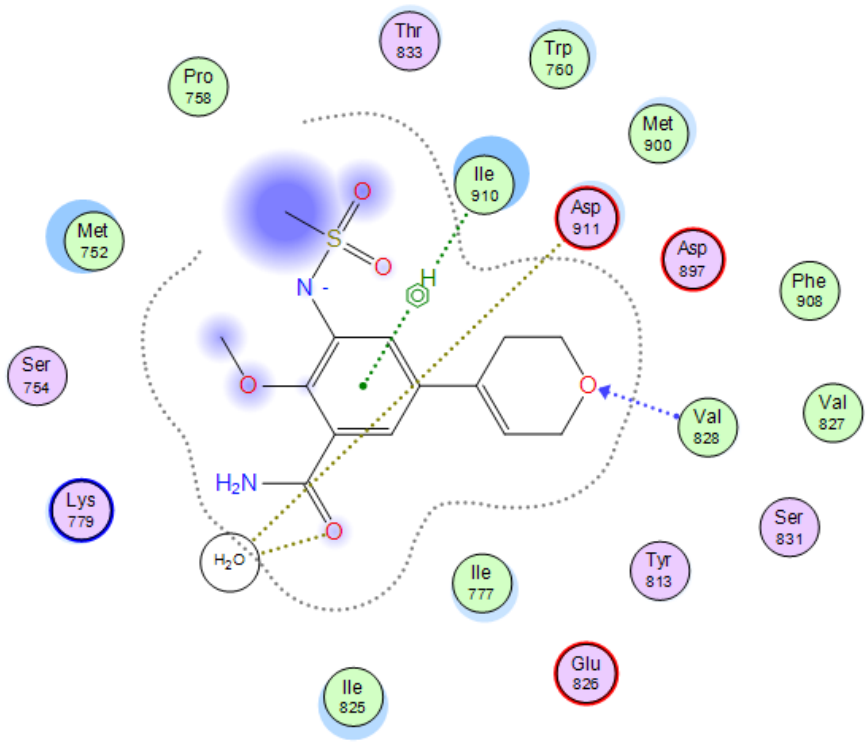


Figure 6.7B 2D Representation of **281** binding to PI3Kδ.

The co-crystal structure highlights some key interactions. The dihydropyran acts as the hinge binding motif, forming a HBA relationship with Val828. In the back pocket region, the amide moiety interacts with the protein backbone via a bridging water molecule, and in comparison to the compound containing the reverse sulfonamide **51**, the amide is 27° out of the plane of the ring. Although the orientation of the amide group cannot be determined due to similar density of the oxygen atom and the NH_2 group, both possible orientations appear to form interactions through the bridging water molecules with Asp911, Tyr813 and Asp787. Additionally Lys779 is within distance to form a hydrogen bond. As observed in forward sulfonamides with a 2-methoxypyridine back pocket, Trp760 does not adopt the 'in' orientation to interact with the S=O bond of the sulfonamide and in **281** the sulfonamide does not appear to make any interactions. An overlay with sulfonamide amide **51** (Figure 6.8) displays a clear movement away from the backbone in order to bind through the bridging water molecule that previous compounds have displaced.

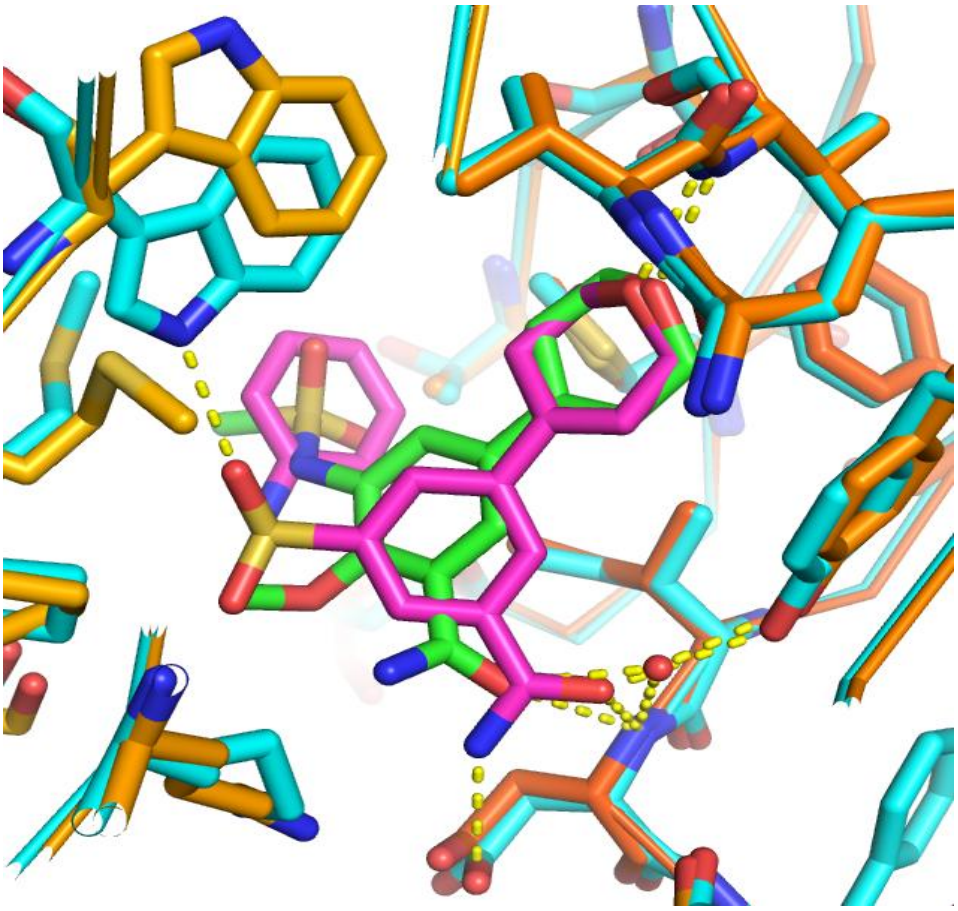
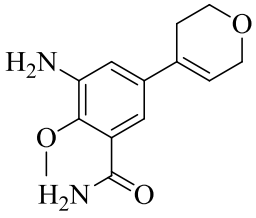


Figure 6.8 Overlay of compounds **51** (magenta) and **281** (green).

The crystal structure unfortunately cannot distinguish between the oxygen and carbon functionality in the sulfonamide. This leads to ambiguity as to the location of the methyl substituent and therefore where the phenyl ring would be in compound **274**. As the sulfonamide does not appear to form any specific interactions (Figure 6.7), intermediate **280** was tested.

Table 6.10 Enzyme inhibition data for compound **280**.[36]



	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
280	<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	N/A
280 High concentration	NT	NT	3.8 (n=2)	4.2 (n=2)	0.32/4.1

The compound was inactive in the 10 mM compound concentration assay and was then tested at higher concentration in order to determine the pIC$_{50}$. Removal of the methylsulfonamide led to an 8-fold drop in potency, indicating that the methyl sulfonamide is having a subtle yet important effect. To further develop SAR on this template, a small number of sulfonamide variants were proposed (Figure 6.9).

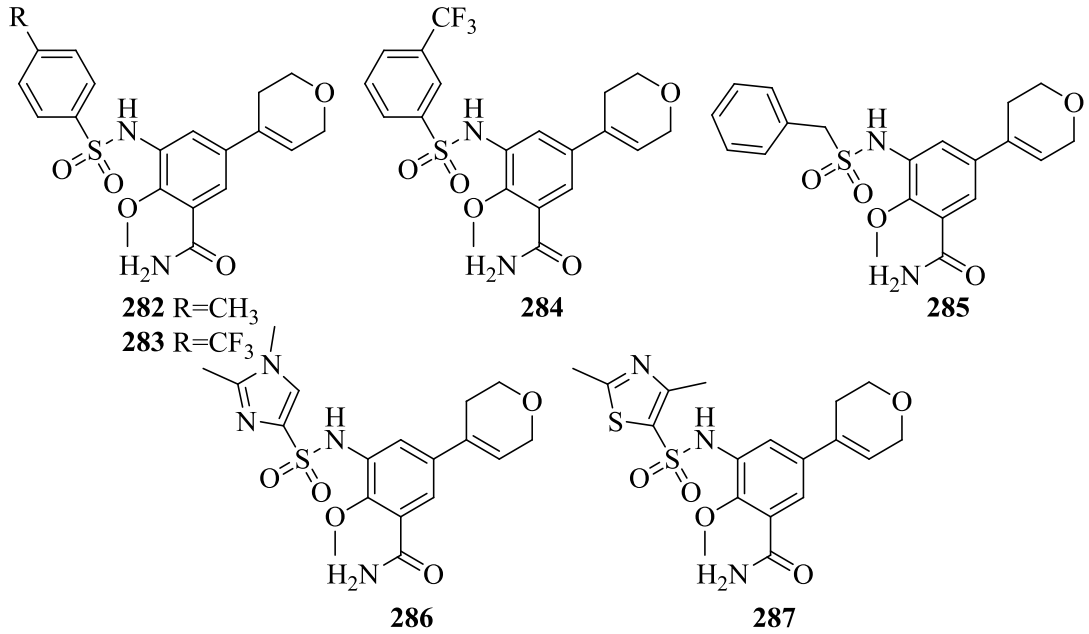
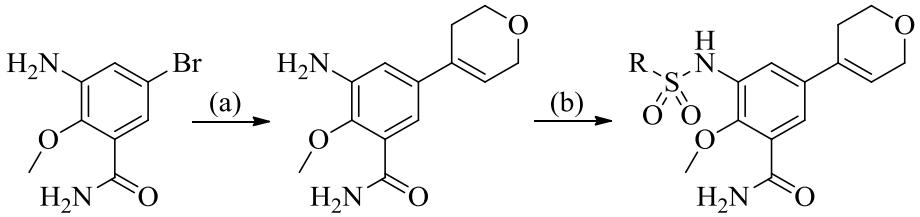


Figure 6.9

Compounds **282**-**284** were selected to test where additional substituents could be tolerated on the phenyl sulfonamide with comparative electron donating (methyl) and withdrawing (trifluoromethyl) effects. Benzyl substituent **285** was chosen to investigate homologation of the phenyl ring as this was previously not tolerated in other series with the dihydropyran. Imidazole **286** and thiazole **287** were chosen to investigate how changing the size of the ring to a 5-membered ring would affect potency. Synthesis and purification of common intermediate **280** was replicated in stoichiometric yield (99 %) and gave a common intermediate from which the compounds were synthesised under standard conditions in varying yields (24-70 %) (Scheme 6.11).



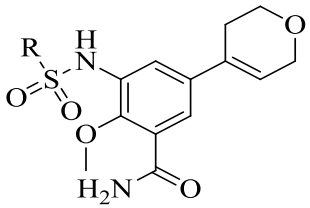
278 **280** **282-287**

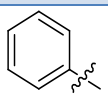
(a) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (98 - 99 %); (b) R-sulfonyl chloride, pyridine, rt, 3 h, (24 – 70 %).

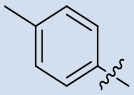
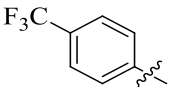
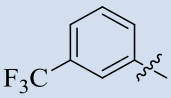
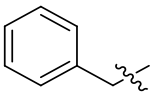
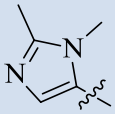
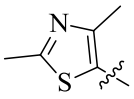
Scheme 6.11

The data for compounds **282**-**287** are shown below (Table 6.11).

Table 6.11 Enzyme inhibition data for compounds **274** and **282**-**287**.[36]



		pIC$_{50}$				
	R =	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
274		5.0[a] (n=3)	4.9 (n=4)	4.6[a] (n=3)	6.3 (n=4)	0.32/4.9

282		4.8[b] (n=1)	4.9 (n=3)	4.6[c,d] (n=1)	6.0 (n=2)	0.29/4.1
283		4.8[a] (n=2)	4.8 (n=3)	4.5[c] (n=2)	6.2 (n=3)	0.27/3.5
284		5.4 (n=3)	5.9 (n=3)	5.2 (n=6)	7.7 (n=3)	0.34/5.1
285		4.9[a] (n=1)	4.8[a] (n=1)	4.3[b] (n=2)	5.5 (n=2)	0.27/4.0
286		5.0 (n=2)	5.3 (n=2)	4.6[b,d] (n=1)	6.5 (n=2)	0.32/6.0
287		5.3 (n=3)	5.6 (n=3)	4.9[d] (n=4)	7.0 (n=3)	0.34/5.3

[a]On one occasion, a result of <4.5 was received. [b]On two occasions, a result of <4.5 was received. [c]On three occasions, a result of <4.5 was received. [d]On one occasion, a result of <4.3 was received.

Substitution in the *para*-position of the aromatic ring (**282** and **283**) has no effect on potency or selectivity, with both compounds similar to phenyl **274**. In comparison, inclusion of the trifluoromethyl-group **284** at the *meta*-position gave an impressive 25-fold increase in potency whilst maintaining good selectivity (63-fold against the nearest isoform). The LE and LLE were good, indicating that inclusion of the lipophilic trifluoromethyl group (cLogP increase of 1.2 unit's) was crucial. Benzyl **285** was poorly active against PI3Kδ, suggesting that homologation of the phenyl was not tolerated within the protein. Imidazole **286** was similar to phenyl **274**, indicating that although it was tolerated; it had no boost in potency. Thiazole **287** boosted potency for the inhibition of PI3Kδ, however also boosted the potency for PI3Kβ in equal measure. Both imidazole **286** and thiazole **287** demonstrated good LE/LLE and substitution from these groups offers a different starting point in regards to lipophilicity and vector to explore the Trp760 interaction compared to a six-membered ring.

As **284** was a standout compound, a crystal structure of **284** in the PI3Kδ protein was collected (Figure 6.10).

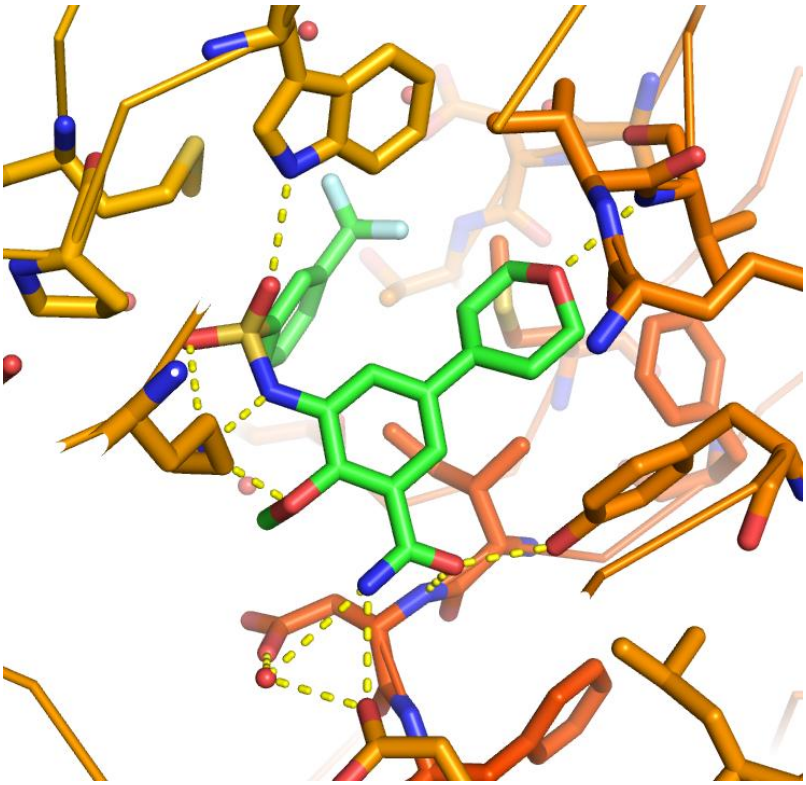


Figure 6.10A Co-crystal structure of **284** in PI3Kδ (Resolution 2.19 Å)[2]

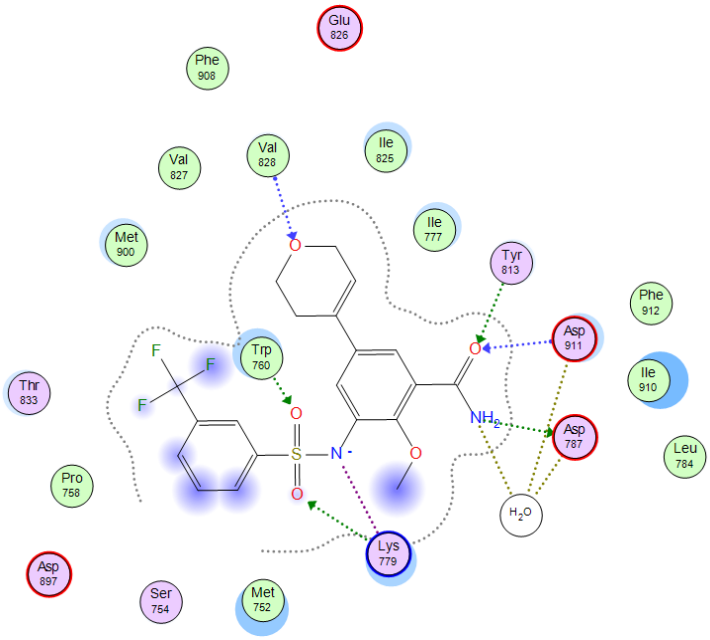


Figure 6.10B 2D Representation of **284** binding to PI3Kδ.

The hinge binding interaction to Val828 from the dihydropyran, the amide making a direct interaction with the phenolic proton of Tyr813 and the N-H of Asp911 and the amide amino group forming an interaction with the carboxylate of the Asp787 residue are all clearly observed. The previously observed water molecule from methylsulfonamide **281** has been clearly displaced from the back pocket region. The orientation of the amide cannot be determined by the density. The conformation shown (Figure 6.10), is the lowest energy conformation computationally, whereas the other conformation of the amide would pay a significant energy barrier (>10 kcal mol^{-1}). Elsewhere in the crystal structure, we observe that the methoxy group, ionised sulfonamide nitrogen and S=O bond interact with a very well defined Lys779 all of which could contribute to the potency increase. The second S=O bond of the sulfonamide forms a HBA interaction from the Trp760 residue. The trifluoromethyl occupies a lipophilic hotspot in the protein the same as compound **16**, a reverse sulfonamide used in the core replacement work in chapter 4 (Figure 6.11).

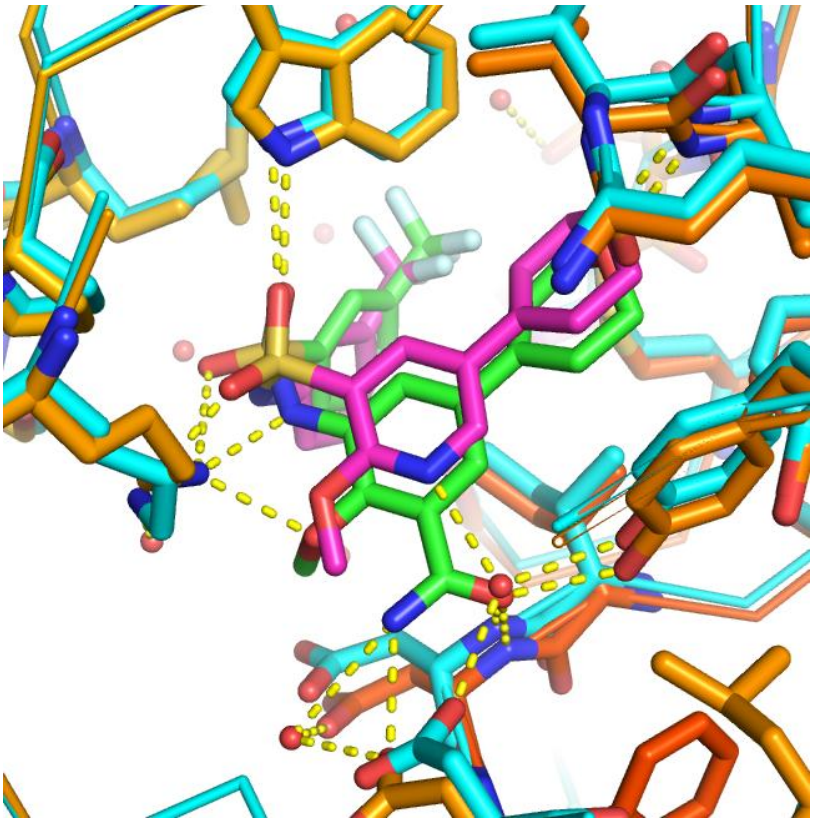


Figure 6.11A Overlay to show the similarity of **16** (magenta) and **284** (green).

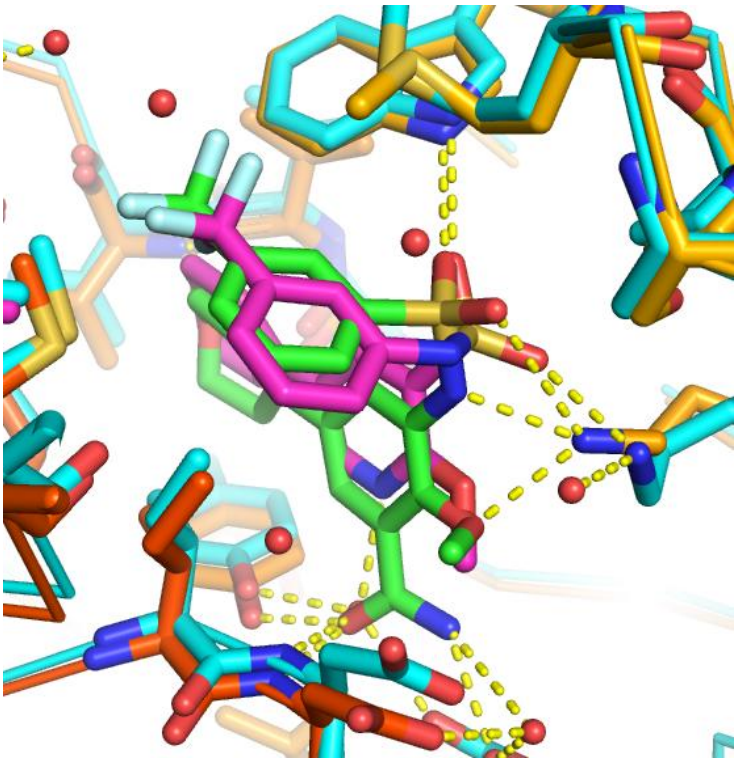


Figure 6.11B Alternative overlay to show the similarity of **16** (magenta) and **284** (green).

These are in vast contrast to the crystallography data of fragment **281** (Figure 6.7), in which the sulfonamide made no interactions with the protein. Instead the trifluoromethylbenzene occupies the lipophilic pocket, allowing the methoxybenzamide to displace the water molecule and directly interact with the backbone. This then forces the sulfonamide into the favoured region for it to form interactions with Lys779 and Trp760, in which it is clearly favourable to flip 'in' (Figure 6.12).

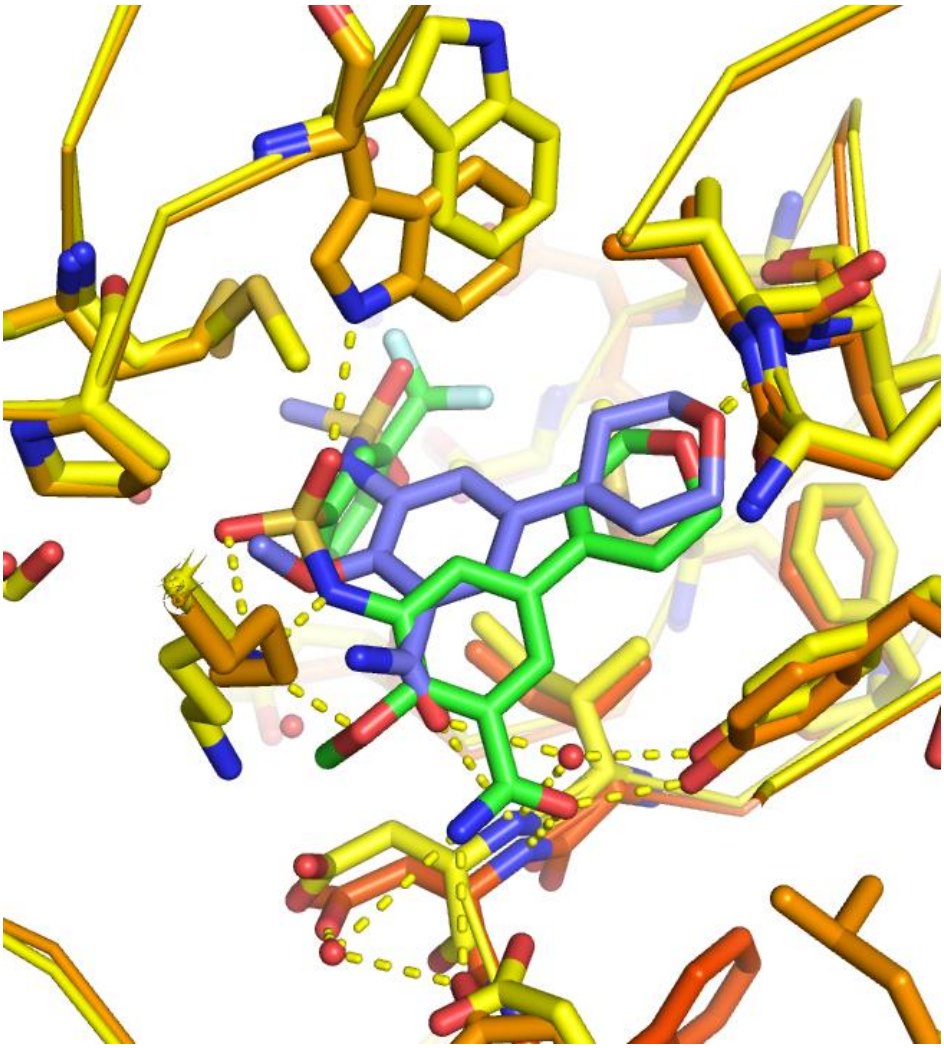


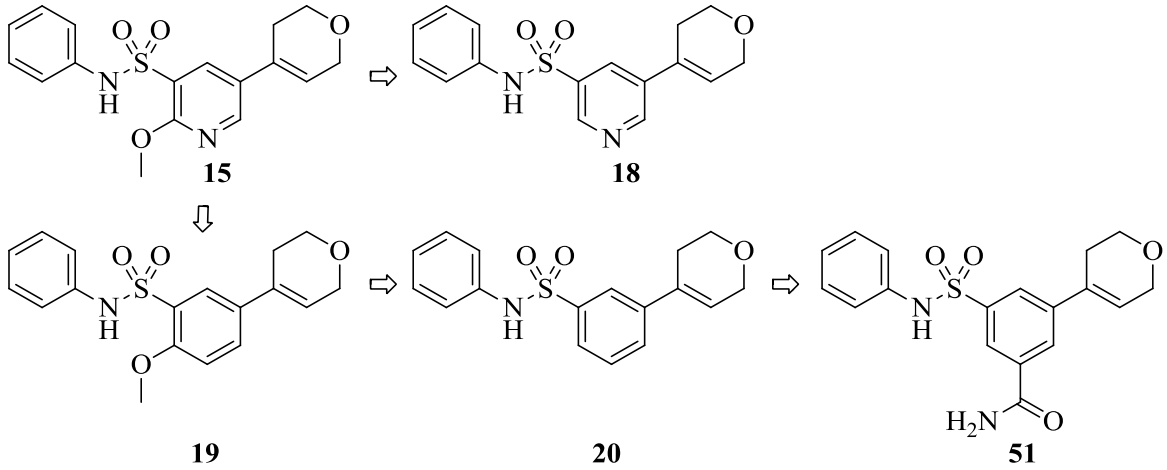
Figure 6.12 Overlay of compounds **280** (blue, yellow protein) and **284** (green, orange protein).

At this stage, no further work would be initiated until intermediate **280** could be tested in the Ames test, as any potential mutagenicity in the intrinsic aniline **280** would halt future work. If the aniline is Ames negative, further work would explore sulfonamide variants utilised in the 2-methoxypyridine series due to the similar vector of **16** and **284** (Figure 6.11).

Chapter 7 Breaking down the starting fragments

The initial focus of this thesis was to replace 2-methoxypyridine, a privileged structure for PI3Kδ. During this **19** and **20** were synthesised that showed that removal of the pyridyl nitrogen was significantly detrimental to potency (Table 7.1).

Table 7.1 Enzyme inhibition data for compounds **15**, **18-20** and **51**.[36]

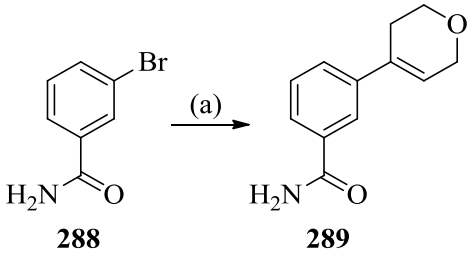


	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
15	5.5 (n=10)	5.6 (n=9)	5.0 (n=7)[a]	6.7 (n=9)	0.38/4.0
18	5.6 (n=4)	5.8 (n=2)	5.5 (n=2)	6.4 (n=2)	0.40/4.1
19	<4.5[b] (n=2)	4.7[b] (n=2)	<4.5[b] (n=2)	5.2 (n=3)	0.32/2.5
20	<4.5 (n=2)	5 (n=2)	4.9 (n=2)	5.5 (n=2)	0.31/3.0
51	5.2 (n=3)	5.6 (n=3)	5.1[a] (n=1)	6.3 (n=3)	0.35/4.5

[a]On two occasions, a result of <4.5 was received. [b]On one occasion, a result of <4.3 was received.

Amide **51** was observed as a compound with some potency and selectivity for PI3Kδ against the other PI3K isoforms and in comparison to phenyl **20** was 12-fold more potent. In historic PI3Kδ programs within GSK, removal of the sulfonamide was significantly detrimental to potency.[78]

However as the amide was significantly different to other previous back pocket groups, amide **289** was synthesised from **288** (Scheme 7.1).



288 **289**

(a) 2-(3,6-Dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (77 %);

Scheme 7.1 Synthesis of fragment compound **289**.

The results are as followed (Table 7.2).

Table 7.2 Enzyme inhibition data for compound **289**.[36]

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
289	<4.5 (n=10)	<4.5 (n=10)	<4.5 (n=10)	<4.5 (n=4)	N/A
289 High concentration	NT	NT	3.4 (n=2)	3.9 (n=2)	0.36/3.1

Amide **289** was inactive against all four PI3K isoforms (Table 7.2). In a high concentration assay, the compound had a pIC_{50} of 3.9, which gave acceptable efficiencies for a fragment of this size. High concentration assays were not requested for all the isoforms because the dihydropyran was a privileged hinge-binding motif for PI3Kδ, so some selectivity was hypothesised. A co-crystal structure of **289** was obtained to evaluate how the fragment binds (Figure 7.1).

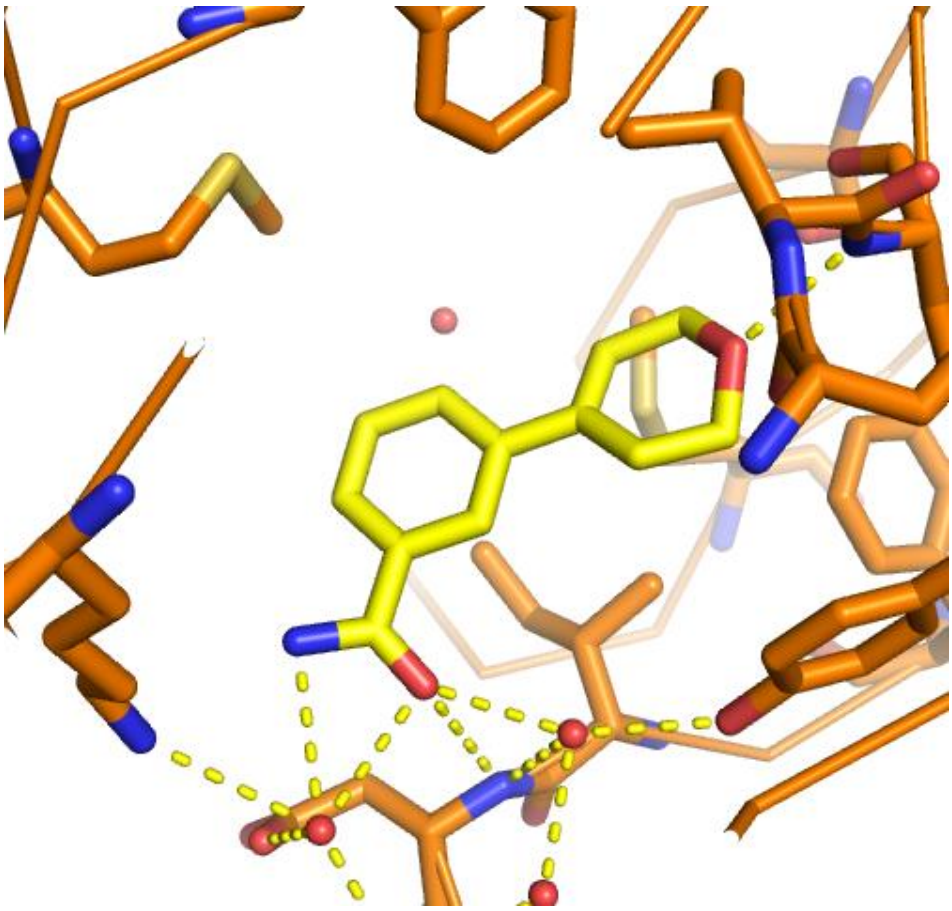


Figure 7.1A Co-crystal structure of **289** in PI3Kδ (Resolution 2.00 Å)[2]

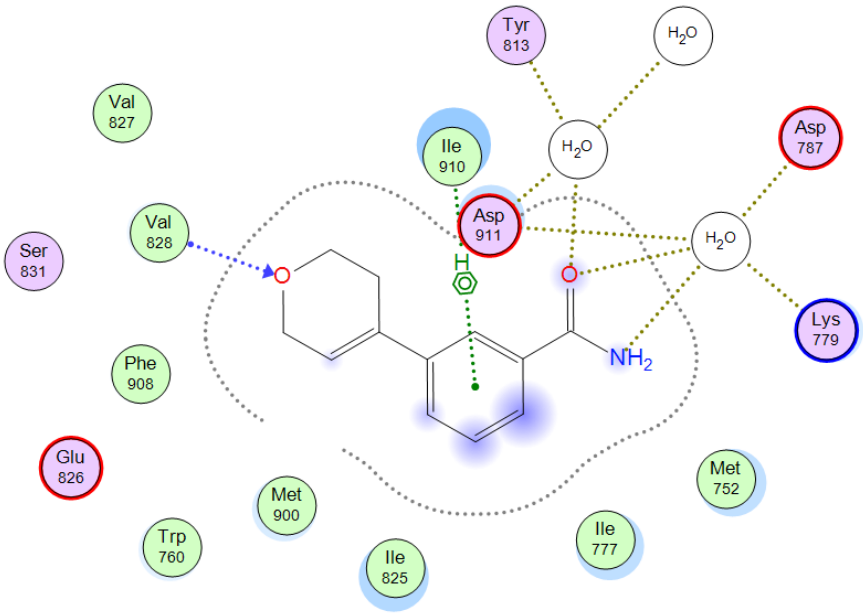


Figure 7.1B 2D Representation of **289** in PI3Kδ.

The co-crystal structure highlights some key interactions. The dihydropyran acts as the hinge binding motif with Val828. In the back pocket region, the amide moiety interacts with the protein backbone via three bridging water molecules and is observed to be 27° out of the plane of the adjoining ring. The orientation of the amide cannot be determined due to the similar density of oxygen and nitrogen, however both orientations could form interactions through the bridging water molecules with Lys779, Asp911, Tyr813 and Asp787. An overlay with sulfonamide amide **51** (Figure 7.2) displays a clear movement away from the backbone in order to bind through the bridging water molecules that **51** displaces.

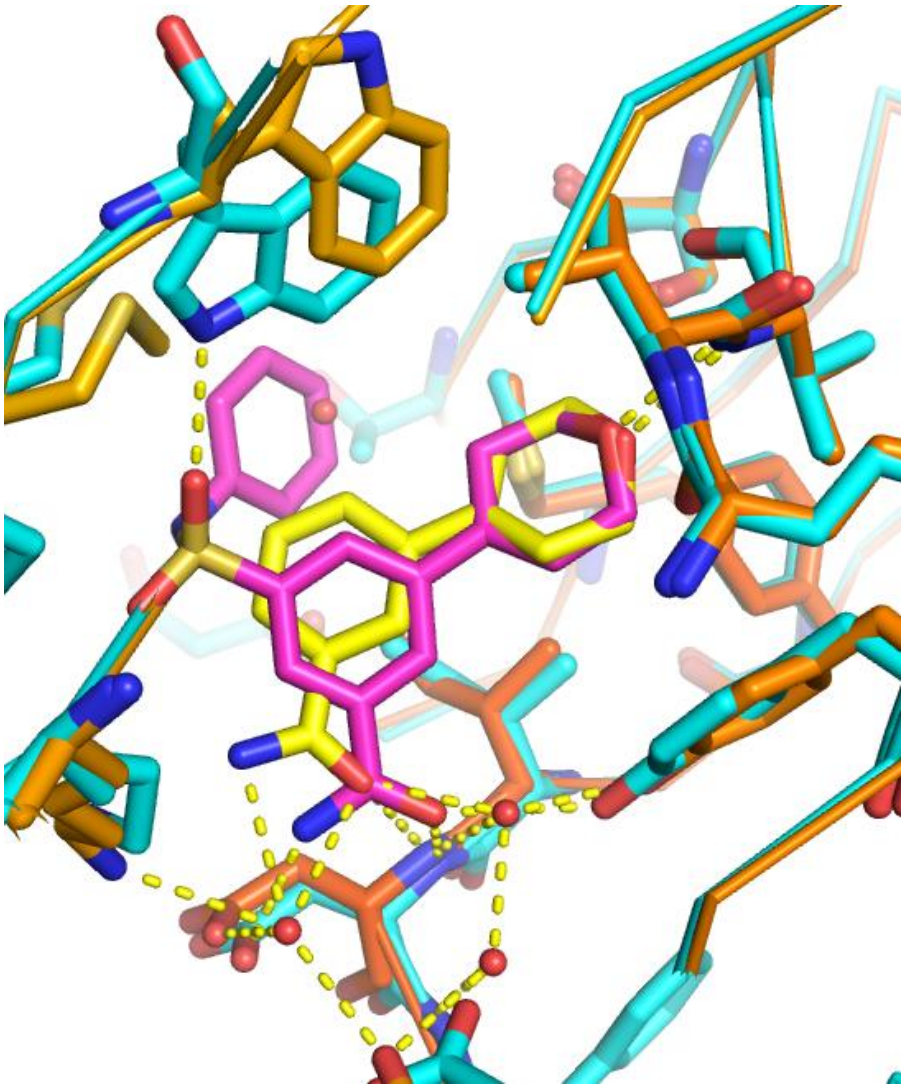


Figure 7.2 Overlay of compounds **51** (magenta, cyan protein) and **289** (yellow, orange protein).

Analysis of the GSK PI3Kδ crystallography database and other published PI3Kδ crystal structures within the literature showed few compounds that had a similar binding mode in comparison to amide **289**. One compound that had some similarities was **290**, a compound from a closely related Sanofi paper[130] (Figure 7.3).

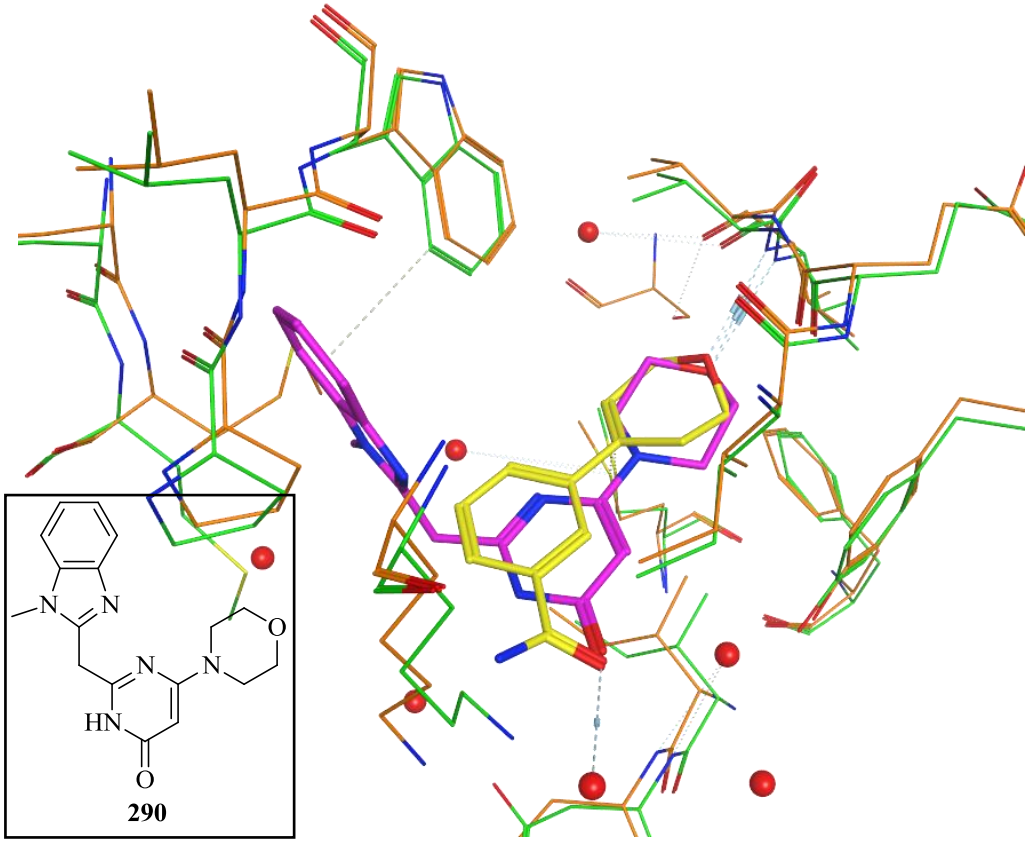
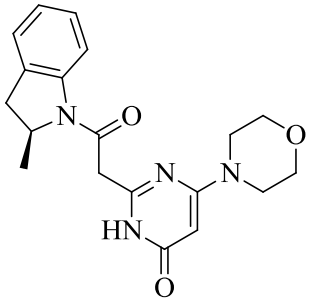


Figure 7.3 Co-crystallography overlay of **289** and **290**.

The overlay shows some similarity in how the hinge binding motif (morpholine and dihydropyran) and back pocket amide and pyrimidone bind. Alongside this, a well defined growth vector from the 5-position in the Sanofi compound **290** could be utilised from amide **289**. A key difference for this vector is that the benzimidazole opens up the induced pocket, a key binding area for Idelalisib, the only PI3Kδ inhibitor on the market. This pocket has only been successfully accessed previously from Idelalisib and compounds with similar chemical structures.

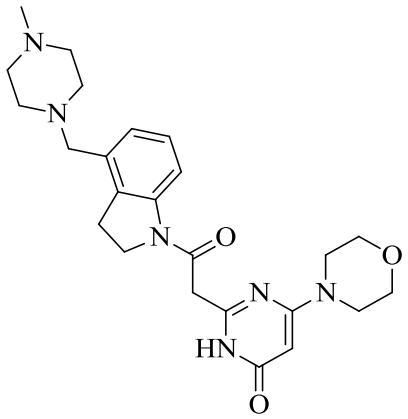
This similarity was encouraging and furthermore, additional papers from Sanofi were published, which outlined their approach in order to discover a selective PI3Kβ inhibitor.[89] The story eventually leads to the discovery of **291**, a potent PI3Kβ inhibitor that possesses modest (20-fold) selectivity over PI3Kδ.



291

Although primarily exploring PI3Kβ, the paper contained a few compounds which were more potent for PI3Kδ than PI3Kβ. An example is **292** (Table 7.3).

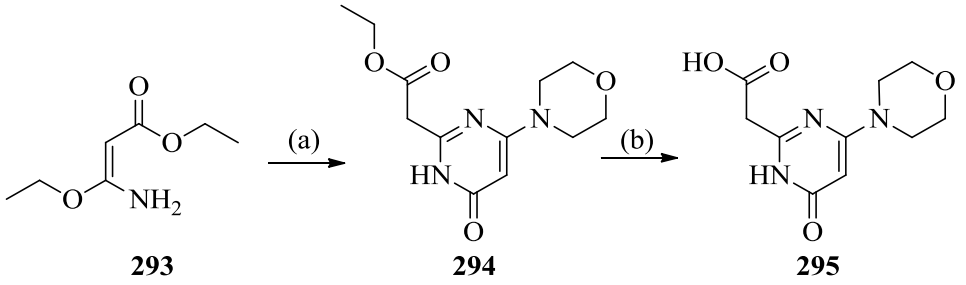
Table 7.3 Reported enzyme inhibition data for compound **292**.



	pIC_{50}			
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**
292	6.1	6.4	5	8.7

Due to the similarities in structure with **290**, the compound could open the induced pocket region of PI3K's. Compound **292** is a potent PI3Kδ inhibitor and was 200-fold selective. It could be hypothesised that the high level of selectivity for PI3Kδ against the other PI3K

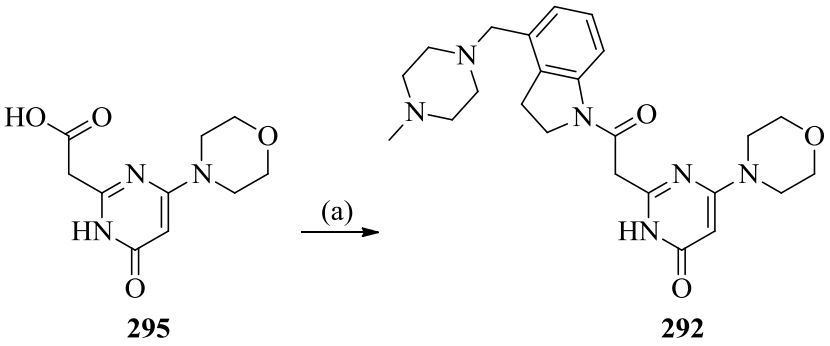
isoforms in **292** is by the piperazine packing close to the Trp760 residue near the Thr750, a known area of the protein in which selectivity can be gained. Unfortunately, there was no protein crystallography of this compound within PI3Kδ reported, with only the crystal structure of **291** in PI3Kβ, where the chiral methyl indoline opened the induced pocket.[89] Sanofi utilised different enzyme concentrations in their PI3K assay, which can lead to different pIC_{50} values. In order to understand any deviation, Sanofi compound **292** was synthesised (Scheme 7.2).



(a) Morpholine, DIPEA, EtOH, 95 °C, 30 h, (7 %); (b) LiOH, THF, MeOH, rt, 3 h, (89 %).

Scheme 7.2 Synthesis of **295**.

Condensation of two equivalents of **293** with morpholine gave **294** in poor yield (7 %) and subsequent hydrolysis gave **295** in good yield (89 %). The literature conditions for the amide coupling utilizing EDCI gave poor conversion. However, amide coupling utilizing T_3P furnished **292** in poor yield (8 %) (Scheme 7.3).

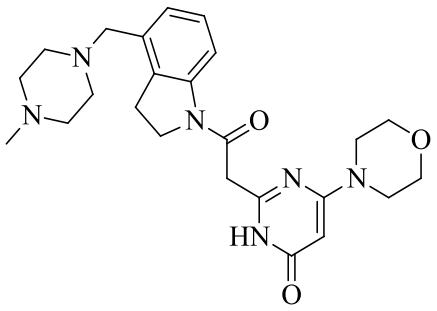


(a) **324**, DIPEA, T_3P, THF, rt, 16 h, (8 %).

Scheme 7.3 Synthesis of **292**.

Results were as follows (Table 7.4).

Table 7.4 Reported and in-house enzyme inhibition data for compound **292**.[36]

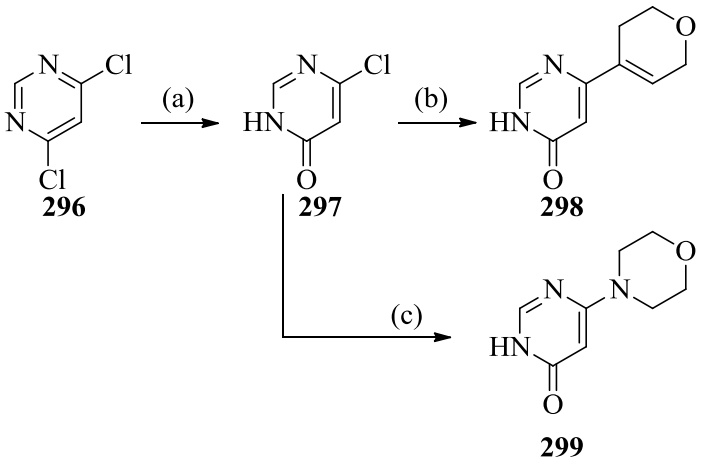


	pIC$_{50}$				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
292	5.4 (n=3)	6.2 (n=3)	4.5[a,b] (n=1)	7.9 (n=3)	0.33/7.7
292 Reported data	6.1	6.4	5	8.7	0.36/8.6

[a]On one occasion, a result of <4.3 was received. [b]On three occasions, a result of <4.5 was received.

In-house data for **292** showed a significant drop in activity and selectivity in comparison to the reported data.[89] The reported data for **292** displayed 200-fold selectivity against PI3Kβ, however in house data showed more modest selectivity (50-fold). Compound **292** was highly efficient with a low cLogP; that may have impacted the AMP permeability (< 10 nm/sec) so the compound was not without issue. Unfortunately the crystal structure of **292** was not sufficiently resolved to be published however it did show that the indoline moiety overlaid perfectly with the published structure **291**.

In order to further investigate this hit, compounds **298** and **299**, fragmented molecules based upon the pyrimidone scaffold used with Sanofi's series, were proposed and synthesised via the following route (Scheme 7.4).



(a) HCl, 1,4-dioxane, H_2O, 70 °C, 5 h, (88 %); (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (45 %); (c) morpholine, DIPEA, 1,4-dioxane, 80 °C, 16 h, (51 %).

Scheme 7.4 Synthesis of fragment compounds **298** and **299**.

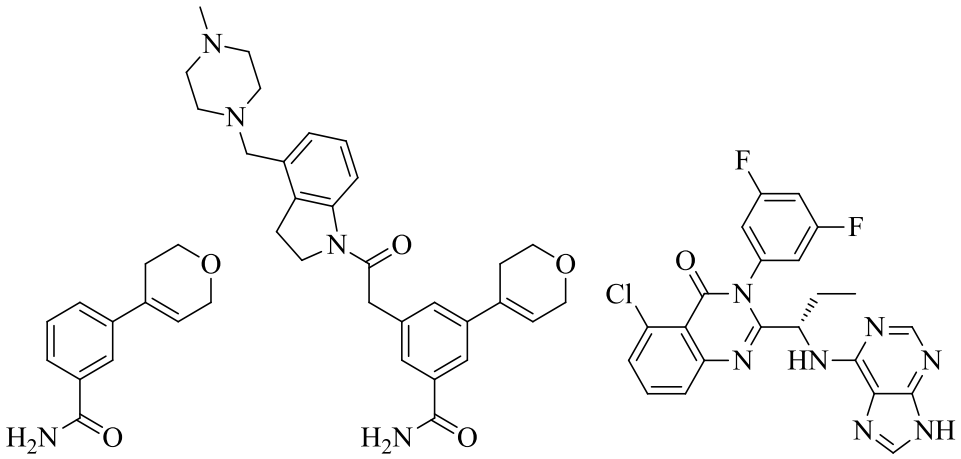
Acid catalysed S_NAr with water gave pyrimidone **297** in good yield (88 %) and this was used in a Suzuki cross-coupling which gave **298** in moderate yield (45 %), due to a problematic purification due to the poor solubility of **298** in DMSO. A second S_NAr with morpholine under basic conditions yielded **299** in moderate yield (51 %). Again due to severe insolubility, the final compound was filtered from the reaction mixture.

Table 7.5 Enzyme inhibition data for compounds **298** and **299**.[36]

	pIC_{50}				
	PI3Kα	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
298	<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	<4.5 (n=2)	N/A
298 High concentration	NT	NT	NT	NT	N/A
299	<4.5 (n=1)	<4.5 (n=1)	<4.5 (n=1)	<4.5 (n=1)	N/A
299 High concentration	NT	NT	NT	NT	N/A

Both compounds (**298** and **299**) were first tested in the normal (10 mM) assay and were found to be below the limit of the assay. However, both **298** and **299** failed to dissolve in the required amount of solvent for the 100 mM high concentration assay, despite heating and sonication. As the value was < 4.5, the highest pIC_{50} value of **298** and **299** could be 4.4. This meant that **298** and **299** were at most three-times more active than amide **289** (pIC_{50} 3.9) and therefore not too dissimilar in potency to start further chemistry from. Pyrimidones **298** and **299** were poorly soluble in organic solvents and in CLND moderate solubility (**298** 60 μg/mL and **299** > 65 μg/mL) was observed. Both pyrimidones had no measurable permeability (**298** < 10 nm/sec and **299** < 3 nm/sec). In contrast amide **289** was readily soluble in organic solvents and moderately soluble in CLND (70 μg/mL). Amide **289** demonstrated superior permeability (157 nm/sec) in comparison to pyrimidones **298** and **299**. This gave encouragement as amide **289** displayed moderate potency and efficiency yet had far superior solubility and permeability than pyrimidones **298** and **299**.

With the crystal structure of fragment **289** and a region of the protein to explore, some initial modelling was conducted to ascertain whether growth from the 5-position of **289** could access the induced pocket.[131] This was initially conducted using **300**, a hybrid mix of fragment **289** and Sanofi compound **292**.



289 **300** **301**

Utilising a known crystal structure of an induced pocket compound **301**[78] with a structural similarity to Idelalisib, a docking protocol was used, with the only requirement being a hinge binding interaction with Val828 (Figure 7.4A with no ligand and Figure 7.4B with ligand).

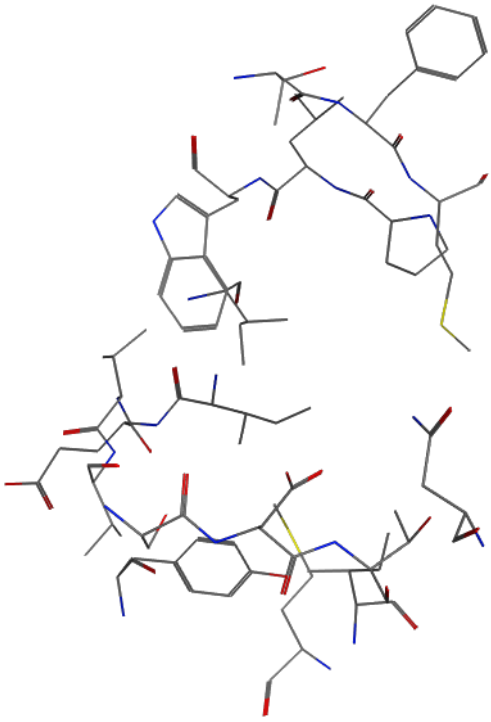


Figure 7.4A Protein without compound **301**.

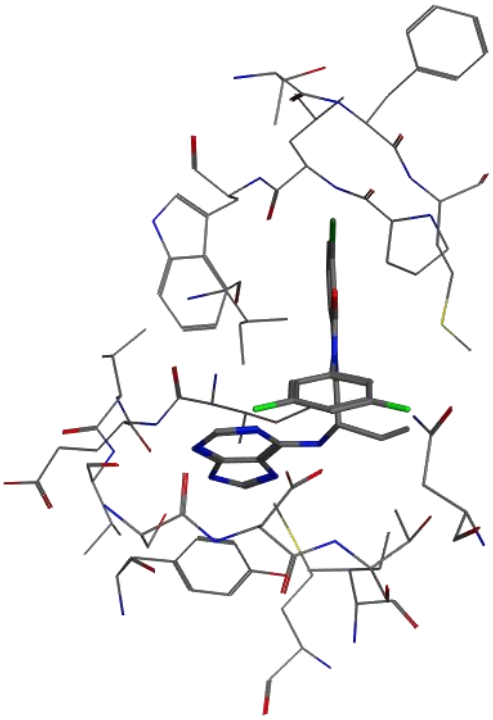


Figure 7.4B Protein with compound **301**.

In order to simplify the docking, no water molecules were retained. Three poses for hybrid compound **300** were obtained (Figure 7.5).

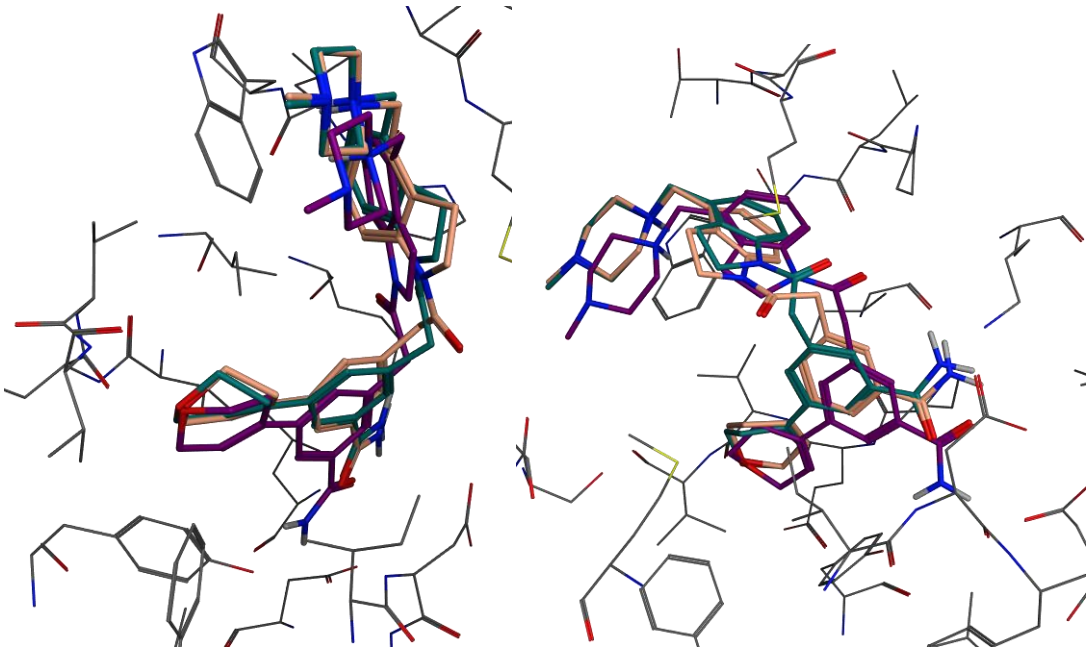


Figure 7.5 Docking poses of **301** obtained.

The docking highlighted that all three poses access the induced fit pocket. The protonated methylpiperazine looks to be loosely associated with the tryptophan, forming a π-cation interaction, whilst occupying space near the threonine residue, a key area of the protein in which selectivity against the other PI3K isoforms can be achieved. The three poses differ from one another around the linker region between the indoline and the benzamide core and also in the location of the benzamide core. All three linkers have different puckers from the benzamide core, and one (shown in teal) 'flips' the amide 180° in comparison to the two other poses (beige and purple). This changes the location and directionality of the benzamide core. In comparison to the fragment **289**, the three poses bind deeper within the protein, appearing to displace the water molecules (Figure 7.6).

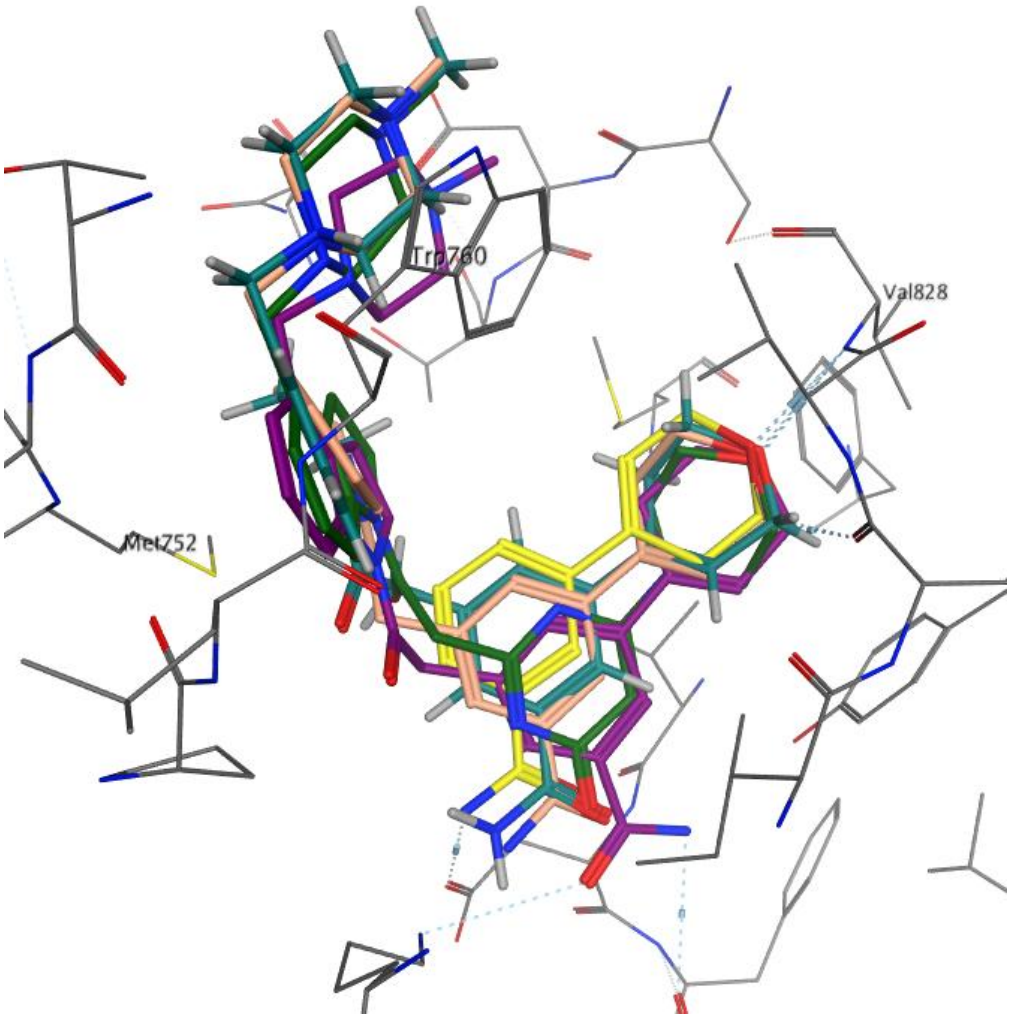
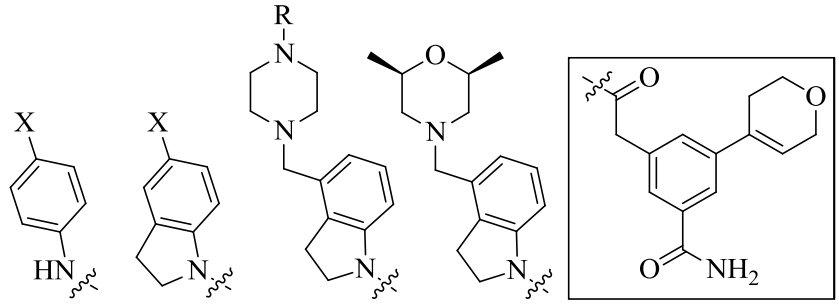


Figure 7.6 Docking poses of **300** and fragment compound **289** demonstrating the deeper binding of the carboxamide back pocket group.

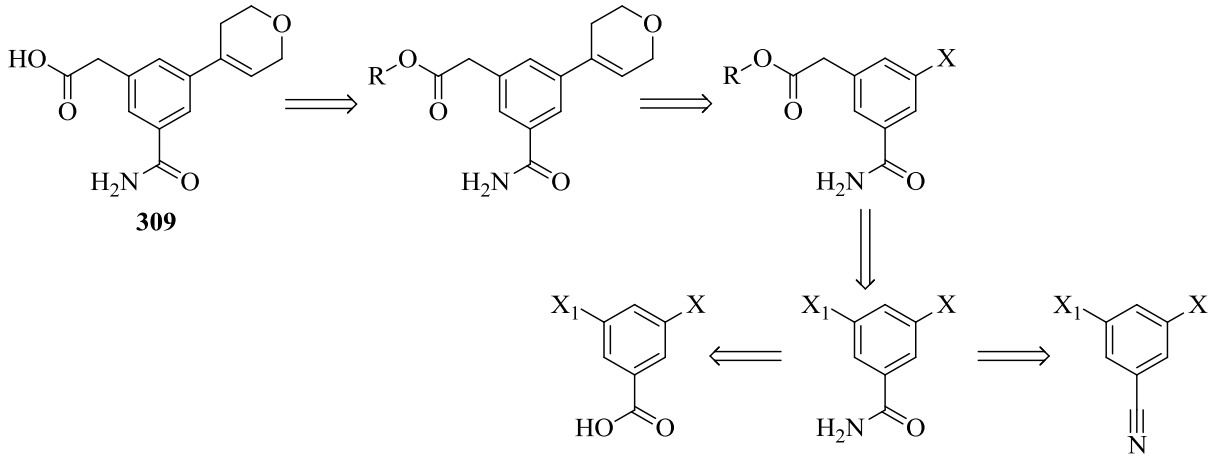
A small selection of compounds were proposed with two major aims, to investigate whether the compounds could induce the pocket from the benzamide fragment, and whether the tryptophan interaction could be accessed from a similar vector (Figure 7.7).



302 X=H **304** X=H **306** R=Me **308**
303 X=F **305** X= F **307** R=*i*Pr

Figure 7.7 Compounds **302-308** selected for synthesis.

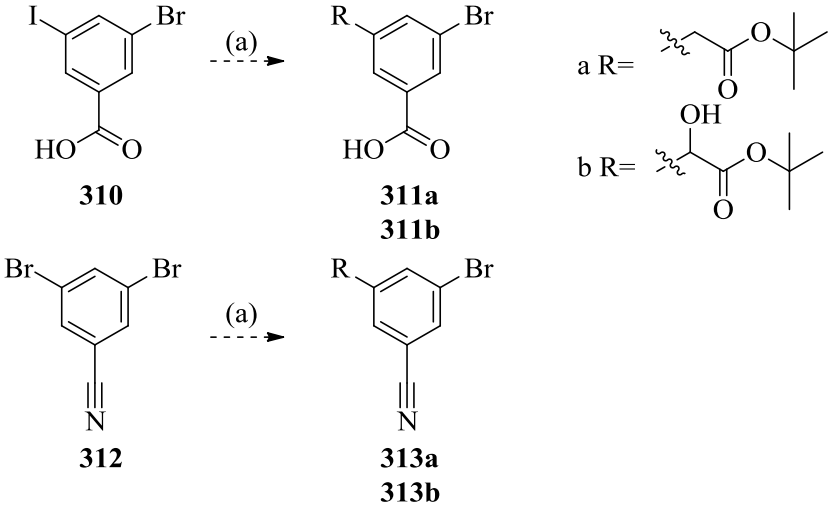
For the synthesis of **302-308**, **309** was a key intermediate. Disconnections identified the key step to be incorporation of the protected phenylacetic ester, from either an acid, primary amide or nitrile, which could be derivatized to the desired product (Scheme 7.5). The dihydropyran would be incorporated by a Suzuki cross-coupling, so the desired starting material would be a 3,5-dihalobenzoic acid derivative.



309

Scheme 7.5 Disconnections **309** leading to a 3,5-dihalobenzoic acid derivative.

Work by Knochel and co-workers had successfully formed Grignard reagents with the iodide of **310** and sequentially quenched with aldehydes.[132] The following reactions were trialled, quenching the pre-formed Grignard reagents with either *tert*-butyl 2-oxoacetate which could be deoxygenated to give the desired phenylacetic ester, or *tert*-butyl 2-bromoacetate, two readily available activated electrophiles (Scheme 7.6).

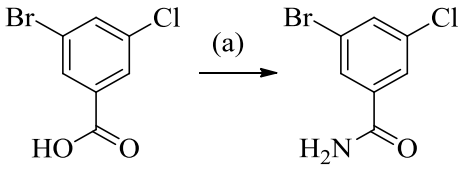


(a) i) MeMgBr, THF, -20 °C, 30 min; ii) *i*PrMgCl.LiCl, 1 h; iii) Electrophile -20 °C to rt, 1 h.

Scheme 7.6 Proposed synthesis of **311a-b** and **313a-b**.

On both **310** and **312**, formation of the Grignard reagent was observed via the disappearance of starting material via LCMS, using isopropyl magnesium chloride lithium chloride. For **310** neither electrophile was attacked by the preformed Grignard reagent on two separate occasions. With dibromo **312**, no reaction was observed using *tert*-butyl 2-bromoacetate and whilst traces of product **313b** were detected with *tert*-butyl 2-oxoacetate, the low conversion and poor reaction profile meant that this was not a viable option.

Alternatively, the use of Negishi cross-couplings between an aryl halide and an α-bromo ester was envisaged. In order to reduce the number of steps required, amide **315** was a key intermediate and would eliminate potential regioselectivity issues by having two esters within the molecule. Amide **315** was synthesised in good yield (89 %) via formation of the acyl chloride *in situ* and quenching in ammonia (Scheme 7.7).



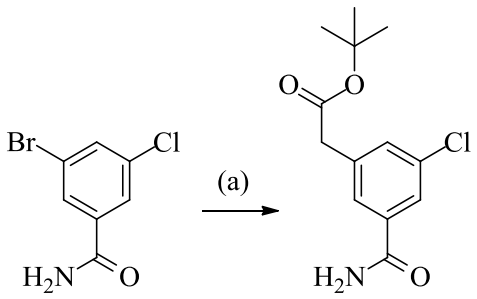
314 **315**

(a) i) $SOCl_2$, 70 °C, 3 h; ii) NH_4OH, THF, 0 °C, (89 %).

Scheme 7.7 Synthesis of intermediate **315**.

Formation of the desired organozinc reagent from *tert*-butyl 2-bromoacetate would give the other coupling partner. Within the literature, there is little precedent for Negishi-cross couplings with a primary amide present, however Hartwig and co-workers used a Pd_2dba_3/QPhos mixture and found many functional groups to be tolerant (Table 7.6).[133]

Table 7.6 Negishi cross-coupling conditions trialled.



315 **316**

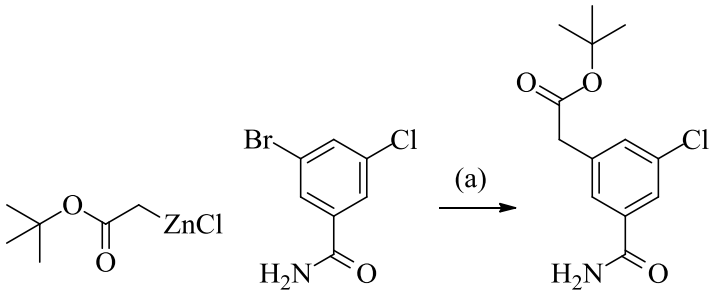
(a) i) Zn (5 eq), TMS-Cl (X eq), 40 °C, 1 h; ii) $BrCH_2COO^tBu$ (5 eq) iii) **315** (1 eq), $Pd_2(dba)_3$ (0.025 eq), QPhos (0.05 eq), rt, 16 h

Equivalents of TMS-Cl	**Product % (SM %)**	**Yield % (Purity %)**
0.3	18-55 (31-70)	0-51 (83)
1	21(31)	17 (90)
5	Complete conversion	73(88)

The initial results were promising as conversion to the product was observed. Utilizing 0.3 equivalents of TMS-Cl gave poorly reproducible conversion of the starting material and the product was isolated in moderate yield. When 1 equivalent of TMS-Cl was utilized, poor conversion and yield were observed, whereas increasing to 3 equivalents, gave dramatically increased yield and purity.

In parallel, the pre-formed organozinc reagent **317** was also investigated as a commercially available coupling partner (Table 7.7).

Table 7.7 Negishi cross-coupling conditions trialled.



317 **315** **316**

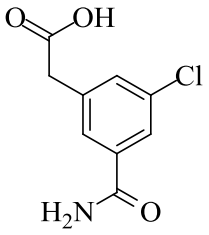
(a) i) **315** (1 eq), $Pd_2(dba)_3$ (0.025 eq), QPhos (0.05 eq) ii) **317** (X eq)

Conditions (a)	**Product %** **(SM %)**	**Yield %** **(Purity %)**
ii) **317** (1.5 eq), rt, 16 h, then 60 °C, 16 h iii) **317** (2 eq), rt, 16 h	23 (47)	31* (62)
i) Degassed prior to addition of organozinc. ii) **317** (1.5 eq), 60 °C, 1 h, μw. iii) **317** (2 eq), 60 °C, 1 h, μw	28 (44)	29* (69)
i) Degassed prior to addition of organozinc. ii) **317** (2.4 eq), 60 °C, 1 h, μw, degassed after the addition of organozinc.	30 (28)	39* (69)
i) Degassed prior to addition of organozinc. ii) **317** (3 eq), 60 °C, 1 h, μw, degassed after the addition of organozinc.	Complete conversion	57-72* (62-88)
i) Degassed prior to addition of organozinc. ii) **317** (3 eq), 60 °C, 1 h, μw, degassed after the addition of organozinc.	Complete conversion	86** (79)

* Purified by normal phase chromatography, ** Purified by reverse phase chromatography

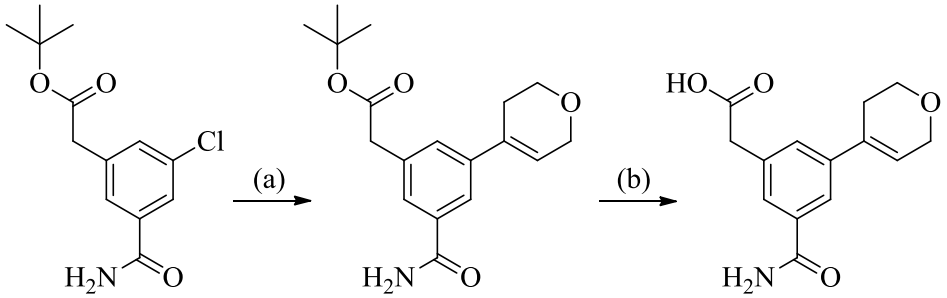
Using the pre-formed organozinc reagent **317** under the conditions used previously yielded some product, however attempts to force conversion including heating and additional equivalents of the organozinc proved unsuccessful. Within the literature, Moloney and co-workers[134] had found that microwave irradiation had previously enhanced the rate of reaction and conversion of this organozinc reagent. This was applied and it was found to give an enhanced reaction rate and yield. Further modifications to the procedure including degassing the vial prior to, and after, the addition of the organozinc and increasing the equivalents of

organozinc to three, both found to enhance the yield and purity of the product after normal phase chromatography. The final modification to this reaction was via the adoption of reverse phase chromatography to give the product in high yield and purity. This was because a small percentage of **318** was isolated after normal phase chromatography, indicating the product may not have been completely stable on silica due to the acid-labile *tert*-butyl ester.



318

With sufficient **316** in hand, the following route to intermediate **309** was undertaken (Scheme 7.8)

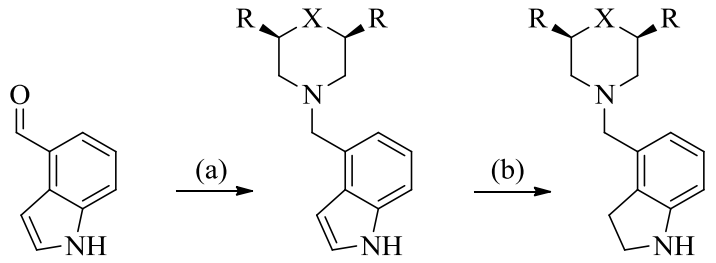


316 **319** **309**

(a) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (74 - 78 %); (b) HCl, DCM, rt, 6 h, (73 - 84 %).

Scheme 7.8 Synthesis of intermediate **309**.

Suzuki cross-coupling of **318** and deprotection of the *tert*-butyl ester proceeded in good yield (74 - 78 % and 73 - 84 % respectively). Monomers **324-326** required synthesis and the following route was used (Scheme 7.9).



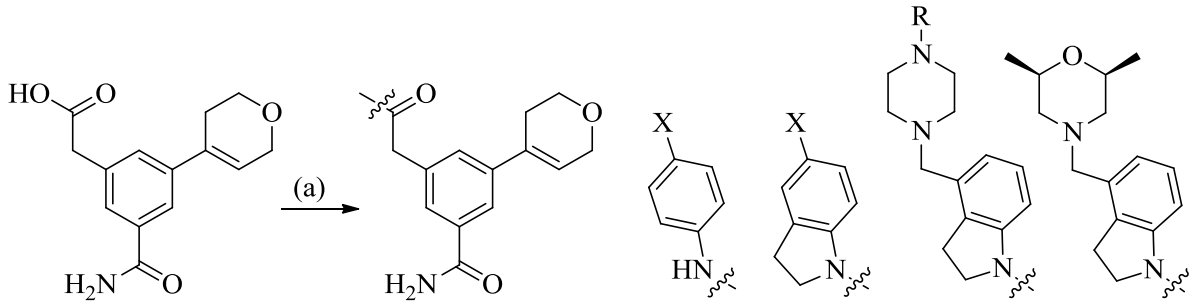
320 **321** R=H, X = NMe; **322** R=H, X = N*i*Pr; **323** R=Me, X = O; **324** R=H, X = NMe; **325** R=H, X = N*i*Pr; **326** R=Me, X = O

(a) R_2NH, $NaBH(OAc)_3$ or $NaBH_3(CN)$, methanol or THF, rt, 16 h (35-89 %); (b) $NaBH_3(CN)$, acetic acid, rt, 2-16 h, (55 - 73 %).

Scheme 7.9 Synthesis of anilines **324**-**326**.

Reductive amination utilizing sodium cyanoborohydride or sodium triacetoxyborohydride proceeded in variable yields (35 - 89 %). The subsequent indole was then reduced using sodium cyanoborohydride in acetic acid to give the monomers **324**-**326** in moderate yields (55 – 73 %).

Amide bond formation utilizing T_3P under basic conditions yielded **302**-**308** in poor yields (14-42 %) (Scheme 7.10).



309

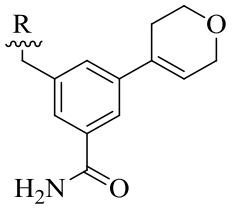
302 X=H; **303** X=F; **304** X=H; **305** X= F; **306** R=Me; **307** R=*i*Pr; **308**

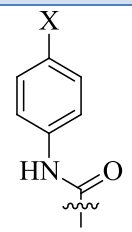
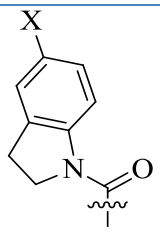
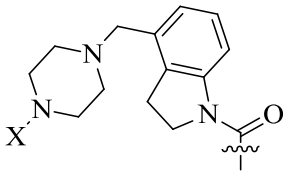
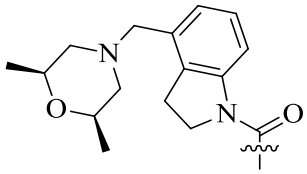
(a) R_2NH, T_3P, DIPEA, THF or DMF, 1-16 h, (14 - 42 %).

Scheme 7.10 Synthesis of amides **302**-**308**.

The table below illustrates the results for amides (**302**-**308**) (Table 7.8).

Table 7.8 Enzyme inhibition data for compounds **302**-**308**.[36]



			pIC_{50}				
	R=	**X=**	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**LE/LLE**
302		H	4.8[a] (n=2)	5.5 (n=3)	<4.5 (n=3)	5.5 (n=3)	0.30/4.1
303		F	4.9[a] (n=2)	6.0 (n=3)	<4.5 (n=3)	6.1 (n=3)	0.32/4.3
304		H	4.8 (n=4)	5.7 (n=4)	<4.5 (n=4)	5.8 (n=4)	0.29/4.0
305		F	4.8 (n=3)	6.7 (n=3)	4.8[b] (n=3)	6.7 (n=3)	0.33/4.8
306		Me	4.9[a] (n=6)	5.4 (n=7)	<4.5[c] (n=7)	6.9 (n=7)	0.27/4.8
307		*i*Pr	5.2 (n=2)	5.1 (n=2)	<4.5[c] (n=2)	7.0 (n=2)	0.26/4.0
308		-	<4.5 (n=2)	4.8 (n=2)	<4.5[c] (n=2)	5.6 (n=2)	0.21/3.0

[a]On one occasion, a result of <4.5 was received. [b]On two occasions, a result of <4.5 was received. [c]On two occasions, a result of <4.3 was received.

For compounds **302**-**308**, we can observe active and efficient compounds. In comparison to amide fragment **289** (pIC_{50} 3.9), incorporation of phenyl or indoline amide increases potency between 40- fold in **302**, to 630- fold in **305**. This potency boost could be caused by induction of the pocket. For **302**-**305** we observed equipotent compounds at both PI3Kβ and

PI3Kδ, which suggests that induction of the pocket from this vector is tolerated in both PI3Kβ and PI3Kδ. Compounds **302**-**305** all demonstrate a drop in potency of between 8- and 50-fold at PI3Kδ in comparison with the matched pairs within Sanofi's reported data.[88,89,135]

More encouragingly, **306** and **307** demonstrated good potency and some selectivity for PI3Kδ. In comparison with indoline **304**, both **306** and **307** compounds are ~10-fold more potent, suggesting that the inclusion of the piperazine group is packing close to Trp760. This in turn imparts some selectivity against the other class I isoforms due to the clash with Arg770 in PI3Kα and Lys777/802 in PI3Kβ and PI3Kγ. Unfortunately, bulking of the terminal amine with the isopropylpiperazine group did not give a boost in potency. In comparison to Sanofi compound **292**, the match pair amide **306** was ten-times less active, signifying that the pyrimidone ring interacts more favourable than the benzamide ring. Co-crystallography of **306** with PI3Kδ was collected (Figure 7.8).

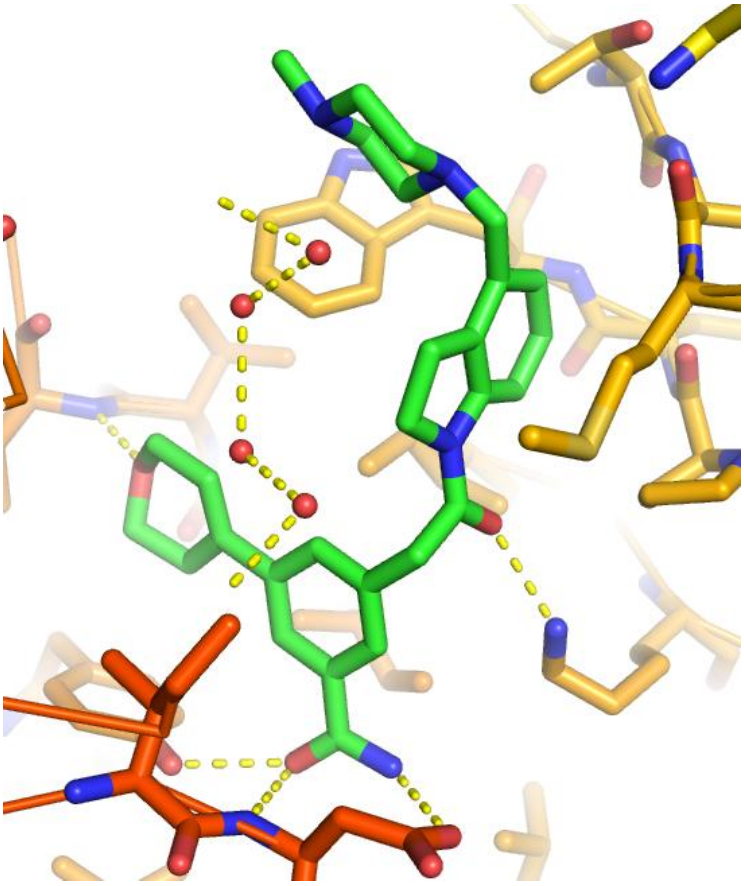


Figure 7.8A Co-crystal structure of **306** in PI3Kδ (Resolution 2.18 Å)[2]

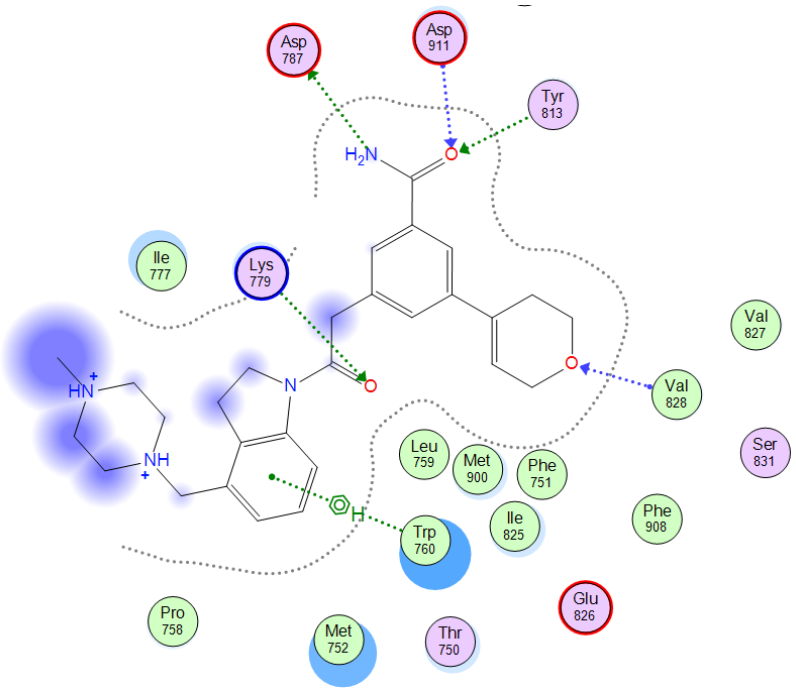


Figure 7.8B 2D Representation of **306** binding to PI3Kδ.

The crystal structure demonstrated the key hinge interaction with Val828 from the dihydropyran hinge binder. In the back pocket region of the protein, the primary amide carbonyl features HBA interactions with Tyr813 and the backbone N-H of Asp911 through the carbonyl, whilst the NH_2 group interacted with the side-chain of Asp911. In the linker region, the tertiary amide carbonyl forms a HBA relationship with Lys779. The indoline clearly induces the pocket between Trp760 and Met752. From this, the methylpiperazine protrudes to pack close to Trp760 and the methyl group extends out into the solvent-exposed region of the protein. The orientation of the piperazine shown is the dominant density observed in the crystal structure; however the exact pucker cannot be determined. The crystal structure however does indicate that further lipophilicity from the piperazine such as isopropyl **307** is simply moving into a solvent exposed region of the protein, in which increased lipophilicity does not result in enhanced potency.

In comparison with the fragment amide **289**, the back pocket group has moved to displace the previously observed water molecules in order to accommodate the indoline fragment (Figure 7.9).

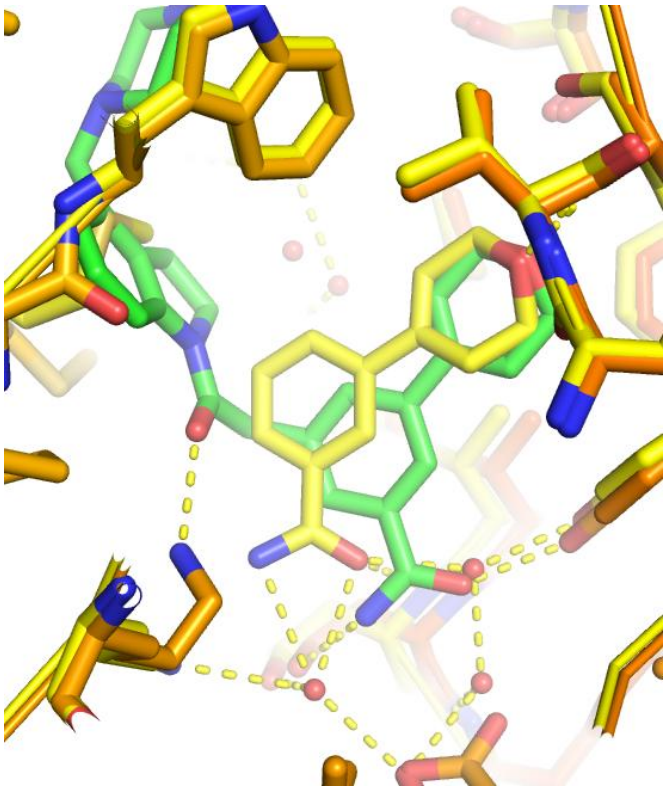
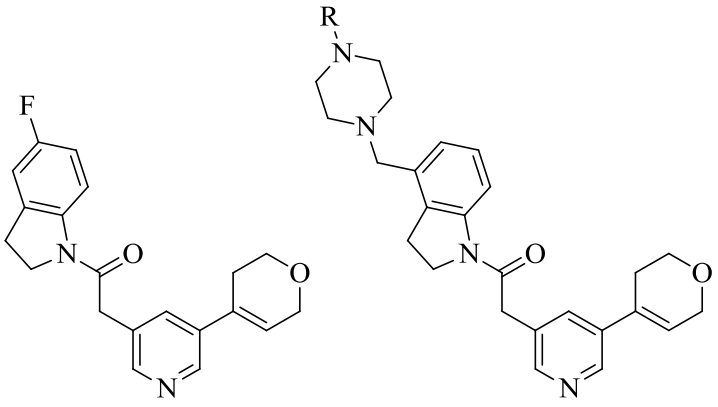


Figure 7.9 Overlay of **289** (yellow) and **306** (green) highlighting the deeper binding of the benzamide back pocket groups of **306**.

Dimethyl morpholine **308** was included in order to probe the requirement for the piperazine and we found this compound to be a poorly efficient compound with modest potency. It could be hypothesised that the flanking two methyl groups clash with the protein or removal of the second basic centre which could interact with Trp760 though a π-cation interaction is detrimental.

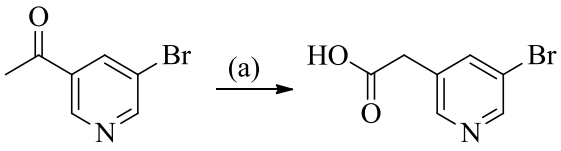
Whilst conducting this work, we wanted to investigate whether an alternative back pocket group could be used. The new induced pocket compounds such as **306** displaced the water molecule, but it could be envisaged that a pyridyl back pocket group would not displace the water molecule. Previously, with both the reverse and forward sulfonamides, 2-methoxypyridine was a privileged structure, which bound to the protein backbone through a bridging water molecule. With this in mind, the following compounds were chosen as a set of matched pairs (in comparison to the benzamide back pocket) to ascertain whether they would induce the pocket, if they would have any selectivity for PI3Kδ and how they differed

(if at all) from the benzamide back pocket. In order to simplify the synthesis, initially the pyridyl back pocket would be used, and if successful, further substituents at the 2-position could be installed.



327 **328** R=Me
329 R=*i*Pr

In order to access this template, ketone **330** was an ideal starting material. Utilising the Willgerodt-Kindler rearrangement, the ketone carbonyl was homologated to give **331** in a one-pot process (Scheme 7.11).



330 **331**

(a) i) Sulfur, morpholine, 130 °C, 16 h; ii) NaOH, 100 °C, 1 h, (40 %).

Scheme 7.11 Synthesis of acid **331**.

The mechanism of the Willgerodt-Kindler re-arrangement is believed to proceed through an cyclic sulfur species **333d** which ring opens to give **333e**. This further reacts with sulfur and morpholine to give the intermediate **333f** which rearranges to give thioamide **333g**. Thioamide **333g** is then hydrolysed upon the addition of a base to give the homologated acid **331** (Figure 7.10).[136]

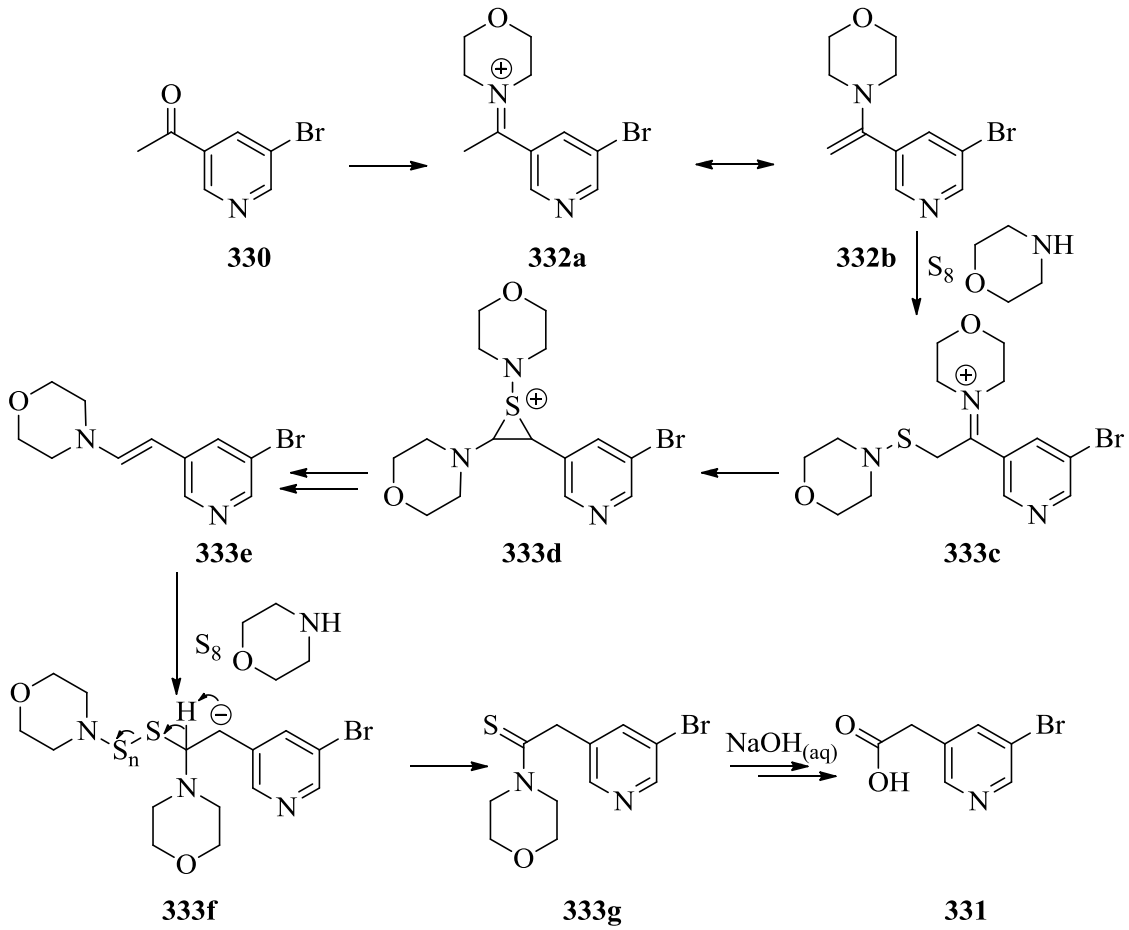
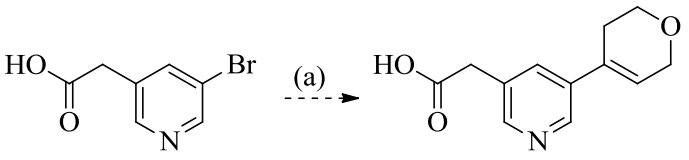


Figure 7.10 Proposed mechanism of Willgerodt-Kindler reaction.[136]

The reaction proceeded well on a small scale, and upon workup and purification, a low yield (40 %) was achieved. Ideally, a Suzuki cross-coupling could yield intermediate **333** from which the 3-amides could be made in parallel (Scheme 7.12).

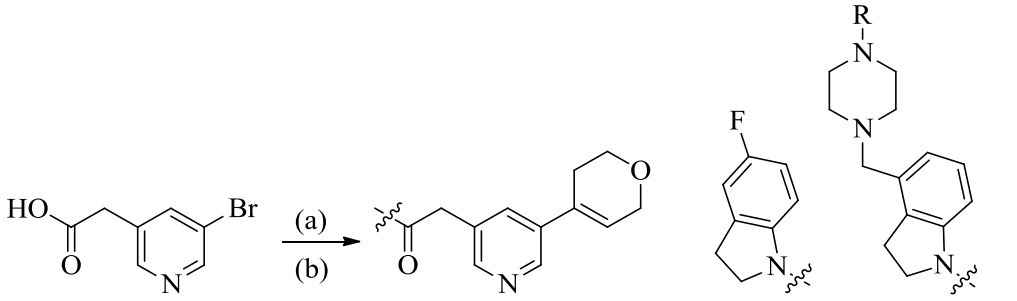


331 **333**

(a) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min.

Scheme 7.12 Proposed synthesis of intermediate **333**.

The reaction was trialled and although the product was observed via LCMS, upon removal of the reaction solvent *in vacuo*, the previously yellow solution degraded to a dark-brown gum which via LCMS showed significant degradation of the product. To overcome this, formation of the desired amides using T_3P and then direct Suzuki cross-coupling yielded **327**-**329** in moderate yields (Scheme 7.13).



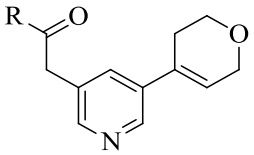
331 **327** **328** R=Me
329 R=*i*Pr

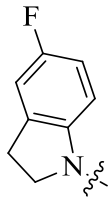
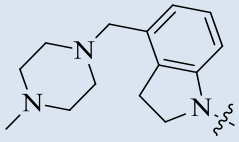
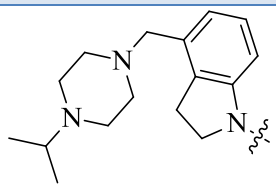
(a) R_2NH, T_3P, DIPEA, DMF, rt, 16 h; (b) 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane, 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex, K_3PO_4, 1,4-dioxane, H_2O, μw, 80 °C, 30 min, (74 - 78 %).

Scheme 7.13 Synthesis of amides **327**-**329**.

The results are as followed (Table 7.9).

Table 7.9 Enzyme inhibition data for compounds **327**-**329** [36]



		pIC$_{50}$					
	R=	**PI3Kα**	**PI3Kβ**	**PI3Kγ**	**PI3Kδ**	**WB IFNγ**	**LE/LLE**
327		4.7 (n=3)	7.1 (n=3)	4.4[a] (n=2)	6.9 (n=3)	-	0.38/5.0
328		<4.5 (n=3)	5.4 (n=3)	<4.5 (n=3)	7.0 (n=3)	6.8 (n=4)	0.30/4.9
329		4.7[b] (n=2)	5.6 (n=4)	<4.5[c] (n=2)	7.5 (n=4)	6.9 (n=4)	0.30/4.6

[a]On three occasions, a result of <4.5 was received. [b]On two occasions, a result of <4.5 was received. [c]On one occasion, a result of <4.3 was received.

All the compounds synthesised with a pyridyl back pocket group were active, signifying that this group can be utilised as the back pocket motif. Fluoroindoline **327** was equipotent at PI3Kβ and PI3Kδ and possessed good efficiency. Both methyl **328** and isopropyl **329** were potent PI3K inhibitors with increasing levels of selectivity for PI3Kδ against PI3Kβ. Both methyl **328** and isopropyl **329** were tested in the relevant whole blood assay and we observe a small drop-off from the isolated enzyme assay. Co-crystallography of methyl **328** with PI3Kδ was collected (Figure 7.11).

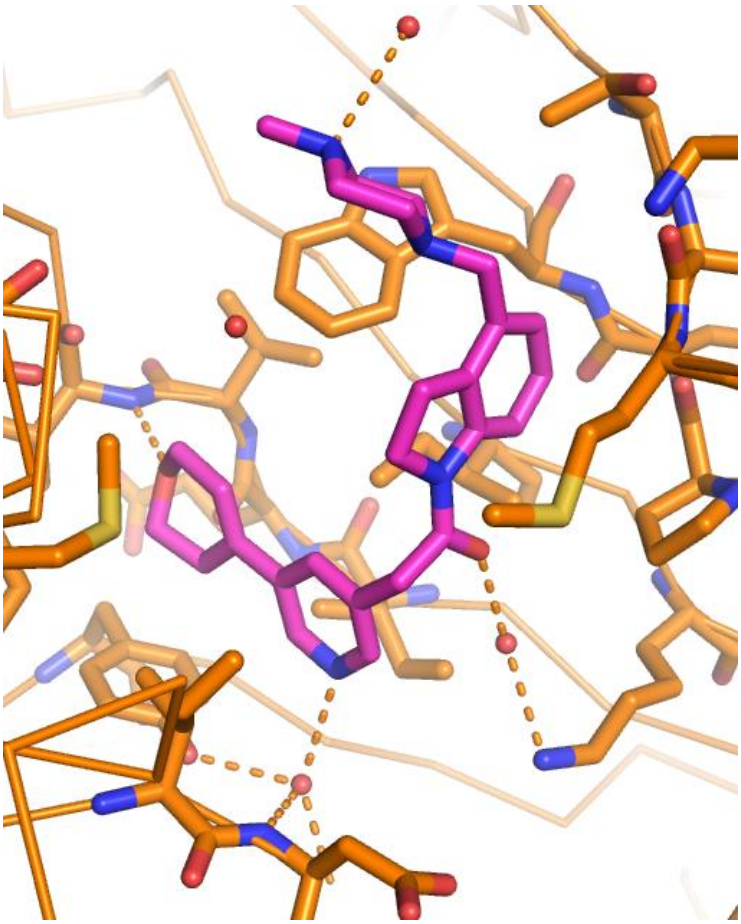


Figure 7.11A Co-crystal structure of **328** in PI3Kδ (Resolution 2.34 Å)[2]

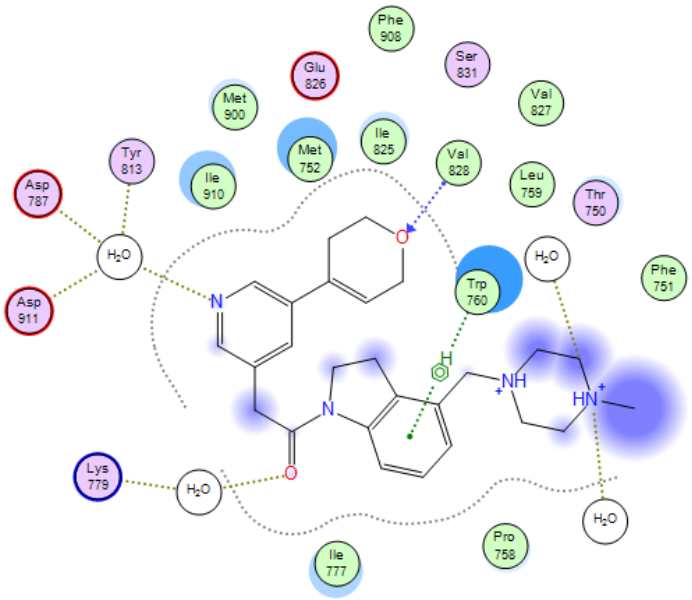


Figure 7.11B 2D Representation of **328** binding to PI3Kδ.

From the crystal structure (Figure 7.11), we observe the key dihydropyran hinge binding interaction to Val828 and the pyridyl back pocket group binding to Asp911 and Tyr813 through a bridging water molecule. Lys779 interacts with the tertiary amide through a linking water molecule. The indoline clearly induces the pocket between Trp760 and Met752. The methylpiperazine protrudes to pack close to Trp760 and the methyl group extends out into the solvent exposed region of the protein. The three pyridyl compounds **327-329** potencies and selectivities are very similar to the matched paired benzamide induced fit compounds **305**, **307** and **308**. By crystallography, the compounds **306** and **328** overlay similarly (Figure 7.12).

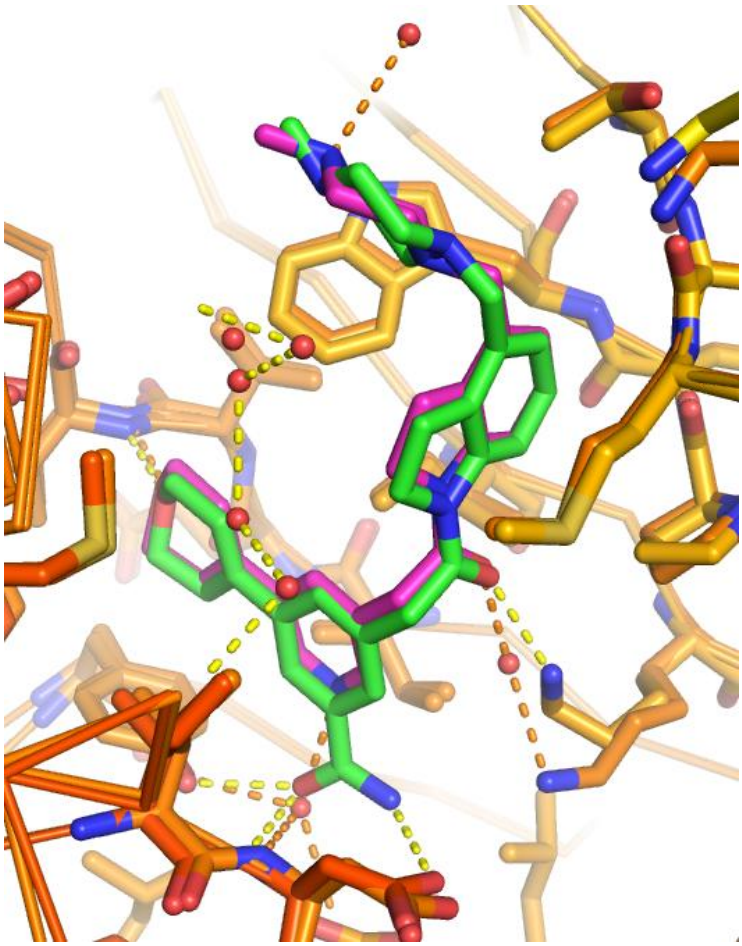


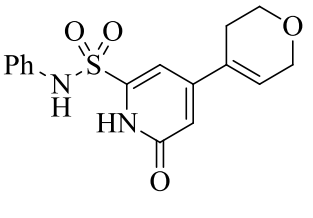
Figure 7.12 Overlay of **306** (green) **328** (magenta).

The work highlighted identified two novel chemical series that reached the selectivity pocket through the induced pocket. This is also an interesting series of compounds to pursue as it did not contain a sulfonamide group, a moiety that has proven crucial in previous endeavours.[78]

Chapter 8 Conclusions and Future Work

8.1 Conclusions

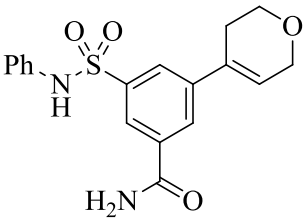
The initial work of this thesis focussed on replacements of a 2-methoxypyridine, a privileged back pocket group which inferred potency and selectivity for PI3Kδ. This initially began with targets being selected from a BROOD screen, which identified compounds which bound through or displaced a water molecule in the back pocket region. It was found that alternative motifs could bind through the water molecule whilst maintaining similar potency such as **29**, or the water molecule could be displaced at a small loss in potency with compounds such as **51** and **52**. This work was crucially supported by the collection of co-crystal structures.



29

pIC_{50} PI3Kδ 6.8

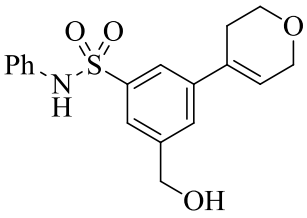
Selec. 4-fold



51

pIC_{50} PI3Kδ 6.3

Selec. 5-fold

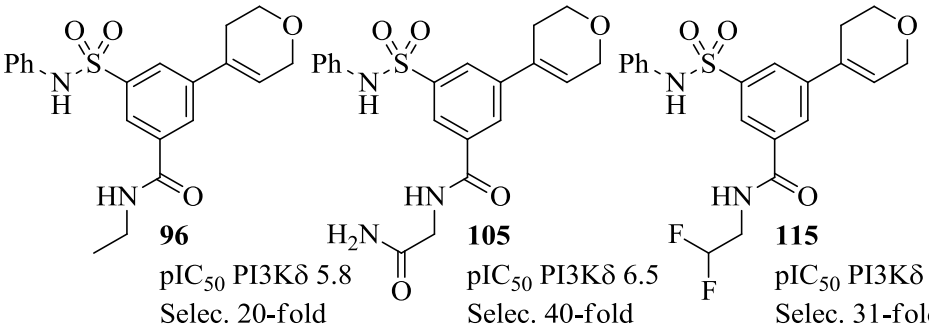


52

pIC_{50} PI3Kδ 6.0

Selec. 8-fold

Despite this, work to replace the 2-methoxypyridine group could not achieve similar levels of potency and selectivity inferred by the group. Work to optimise amide **51** was carried out in order improve the potency and selectivity with the aim of entering initial studies *in vitro* with the overall aim to find a novel chemical motif with comparable properties to Idelalisib. This was broken down into two key areas, substitution from the amide N-H and substitution from the sulfonamide substituent of **51**. Work from the amide N-H explored a previously unexplored vector within PI3Kδ. Initially, substitution from this position caused a drop in potency, however further substitution with a variety of groups to give compounds such as **105** and **115** maintained potency and boosted selectivity, in a region of the protein which is conserved between all four PI3K isoforms.



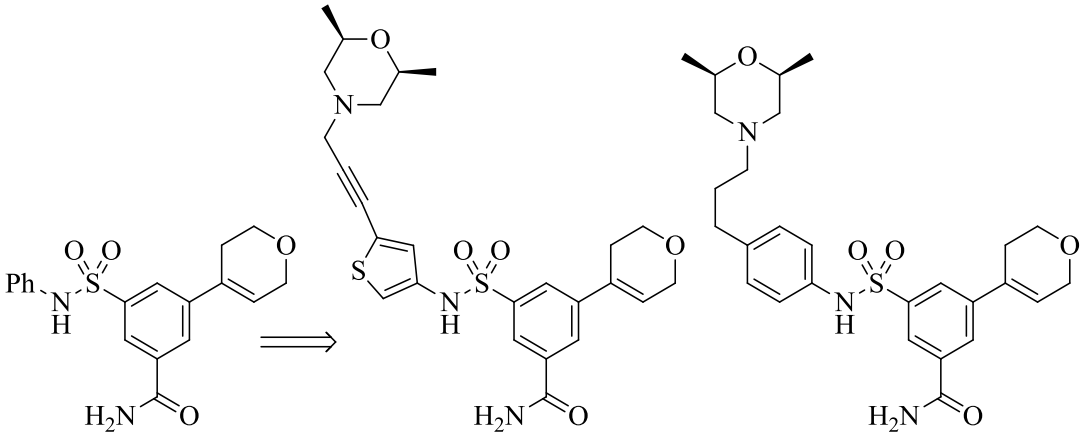
96
pIC_{50} PI3Kδ 5.8
Selec. 20-fold

105
pIC_{50} PI3Kδ 6.5
Selec. 40-fold

115
pIC_{50} PI3Kδ 6.5
Selec. 31-fold

Unfortunately from this position, further gains in potency and selectivity were not possible from the thirty one substituted amides synthesised and reported in this thesis. However, this position can be used in order to manipulate physical properties of the molecules such as permeability, HBA/HBD, solubility and lipophilicity whilst maintaining moderate potency and selectivity.

An area in which more success was achieved was via manipulation of the sulfonamide group. The synthesis of a number of sulfonamides which probed various functionalities demonstrated that alkyl- and pyridyl- sulfonamides were not tolerated. Some functionality such as methoxy or methyl groups provided vectors to substitute from whilst either maintaining or boosting potency. From this position, computational modelling of a morpholine group into an area of the protein close to Trp760 and Thr750, an area known to impart potency and selectivity for PI3Kδ led to the discovery of motifs such as **240** and **214**, which possessed excellent potency and selectivity for PI3Kδ.

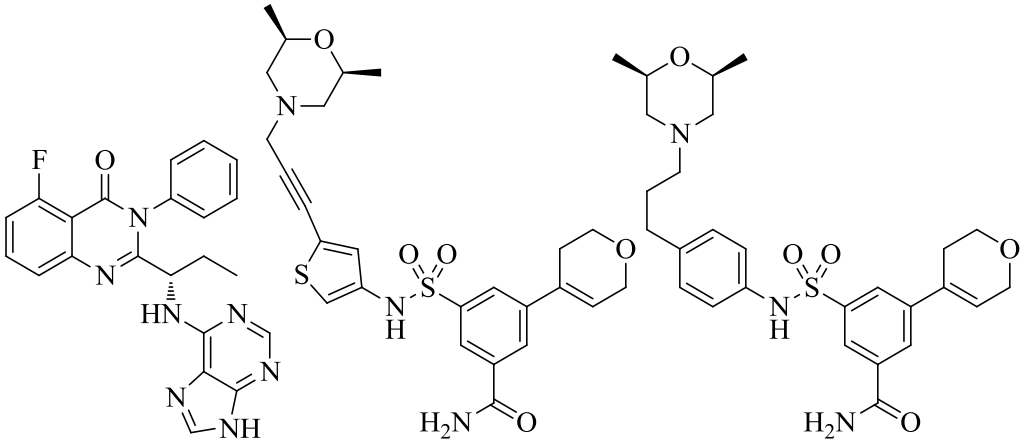


51
pIC_{50} PI3Kδ 6.3
Selec. 5-fold

240
pIC_{50} PI3Kδ 7.8
Selec. 316-fold

214
pIC_{50} PI3Kδ 7.5
Selec. 251-fold

Preliminary data demonstrated that the compounds were active within the whole blood assay however were moderately to highly cleared in two species of microsomes. The comparisons of Idelalisib **4**, compounds **240** and **214** are shown below.

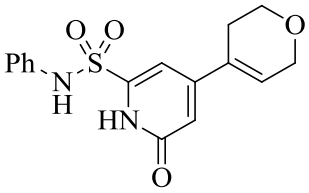


4 **240** **214**

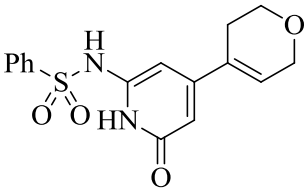
	4	**240**	**214**
PI3Kδ	8.1	7.8	7.5
PI3Kα/β/γ	5.0/5.8/6.6	5.3/5.1/5.0	4.9/5.1/4.6
LE/LLE	0.36/4.4	0.31/5.6	0.29/4.3
WB IFN γ	6.7	5.5	6.5
Solubility	126 µg/mL	>194 µg/mL	>195 µg/mL
Permeability	410 nm/sec	38 nm/sec	78 nm/sec
cLogP	3.6	2.1	3.2
Aromatic rings	5	2	2
PFI	8.2	5.8	5.9

It can be clearly observed that the enzyme potency of **4**, **240** and **214** are in a similar region. Selectivity with the carboxamide back pocket is comparable at PI3Kα and PI3Kβ, however **240** and **214** infer far greater selectivity against PI3Kγ (630- fold and 794- fold) than **4** (31-fold). The solubilities of the compounds are comparably moderate to high, and whilst permeability has decreased, it is still moderate. Other physical properties such as lipophilicity and aromatic ring count have decreased in comparison to Idelalisib which infers a lower value of PFI.

Building on compounds such as amide **51**, alcohol **52**, acid **56** and pyridone **29**, the 're-reversal' of the sulfonamide was carried out in order to ascertain whether the reversal would be tolerated. Initially, it was found that reversal of the pyridone sulfonimide **29** with **246** was tolerated, but the drop in potency and wider physical properties of this molecule stopped further work on the template.

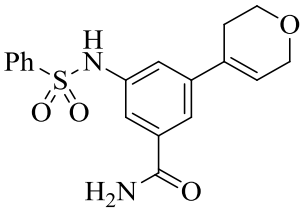


29
PI3Kδ 6.8
Selec. 4-fold

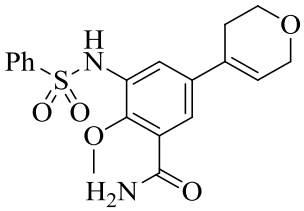


246
PI3Kδ 6.3
Selec. 10-fold

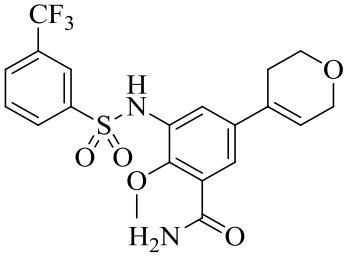
Reversal of the sulfonamide in amide **51**, alcohol **52** and acid **56** led to compounds with poor activity for PI3Kδ such as compound **248**. However, during the progress of this work, reports in the literature suggested that incorporation of a methoxy substituent at the 2-position would be beneficial and compounds **274** and **284** successfully achieved this reported effect to give high potency and selectivity.



248
PI3Kδ 4.9
Selec. 1-fold

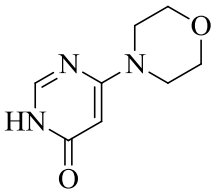


274
PI3Kδ 6.3
Selec. 20-fold

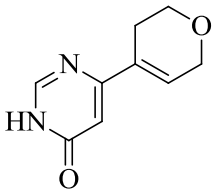


284
PI3Kδ 7.7
Selec. 63-fold

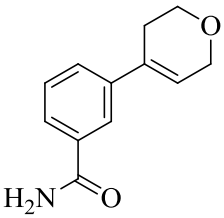
Finally using the phenyl carboxamide back pocket group, work conducted was to observe how complete removal of the sulfonamide moiety could then be grown into a novel chemical template. Upon gaining X-ray co-crystallography data, similarities to a series of compounds published by Sanofi were observed. In comparison to the Sanofi pyrimidone fragments **298** and **299**, fragments such as amide **289** were comparable and had improved physicochemical properties.



298
PI3Kδ < 4.5

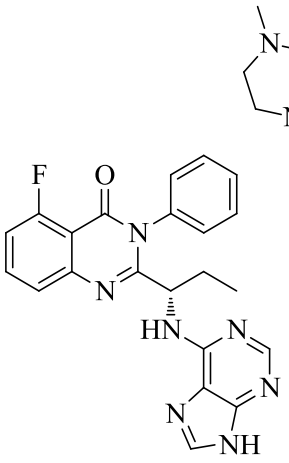


299
PI3Kδ < 4.5

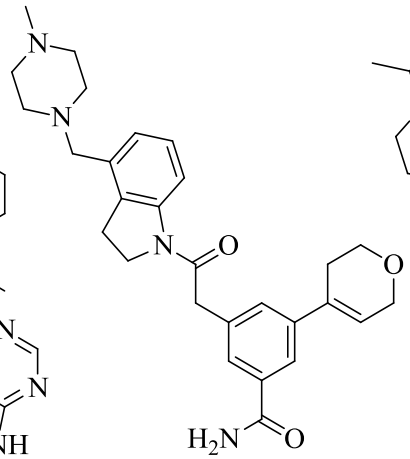


289
PI3Kδ 3.9

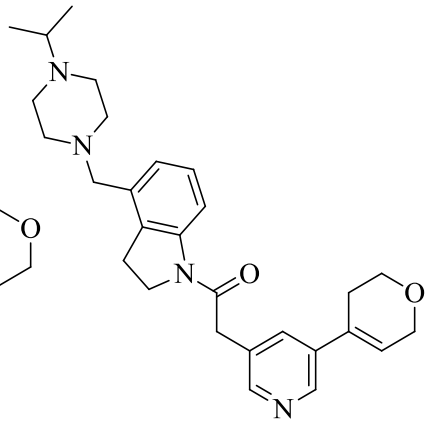
This led to the design and discovery of compounds such as **306**; potent PI3Kδ inhibitors with moderate selectivity. Furthermore, through knowledge of historical GSK programs, a number of pyridyl containing compounds were synthesised with **329** the highlight compound. The comparison of Idelalisib **4**, amide **306** and pyridyl **329** is demonstrated below.



4



306



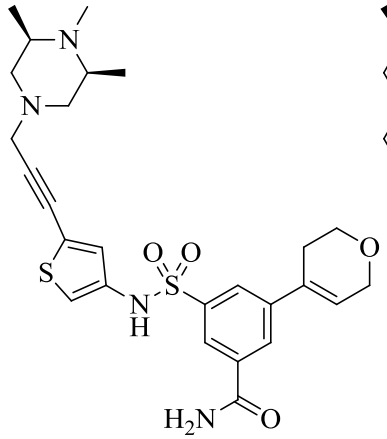
329

	4	**306**	**329**
PI3Kδ	8.1	6.9	7.5
PI3Kα/β/γ	5.0/5.8/6.6	4.9/5.4/<4.5	4.7/5.6/<4.5
LE/LLE	0.36/4.4	0.27/4.8	0.30/4.6
WB IFN γ	6.7	6.3	6.9
Solubility	126 μg/mL	103 μg/mL	178 μg/mL
Permeability	410 nm/sec	19 nm/sec	150 nm/sec
cLogP	3.6	2.1	2.9
Aromatic rings	5	2	2
PFI	8.2	3.5	4.6

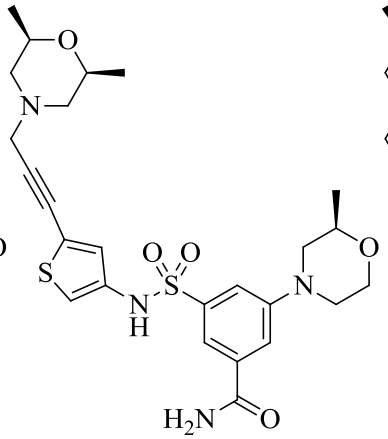
This demonstrates that **306** and **329** are not as potent inhibitors of PI3Kδ compared to **4**. Although **306** and **329** possess less selectivity against PI3Kβ than **4**, this initial small set of compounds could be further elaborated to investigate boosting the PI3Kβ selectivity. A key improvement in both the amide and pyridyl containing compounds is the low drop in potency between the enzymatic and whole blood assay. In both **306** and **329** a small 4-fold drop-off is observed between enzymatic and whole blood assays whereas a larger 25-fold drop-off is observed in **4**. This results in compounds **306** and **329** demonstrating comparable whole blood potency to compound 4. Further improvements observed in compounds **306** and **329** in comparison to **4** are some physicochemical properties such as reduced lipophilicity and aromatic ring count that infers a small PFI value.

8.2 Future Work

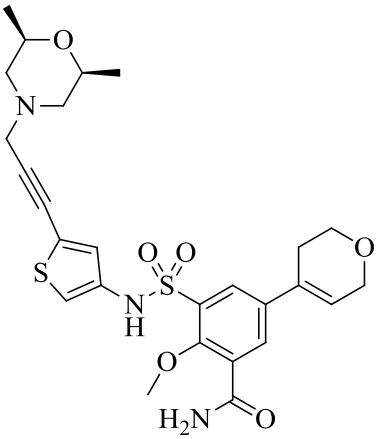
In chapter four, the discovery of a number of compounds with high potency and selectivity is reported; however initial results in WB demonstrated a drop off in potency and initial IVC data demonstrated moderate and high clearance. Future work in this area would be to synthesise and test further novel compounds with the alkyne group either on a thiophene or phenyl ring. Such changes could include changes of the dimethylmorpholine group to a piperazine (**334**) or by changing the hinge binder to a methylmorpholine (**335**). An alternative area to modify could be the back pocket group. As the alkyne moves the carboxamide back pocket group deeper into the protein, this could then make changes such as incorporation of methoxy group (**336**) more favourable. These would all probe parts of the molecule that could attribute to the high whole blood drop-off or high IVC.



334

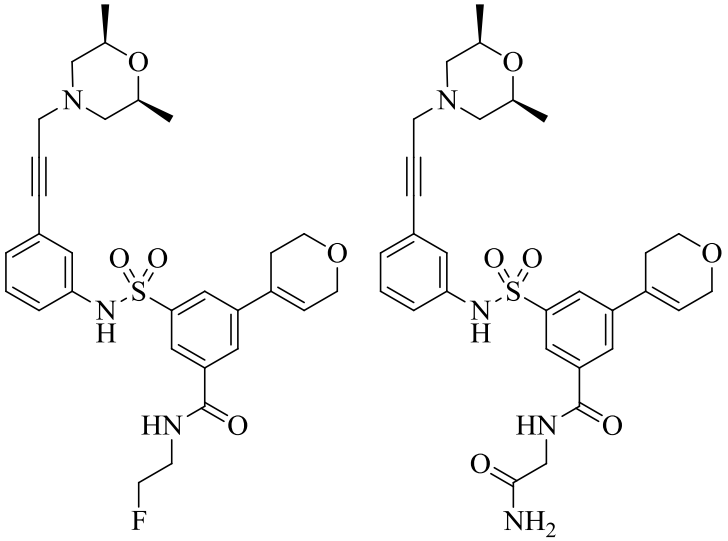


335



336

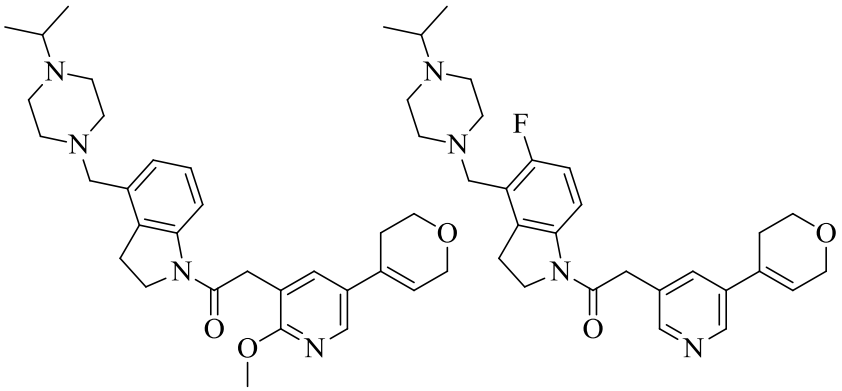
With compounds that do not move the back pocket group deeper into the protein, the potential to form a compound that combines modification to the amide and sulfonamide variant is possible. Compounds such as **337** and **338** would boost the selectivity of the compounds; however it would be crucial to balance the physicochemical properties of the compound while matching a sulfonamide modification with an amide modification.



337 **338**

For the forward sulfonamide compounds investigated in chapter 6, the initial future work relies on the critical Ames test of the corresponding aniline. If this aniline is negative, further iterations of sulfonamide arrays coupled with protein crystallography and computational modelling would be required in order to grow the compounds into a similar region as the reverse sulfonamide benzamides highlighted in chapter 5.

Future work for the induced fit compounds discussed in chapter 7 will mainly focus on the synthesis of more compounds, specifically with the pyridyl back pocket due to its low WB drop-off. Incorporation of a 2-methoxy group **339** is of significant interest, as this is a privileged back pocket group used routinely for PI3Kδ inhibitors. Also, in order to further boost potency, incorporation of a fluorine at the 5-position of the indoline ring is of interest **340**.



339 **340**

Chapter 9 Experimental

Chemistry

All solvents used were of HPLC quality and were purchased from Fisher Scientific. Anhydrous solvents used were obtained from Sigma Aldrich or Alfa Aesar and used without further purification. Other starting materials were purchased from commercial sources unless indicated otherwise.

All NMR spectra were recorded on a Bruker AV-400, AV-500 or AV-600 spectrometer at 298 K unless otherwise stated. Chemical shifts are quoted in parts per million to the nearest 0.01 ppm for ^{1}H and the nearest 0.1 ppm for ^{13}C spectra. Coupling constants are quoted in hertz to the nearest 0.5 Hz. All NMR data are rationalised and NMR spectra were recorded in $CDCl_3$, d^4-methanol or d^6-dimethylsulfoxide with TMS as an internal standard. Homocouplings (H-H) are given in hertz and specified by *J*; the nuclei involved in heteronuclear couplings are defined with the observed nucleus given first. Unless stated otherwise, all refer to ^{3}J couplings. Infrared spectra were recorded from solid or oil using a Perkin Elmer "Spectrum One" Spectrometer. Melting points were measured on a Stuart – automatic melting point SMP40 machine and are uncorrected. High resolution mass spectra (HRMS) were recorded on a Micromass Autospec 500 OAT spectrometer. HRMS was recorded by Bill Leavens, Analytical Chemistry, GSK, Stevenage and the dominant isotopic mass (^{79}Br and ^{35}Cl) is recorded. Flash column chromatography was performed on either FlashMaster II automated purification machine or on CombiFlash Companion automated purification machine collecting via UV at 256 nm unless stated. TLC was performed using pre-coated silica on aluminium backed plates. TLC plates were visualised by $KMnO_4$ dissolved in H_2O, UV or Iodine staining. Crude products further used in reactions are assumed to be 100 % pure for yield calculations.

Liquid Chromatography Mass Spectrometry (LCMS) was carried out on an Acquity Ultra Performance Liquid Chromatography (UPLC) machine on a C_{18} column (50 mm x 2.1 mm, 1.7 µm packing diameter) at 40 °C. The solvents employed were (with modifiers formic acid, trifluoroacetic acid, or ammonium bicarbonate):

A = 0.1 % v/v solution of modifier in water.

B = 0.1 % v/v solution of modifier in acetonitrile.

Time (min)	Flow Rate (mL/min)	% A	% B
0	1	97	3
1.5	1	0	100
1.9	1	0	100
2.0	1	97	3

The UV wavelength detection range was between 210 nm to 350 nm. Mass spectrometry was performed by Waters ZQ ionisation, by alternate-scan positive and negative electrospray.

Mass Directed Auto Preparative HPLC (MDAP) was performed on either a Sunfire (formic modifier) or XBridge (ammonium bicarbonate modifier) C_{18} columns (150 mm x 30 mm, 5 µm packing diameter) at ambient temperature. The gradient employed was selected from 5 pre-set methods (A, B, C, D, or E) with the following solvents:

A = 0.1 % v/v solution of modifier in water.

B = 0.1 % v/v solution of modifier in acetonitrile.

Method A

Time (min)	Flow Rate (mL/min)	% A	% B
0	40	95	5
1	40	95	5
20	40	70	30
20.5	40	1	99
25	40	1	99

Method B

Time (min)	Flow Rate (mL/min)	% A	% B
0	40	85	15
1	40	85	15
20	40	45	55
20.5	40	1	99
25	40	1	99

Method C

Time (min)	Flow Rate (mL/min)	% A	% B
0	40	70	30
1	40	70	30
20	40	15	85
20.5	40	1	99
25	40	1	99

Method D

Time (min)	Flow Rate (mL/min)	% A	% B
0	40	50	50
1	40	50	50

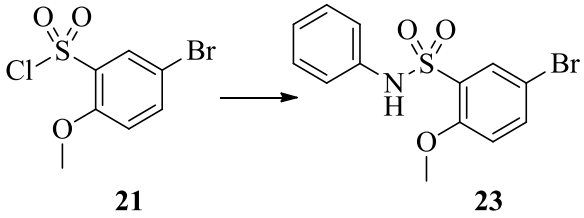
20	40	1	99
20.5	40	1	99
25	40	1	99

Method E

Time (min)	Flow Rate (mL/min)	% A	% B
0	40	70	30
1	40	70	30
20	40	15	85
20.5	40	1	99
25	40	1	99

The UV wavelength detection range was between 210 nm to 350 nm. Mass spectrometry was performed by Waters ZQ ionisation, by alternate-scan positive and negative electrospray.

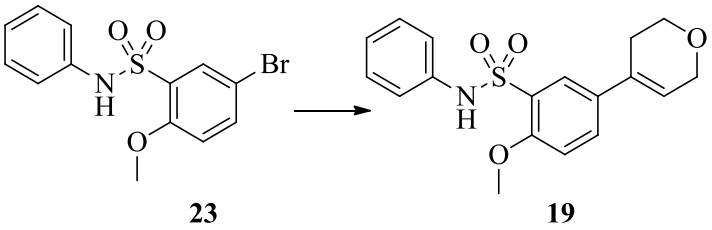
5-Bromo-2-methoxy-*N*-phenylbenzenesulfonamide 23



21 **23**

5-Bromo-2-methoxybenzene-1-sulfonyl chloride **21** (220 mg, 0.77 mmol) and aniline (0.07 mL, 0.77 mmol) were combined in pyridine (1 mL). The resulting mixture was stirred at room temperature for 3 h and then diluted with water (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed with hydrochloric acid (5 mL, 2 M), brine (2 x 10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 – 40 % ethyl acetate/cyclohexane) provided 5-bromo-2-methoxy-*N*-phenylbenzene-sulfonamide **23** (221 mg, 84 %) as a white solid. M.P. 205 °C (decomposition); LCMS (Formic acid): 100 % purity, R_t=1.07, $[MH]^-$ =340, 342; δ_H (400 MHz, $CDCl_3$) 7.95 (1H, d, *J* = 2.5 Hz), 7.59 (1H, dd, *J* = 8.8, 2.5 Hz), 7.27 - 7.32 (2H, m), 7.19 – 7.02 (3H, m), 6.94 (1H, s), 6.91 (1H, d, *J* = 8.8 Hz), 4.05 (3H, s); δ_C (101 MHz, $CDCl_3$) 155.4, 137.6, 136.3, 133.3, 129.3, 128.1, 125.4, 121.2, 113.9, 112.7, 56.7; ν_{max} (liquid film)/cm^{-1}; 3249, 1583, 1480, 1417, 1341, 1273, 1155, 1066, 1017, 814; *m/z* (ES) Found: $[MH]^+$ 341.9794, $C_{13}H_{13}BrNO_3S$ is $[MH]^+$ 341.9800.

5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-*N*-phenylbenzenesulfonamide 19

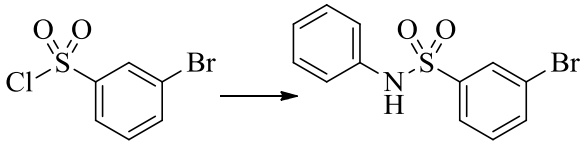


23 **19**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (34 mg, 0.162 mmol), 5-bromo-2-methoxy-*N*-phenylbenzenesulfonamide **23** (50 mg, 0.146 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (8 mg, 0.015 mmol) and tripotassium phosphate (68 mg, 0.321 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 120 °C for 30 min. Additional 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (34 mg, 0.162 mmol) was added and was further heated and stirred in a Biotage microwave

reactorreactorat 120 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and was concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-*N*-phenylbenzenesulfonamide **19** (21 mg, 41 %) as a white solid. M.P. 184-186 °C ; LCMS (Formic acid): 98 % purity, R_t= 0.97, $[MH]^+$= 346; δ_H (400 MHz, $CDCl_3$) 7.86 (1H, d, *J* = 2.4 Hz), 7.51 (1H, dd, *J* = 8.7, 2.4 Hz), 7.25 – 7.20 (2H, m), 7.13 – 7.06 (3H, m) 6.97 (1H, d, *J* = 8.7 Hz) 6.96 (1H, s), 6.13 – 5.98 (1H, m), 4.30 (2H, m), 4.06 (3H, s), 3.91 (2H, t, *J* = 5.5 Hz), 2.44 (2H, m); δ_C (101 MHz, $CDCl_3$) 155.1, 136.6, 133.2, 132.1, 130.7, 129.2, 126.9, 126.4, 125.3, 122.9, 121.5, 111.8, 65.7, 64.3, 56.6, 26.9; ν_{max} (liquid film)/cm^{-1}; 3272, 2924, 1601, 1493, 1420, 1335, 1289, 1147, 1017, 924, 761; *m/z* (ES) Found: $[MH]^+$ 346.1106, $C_{18}H_{20}NO_4S$ is $[MH]^+$ 346.1108.

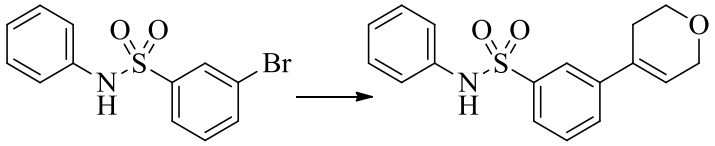
3-Bromo-*N*-phenylbenzenesulfonamide 24



22 **24**

3-Bromobenzene-1-sulfonyl chloride **22** (0.113 mL, 0.783 mmol) and aniline (0.071 mL, 0.783 mmol) were combined in pyridine (1 mL). The resulting mixture was stirred at room temperature for 5 h and then diluted with water (10 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed with hydrochloric acid (10 mL, 2 M), brine (2 x 10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (30 – 70 % TBME/cyclohexane at 222 nm) provided 3-bromo-*N*-phenylbenzenesulfonamide **24** (198 mg, 81 %) as a white solid. M.P. 110-112 °C (lit. 98-100 °C);[137] LCMS (Formic acid) 100 % purity, R_t= 0.97, $[MH]^-$= 310, 312; δ_H (400 MHz, $CDCl_3$) 7.96 (1H, t, *J* = 1.8 Hz), 7.69 (2H, m), 7.36 – 7.25 (3H, m), 7.21 – 7.14 (1H, m), 7.13 – 7.07 (2H, m), 6.84 (1H, s); δ_C (101 MHz, $CDCl_3$) 140.9, 136.0, 135.8, 130.5, 130.1, 129.5, 126.0, 125.8, 123.0, 122.1; ν_{max} (liquid film)/cm^{-1}; 3250, 1597, 1481, 1416, 1334, 1155, 918; *m/z* (ES) Found: $[MH]^+$ 311.9692, $C_{12}H_{11}BrNO_2S$ is $[MH]^+$ 311.9688.

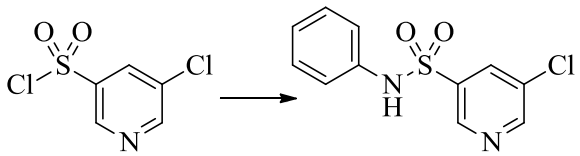
3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide 20



24 **20**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (37 mg, 0.176 mmol), 3-bromo-*N*-phenylbenzenesulfonamide **24** (50 mg, 0.16 mmol), $PdCl_2$(dppf) (11 mg, 0.016 mmol) and sodium carbonate (37 mg, 0.352 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorreactorat 120 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide **20** (29 mg, 57 %) as a brown solid. M.P. 148-149 °C; LCMS (Formic acid) 100 % purity, R_t= 0.99, $[MH]^-$= 314 ; δ_H (400 MHz, $CDCl_3$) 7.75 (1H, t, *J* = 1.7 Hz), 7.68 – 7.65 (1H, m), 7.59 – 7.53 (1H, m), 7.42 (1H, t, *J* = 7.8 Hz), 7.32 – 7.23 (2H, m), 7.19 – 7.12 (1H, m), 7.12 – 7.07 (2H, m), 6.66 (1H, s), 6.18 – 6.08 (1H, m), 4.33 - 4.30 (2H, m), 3.94 – 3.90 (2H, m), 2.48 – 2.39 (2H, m); δ_C NMR (101 MHz, $CDCl_3$) 141.2, 139.4, 136.4, 132.7, 129.4, 129.1, 129.0, 125.7, 125.6, 124.8, 123.3, 122.0, 65.6, 64.2, 26.9; ν_{max} (liquid film)/cm^{-1} 3126, 2902, 1600, 1493, 1346, 1155, 1119, 922; *m/z* (ES) Found: $[MH]^+$ 316.1003, $C_{17}H_{18}NO_3S$ is $[MH]^+$ 316.1002.

5-Chloro-*N*-phenylpyridine-3-sulfonamide 27

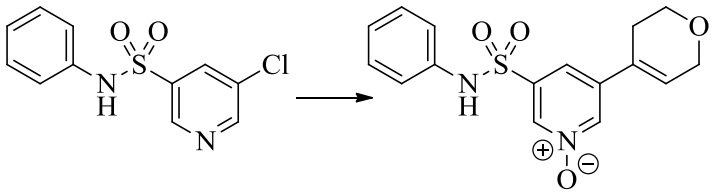


26 **27**

5-Chloropyridine-3-sulfonyl chloride **26** (278 mg, 1.311 mmol) and aniline (0.12 mL, 1.311 mmol) were combined in pyridine (2 mL). The resulting mixture was stirred at room temperature for 1 h and then diluted with water (30 mL) and extracted with ethyl acetate (30 mL). The organic layer was washed with hydrochloric acid (20 mL, 2 M), brine (2 x 15 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 50 % TBME/cyclohexane at 222 nm) provided 5-chloro-*N*-

phenylpyridine-3-sulfonamide **27** (58 mg, 16 %) as an orange oil. LCMS (Formic acid) 81 % , R_t= 0.95, $[MH]^-$= 267, 269; δ_H (400 MHz, $CDCl_3$) 8.83 (1H, d, *J* = 1.9 Hz), 8.73 (1H, d, *J* = 2.3 Hz), 8.02 (1H, m), 7.36 – 7.30 (2H, m), 7.25 – 7.20 (1H, m), 7.15 – 7.10 (2H, m), 6.99 (1H, s). Product was used without further purification due to low yield and purity.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)pyridine 1-oxide 25

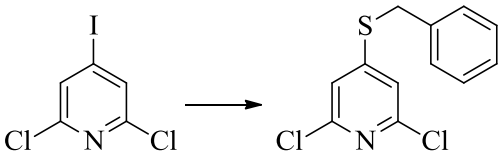


27 **25**

5-Chloro-*N*-phenylpyridine-3-sulfonamide **27** (59 mg, 0.22 mmol) was dissolved in acetic acid (2 mL) and to this 30 % hydrogen peroxide in water (0.1 mL, 0.979 mmol) was added and stirred at 85 °C for 2 h. The reaction mixture was quenched with aqueous saturated sodium sulfite (1 mL), diluted with water (5 mL) and then extracted into ethyl acetate (2 x 10 mL). The organic layer was washed with brine (5 mL), passed through a hydrophobic frit and concentrated *in vacuo.* The residue was combined with 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (55 mg, 0.262 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (8 mg, 0.014 mmol) and tripotassium phosphate (117 mg, 0.551 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) and was heated and stirred in a Biotage microwave reactorreactorat 100 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give an impure brown oil. The oil was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)pyridine 1-oxide **25** (11 mg, 15 %) as a white solid. M.P. 190 °C (decomposition); LCMS (Formic acid) 100 %, R_t= 0.74, $[MH]^-$= 331; δ_H (400 MHz, DMSO-d_6) 8.48 - 8.46 (1H, m), 8.24 – 8.22 (1H, m), 7.56 – 7.54 (1H, m), 7.30 – 7.24 (2H, m), 7.13 – 7.04 (3H, m), 6.52 – 6.49 (1H, m), 4.24 – 4.21 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 2.39 – 2.34 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 139.3, 138.3, 134.9, 129.7, 129.2, 128.6, 124.7, 124.6, 121.7, 119.5, 118.8, 65.3,

63.66, 26.0; ν_{max} (liquid film)/cm^{-1} 3090, 3054, 2809, 1593, 1487, 1411, 1355, 1192, 1162; *m/z* (ES) Found: $[MH]^+$ 333.0909, $C_{16}H_{16}N_2O_4S$ is $[MH]^+$ 333.0904.

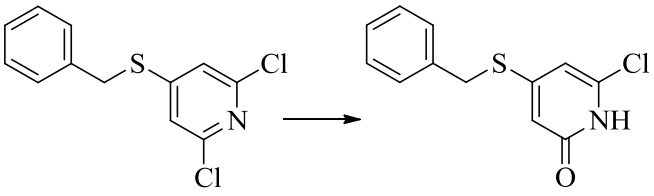
4-(Benzylthio)-2,6-dichloropyridine 31



31

A mixture of tetrabutylammonium bromide (0.6 g, 1.861 mmol), 2,6-dichloro-4-iodopyridine (2 g, 7.3 mmol) and phenylmethanethiol (0.84 mL, 7.3 mmol) were dissolved in toluene (40 mL) and water (20 mL). Aqueous sodium hydroxide (10 mL, 20 mmol, 2 M) was added and stirred at reflux for 19 h. The reaction was cooled to room temperature and then diluted with water (100 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with brine (2 x 100 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in 1:1 DMSO:acetonitrile and was purified by reverse phase chromatography, eluting with 45-100 % acetonitrile in water with a formic acid modifier on a 330 g C_{18} column to give 4-(benzylthio)-2,6-dichloropyridine **31** (1.238 g, 62 %) as an off-white solid. M.P. 80-82 °C; LCMS (Formic acid) 100 % purity, R_t= 1.35, $[MH]^+$= 270, 272; δ_H (400 MHz, $CDCl_3$) 7.46 – 7.30 (5H, m), 7.08 (2H, s), 4.24 (2H, s); δ_C (101 MHz, $CDCl_3$) 154.9, 150.4, 134.3, 129.0, 128.7, 128.1, 118.9, 36.0; ν_{max} (liquid film)/cm^{-1} 3060, 3031, 2931, 1556, 1512, 1361, 1170, 1087, 978; *m/z* (ES) Found: $[MH]^+$ 269.9913, $C_{12}H_{10}Cl_2NS$ is $[MH]^+$ 269.9906.

4-(Benzylthio)-6-chloropyridin-2(1*H*)-one 35

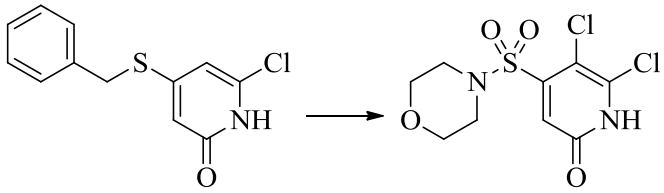


31 **35**

A mixture of 4-(benzylthio)-2,6-dichloropyridine **31** (300 mg, 1.11 mmol)) and 1 M potassium *tert*-butoxide in THF (1.77 mL, 1.77 mmol) was dissolved in *tert*-butanol (5 mL) and stirred at 100 °C for 17 h. Hydrochloric acid (0.35 ml, 1.77 mmol, 5 M) was then added

and stirred for 20 min. The reaction was filtered under vacuum, washing the white solid with further water to give 4-(benzylthio)-6-chloropyridin-2(1*H*)-one **35** (238 mg, 85 %) as a white solid. LCMS (Formic acid) 76 % purity, R_t= 1.02, $[MH]^+$= 270, 272; δ_H (400 MHz, $CDCl_3$) 7.43 – 7.30 (5H, m), 6.45 (1H, d, *J* = 1.5 Hz), 6.40 (1H, d, *J* = 1.5 Hz), 4.19 (2H, s). Pyridone N-H not observed.

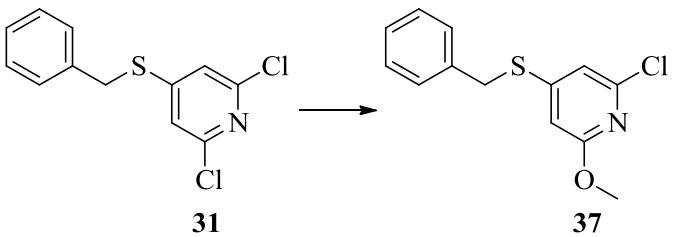
5,6-Dichloro-4-(morpholinosulfonyl)pyridin-2(1*H*)-one 36



35 **36**

Crude 4-(benzylthio)-6-chloropyridin-2(1*H*)-one **35** (238 mg, 0.945 mmol, 85 % purity) was dissolved in aqueous hydrochloric acid (1.418 mL, 2.84 mmol, 2 M) and acetonitrile (4 mL) and to this, NCS (505 mg, 3.78 mmol) was added portionwise over 1 h and then stirred for 1 h. The reaction was diluted with water (30 mL) and extracted with ethyl acetate (30 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give a yellow solid. A portion of the solid (50 mg) was re-dissolved in neat morpholine (0.412 mL, 4.73 mmol) and stirred for 16 h. The excess morpholine was removed *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5,6-dichloro-4-(morpholinosulfonyl)pyridin-2(1*H*)-one **36** (20 mg, 6 %) as a white solid. LCMS (Formic acid) 100 % purity, R_t= 0.85, $[MH]^-$= 311, 313, 315; δ_H (400 MHz, DMSO-d_6) 7.08 (1 H, s) 3.60 - 3.65 (4H, m) 3.25 - 3.29 (4H, m). Pyridone N-H not observed.

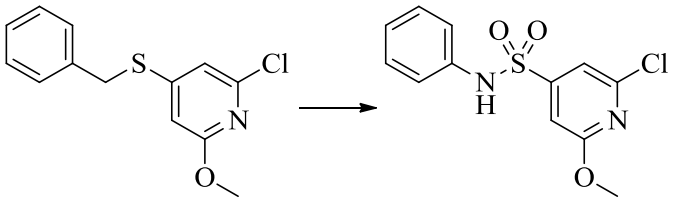
4-(Benzylthio)-2-chloro-6-methoxypyridine 37



31 **37**

A mixture of 4-(benzylthio)-2,6-dichloropyridine **31** (500 mg, 1.851 mmol) and sodium methoxide (1 g, 18.51 mmol) was dissolved in methanol (10 mL) and stirred at 65 °C for 106 h. The reaction was cooled to room temperature and then diluted with water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed with brine (2 x 25 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give 4-(benzylthio)-2-chloro-6-methoxypyridine **37** (453 mg, 92 %) as a crude colourless oil used without further purification. LCMS (Formic acid) 78 % purity, R_t= 1.34, $[MH]^+$= 266, 268; δ_H (400 MHz, $CDCl_3$) 7.44 – 7.24 (5H, m), 6.79 (1H, d, *J* = 1.3 Hz), 6.49 (1H, d, *J* = 1.3 Hz), 4.17 (2H, s), 3.92 (3H, s).

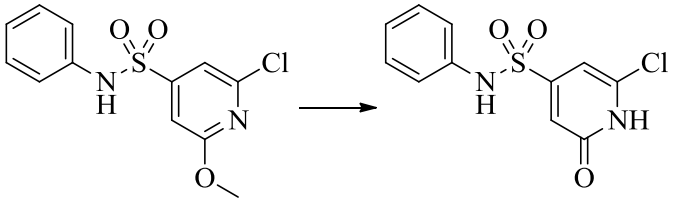
2-Chloro-6-methoxy-*N*-phenylpyridine-4-sulfonamide 38



37 **38**

4-(Benzylthio)-2-chloro-6-methoxypyridine **37** (453 mg, 1.278 mmol) was dissolved in aqueous hydrochloric acid (1.918 mL, 3.84 mmol, 2 M) and acetonitrile (10 mL) and to this, NCS (683 mg, 5.11 mmol) was added portionwise over 1 h and then stirred for 1 h. The reaction was diluted with water (20 mL) and extracted with ethyl acetate (20 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give a colourless oil. The oil was re-dissolved in anhydrous pyridine (2 mL) and to this aniline (0.128 mL, 1.404 mmol) was added and the reaction mixture was stirred for 72 h. The reaction was diluted with water (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed with aqueous hydrochloric acid (2 x 5 mL, 2 M), brine (2 x 10 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (5 – 55 % TBME/cyclohexane at 222 nm) provided 2-chloro-6-methoxy-*N*-phenylpyridine-4-sulfonamide **38** (110 mg, 28 %) as an orange oil. LCMS (Formic acid) 98 % purity, R_t= 1.09, $[MH]^-$= 297, 299; δ_H (400 MHz, $CDCl_3$) 7.36 – 7.25 (2H, m), 7.26 – 7.16 (3H, m), 7.16 – 7.10 (2H, m), 6.99 (1H, d, *J* = 1.2 Hz), 3.97 (3H, s).

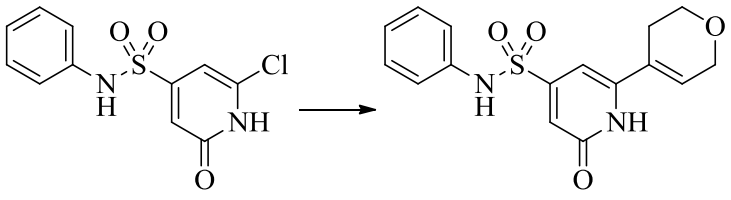
6-Chloro-2-oxo-*N*-phenyl-1,2-dihydropyridine-4-sulfonamide 39



38 **39**

2-Chloro-6-methoxy-*N*-phenylpyridine-4-sulfonamide **38** (110 mg, 0.368 mmol) was dissolved in acetonitrile (0.5 mL) and TMSI (0.351 mL, 2.58 mmol) and was heated and stirred in a Biotage microwave reactorreactorat 65 °C for 60 min. The reaction was diluted with 10 % aqueous ammonium thiosulfate (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 6-chloro-2-oxo-*N*-phenyl-1,2-dihydropyridine-4-sulfonamide **39** (28 mg, 27 %) as a white solid. M.P. 210 °C (decomposition); LCMS (Formic acid) 99 % purity, rt= 0.84, $[MH]^+$= 283, 285; δ_H (400 MHz, CD_3OD) 7.34 – 7.27 (2H, m), 7.21 – 7.10 (3H, m), 7.02 (1H, d, *J* = 1.2 Hz), 6.79 (1H, d, *J* = 1.2 Hz). Sulfonamide N-H and pyridone N-H not observed. δ_C (101 MHz, CD_3OD) 164.3, 152.8, 136.6, 128.9, 125.2, 121.5, 110.3, 106.6. One carbon is not observed. ν_{max} (liquid film)/cm^{-1} 3060, 3031, 2931, 1556, 1512, 1361, 1170, 1087, 978; *m/z* (ES) Found: $[MH]^+$ 285.0097, $C_{11}H_{10}ClN_2O_3S$ is $[MH]^+$ 285.0095.

6-(3,6-Dihydro-2*H*-pyran-4-yl)-2-oxo-*N*-phenyl-1,2-dihydropyridine-4-sulfonamide 28

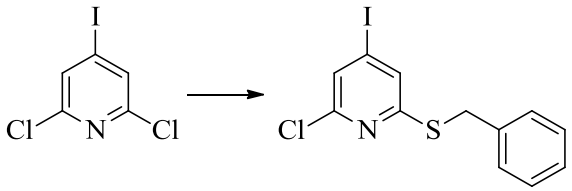


39 **28**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (20 mg, 0.095 mmol), 6-chloro-2-oxo-*N*-phenyl-1,2-dihydropyridine-4-sulfonamide **39** (22 mg, 0.077 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (4 mg, 7 µmol) and tripotassium phosphate (33 mg, 0.155 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorreactorat 120 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with

methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 6-(3,6-dihydro-2*H*-pyran-4-yl)-2-oxo-*N*-phenyl-1,2-dihydropyridine-4-sulfonamide **28** (13 mg, 53 %) as a white solid. M.P. 200-202 °C (decomposition); LCMS (Formic acid) 97 % purity, R_t= 0.75, $[MH]^-$= 331; δ_H (400 MHz, CD_3OD) 7.39 – 7.23 (2H, m), 7.15 (3H, m), 6.70 (1H, d, *J* = 1.5 Hz), 6.48 (2H, m), 4.39 – 4.22 (2H, m), 3.89 (2H, m), 2.36 (2H, m). Sulfonamide N-H and pyridone N-H not observed. δ_C (101 MHz, CD_3OD) 163.6, 152.4, 137.0, 129.6, 128.9, 127.8, 125.0, 121.5, 115.8, 99.5, 64.9, 63.4, 25.1. One carbon is not observed. ν_{max} (liquid film)/cm^{-1} 3413, 3201, 3068, 2955, 1641, 1586, 1338, 1156; *m/z* (ES) Found: $[MH]^+$ 333.0897, $C_{16}H_{17}N_2O_4S$ is $[MH]^+$ 333.0904.

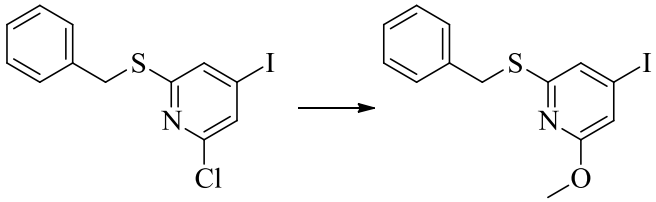
2-(Benzylthio)-6-chloro-4-iodopyridine 32



32

A mixture of tetrabutylammonium bromide (4.5 g, 13.97 mmol), 2,6-dichloro-4-iodopyridine (15 g, 54.8 mmol) and phenylmethanethiol (6.48 mL, 54.8 mmol) were dissolved in toluene (300 mL) and water (150 mL). 2 M aqueous sodium hydroxide (75 mL, 15 mmol) was added and stirred at reflux for 18 h. The reaction was cooled to room temperature and then diluted with water (300 mL) and extracted with ethyl acetate (2 x 500 mL). The organic layer was dried through a hydrophobic frit, over sodium sulfate and concentrated *in vacuo*. Purification by flash chromatography (0 – 3 % ethyl acetate/cyclohexane) gave 2-(benzylthio)-6-chloro-4-iodopyridine **32** (3.5 g, 16 %) as a white solid. M.P. 52-54 °C; LCMS (Formic acid) 98 %, R_t= 1.51, $[MH]^+$= 362, 364; δ_H (400 MHz, $CDCl_3$) 7.46 (1H, d, *J* = 1.1 Hz), 7.44 – 7.40 (2H, m), 7.40 (1H, d, *J* = 1.1 Hz), 7.35 – 7.29 (2H, m), 7.28 – 7.24 (1H, m), 4.40 (2H, s); δ_C (101 MHz, $CDCl_3$) 160.6, 150.7, 137.0, 129.1, 128.7, 128.5, 127.8, 127.3, 105.7, 34.6; ν_{max} (liquid film)/cm^{-1} 3098, 3031, 1532, 1520, 1510, 1352, 1341, 1160, 1100.

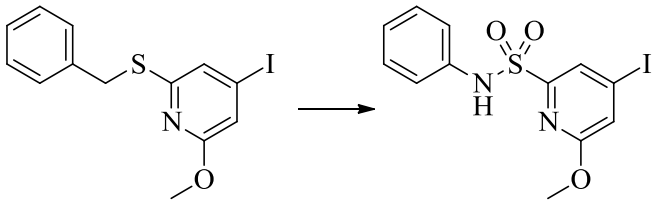
2-(Benzylthio)-4-iodo-6-methoxypyridine 40



32 **40**

2-(Benzylthio)-6-chloro-4-iodopyridine **32** (3 g, 8.3 mmol) was dissolved in sodium methoxide (5 mL, 8.3 mmol, 25 % by weight in methanol) and methanol (5 mL) and stirred at 100 °C for 2 h in a sealed tube. The reaction was cooled to room temperature and concentrated *in vacuo*. Purification by flash chromatography (0 – 3 % ethyl acetate/cyclohexane) gave 2-(benzylthio)-4-iodo-6-methoxypyridine **40** (400 mg, 13 %) as a pale yellow oil. LCMS (Formic acid) 99 %, R_t= 1.61, $[MH]^+$= 358; δ_H (400 MHz, $CDCl_3$) 7.40 (2H, m), 7.35 – 7.29 (2H, m), 7.29 – 7.24 (1H, m), 7.15 (1H, d, *J* = 1.1 Hz), 6.86 (1H, d, *J* = 1.1 Hz), 4.42 (2H, s), 3.93 (3H, s); δ_C (101 MHz, $CDCl_3$) 163.4, 157.2, 137.7, 128.7, 128.5, 127.1, 122.6, 115.1, 106.1, 53.7, 34.3; ν_{max} (liquid film)/cm^{-1} 3063, 3010, 2943, 2852, 1552, 1538, 1450, 1362, 1283, 1143, 1027.

4-Iodo-6-methoxy-*N*-phenylpyridine-2-sulfonamide 41

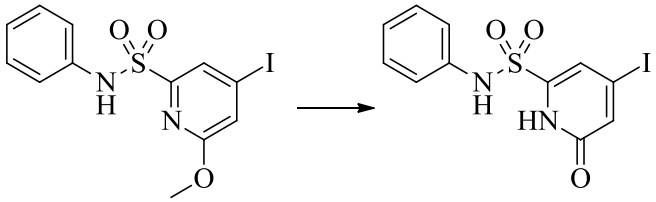


40 **41**

2-(Benzylthio)-4-iodo-6-methoxypyridine **40** (250 mg, 0.7 mmol) was dissolved in aqueous hydrochloric acid (1.05 mL, 2.1 mmol, 2 M) and acetonitrile (4 mL) and to this, NCS (374 mg, 2.8 mmol) was added portionwise over 1 h and then stirred for 1 h. The reaction was diluted with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give a brown oil. The oil was dissolved in 1,4-dioxane (2 mL) and anhydrous pyridine (0.2 mL) and to this aniline (0.063 mL, 0.69 mmol) was added and the reaction mixture was stirred for 2 h. The reaction was diluted with water (10 mL) and extracted with ethyl acetate (2 x 15 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 % ethyl acetate/cyclohexane) provided 4-iodo-6-methoxy-*N*-phenylpyridine-2-sulfonamide **41** (120 mg, 43 %) as an off-white solid. This reaction was

repeated with 2-(benzylthio)-4-iodo-6-methoxypyridine **40** (800 mg, 0.7 mmol) to give 4-iodo-6-methoxy-*N*-phenylpyridine-2-sulfonamide **41** (410 mg, 55 %) as an off-white solid. M.P. 144-146 °C; LCMS (Formic acid) 97 %, R_t= 1.22, $[MH]^+$= 390; δ_H (400 MHz, $CDCl_3$) 7.85 (1H, d, *J* = 1.2 Hz), 7.32 (1H, d, *J* = 1.2 Hz), 7.31 – 7.25 (2H, m), 7.20 (2H, m), 7.17 – 7.12 (1H, m), 7.00 (1H, s), 3.89 (3H, s); δ_C (101 MHz, $CDCl_3$) 163.9, 153.4, 136.2, 129.2, 125.6, 124.5, 124.4, 121.7, 106.7, 54.4; ν_{max} (liquid film)/cm^{-1} 3225, 3064, 1573, 1480, 1417, 1461, 1377, 1168, 1141, 1020; *m/z* (ES) Found: $[MH]^+$ 390.9611, $C_{12}H_{12}IN_2O_3S$ is $[MH]^+$ 390.9608.

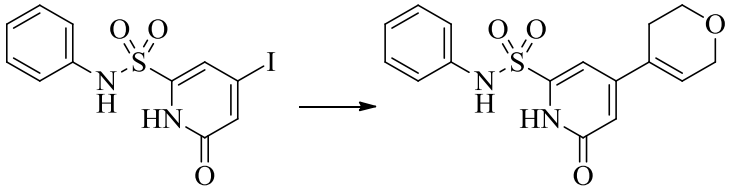
4-Iodo-6-oxo-*N*-phenyl-1,6-dihydropyridine-2-sulfonamide 42



41 **42**

4-Iodo-6-methoxy-*N*-phenylpyridine-2-sulfonamide **41** (500 mg, 1.281 mmol) was dissolved in acetonitrile (25 mL) and TMSI (1.221 mL, 8.97 mmol) and was heated and stirred in a Biotage microwave reactorreactorat 65 °C for 60 min. The reaction was diluted with 10 % aqueous ammonium thiosulfate (50 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (20 % ethyl acetate/cyclohexane) provided 4-iodo-6-methoxy-*N*-phenylpyridine-2-sulfonamide **42** (280 mg, 53 %) as a pale yellow solid. M.P. 200 °C (decomposition); LCMS (Formic acid) 96 %, R_t= 1.04, $[MH]^-$= 375; δ_H (400 MHz, $CDCl_3$) 11.98 (1H, s), 10.37 (1H, s), 7.67 – 7.65 (1H, m), 7.31 (1H, d, *J* = 1.0 Hz), 7.29 – 7.22 (2H, m), 7.16 - 7.12 (2H, m), 7.08 - 7.04 (1H, m); δ_C (101 MHz, DMSO-d_6) 164.1, 137.7, 129.5, 124.5, 122.9, 122.8, 122.7, 120.7, 109.7; ν_{max} (liquid film)/cm^{-1} 3272, 3021, 1646, 1587, 1548, 1480, 1419, 1348, 1170, 1139; *m/z* (ES) Found: $[MH]^+$ 376.9452, $C_{11}H_{10}IN_2O_3S$ is $[MH]^+$ 376.9451.

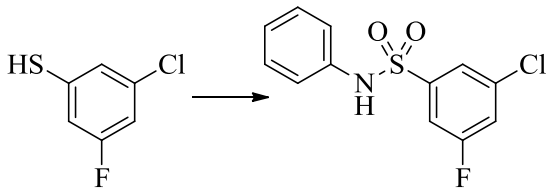
4-(3,6-Dihydro-2*H*-pyran-4-yl)-6-oxo-*N*-phenyl-1,6-dihydropyridine-2-sulfonamide 29



42 **29**

A mixture of 4-iodo-6-oxo-*N*-phenyl-1,6-dihydropyridine-2-sulfonamide **42** (160 mg, 0.425 mmol), 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (107 mg, 0.51 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (22 mg, 0.039 mmol) and tripotassium phosphate (181 mg, 0.851 mmol) in 1,4-dioxane (2 mL) and water (0.4 mL) was heated and stirred in a Biotage microwave reactorat 120 °C for 60 min. The reaction was passed through a pad of Celite, eluting with methanol and concentrated *in vacuo*. The oil was dissolved in 1:1 MeCN:DMSO (1 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 4-(3,6-dihydro-2*H*-pyran-4-yl)-6-oxo-*N*-phenyl-1,6-dihydropyridine-2-sulfonamide **29** (22 mg, 15 %) as an off-white solid. M.P. 190 °C (decomposition); LCMS (Formic acid) 99 %, R_t= 0.91, $[MH]^-$= 331; δ_H (600 MHz, DMSO-d_6) 7.10 – 7.04 (2H, m), 7.03 – 6.99 (2H, m), 6.88 – 6.84 (1H, m), 6.79 – 6.74 (2H, m), 6.40 – 6.36 (1H, m), 6.33 – 6.29 (1H, m), 4.22 – 4.19 (2H, m), 3.77 (2H, t, *J* = 5.4 Hz), 2.34 – 2.28 (2H, m). Sulfonamide N-H not observed. δ_C (151 MHz, DMSO-d_6) 149.1, 131.3, 128.3, 126.8, 120.6, 112.5, 102.2, 101.7, 64.9, 63.3, 25.6. Three carbons are not observed. v_{max} (liquid film)/cm^{-1} 3335, 1601, 1460, 1427, 1260, 1095, 1001; *m/z* (ES) Found: $[MH]^+$ 333.0905, $C_{16}H_{17}N_2O_4S$ is $[MH]^+$ 333.0904.

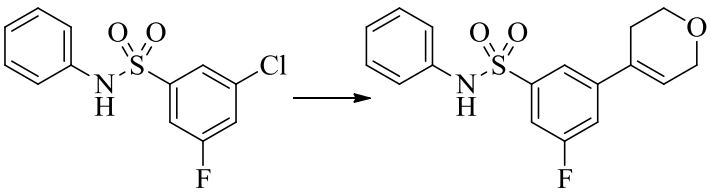
3-Chloro-5-fluoro-*N*-phenylbenzenesulfonamide 47



46 **47**

3-Chloro-5-fluorobenzenethiol **46** (120 mg, 0.738 mmol) was dissolved in aqueous hydrochloric acid (1.2 mL, 2.4 mmol, 2 M) and acetonitrile (5 mL), cooled to 12-16 °C and to this, NCS (394 mg, 2.95 mmol) was added portionwise over 1 h and then stirred for 1 h. The reaction was diluted with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give a colourless oil. The oil was re-dissolved in pyridine (3 mL) and to this aniline (0.067 mL, 0.738 mmol) was added and the reaction mixture was stirred for 3 h. The reaction was diluted with water (25 mL) and extracted with ethyl acetate (3 x 25 mL). The organic layer was washed with aqueous hydrochloric acid (2 x 25 mL, 2 M), brine (2 x 50 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 – 60 % TBME/cyclohexane) provided 3-chloro-5-fluoro-*N*-phenylbenzenesulfonamide **47** (162 mg, 77 %) as a white solid. M.P. 143-145 °C; LCMS (Formic acid) 100 %, R_t= 1.16, $[MH]^-$= 284, 286; δ_H (400 MHz, DMSO-d_6) 10.45 (1H, s), 7.79 – 7.73 (1H, m), 7.61 – 7.58 (1H, m), 7.52 (1H, ddd, *J* = 7.9, 2.3, 1.5 Hz), 7.32 – 7.24 (2H, m), 7.14 – 7.07 (3H, m); δ_C (101 MHz, DMSO-d_6) 162.1 (d, $^1J_{C\text{-}F}$ = 253.1 Hz), 143.1 (d, $^3J_{C\text{-}F}$ = 7.6 Hz), 137.2, 135.5 (d, $^3J_{C\text{-}F}$ = 10.2 Hz), 129.8, 125.3, 123.28 (d, $^4J_{C\text{-}F}$ = 3.4 Hz), 121.3, 121.1 (d, $^2J_{C\text{-}F}$ = 25.2 Hz), 113.3 (d, $^2J_{C\text{-}F}$ = 24.5 Hz); δ_F (376 MHz, DMSO-d_6) -107.3 – -107.4 (m); ν_{max} (liquid film)/cm^{-1} 3213, 3073, 1589, 1338, 1282, 1158, 1151.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-fluoro-*N*-phenylbenzenesulfonamide 44

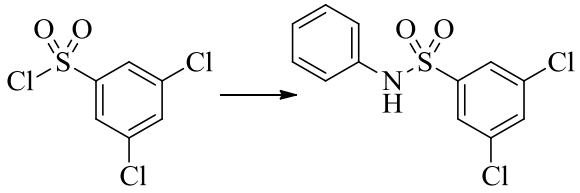


47 **44**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (25 mg, 0.119 mmol), 3-chloro-5-fluoro-*N*-phenylbenzenesulfonamide **47** (30 mg, 0.105 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (5 mg, 8.92 µmol) and tripotassium phosphate (70 mg, 0.33 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 3-(3,6-

dihydro-2*H*-pyran-4-yl)-5-fluoro-*N*-phenylbenzenesulfonamide **44** (13 mg, 37 %) as a white solid. M.P. 158-160 °C; LCMS (Formic acid) 100 %, R_t= 1.12, $[MH]^-$= 332; δ_H (400 MHz, DMSO-d_6) 10.37 (1H, s), 7.62 – 7.59 (1H, m), 7.59 – 7.53 (1H, m), 7.45 – 7.34 (1H, m), 7.30 – 7.21 (2H, m), 7.13 – 7.07 (2H, m), 7.07 – 7.02 (1H, m), 6.44 – 6.38 (1H, m), 4.27 – 4.19 (2H, m), 3.81 (2H, t, *J* = 5.3 Hz), 2.42 – 2.34 (2H, m); δ_C (101 MHz, DMSO-d_6) 162.36 (d, $^1J_{C\text{-}F}$ = 247.7 Hz), 143.55 (d, $^3J_{C\text{-}F}$ = 7.5 Hz), 142.41 (d, $^4J_{C\text{-}F}$ = 7.4 Hz), 138.26, 131.52 (d, $^4J_{C\text{-}F}$ = 2.1 Hz), 129.66, 126.94, 124.73, 121.15, 118.97 (d, $^4J_{C\text{-}F}$ = 2.6 Hz), 115.98 (d, $^2J_{C\text{-}F}$ = 22.4 Hz), 112.47 (d, $^2J_{C\text{-}F}$ = 24.8 Hz), 65.37, 63.80, 26.53; δ_F (376 MHz, DMSO-d_6) -110.6 (dd, $^2J_{F\text{-}H}$ = 10.2, $^2J_{F\text{-}H}$ = 8.1); ν_{max} (liquid film)/cm^{-1} 3136, 3065, 2967, 2902, 1600, 1583, 1492, 1430, 1348, 1287, 1154.

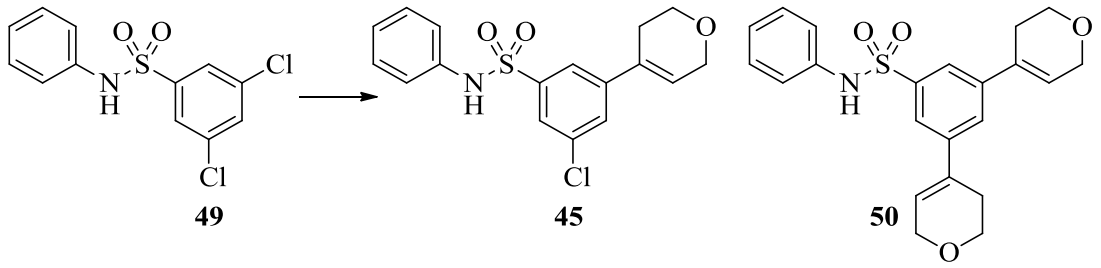
3,5-Dichloro-*N*-phenylbenzenesulfonamide 49



48 **49**

3,5-Dichlorobenzene-1-sulfonyl chloride **48** (1.07 g, 4.36 mmol) and aniline (0.418 mL, 4.58 mmol) were added to pyridine (2 mL). The resulting mixture was stirred at room temperature for 2 h and then diluted with water (25 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layer was washed with aqueous hydrochloric acid (2 x 25 mL, 2 M), brine (50 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 – 60 % TBME/cyclohexane) provided 3,5-dichloro-*N*-phenylbenzenesulfonamide **49** (1.05 g, 80 %) as a white solid. LCMS (Formic acid) 98 %, R_t= 1.18, $[MH]^-$= 348, 350; δ_H (400 MHz, DMSO-d_6) 10.43 (1H, s), 7.93 (1H, t, *J* = 1.9 Hz), 7.69 (2H, d, *J* = 1.9 Hz), 7.33 – 7.25 (2H, m), 7.15 – 7.07 (3H, m).[138]

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-chloro-*N*-phenylbenzenesulfonamide 45

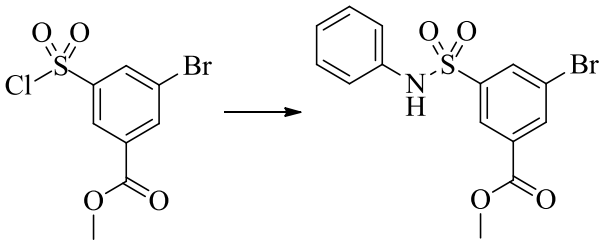


49 **45** **50**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (76 mg, 0.364 mmol), 3,5-dichloro-*N*-phenylbenzenesulfonamide **49** (100 mg, 0.331 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (211 mg, 0.993 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method D) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-chloro-*N*-phenylbenzenesulfonamide **45** (54 mg, 46 %) as a white solid. M.P. 129-131 °C; LCMS (Formic acid) 98 %, R_t= 1.18, $[MH]^-$= 348, 350; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 7.74 – 7.71 (1H, m), 7.71 – 7.68 (1H, m), 7.63 – 7.58 (1H, m), 7.31 – 7.22 (2H, m), 7.16 – 7.10 (2H, m), 7.10 – 7.03 (1H, m), 6.43 – 6.35 (1H, m), 4.27 – 4.19 (2H, m), 3.80 (2H, t, *J* = 5.4 Hz), 2.42 – 2.32 (2H, m); δ_C (101 MHz, DMSO-d_6) 143.0, 141.9, 137.7, 134.6, 131.3, 129.7, 128.8, 127.1, 125.0, 124.9, 121.4, 121.2, 65.3, 63.8, 26.4; v_{max} (liquid film)/cm^{-1} 3135, 2961, 2921, 1490, 1344, 1162, 1120.

And 3,5-bis(3,6-dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide **50** (21 mg, 16 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 1.10, $[MH]^+$= 398; δ_H (400 MHz, DMSO-d_6) 10.24 (1H, s), 7.70 – 7.61 (3H, m), 7.29 – 7.18 (2H, m), 7.13 – 7.08 (2H, m), 7.08 – 6.98 (1H, m), 6.38 – 6.28 (2H, m), 4.28 – 4.18 (4H, m), 3.82 (4H, d, *J* = 10.9 Hz), 2.45 – 2.38 (4H, m).

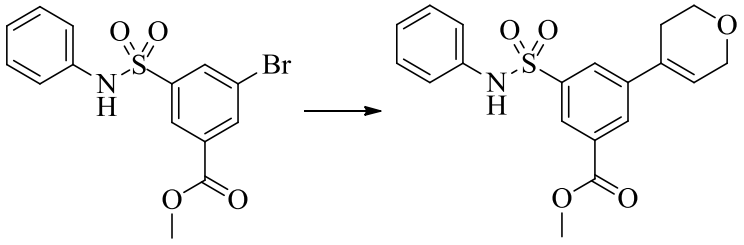
Methyl 3-bromo-5-(*N*-phenylsulfamoyl) benzoate 54



53 **54**

Methyl 3-bromo-5-(chlorosulfonyl)benzoate **53** (501 mg, 1.598 mmol) and aniline (0.16 mL, 1.758 mmol) were added to pyridine (5 mL). The resulting mixture was stirred at room temperature for 3 h and then diluted with water (50 mL) and extracted with ethyl acetate (50 mL). The organic layer was washed with aqueous hydrochloric acid (10 mL, 2 M), brine (2 x 25 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (20 – 60 % TBME/cyclohexane at 214 nm) provided methyl 3-bromo-5-(*N*-phenylsulfamoyl)benzoate **54** (455 mg, 77 %) as a yellow solid. M.P. 123-125 °C; LCMS (Formic acid) 98 % purity, R_t= 1.14, $[MH]^-$= 368, 370 ; δ_H (400 MHz, $CDCl_3$) 8.37 (1H, t, *J* = 1.5 Hz), 8.33 (1H, t, *J* = 1.5 Hz), 8.06 (1H, t, *J* = 1.8 Hz), 7.34 – 7.27 (2H, m), 7.23 – 7.17 (1H, m), 7.12 – 7.07 (2H, m), 6.68 (1H, s), 3.96 (3H, s); δ_C NMR (101 MHz, $CDCl_3$) 164.0, 141.4, 136.8, 135.4, 133.9, 132.9, 129.7, 126.8, 126.2, 123.2, 122.3, 52.9; ν_{max} (liquid film)/cm^{-1} 3194, 1705, 1421, 1343, 1290, 1169; *m/z* (ES) Found: $[MH]^+$ 369.9742, $C_{14}H_{13}BrNO_4S$ is $[MH]^+$ 369.9743.

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoate 55

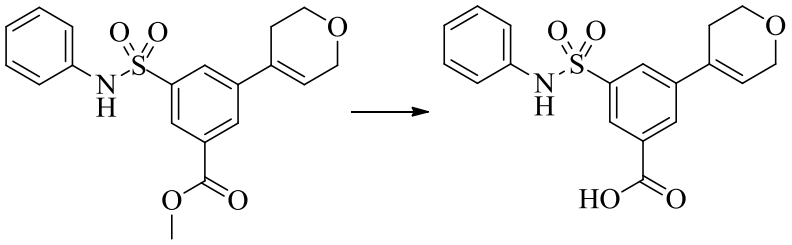


54 **55**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (187 mg, 0.891 mmol), methyl 3-bromo-5-(*N*-phenylsulfamoyl)benzoate **54** (300 mg, 0.81 mmol), $PdCl_2$(dppf) (59 mg, 0.081 mmol) and sodium carbonate (189 mg, 1.783 mmol) in 1,4-dioxane (3 mL) and water (0.6 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. Purification by flash chromatography (20 – 100 % TBME/cyclohexane at 222 nm) provided methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoate **55** (200 mg, 66 %) as a white solid. M.P. 112-114 °C; LCMS

(Formic acid) 98 % purity, R_t= 1.05, $[MH]^-$= 372.; δ_H (400 MHz, $CDCl_3$) 8.35 (1H, t, *J* = 1.6 Hz), 8.22 (1H, t, *J* = 1.6 Hz), 7.87 (1H, t, *J* = 1.6 Hz), 7.31 – 7.26 (2H, m), 7.20 – 7.14 (1H, m), 7.12 – 7.08 (2H, m), 6.68 (1H, s), 6.23 – 6.18 (1H, m), 4.32 (2H, m), 4.00 – 3.87 (5H, m), 2.52 – 2.36 (2H, m); δ_C (101 MHz, $CDCl_3$) 165.6, 141.7, 140.0, 136.0, 132.0, 131.5, 129.8, 129.5, 127.2, 126.5, 125.9, 125.8, 122.2, 65.7, 64.0, 52.6, 26.8; ν_{max} (liquid film)/cm^{-1} 3259, 3040, 2955, 1726, 1493, 1305, 1245, 1160; *m/z* (ES) Found: $[MH]^+$ 374.1061, $C_{19}H_{20}NO_5S$ is $[MH]^+$ 374.1057.

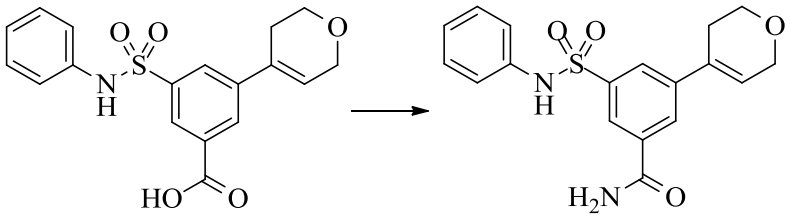
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid 56



55 **56**

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoate **55** (200 mg, 0.536 mmol) was dissolved in THF (2 mL) and water (2 mL). To this, 2 M aqueous lithium hydroxide (0.536 mL, 1.071 mmol) was added and stirred for 16 h. The THF was concentrated *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid and extracted with ethyl acetate (5 mL). The organic layer was washed with brine (5 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (189 mg, 98 %) as a white solid. M.P. 210-212 °C; LCMS (Formic acid) 98 % purity, R_t= 1.05, $[MH]^-$= 358; δ_H (400 MHz, DMSO-d_6) 13.51 (1H, s), 10.32 (1H, s), 8.16 (1H, t, *J* = 1.5 Hz), 8.13 (1H, t, *J* = 1.6 Hz), 7.94 (1H, t, *J* = 1.7 Hz), 7.29 – 7.20 (2H, m), 7.12 – 7.04 (3H, m), 6.39 (1H, s), 4.24 – 4.20 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 2.41 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.3, 141.5, 140.8, 137.8, 132.7, 131.7, 129.7, 129.3, 126.6, 126.5, 126.1, 125.0, 121.2, 65.4, 63.8, 26.6; ν_{max} (liquid film)/cm^{-1} 3264, 3049, 2899, 1693, 1492, 1341, 1221, 1139, 932; *m/z* (ES) Found: $[MH]^+$ 360.0905, $C_{18}H_{18}NO_5S$ is $[MH]^+$ 360.0900.

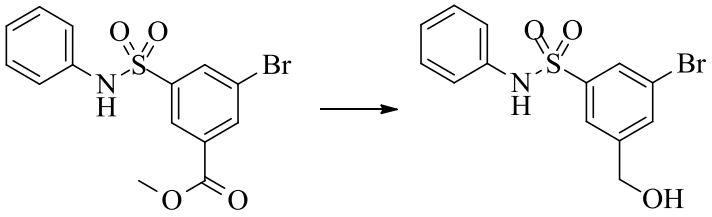
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide 51



56 **51**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (100 mg, 0.278 mmol), DIPEA (0.097 mL, 0.556 mmol), and HATU (106 mg, 0.278 mmol) were dissolved in DMF (1 mL) and stirred for 30 min. To this, ammonia (1.53 mL, 0.765 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at room temperature for 24 h and then diluted with water (10 mL) and extracted with ethyl acetate (10 mL). The organic layer was washed with brine (2 x 5 mL), aqueous lithium chloride (2 x 5 mL, 9 %), dried through a hydrophobic frit and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide **51** (41 mg, 41 %) as a white solid. M.P. 190-192 °C; LCMS (Formic acid) 100 % purity, R_t= 0.81, $[MH]^-$= 357; δ_H (400 MHz, DMSO-d_6) 8.18 (1H, s), 8.14 (1H, t, *J* = 1.5 Hz), 8.06 (1H, t, *J* = 1.6 Hz), 7.85 (1H, t, *J* = 1.6 Hz), 7.49 (1H, s), 7.15 – 7.10 (2H, m), 7.04 – 6.95 (2H, m), 6.90 – 6.79 (1H, m), 6.40 – 6.32 (1H, m), 6.03 (1H, s), 4.25 – 4.23 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 2.42 – 2.40 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.1, 143.2, 141.9, 140.7, 135.5, 132.3, 129.3, 126.6, 125.7, 125.0, 124.9, 122.3, 120.9, 65.5, 63.9, 26.8; ν_{max} (liquid film)/cm^{-1} 3194, 3049, 1705, 1412, 1290, 1169, 1140; *m/z* (ES) Found: $[MH]^+$ 359.1068, $C_{18}H_{19}N_2O_4S$ is $[MH]^+$ 359.1060.

3-Bromo-5-(hydroxymethyl)-*N*-phenylbenzenesulfonamide 57

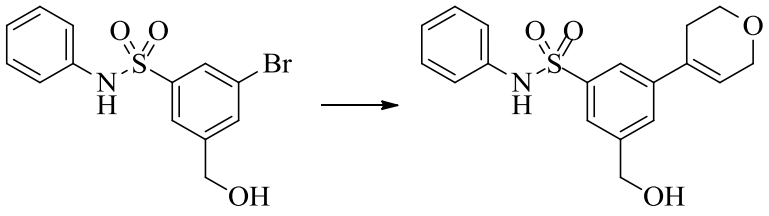


54 **57**

Lithium aluminium hydride (0.9 mL, 0.9 mmol, 1 M in THF) diluted with THF (1 mL) was cooled to 0 °C under nitrogen, and to this, methyl 3-bromo-5-(*N*-phenylsulfamoyl)benzoate **54** (100 mg, 0.27 mmol) in THF (2 mL) was added dropwise over 30 min. The reaction was further stirred for 30 min and was then quenched by the addition of THF:water (4 mL:1 mL)

and water (10 mL) and then extracted into ethyl acetate (5 x 10 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 3-bromo-5-(hydroxymethyl)-*N*-phenylbenzenesulfonamide **57** (77 mg, 83 %) as a colourless oil. LCMS (Formic acid) 94 % purity, R_t= 0.94, $[MH]^-$= 340, 342; δ_H (400 MHz, $CDCl_3$) 7.80 – 7.75 (2H, m), 7.64 (1H, m), 7.33 – 7.21 (3H, m), 7.19 – 7.12 (1H, m), 7.12 – 7.06 (2H, m), 4.68 (2H, m). Alcohol O-H not observed. δ_C (101 MHz, $CDCl_3$) 144.4, 140.7, 135.8, 134.0, 129.4, 129.0, 125.9, 123.5, 122.9, 122.0, 63.4; ν_{max} (liquid film)/cm^{-1} 3447, 3153, 2979, 2914, 1494, 1343, 1150, 1020; *m/z* (ES) Found: $[MH]^+$ 341.9794, $C_{13}H_{13}BrNO_3S$ is $[MH]^+$ 341.9794.

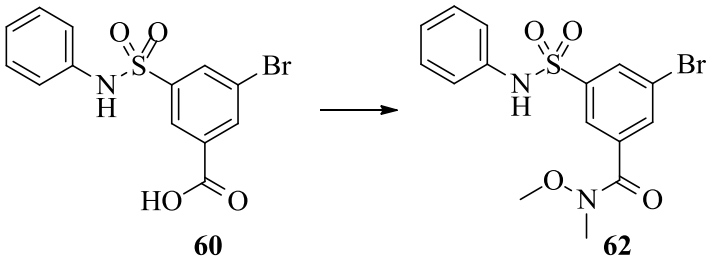
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(hydroxymethyl)-*N*-phenylbenzenesulfonamide 52



57 **52**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (40 mg, 0.193 mmol), 3-bromo-5-(hydroxymethyl)-*N*-phenylbenzenesulfonamide **57** (60 mg, 0.175 mmol), $PdCl_2$(dppf) (13 mg, 0.018 mmol) and sodium carbonate (41 mg, 0.386 mmol) in 1,4-dioxane (1 mL) and water (0.2 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 20 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give a black impure oil. The oil was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(hydroxymethyl)-*N*-phenylbenzenesulfonamide **52** (10 mg, 17 %) as a white solid. M.P. 162-164 °C; LCMS (Formic acid) 100 % purity, R_t= 0.85, $[MH]^-$= 344; δH (400 MHz, CD_3OD) 7.70 -7.68 (1H, m), 7.66 - 7.56 (2H, m), 7.29 – 7.16 (2H, m), 7.14 – 7.03 (3H, m), 6.25 – 6.15 (1H, m), 4.63 (2H, s), 4.29 - 4.27 (2H, m), 3.91 (2H, t, *J* = 5.5 Hz), 2.44 - 2.42 (2H, m). Sulfonamide N-H and alcohol O-H not observed. δ_C (101 MHz, CD_3OD) 143.2, 141.0, 140.0, 137.6, 132.7, 128.7, 126.6, 124.4, 124.1, 123.4, 121.7, 121.1, 65.2, 63.9, 62.9, 26.6; ν_{max} (liquid film)/cm^{-1} 3260, 3161, 2914, 1498, 1353, 1154; *m/z* (ES) Found: $[MH]^+$ 346.1114, $C_{18}H_{20}NO_4S$ is $[MH]^+$ 346.1108.

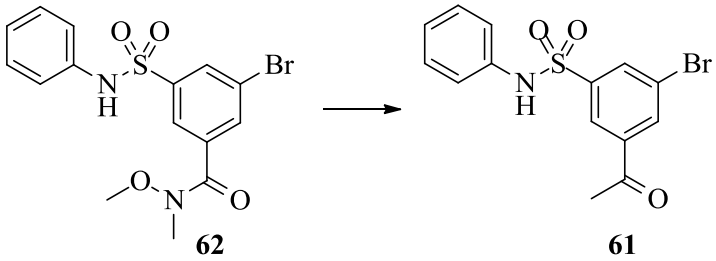
3-Bromo-*N*-methoxy-*N*-methyl-5-(*N*-phenylsulfamoyl)benzamide 62



60 **62**

1*H*-Benzo[*d*][1,2,3]triazol-1-ol hydrate (206 mg, 1.342 mmol), 3-bromo-5-(*N*-phenylsulfamoyl)benzoic acid **60** (239 mg, 0.671 mmol), *N*-((ethylimino)methylene)-*N*,*N*-dimethylpropane-1,3-diamine hydrochloride (257 mg, 1.342 mmol) and DIPEA (0.352 mL, 2.013 mmol) were dissolved in DMF (3 mL) and stirred for 5 min. To this, *N*,*O*-dimethylhydroxylamine hydrochloride (131 mg, 1.342 mmol) in DMF (2 mL) was added dropwise. The resulting mixture was stirred at room temperature for 48 h and then diluted with water (5 mL) and extracted with ethyl acetate (5 mL). The organic layer was washed with brine (2 x 5 mL), aqueous lithium chloride (2 x 5 mL, 9 %), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (20 – 100 % TBME/cyclohexane) provided 3-bromo-*N*-methoxy-*N*-methyl-5-(*N*-phenylsulfamoyl) benzamide **62** (39 mg, 14 %) as a colourless oil. LCMS (Formic acid) 99 % purity, R_t= 1.00, $[MH]^-$ = 397, 399; δ_H (400 MHz, $CDCl_3$) 8.10 (1H, t, *J* = 1.5 Hz), 8.00 (1H, t, *J* = 1.5 Hz), 7.97 (1H, t, *J* = 1.5 Hz), 7.49 (1H, s), 7.30 – 7.23 (2H, m), 7.18 – 7.10 (3H, m), 3.44 (3H, s), 3.36 (3H, s).

3-Acetyl-5-bromo-*N*-phenylbenzenesulfonamide 61

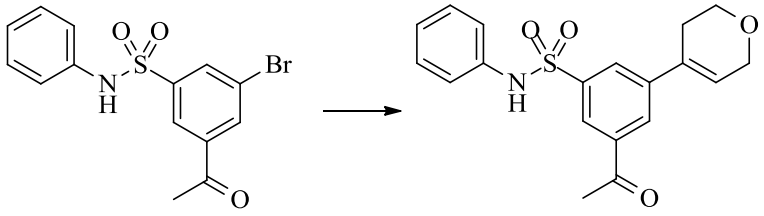


62 **61**

3-Bromo-*N*-methoxy-*N*-methyl-5-(*N*-phenylsulfamoyl)benzamide **62** (53 mg, 0.133 mmol) in THF (1 mL) was cooled to 0 ºC. To this methylmagnesium bromide (0.279 mL, 0.279

mmol, 1 M in di-*N*-butyl ether) was added dropwise at 0 °C under nitrogen. The reaction was stirred for 30 min and was then quenched by the addition of saturated aqueous ammonium chloride (2 mL) and stirred for 30 min. The residue was diluted with water (10 mL) and then extracted into ethyl acetate (10 mL). The organic layer was washed with brine (2 x 10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 3-acetyl-5-bromo-*N*-phenylbenzenesulfonamide **61** (39 mg, 64 %) as a crude colourless oil. LCMS (Formic acid) 76 % purity, R_t= 1.06, $[MH]^-$ = 352, 354; δ_H (400 MHz, $CDCl_3$) 8.23 (1H, t, *J* = 1.5 Hz), 8.18 (1H, t, *J* = 1.5 Hz), 8.09 (1H, t, *J* = 1.5 Hz), 7.34 – 7.26 (2H, m), 7.24 – 7.16 (2H, m), 7.15 – 7.09 (1H, m), 7.01 (1H, s), 2.54 (3H, s). Used without further purification in the next step.

3-Acetyl-5-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide 59

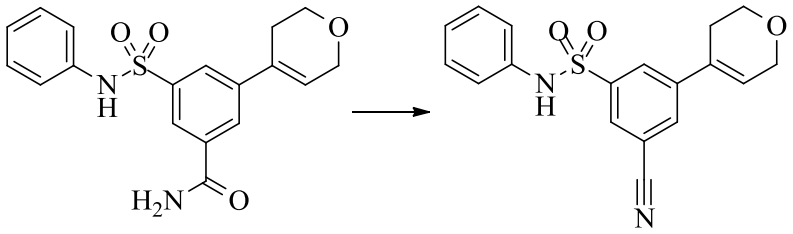


61 **59**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (36 mg, 0.171 mmol), 3-acetyl-5-bromo-*N*-phenylbenzenesulfonamide **61** (30 mg, 0.085 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (5 mg, 9 µmol) and tripotassium phosphate (58 mg, 0.273 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on XBridge C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method B) then purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 3-acetyl-5-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide **59** (15 mg, 49 %) as an off-white solid. M.P. 166-168 °C; LCMS (Formic acid) 100 % purity, R_t= 0.98, $[MH]^-$= 356; δ_H (400 MHz, CD_3OD) 8.18 - 8.17 (2H, m), 7.91 (1H, t, *J* = 1.7 Hz), 7.29 – 7.21 (2H, m), 7.16 – 7.08 (3H, m), 6.30 (1H, m) 4.32 (2H, m), 3.93 (2H, t, *J* = 5.4 Hz), 2.59 (3H, s), 2.50 – 2.44 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, CD_3OD) 197.0, 141.7, 140.6, 137.7, 137.3, 132.1, 128.8, 127.7,

126.6, 125.4, 124.9, 124.8, 121.5, 65.2, 63.8, 26.4, 25.3; ν_{max} (liquid film)/cm^{-1} 3062, 2990, 2884, 1688, 1349, 1160; *m/z* (ES) Found: $[MH]^+$ 358.1109, $C_{19}H_{20}NO_4S$ is $[MH]^+$ 358.1108.

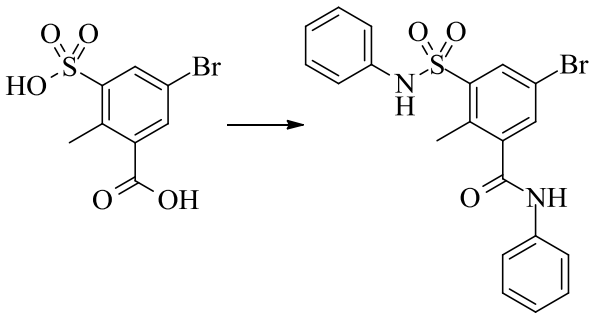
3-Cyano-5-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide 63



51 **63**

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **51** (30 mg, 0.084 mmol) was dissolved in DCM (1 mL) and triethylamine (0.035 mL, 0.251 mmol). To this, triflic anhydride (0.023 mL, 0.134 mmol) was added and stirred for 16 h. The solvent was removed *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 3-cyano-5-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-phenylbenzenesulfonamide **63** (15 mg, 52 %) as an off-white solid. M.P. 140-142 °C; LCMS (Formic acid) 100 % purity, R_t= 1.01, $[MH]^-$= 339; δ_H (400 MHz, CD_3OD) 8.00 (1H, t, *J* = 1.5 Hz), 7.94 – 7.91 (2H, m), 7.31 – 7.24 (2H, m), 7.16 – 7.08 (3H, m), 6.35 – 6.29 (1H, m), 4.30 (2H, m), 3.91 (2H, t, *J* = 5.4 Hz), 2.43 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, CD_3OD) 142.4, 141.3, 137.0, 131.7, 131.2, 128.9, 128.3, 126.7, 126.6, 125.1, 121.6, 116.8, 113.3, 65.2, 63.7, 26.1; ν_{max} (liquid film)/cm^{-1} 3250, 3052, 2995, 2240, 1560, 1422, 1361, 1156, 927.

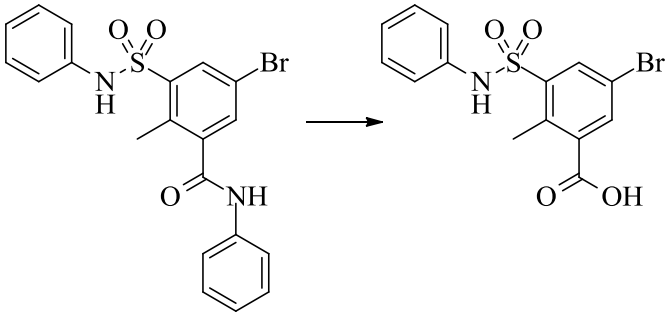
5-Bromo-2-methyl-*N*-phenyl-3-(*N*-phenylsulfamoyl)benzamide 73



72 **73**

Thionyl chloride (2 ml, 27.4 mmol) was stirred at 0 °C under nitrogen and to this 5-bromo-2-methyl-3-sulfobenzoic acid **72** (1g, 3.19 mmol) was added and stirred for 2 days. The solvent was concentrated *in vacuo*. The oil was re-dissolved in anhydrous pyridine (0.8 mL) and 1,4-dioxane (4 mL), and aniline (0.349 mL, 3.83 mmol) was added and the reaction mixture was stirred for 18 h. The reaction was diluted with water (100 mL) and extracted with ethyl acetate (100 mL). The organic layer was washed with aqueous hydrochloric acid (50 mL, 2 M), brine (2 x 25 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 100 % TBME/cyclohexane at 222 nm) gave methyl 5-chloro-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **73** (530 mg, 37 %) as white solid. M.P. 150-152 °C; LCMS (Formic acid) 95 % , R_t= 1.17, $[MH]^+$= 445, 447; δ_H (400 MHz, DMSO-d_6) 10.56 (1H, s), 8.09 (1H, d, *J* = 2.1 Hz), 7.91 (1H, d, *J* = 2.1 Hz), 7.69 (2H, m), 7.36 (2H, m), 7.32 – 7.24 (2H, m), 7.16 – 7.09 (3H, m), 7.05 (1H, m), 2.57 (3H, s). Amide *N*-H not observed. δ_C (101 MHz, DMSO-d_6) 165.4, 142.8, 141.4, 139.1, 137.9, 134.2, 133.8, 132.4, 129.8, 129.2, 124.5, 124.3, 120.2, 119.9, 118.8, 16.3; ν_{max} (liquid film)/cm^{-1} 3063, 3010, 3987, 2943, 2852, 1552, 1538, 1480, 1362, 1283, 1143, 1027; *m/z* (ES) Found: $[MH]^+$ 445.0218, $C_{20}H_{18}BrN_2O_3S$ is $[MH]^+$ 445.0216.

5-Bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoic acid 74

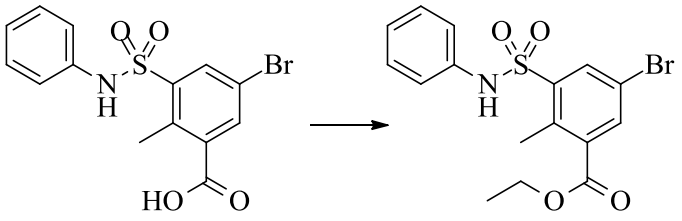


73 **74**

5-Bromo-2-methyl-*N*-phenyl-3-(*N*-phenylsulfamoyl)benzamide **73** (400 mg, 0.898 mmol) was dissolved in methanol (20 mL) and to this 1 M aqueous lithium hydroxide (5 mL, 5 mmol)was added and stirred at 100 °C for 4 days. The reaction mixture was concentrated *in vacuo*. The residue was acidified to pH 5 with 2 M aqueous hydrochloric acid and filtered. The solid filtered was dried in a vacuum oven (40 °C, 1 mbar) for 3 h to give 5-bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoic acid **74** as a white solid (517 mg). LCMS (Formic acid) 90 %, R_t= 0.94, $[MH]^-$= 368, 370; δ_H (400 MHz, DMSO-d_6) 13.54 (1H, s), 10.68 (1H,

s), 8.10 (1H, d, *J* = 2.2 Hz), 8.01 (1H, d, *J* = 2.2 Hz), 7.30 – 7.23 (2H, m), 7.12 – 7.01 (3H, m), 2.65 (3H, s). The product was used without further purification.

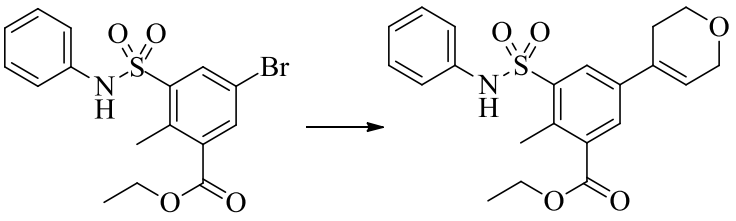
Ethyl 5-bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoate 75



74 **75**

5-Bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoic acid **74** (517 mg, 1.396 mmol) was dissolved in ethanol (5 mL). To this, thionyl chloride (0.306 mL, 4.19 mmol) was added and stirred for 96 h. The solvent and thionyl chloride were removed *in vacuo*. The residue was dissolved in water (100 mL) and then extracted into ethyl acetate (2 x 100 mL). The organic layer was washed with brine (100 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 50 % TBME/cyclohexane at 222 nm) gave ethyl 5-bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoate **75** (349 mg, 62 % over 2 steps) as a dark brown oil. LCMS (Formic acid) 97 %, R_t= 1.22, $[MH]^-$= 396, 398; δ_H (400 MHz, $CDCl_3$) 8.28 (1H, d, *J* = 2.2 Hz), 8.03 (1H, d, *J* = 2.2 Hz), 7.31 – 7.25 (2H, m), 7.18 – 7.12 (1H, m), 7.08 – 7.03 (2H, m), 6.79 (1H, s), 4.40 (2H, q, *J* = 7.1 Hz), 2.80 (3H, s), 1.42 (3H, t, *J* = 7.1 Hz); δ_C (101 MHz, $CDCl_3$) 165.7, 140.9, 136.9, 136.7, 135.6, 135.5, 135.4, 129.5, 125.8, 121.3, 119.2, 62.0, 16.6, 14.1; ν_{max} (liquid film)/cm^{-1} 3257, 3072, 1716, 1648, 1596, 1482, 1413, 1320, 1283, 1149, 1011.

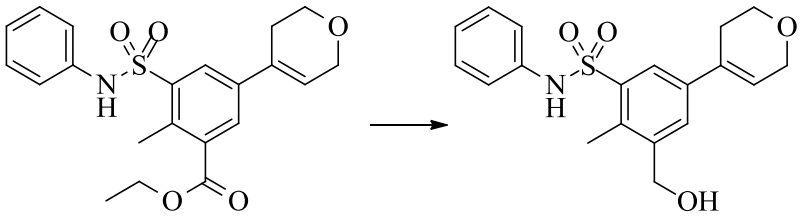
Ethyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzoate 76



75 **76**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (174 mg, 0.829 mmol), ethyl 5-bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoate **75** (300 mg, 0.753 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (42 mg, 0.075 mmol) and tripotassium phosphate (512 mg, 2.41 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) were heated and stirred in a Biotage microwave reactorat 80 °C for 60 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. Purification by flash chromatography (0 – 100 % TBME/cyclohexane at 222 nm) gave ethyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzoate **76** (170 mg, 56 %) as an orange solid. M.P. 100-102 °C; LCMS (Formic acid) 98 %, R_t= 1.11, $[MH]^-$= 400; δ_H (400 MHz, $CDCl_3$) 8.14 (1H, d, *J* = 2.1 Hz), 7.88 (1H, d, *J* = 2.1 Hz), 7.28 – 7.22 (2H, m), 7.15 – 7.09 (1H, m), 7.06 – 7.03 (2H, m), 6.71 (1H, s), 6.20 – 6.18 (1H, m), 4.41 (2H, q, *J* = 7.1 Hz), 4.35 – 4.32 (2H, m), 3.93 (2H, t, *J* = 5.4 Hz), 2.84 (3H, s), 2.50 – 2.41 (2H, m), 1.42 (3H, t, *J* = 7.1 Hz); δ_C (101 MHz, $CDCl_3$) 167.3, 139.4, 138.0, 136.1, 136.0, 134.5, 131.9, 130.0, 129.4, 128.8, 125.4, 124.8, 121.1, 65.6, 64.1, 61.6, 26.7, 16.8, 14.2; ν_{max} (liquid film)/cm^{-1} 3279, 3034, 2951, 1722, 1491, 1450, 1333, 1215, 1145; 1101; *m/z* (ES) Found: $[MH]^+$ 402.1372, $C_{21}H_{24}NO_5S$ is $[MH]^+$ 402.1370.

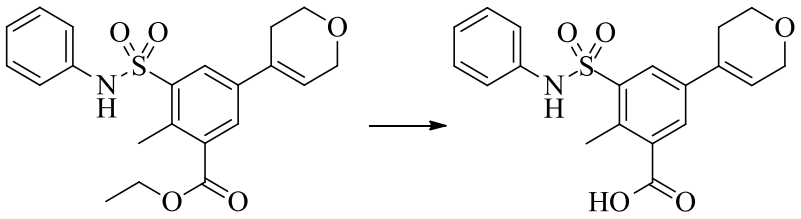
5-(3,6-Dihydro-2*H*-pyran-4-yl)-3-(hydroxymethyl)-2-methyl-*N*-phenylbenzene sulfonamide 65



Sodium borohydride (25 mg, 0.661 mmol), calcium chloride (35 mg, 0.315 mmol) and THF (10 mL) were stirred under nitrogen for 1 h. To this, ethyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzoate **76** (40 mg, 0.1 mmol) in THF (0.5 mL) was added dropwise at 0 °C under nitrogen. The reaction was further stirred for 5 days and was then quenched by the addition of methanol (10 mL) and concentrated *in vacuo*. The residue was dissolved in water (10 mL) and then extracted into ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on XBridge

column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-3-(hydroxymethyl)-2-methyl-*N*-phenylbenzene sulfonamide **65** (18.6 mg, 52 %) as a white solid. M.P. 185-187 °C; LCMS (Formic acid) 100 %, R_t= 0.88, $[MH]^-$= 358; δ_H (400 MHz, DMSO-d_6) 10.39 (1H, s), 7.84 (1H, d, *J* = 1.9 Hz), 7.70 (1H, d, *J* = 1.7 Hz), 7.24 – 7.18 (2H, m), 7.08 (2H, m), 6.97 (1H, m) 6.26 – 6.19 (1H, m), 5.25 (1H, t, *J* = 5.4 Hz), 4.53 (2H, d, *J* = 5.4 Hz), 4.22 (2H, m), 3.82 (2H, t, *J* = 5.4 Hz), 2.51 (3H, s), 2.38 (2H, m); δ_C (101 MHz, DMSO-d_6) 143.6, 138.9, 138.2, 137.1, 133.6, 132.4, 129.6, 127.5, 124.2, 123.9, 119.2, 65.4, 63.9, 61.4, 26.7, 14.6. One carbon is not observed. ν_{max} (liquid film)/cm^{-1} 3536, 3129, 2887, 1600, 1493, 1421, 1337, 1229, 1151, 1121, 1061; *m/z* (ES) Found: $[MH]^+$ 360.1264, $C_{19}H_{22}NO_4S$ is $[MH]^+$ 360.1264.

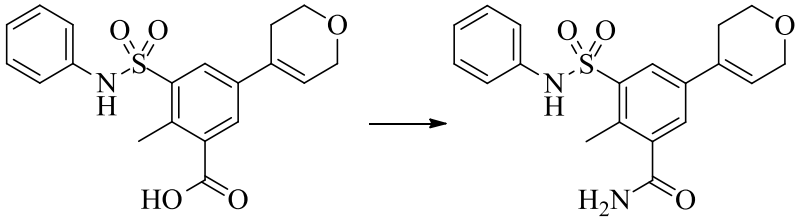
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzoic acid 66



76 **66**

Ethyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzoate **76** (85 mg, 0.212 mmol) was dissolved in THF (0.5 mL) and water (0.5 mL). To this, 2 M aqueous lithium hydroxide (0.233 mL, 0.466 mmol) was added and stirred for 16 h. The THF was removed under a flow of nitrogen. The solution was acidified to pH 5, filtered and washed with further water (5 mL) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoic acid **66** (74 mg, 94 %) as a pale yellow solid. M.P. 191 °C (decomposition); LCMS (Formic acid) 99 %, R_t= 0.88, $[MH]^-$= 372; δ_H (400 MHz, DMSO-d_6) 13.41 (1H, s), 10.53 (1H, s), 8.04 (1H, d, *J* = 2.0 Hz), 7.86 (1H, d, *J* = 1.9 Hz), 7.25 (2H, m), 7.10 (2H, m), 7.01 (1H, m), 6.32 (1H, m), 4.22 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 2.70 (3H, s), 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 169.0, 139.9, 137.8, 137.6, 136.3, 134.9, 131.5, 129.7, 129.0, 127.4, 125.5, 124.3, 119.6, 65.4, 63.8, 26.5, 16.7; ν_{max} (liquid film)/cm^{-1} 3297, 3089, 2849, 1723, 1603, 1488, 1424, 1335, 1318, 1192, 1136, 1092; *m/z* (ES) Found: $[MH]^+$ 374.1060, $C_{19}H_{20}NO_5S$ is $[MH]^+$ 374.1057.

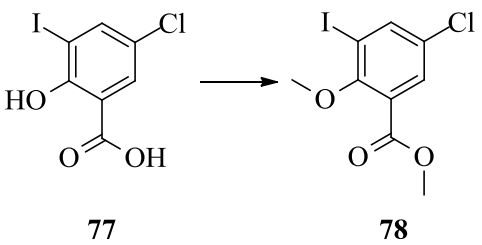
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzamide 67



66 **67**

A mixture of 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzoic acid **66** (50 mg, 0.134 mmol), DIPEA (0.047 mL, 0.268 mmol), and HATU (56 mg, 0.147 mmol) were dissolved in DMF (1 mL) and stirred for 30 min. To this, ammonia (1.339 mL, 0.669 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture as stirred at room temperature for 72 h and then diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), aqueous lithium chloride (2 x 10 mL, 9 %), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methyl-3-(*N*-phenylsulfamoyl)benzamide **67** (20 mg, 40 %) as a cream coloured solid. M.P. 184-186 °C; LCMS (Formic acid) 100 %, R_t= 0.79, $[MH]^-$= 371; δ_H (400 MHz, DMSO-d_6) 7.93 (1H, d, *J* = 2.6 Hz), 7.92 (1H, s), 7.56 (1H, s), 7.53 (1H, d, *J* = 1.9 Hz), 7.26 – 7.19 (2H, m), 7.12 – 7.08 (2H, m), 7.01 – 6.96 (1H, m), 6.32 – 6.29 (1H, m), 4.24 – 4.20 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 2.58 (3H, s), 2.39 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 170.5, 141.3, 139.7, 137.4, 132.7, 131.8, 129.6, 127.1, 125.5, 125.1, 123.9, 123.8, 119.4, 65.4, 63.9, 26.5, 16.4; ν_{max} (liquid film)/cm^{-1} 3363, 3263, 3073, 2957, 2827, 1660, 1599, 1490, 1426, 1382, 1343, 1310, 1291, 1152; *m/z* (ES) Found: $[MH]^+$ 373.1218, $C_{19}H_{21}N_2O_4S$ is $[MH]^+$ 373.1217.

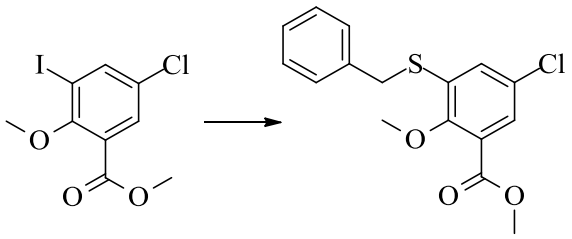
Methyl 5-chloro-3-iodo-2-methoxybenzoate 78



77 **78**

5-Chloro-2-hydroxy-3-iodobenzoic acid **77** (500 mg, 1.675 mmol) and potassium carbonate (718 mg, 5.19 mmol) were dissolved in acetone (5 mL) and to this methyl iodide (0.314 mL, 5.03 mmol) was added and stirred at 60 °C for 6 h. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (50 mL) and then extracted into ethyl acetate (50 mL). The organic layer was washed with brine (25 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 50 % TBME/cyclohexane) provided methyl 5-chloro-3-iodo-2-methoxybenzoate **78** (310 mg, 57 %) as a crude brown oil. LCMS (Formic acid) 80 %, R_t= 1.24, $[MH]^+$= 327, 329; δ_H (400 MHz, $CDCl_3$) 7.93 (1H, d, *J* = 2.6 Hz), 7.78 (1H, d, *J* = 2.6 Hz), 3.94 (3H, s), 3.89 (3H, s). The product was used as a crude component in further reactions.

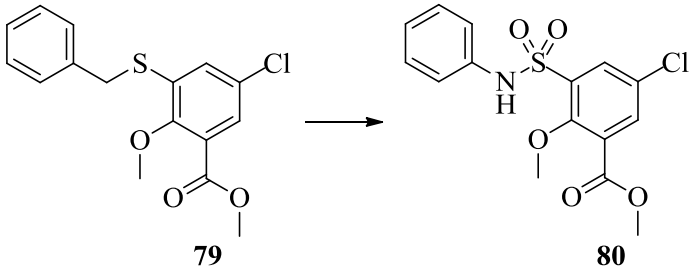
Methyl 3-(benzylthio)-5-chloro-2-methoxybenzoate 79



78 **79**

A mixture of methyl 5-chloro-3-iodo-2-methoxybenzoate **78** (200 mg, 0.613 mmol), benzyl mercaptan (0.08 mL, 0.696 mmol), $Pd_2(dba)_3$ (28 mg, 0.031 mmol), XantPhos (35 mg, 0.06 mmol) and DIPEA (0.055 mL, 0.315 mmol) in toluene (2 mL) was heated and stirred in a Biotage microwave reactorat 100 °C for 60 min. The reaction was passed through a pad of Celite, eluting with methanol and concentrated *in vacuo*. The residue was dissolved in water (100 mL) and then extracted into ethyl acetate (100 mL). The organic layer was washed with brine (2 x 100 mL) passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 20 % TBME/cyclohexane) provided methyl 3-(benzylthio)-5-chloro-2-methoxybenzoate **79** (153 mg, 77 %) as a crude orange oil. LCMS (Formic acid) 66 %, R_t= 1.35, $[MH]^+$= 323, 325; δ_H (400 MHz, $CDCl_3$) 7.59 (1H, d, *J* = 2.8 Hz), 7.41 – 7.29 (6H, m), 4.14 (2H, s), 3.94 (3H, s), 3.91 (3H, s). he product was used as a crude component in further reactions.

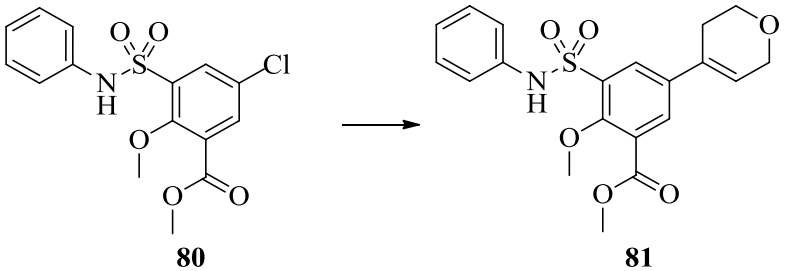
5-Chloro-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate 80



79 **80**

Methyl 3-(benzylthio)-5-chloro-2-methoxybenzoate **79** (153 mg, 0.474 mmol) was dissolved in aqueous hydrochloric acid (0.948 mL, 1.896 mmol, 2 M) and acetonitrile (10 mL) and to this, NCS (190 mg, 1.422 mmol) was added portionwise over 1 h and then stirred for 1 h. The reaction was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give a colourless oil. The oil was re-dissolved in anhydrous pyridine (1 mL) and to this aniline (0.052 mL, 0.57 mmol) was added and the reaction mixture was stirred for 21 h. The reaction was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with aqueous hydrochloric acid (10 mL, 2 M), brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 70 % TBME/cyclohexane at 222 nm) provided methyl 5-chloro-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **80** (65 mg, 38 %) as a colourless oil. LCMS (Formic acid) 100 %, R_t= 1.11, $[MH]^+$= 354, 356; δ_H (400 MHz, CD_3OD) 7.97 (1H, d, *J* = 2.8 Hz), 7.91 (1H, d, *J* = 2.8 Hz), 7.25 – 7.18 (2H, m), 7.16 – 7.13 (2H, m), 7.09 – 7.04 (1H, m), 4.01 (3H, s), 3.95 (3H, s). Sulfonamide N-H not observed.

Methyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate 81

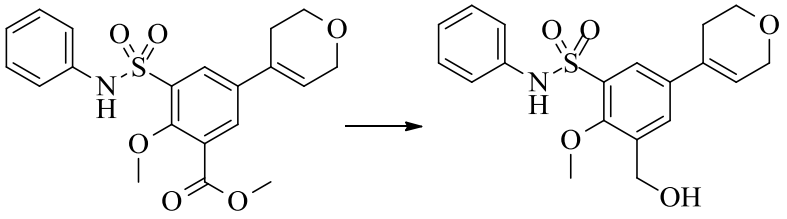


80 **81**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (39 mg, 0.186 mmol), methyl 5-chloro-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **80** (60 mg,

0.169 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (9 mg, 0.016 mmol) and tripotassium phosphate (115 mg, 0.54 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) were heated and stirred in a Biotage microwave reactorat 100 °C for 90 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. Purification by flash chromatography (0 – 100 % TBME/cyclohexane then 0 – 30 % methanol/TBME at 222 nm) provided methyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **81** (67 mg, 98 %) as a white solid. M.P. 161-163 °C; LCMS (Formic acid) 95 %, R_t= 1.01, $[MH]^-$= 402; δ_H (400 MHz, $CDCl_3$) 8.03 (1H, d, *J* = 2.5 Hz), 7.93 (1H, d, *J* = 2.5 Hz), 7.26 – 7.19 (2H, m), 7.14 – 7.09 (3H, m), 7.02 (1H, s), 6.17 – 6.15 (1H, m), 4.32 – 4.29 (2H, m), 4.12 (3H, s), 4.00 (3H, s), 3.91 (2H, t, *J* = 5.4 Hz), 2.48 – 2.39 (2H, m); δ_C (101 MHz, $CDCl_3$) 164.9, 156.5, 136.1, 136.1, 133.9, 132.6, 131.5, 130.0, 129.2, 126.0, 125.1, 125.0, 122.8, 65.6, 64.5, 64.1, 52.7, 26.8; ν_{max} (liquid film)/cm^{-1} 3279, 3034, 2951, 1722, 1491, 1450, 1333, 1215, 1145; 1101; *m/z* (ES) Found: $[MH]^+$ 404.1166, $C_{20}H_{22}NO_6S$ is $[MH]^+$ 404.1163.

5-(3,6-Dihydro-2*H*-pyran-4-yl)-3-(hydroxymethyl)-2-methoxy-*N*-phenylbenzene sulfonamide 68

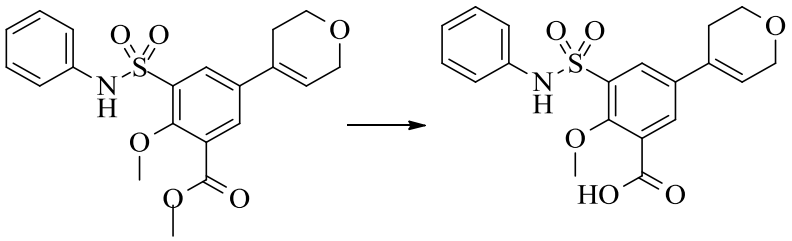


81 **68**

Sodium borohydride (12 mg, 0.312 mmol), calcium chloride (17 mg, 0.156 mmol) and THF (10 mL) were stirred under nitrogen for 1 h. To this, methyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **81** (30 mg, 0.074 mmol) in THF (0.5 mL) was added dropwise at 0 °C under nitrogen. The reaction was further stirred for 21 h and was then quenched by the addition of methanol (10 mL) and concentrated *in vacuo*. The residue was dissolved in water (10 mL) and then extracted into ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-3-(hydroxymethyl)-2-methoxy-*N*-phenyl benzene-sulfonamide **68** (5.1 mg, 18 %) as an off-white solid. M.P. 169-171 °C; LCMS (Formic acid) 100 % , R_t= 0.86, $[MH]^-$= 374; δ_H (400 MHz, DMSO-d_6) 7.74 (1H, d, *J* = 2.4 Hz), 7.71 (1H,

d, *J* = 2.4 Hz), 7.23 – 7.15 (2H, m), 7.08 (2H, m), 6.96 – 6.92 (1H, m), 6.23 – 6.20 (1H, m), 5.46 – 5.27 (1H, m), 4.62 – 4.49 (2H, m), 4.23 – 4.20 (2H, m), 3.84 (3H, s), 3.83 – 3.79 (2H, m), 2.41 – 2.36 (2H, m). Sulfonamide N-H not observed. δ_C (126 MHz, DMSO-d_6) 154.2, 138.0, 135.4, 133.4, 132.2, 129.9, 129.4, 124.5, 124.2, 123.5, 119.5, 119.3, 65.4, 63.9, 63.1, 57.8, 26.8; ν_{max} (liquid film)/cm^{-1} 3553, 3455, 3139, 2897, 1602, 1473, 1423, 1228, 1157, 1128; *m/z* (ES) Found: $[MH]^+$ 376.1213, $C_{19}H_{22}NO_5S$ is $[MH]^+$ 376.1213.

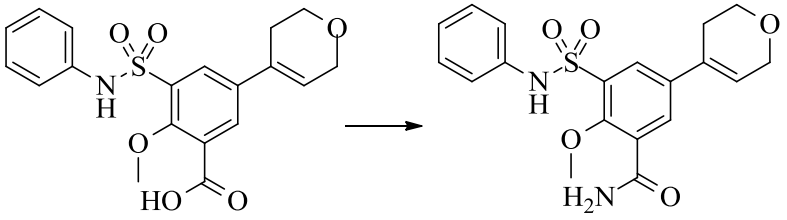
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoic acid 69



81 **69**

Methyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **81** (36 mg, 0.089 mmol) was dissolved in THF (0.5 mL) and water (0.5 mL). To this, 2 M aqueous lithium hydroxide (0.098 mL, 0.196 mmol) was added and stirred for 16 h. The THF was removed *in vacuo*. The solution was acidified to pH 5, filtered and washed with further water (5 mL) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoic acid **69** (26 mg, 75 %) as a cream-coloured solid. M.P. 202 °C (decomposition); LCMS (Formic acid) 95 %, R_t= 0.88, $[MH]^-$= 388; δ_H (400 MHz, DMSO-d_6) 13.51 (1H, s), 10.23 (1H, s), 7.94 (1H, d, *J* = 2.5 Hz), 7.91 (1H, d, *J* = 2.5 Hz), 7.22 (2H, m), 7.12 (2H, m), 6.99 (1H, m), 6.29 (1H, m), 4.21 (2H, m), 3.90 (3H, s), 3.81 (2H, t, *J* = 5.4 Hz), 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 156.3, 138.0, 134.9, 133.9, 131.9, 131.3, 129.6, 128.9, 128.8, 125.2, 124.1, 119.7, 65.4, 63.9, 63.5, 26.6; ν_{max} (liquid film)/cm^{-1} 3139, 2972, 2889, 2856, 1708, 1599, 1488, 1417, 1343, 1226, 1156; *m/z* (ES) Found: $[MH]^+$ 390.1007, $C_{19}H_{20}NO_6S$ is $[MH]^+$ 390.1006.

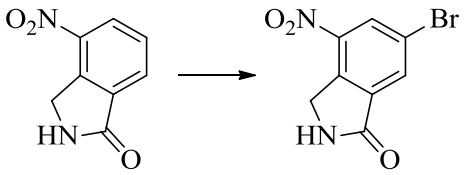
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzamide 70



69 **70**

A mixture of 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoic acid **69** (20 mg, 0.051 mmol), DIPEA (0.018 mL, 0.103 mmol), and HATU (22 mg, 0.058 mmol) were dissolved in DMF (1 mL) and stirred for 30 min. To this, ammonia (0.514 mL, 0.257 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at room temperature for 72 h and then diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), aqueous lithium chloride (2 x 10 mL, 9 %), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzamide **70** (9.2 mg, 46 %) as a white solid. M.P. 177-179 °C; LCMS (Formic acid) 100 %, R_t= 0.82, $[MH]^+$= 389; δ_H (400 MHz, DMSO-d_6) 7.96 (1H, s), 7.86 (1H, t, *J* = 2.5 Hz), 7.70 (1H, s), 7.65 (1H, t, *J* = 2.5 Hz), 7.24 – 7.19 (2H, m), 7.15 – 7.11 (2H, m), 6.99 – 6.95 (1H, m), 6.30 – 6.27 (1H, m), 4.23 – 4.20 (2H, m), 3.89 (3H, s), 3.81 (2H, t, *J* = 4.3 Hz), 2.40 (2H, m). Sulfonamide N-H not observed. δ_C (126 MHz, DMSO-d_6) 168.0, 154.4, 138.5, 134.5, 133.1, 131.6, 131.5, 130.5, 129.5, 127.0, 124.8, 123.8, 119.5, 65.4, 63.9, 62.5, 26.6; ν_{max} (liquid film)/cm^{-1} 3383, 3194, 3049, 2940, 1685, 1574, 1412, 1360, 1310, 1290, 1169, 1140; *m/z* (ES) Found: $[MH]^+$ 389.1164, $C_{19}H_{21}N_2O_5S$ is $[MH]^+$ 389.1166.

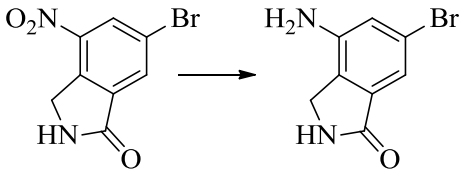
6-Bromo-4-nitroisoindolin-1-one 84



83 **84**

A mixture of 4-nitroisoindolin-1-one **83** (1 g, 5.61 mmol) and NBS (1.2 g, 6.74 mmol) were dissolved in concentrated sulfuric acid (2 mL, 37.5 mmol) and stirred at 70 °C for 2 h. The reaction was cooled, diluted with water (50 mL), neutralised to pH 7 with saturated sodium carbonate (50 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layer was washed with brine (100 mL), passed through a hydrophobic frit and the solvent concentrated *in vacuo* to give 6-bromo-4-nitroisoindolin-1-one **84** (1.4 g, 97 %) as a yellow solid. M.P. 205 °C decomposition; LCMS (Formic acid) 98 %, R_t= 0.76, $[MH]^+$= 257, 259; δ_H (400 MHz, DMSO-d_6) 9.16 – 9.03 (1H, m), 8.54 (1H, d, *J* = 1.7 Hz), 8.24 (1H, d, *J* = 1.6 Hz), 4.76 – 4.72 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 144.5, 138.9, 138.1, 132.3, 129.4, 121.9, 46.3; ν_{max} (liquid film)/cm^{-1} 3135, 3079, 2964, 2887, 1705, 1676, 1524, 1344, 1162.

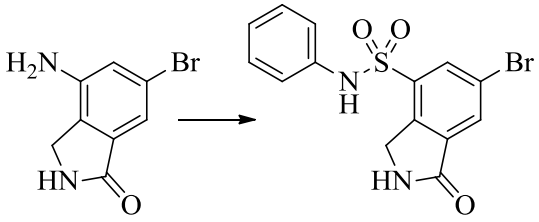
4-Amino-6-bromoisoindolin-1-one 85



84 **85**

A mixture of 6-bromo-4-nitroisoindolin-1-one **84** (1.35 g, 5.25 mmol, ammonium chloride (0.140 g, 2.63 mmol) and iron (0.880 g, 15.76 mmol) was dissolved in ethanol (5 mL) and water (2 mL) and stirred at 80 °C for 17 h. The reaction was cooled and the solvent concentrated *in vacuo*. The residue was diluted with water (50 mL) and ethyl acetate (50 mL) and filtered through Celite, further washing the Celite with water (50 mL) and ethyl acetate (50 mL). The organic layer was separated, washed with brine (50 mL), passed through a hydrophobic frit and the solvent concentrated *in vacuo* to give 4-amino-6-bromoisoindolin-1-one **85** (708 mg, 59 %) as a yellow solid. M.P. 228 °C decomposition; LCMS (Formic acid) 88 %, R_t= 0.76, $[MH]^+$= 227, 229; δ_H (400 MHz, DMSO-d_6) 8.51 – 8.43 (1H, m), 6.93 – 6.90 (2H, m), 4.10 (2H, d, *J* = 0.8 Hz). Aniline NH_2 is not observed. δ_C (101 MHz, DMSO-d_6) 169.7, 145.9, 135.6, 127.5, 121.8, 117.9, 112.6, 43.7; ν_{max} (liquid film)/cm^{-1} 3334, 3179, 3064, 1685, 1637, 1599, 1333, 1162; *m/z* (ES) Found: $[MH]^+$ 226.9818, $C_8H_8BrN_2O$ is $[MH]^+$ 226.9815.

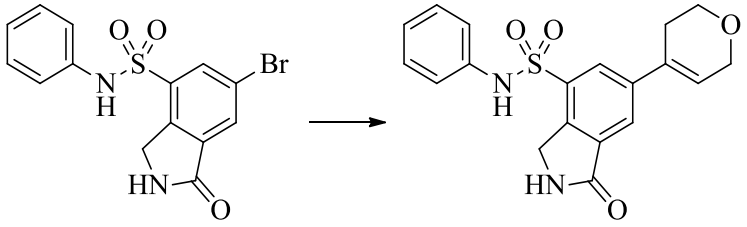
6-Bromo-1-oxo-*N*-phenylisoindoline-4-sulfonamide 86



85 **86**

In a 2-necked flask, thionyl chloride (10 mL, 137 mmol) was added dropwise to water (10 mL) over 60 min and then stirred for 24 h. Copper (I) chloride (6 mg, 0.061 mmol) was added to the mixture and the resultant yellow green solution was cooled to 0 °C. In a separate 3-necked flask, 37.5 % aqueous hydrochloric acid (4 mL, 132 mmol) was added with vigorous stirring to 4-amino-6-bromoisoindolin-1-one **85** (700 mg, 3.08 mmol) and then cooled to -5 °C. To this, sodium nitrite (425 mg, 6.17 mmol) in water (10 mL) was added dropwise over 1 h at -5 °C, and then further stirred for 10 min. This mixture was added via cannula to the 2-necked flask over 30 min at -5 °C and further stirred for 1 h. The precipitated solid was collected via vacuum filtration and washed with aqueous hydrochloric acid (2 x 10 mL, 2 M) to give 6-bromo-1-oxoisoindoline-4-sulfonyl chloride (730 mg, 76 %, 76 % pure via LCMS) of a yellow solid. A mixture of 6-bromo-1-oxoisoindoline-4-sulfonyl chloride (260 mg, 0.837 mmol) and aniline (0.080 mL, 0.879 mmol) were combined in pyridine (1 mL). The resulting mixture was stirred at room temperature for 2 h, diluted with water (25 mL) and extracted with ethyl acetate (3 x 25 mL). The organic layer was washed with aqueous hydrochloric acid (2 x 25 mL, 2 M), brine (50 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (75 – 100 % TBME/cyclohexane then 0 – 10 % methanol in TBME) provided 6-bromo-1-oxo-*N*-phenylisoindoline-4-sulfonamide **86** (191 mg, 62 %) as a white solid. LCMS (Formic acid) 99 %, R_t= 0.92, $[MH]^+$= 367, 369; δ_H (400 MHz, DMSO-d_6) 10.44 (1H, s), 8.95 – 8.87 (1H, m), 8.03 (1H, d, *J* = 1.7 Hz), 7.95 (1H, d, *J* = 1.7 Hz), 7.31 – 7.22 (2H, m), 7.14 – 7.08 (1H, m), 7.08 – 7.02 (2H, m), 4.38 – 4.34 (2H, m).

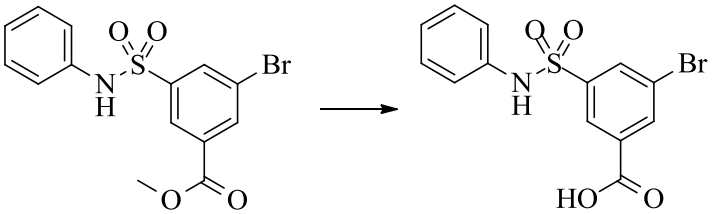
6-(3,6-Dihydro-2*H*-pyran-4-yl)-1-oxo-*N*-phenylisoindoline-4-sulfonamide 82



86 **82**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (23 mg, 0.112 mmol), 6-bromo-1-oxo-*N*-phenylisoindoline-4-sulfonamide **86** (39 mg, 0.106 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (6 mg, 10.71 µmol) and tripotassium phosphate (72 mg, 0.34 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 6-(3,6-dihydro-2*H*-pyran-4-yl)-1-oxo-*N*-phenylisoindoline-4-sulfonamide **82** (25 mg, 63 %) as a white solid. M.P. 239-241 °C; LCMS (Formic acid) 100 %, R_t= 0.85, $[MH]^+$= 371; δ_H (400 MHz, DMSO-d_6) 10.34 (1H, s), 8.79 (1H, s), 7.97 – 7.80 (2H, m), 7.32 – 7.17 (2H, m), 7.13 – 7.02 (3H, m), 6.44 – 6.34 (1H, m), 4.43 (2H, s), 4.28 – 4.19 (2H, m), 3.83 (2H, t, *J* = 5.3 Hz), 2.48 – 2.37 (2H, m); δ_C (101 MHz, DMSO-d_6) 168.3, 141.0, 140.4, 137.5, 135.4, 135.2, 131.8, 129.7, 126.7, 126.1, 125.3, 123.1, 121.6, 65.4, 63.9, 45.4, 26.7; ν_{max} (liquid film)/cm^{-1} 3251, 3192, 3079, 1709, 1597, 1336, 1152, 1134; *m/z* (ES) Found: $[MH]^+$ 371.1075, $C_{19}H_{19}N_2O_4S$ is $[MH]^+$ 371.1060.

3-Bromo-5-(*N*-phenylsulfamoyl)benzoic acid 60

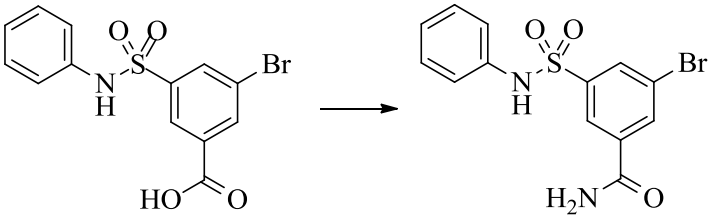


54 **60**

Methyl 3-bromo-5-(*N*-phenylsulfamoyl)benzoate **54** (1.1 g, 2.97 mmol) was dissolved in THF (4 mL) and water (4 mL). To this, aqueous lithium hydroxide (4.46 mL, 8.91 mmol, 2

M) was added and stirred for 16 h. The THF was removed *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid, filtered and washed with further water (5 mL) to give 3-bromo-5-(*N*-phenylsulfamoyl)benzoic acid **60** (1.02 g, 96 %) as a white solid. LCMS (Formic acid) 95 %, R_t= 1.07, $[MH]^-$= 354, 356; δ_H (400 MHz, DMSO-d_6) 13.81 (1H, s), 10.43 (1H, s), 8.25 – 8.20 (2H, m), 8.08 – 8.03 (1H, m), 7.33 – 7.23 (2H, m), 7.14 – 7.05 (3H, m).

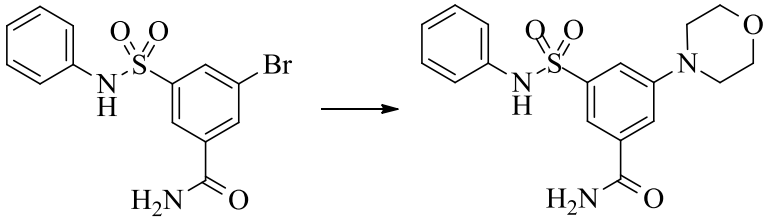
3-Bromo-5-(*N*-phenylsulfamoyl)benzamide 91



60 **91**

3-Bromo-5-(*N*-phenylsulfamoyl)benzoic acid **60** (1 g, 2.81 mmol) was dissolved in thionyl chloride (5 mL, 68.5 mmol) and stirred at 70 °C for 3 h. The thionyl chloride was concentrated *in vacuo*. The residue was diluted with THF (5 mL) and added dropwise to ammonium hydroxide (10 mL, 71.9 mmol, 28 %) at 0 °C. The THF was concentrated *in vacuo*. The residue was neutralised to pH 7 with 2 M aqueous hydrochloric acid and extracted into ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and concentrated *in vacuo.* The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-55 % acetonitrile in water with an ammonium carbonate modifier on a 130 g C_{18} column to give to give 3-bromo-5-nitrobenzamide **91** (791 mg, 79 %) a cream solid. LCMS (Formic acid) 82 %, R_t= 0.90, $[MH]^+$= 355, 357; δ_H (400 MHz, DMSO-d_6) 10.40 (1H, s), 8.30 – 8.28 (1H, m), 8.26 (1H, s), 8.24 – 8.23 (1H, m), 8.00 – 7.95 (1H, m), 7.70 (1H, s), 7.31 – 7.23 (2H, m), 7.13 – 7.04 (3H, m).

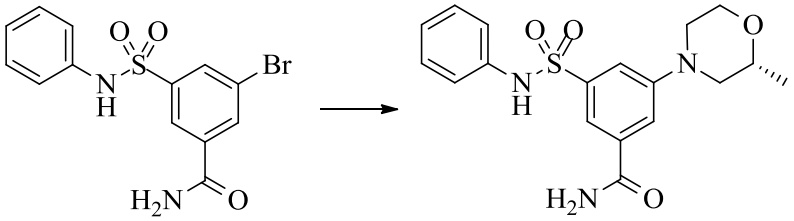
3-Morpholino-5-(*N*-phenylsulfamoyl)benzamide 88



91 **88**

A mixture of 3-bromo-5-(*N*-phenylsulfamoyl)benzamide **91** (100 mg, 0.282 mmol)XPhos (13 mg, 0.028 mmol), LHMDS (1.12 ml, 1.126 mmol, 1 M in THF), $Pd_2(dba)_3$ (13 mg, 0.014 mmol) and morpholine (0.034 mL, 0.394 mmol) were combined and stirred at 65 °C for 17 h. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (0.6 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) then further purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-morpholino-5-(*N*-phenylsulfamoyl)benzamide **88** (24 mg, 23 %) as a white solid. M.P. 178-180 °C; LCMS (Formic acid) 100 %, R_t= 0.77, $[MH]^+$= 362; δ_H (400 MHz, DMSO-d_6) 10.22 (1H, s), 8.09 (1H, s), 7.71 – 7.65 (1H, m), 7.60 – 7.56 (1H, m), 7.45 (1H, s), 7.33 – 7.29 (1H, m), 7.26 – 7.17 (2H, m), 7.12 – 7.06 (2H, m), 7.04 – 6.97 (1H, m), 3.78 – 3.69 (4H, m), 3.20 – 3.10 (4H, m); δ_C (101 MHz, DMSO-d_6) 167.1, 151.5, 141.4, 138.7, 136.2, 129.5, 124.2, 120.7, 117.7, 116.4, 114.5, 66.2, 48.2; ν_{max} (liquid film)/cm^{-1} 3493, 3349, 3111, 2968, 2890, 2843, 1663, 1586, 1245, 1163, 1135, 1111, 932; *m/z* (ES) Found: $[MH]^+$ 362.1161, $C_{17}H_{20}N_3O_4S$ is $[MH]^+$ 362.1169.

(*R*)-3-(2-Methylmorpholino)-5-(*N*-phenylsulfamoyl)benzamide 89

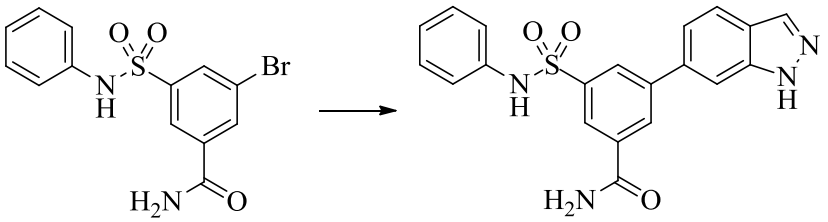


91 **89**

A mixture of 3-bromo-5-(*N*-phenylsulfamoyl)benzamide **91** (100 mg, 0.282 mmol), dicyclohexyl(2',4',6'-triisopropyl-[1,1'-biphenyl]-2-yl)phosphine (13 mg, 0.028 mmol)LHMDS (1.12 mL, 1.126 mmol, 1 M in THF), $Pd_2(dba)_3$ (13 mg, 0.014 mmol) and (*R*)-2-methylmorpholine (40 mg, 0.394 mmol) were combined and stirred at 65 °C for 17 h.

The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1.2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give (*R*)-3-(2-methylmorpholino)-5-(*N*-phenylsulfamoyl)benzamide **89** (39 mg, 36 %) as a white solid. M.P. 161-163 °C; LCMS (Formic acid) 100 %, R_t= 0.83, $[MH]^+$= 376; δ_H (400 MHz, DMSO-d_6) 8.10 (1H, s), 7.70 – 7.67 (1H, m), 7.61 – 7.56 (1H, m), 7.46 (1H, s), 7.31 – 7.28 (1H, m), 7.26 – 7.20 (2H, m), 7.15 – 7.08 (2H, m), 7.06 – 6.99 (1H, m), 3.93 (1H, dd, *J* = 11.5, 2.3 Hz), 3.68 – 3.55 (3H, m), 3.52 (1H, m), 2.70 (1H, td, *J* = 11.9, 3.5 Hz), 2.37 (1H, dd, *J* = 11.7, 10.2 Hz), 1.17 (3H, d, *J* = 6.1 Hz). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 167.1, 151.3, 141.2, 138.4, 136.2, 129.5, 124.4, 120.8, 117.8, 116.3, 114.5, 71.3, 65.9, 54.1, 47.4, 19.2; ν_{max} (liquid film)/cm^{-1} 3437, 3177, 2965, 2886, 2834, 1661, 1593, 1572, 1249, 1165, 1134, 1015; *m/z* (ES) Found: $[MH]^+$ 376.1314, $C_{18}H_{22}N_3O_4S$ is $[MH]^+$ 376.1326; $[\alpha]_D^{22}$ -44 (*c* 0.01, MeOH).

3-(1*H*-Indazol-6-yl)-5-(*N*-phenylsulfamoyl)benzamide 90

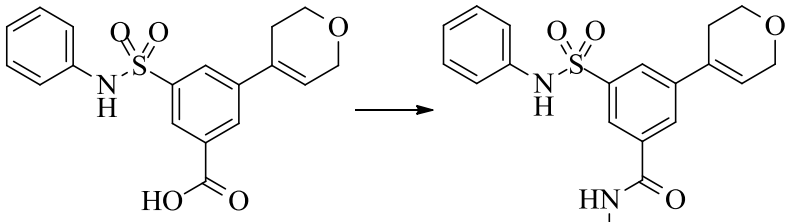


91 **90**

A mixture of 3-bromo-5-(*N*-phenylsulfamoyl)benzamide **91** (50 mg, 0.141 mmol), 6-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)-1*H*-indazole (38 mg, 0.155 mmol), potassium phosphate (93 mg, 0.436 mmol), and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (8 mg, 0.014 mmol) in 1,4-dioxane (1 mL) and water (0.2 mL) were heated and stirred in a Biotage microwave reactorat 120 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(1*H*-indazol-6-yl)-5-(*N*-phenylsulfamoyl)benzamide **90** (19 mg, 34 %) as a white solid. M.P. 200-202 °C; LCMS (Formic acid) 100 %, R_t= 0.88, $[MH]^+$ = 393; δ_H (400 MHz, DMSO-d_6) 13.27 (1H, s), 10.37 (1H, s), 8.46 – 8.41 (1H, m), 8.34 (1H, s), 8.28 – 8.24 (1H, m), 8.17 – 8.12 (2H, m), 7.91 (1H, dd, *J* = 8.4, 0.4 Hz), 7.82 – 7.76 (1H, m), 7.64 (1H, s), 7.41 (1H, dd, *J* = 8.4, 1.5 Hz),

7.30 – 7.22 (2H, m), 7.18 – 7.13 (2H, m), 7.10 – 7.01 (1H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 142.1, 141.3, 140.8, 138.4, 136.4, 136.3, 133.9, 130.2, 129.6, 127.6, 125.3, 124.6, 123.2, 121.8, 121.0, 120.2, 108.8; ν_{max} (liquid film)/cm^{-1} 3396, 3257, 3143, 2971, 1697, 1392, 1334, 1162, 1124; *m/z* (ES) Found: $[MH]^+$ 393.1006, $C_{20}H_{17}N_4O_3S$ is $[MH]^+$ 393.1016.

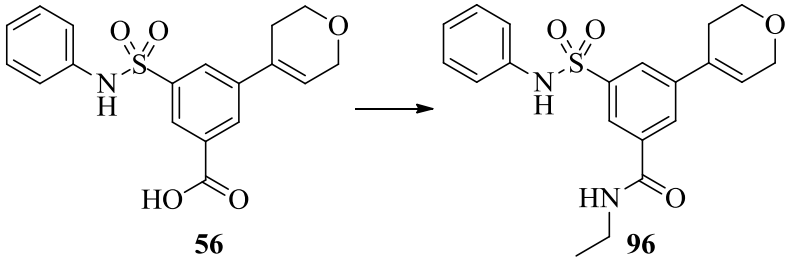
3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-methyl-5-(*N*-phenylsulfamoyl)benzamide 95



56 **95**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) DIPEA (0.058 mL, 0.334 mmol) and HATU (35 mg, 0.092 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 minutes. To this, methanamine (0.125 mL, 0.25 mmol, 2 M in THF) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated under a flow of nitrogen. The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-methyl-5-(*N*-phenylsulfamoyl)benzamide **95** (17 mg, 54 %) as a white solid. M.P. 127-129 °C; LCMS (Formic acid) 99 %, R_t= 0.89, $[MH]^+$= 373; δ_H (400 MHz, DMSO-d_6) 10.21 (1H, s), 8.72 – 8.65 (1H, m), 8.15 – 8.11 (1H, m), 8.10 – 8.07 (1H, m), 7.87 – 7.85 (1H, m), 7.26 – 7.19 (2H, m), 7.11 – 7.05 (2H, m), 7.05 – 6.97 (1H, m), 6.41 – 6.36 (1H, m), 4.27 – 4.24 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.79 (3H, d, *J* = 4.5 Hz), 2.45 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 165.4, 141.0, 140.8, 138.2, 135.9, 132.0, 129.6, 127.1, 126.2, 124.8, 124.7, 124.6, 120.9, 65.4, 63.8, 26.8, 26.7; ν_{max} (liquid film)/cm^{-1} 3286, 3073, 2887, 1640, 1598, 1545, 1491, 1412, 1339, 1147; *m/z* (ES) Found: $[MH]^+$ 373.1226, $C_{19}H_{21}N_2O_4S$ is $[MH]^+$ 373.1217.

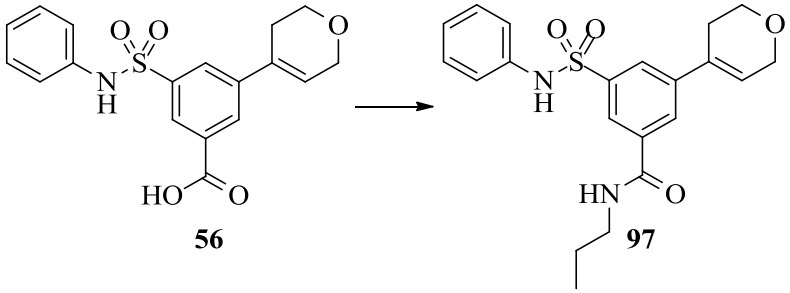
3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-ethyl-5-(*N*-phenylsulfamoyl)benzamide 96



56 **96**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (40 mg, 0.111 mmol), DIPEA (0.097 mL, 0.334 mmol), and HATU (42 mg, 0.111 mmol) were dissolved in DMF (0.5 mL) and stirred for 10 minutes. To this, ethylamine (0.067 mL, 0.134 mmol, 2 M in THF) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated *in vacuo*. The product was dissolved in DMSO (0.5 mL) and purified by MDAP on XBridge column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-ethyl-5-(*N*-phenylsulfamoyl)benzamide **96** (21.6 mg, 47 %) as a white solid. M.P. 180-182 °C; LCMS (Ammonium carbonate) 100 %, R_t= 0.84, $[MH]^-$= 385; δ_H (600 MHz, DMSO-d_6) 10.31 (1H, s), 8.73 (1H, t, *J* = 5.1 Hz), 8.14 – 8.11 (1H, m), 8.10 – 8.07 (1H, m), 7.86 – 7.83 (1H, m), 7.26 – 7.19 (2H, m), 7.11 – 7.06 (2H, m), 7.04 – 7.00 (1H, m), 6.43 – 6.34 (1H, m), 4.29 – 4.20 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.30 – 3.26 (2H, m), 2.45 – 2.36 (2H, m), 1.12 (3H, t, *J* = 7.2 Hz); δ_C (151 MHz, DMSO-d_6) 164.7, 141.0, 140.7, 138.1, 136.1, 132.0, 129.6, 127.2, 126.2, 124.8, 124.7, 124.6, 120.9, 65.3, 63.8, 34.7, 26.7, 15.0; ν_{max} (liquid film)/cm^{-1} 3337, 3062, 2974, 2894, 2860, 1637, 1546, 1497, 1350, 1166, 1114, 936; *m/z* (ES) Found: $[MH]^+$ 387.1381, $C_{20}H_{23}N_2O_4S$ is $[MH]^+$ 387.1373.

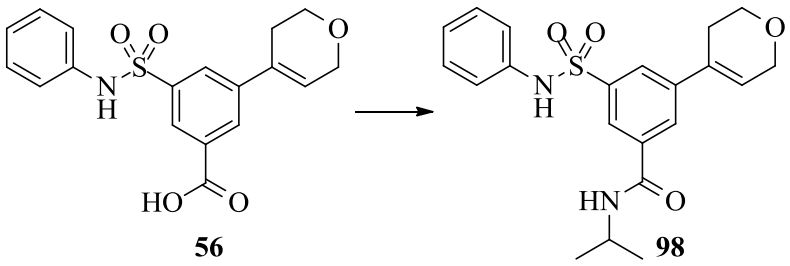
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)-*N*-propylbenzamide 97



56 **97**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) 50 % T_3P in ethyl acetate (0.06 mL, 0.1 mmol, DIPEA (0.058 mL, 0.334 mmol) and propan-1-amine (0.014 mL, 0.167 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 15 h and concentrated under a flow of nitrogen. The product was dissolved in 1:1 MeOH:DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)-*N*-propylbenzamide **97** (18 mg, 53 %) as a white solid. M.P. 193-195 °C; LCMS (Formic acid) 100 %, R_t= 1.05, $[MH]^+$= 401; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 8.71 (1H, t, *J* = 5.6 Hz), 8.14 – 8.12 (1H, m), 8.12 – 8.08 (1H, m), 7.86 – 7.83 (1H, m), 7.26 – 7.19 (2H, m), 7.12 – 7.06 (2H, m), 7.06 – 7.00 (1H, m), 6.41 – 6.37 (1H, m), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.26 – 3.19 (2H, m), 2.46 – 2.37 (2H, m), 1.59 – 1.48 (2H, m), 0.89 (3H, t, *J* = 7.4 Hz); δ_C (101 MHz, DMSO-d_6) 164.9, 141.0, 140.8, 136.1, 132.0, 129.6, 127.2, 126.2, 124.8, 124.6, 124.5, 120.9, 119.6, 65.4, 63.8, 41.65, 26.73, 22.72, 11.91; ν_{max} (liquid film)/cm^{-1} 3328, 3057, 2962, 2859, 1636, 1597, 1496, 1347, 1336, 1227, 931; *m/z* (ES) Found: $[MH]^+$ 401.1541, $C_{21}H_{25}N_2O_4S$ is $[MH]^+$ 401.1530.

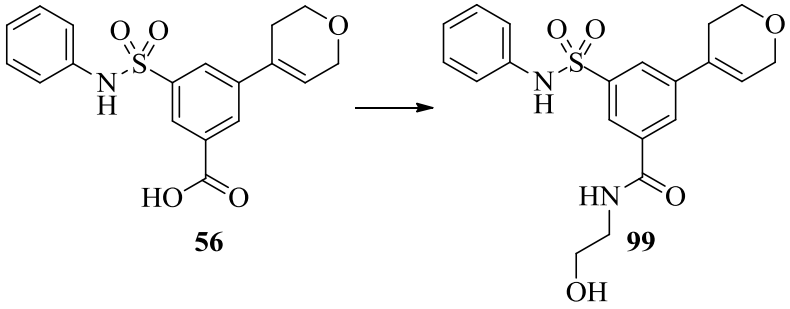
3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-isopropyl-5-(*N*-phenylsulfamoyl)benzamide 98



A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) DIPEA (0.073 mL, 0.417 mmol) and HATU (35 mg, 0.092 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 minutes. To this, propan-2-amine (0.021 mL, 0.125 mmol) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated under a flow of nitrogen. The product was dissolved in 1:1 MeOH:DMSO (0.6 mL) and purified by MDAP on XBridge column using acetonitrile/water with a formic acid modifier (method C) to 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-isopropyl-5-(*N*-phenylsulfamoyl)benzamide **98** (15 mg, 44 %) as a white solid. M.P. 200-202 °C; LCMS (Formic acid) 100 %, R_t= 1.04, $[MH]^+$= 401; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 8.48

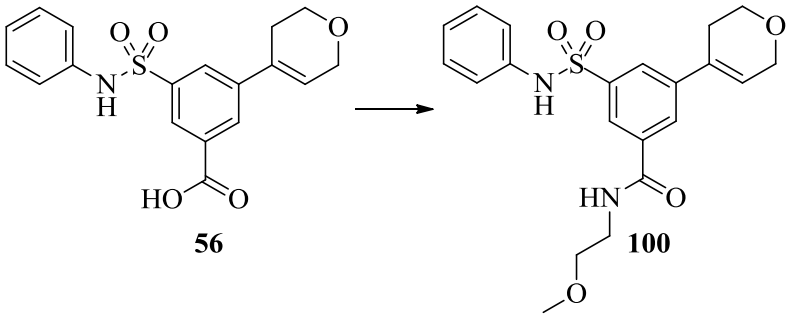
(1H, d, *J* = 7.6 Hz), 8.15 – 8.10 (1H, m), 8.10 – 8.06 (1H, m), 7.85 – 7.82 (1H, m), 7.27 – 7.19 (2H, m), 7.12 – 7.06 (2H, m), 7.06 – 7.00 (1H, m), 6.43 – 6.35 (1H, m), 4.27 – 4.23 (2H, m), 4.16 – 4.04 (1H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.44 – 2.38 (2H, m), 1.17 (6H, d, *J* = 6.6 Hz); δ_C (101 MHz, DMSO-d_6) 164.2, 141.0, 140.7, 136.3, 132.1, 129.6, 127.3, 126.2, 124.8, 124.8, 124.7, 120.9, 119.6, 65.4, 63.8, 41.8, 26.7, 22.7; v_{max} (liquid film)/cm^{-1} 3357, 3079, 2967, 2889, 1658, 1531, 1495; 1227, 1154, 1142, 935; *m/z* (ES) Found: $[MH]^+$ 401.1541, $C_{21}H_{25}N_2O_4S$ is $[MH]^+$ 401.1530.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-(2-hydroxyethyl)-5-(*N*-phenylsulfamoyl)benzamide 99



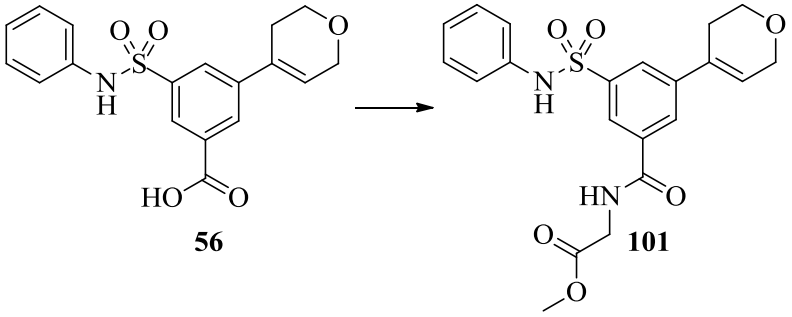
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (40 mg, 0.111 mmol) DIPEA (0.097 mL, 0.334 mmol) and HATU (42 mg, 0.111 mmol) were dissolved in DMF (0.5 mL) and stirred for 10 minutes. To this, ethanolamine (0.008 mL, 0.134 mmol) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated *in vacuo*. The product was dissolved in DMSO (0.5 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-(2-hydroxyethyl)-5-(*N*-phenylsulfamoyl)benzamide **99** (14.5 mg, 30 %) as a white solid. M.P. 163-165 °C; LCMS (Ammonium carbonate) 99 %, R_t= 0.70, $[MH]^+$= 403; δ_H (600 MHz, DMSO-d_6) 10.28 (1H, s), 8.74 (1H, t, *J* = 4.8 Hz), 8.15 – 8.14 (1H, m), 8.13 – 8.12 (1H, m), 7.87 – 7.83 (1H, m), 7.26 – 7.20 (2H, m), 7.11 – 7.07 (2H, m), 7.05 – 7.00 (1H, m), 6.41 – 6.37 (1H, m), 4.73 (1H, s), 4.27 – 4.22 (2H, m), 3.83 (2H, t, *J* = 5.2 Hz), 3.54 – 3.47 (2H, m), 3.36 – 3.33 (2H, m), 2.43 – 2.39 (2H, m); δ_C (151 MHz, DMSO-d_6) 165.1, 141.0, 140.7, 138.0, 136.0, 132.0, 129.6, 127.3, 126.2, 124.8, 124.7, 120.9, 65.3, 63.8, 60.0, 42.8, 26.7. One carbon is not observed. v_{max} (liquid film)/cm^{-1} 3358, 2079, 2967, 2890, 1659, 1637, 1340, 1317, 1151, 1120, 934; *m/z* (ES) Found: $[MH]^+$ 403.1335, $C_{20}H_{23}N_2O_5S$ is $[MH]^+$ 403.1322.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-(2-methoxyethyl)-5-(*N*-phenylsulfamoyl)benzamide 100



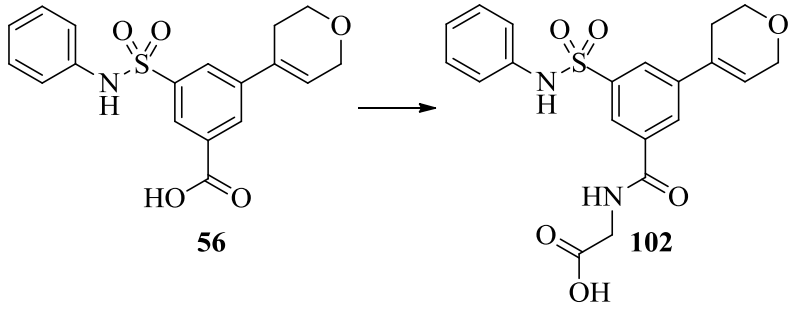
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) 50 % T_3P in ethyl acetate (0.060 mL, 0.1 mmol) DIPEA (0.058 mL, 0.334 mmol) and 2-methoxyethanamine (0.015 mL, 0.167 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 15 h and concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method A) and then XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-(2-methoxyethyl)-5-(*N*-phenylsulfamoyl) benzamide **100** (31 mg, 89 %) as a yellow oil. LCMS (Formic acid) 100 %, R_t= 0.82, $[MH]^+$= 417; δ_H (400 MHz, DMSO-d_6) 10.29 (1H, s), 8.83 (1H, t, *J* = 4.6 Hz), 8.16 – 8.14 (1H, m), 8.14 – 8.10 (1H, m), 7.89 – 7.83 (1H, m), 7.28 – 7.19 (2H, m), 7.13 – 7.07 (2H, m), 7.07 – 7.00 (1H, m), 6.44 – 6.36 (1H, m), 4.28 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.49 – 3.41 (4H, m), 3.27 (3H, s), 2.45 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 165.1, 141.1, 140.6, 137.9, 135.8, 132.0, 129.6, 127.3, 126.3, 124.9, 124.8, 121.1, 120.9, 70.8, 65.4, 63.8, 58.4, 58.3, 26.7; ν_{max} (liquid film)/cm^{-1} 3358, 3077, 2967, 3930, 2892, 1639, 1542, 1495, 1426, 1164, 1151, 1121; *m/z* (ES) Found: $[MH]^+$ 417.1493, $C_{21}H_{25}N_2O_5S$ is $[MH]^+$ 417.1479.

Methyl 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetate 101



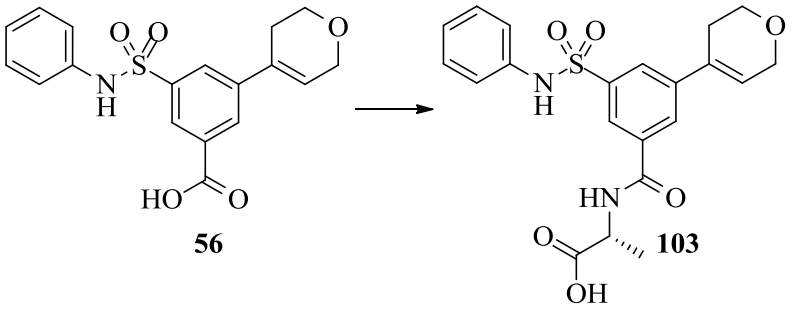
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) 50 % T_3P in ethyl acetate (0.060 mL, 0.1 mmol) DIPEA (0.087 mL, 0.501 mmol) and methyl 2-aminoacetate hydrochloride (11 mg, 0.088 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 15 h and concentrated *in vacuo*. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) and concentrated *in vacuo*. The residue was dissolved in water (10 mL) and extracted with DCM (10 mL). The organic layer was washed with water (3 x 10 mL), passed through a hydrophobic frit and the solvent concentrated *in vacuo*. The residue was dried on a high vacuum line overnight and then dried in a vacuum oven (40 °C, 1 mbar) for 3 days to give methyl 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido) acetate **101** (31 mg, 89 %) as a white solid. M.P. 166-168 °C; LCMS (Formic acid) 95 %, R_t= 0.93, $[MH]^+$= 431;δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 9.25 (1H, t, *J* = 5.8 Hz), 8.21 – 8.12 (2H, m), 7.93 – 7.83 (1H, m), 7.28 – 7.19 (2H, m), 7.13 – 7.07 (2H, m), 7.07 – 7.02 (1H, m), 6.46 – 6.36 (1H, m), 4.28 – 4.23 (2H, m), 4.04 (2H, d, *J* = 5.8 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 3.67 (3H, s), 2.45 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 170.5, 165.5, 141.2, 140.8, 137.8, 135.1, 131.9, 129.7, 127.4, 126.4, 125.2, 124.9, 124.8, 120.9, 65.4, 63.8, 52.2, 41.7, 26.6; v_{max} (liquid film)/cm^{-1} 3360, 3195, 2924, 1726, 1675, 1650, 1619, 1492, 1406, 1226, 1154; *m/z* (ES) Found: $[MH]^+$ 431.1264, $C_{21}H_{23}N_2O_6S$ is $[MH]^+$ 431.1271.

2-(3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetic acid 102



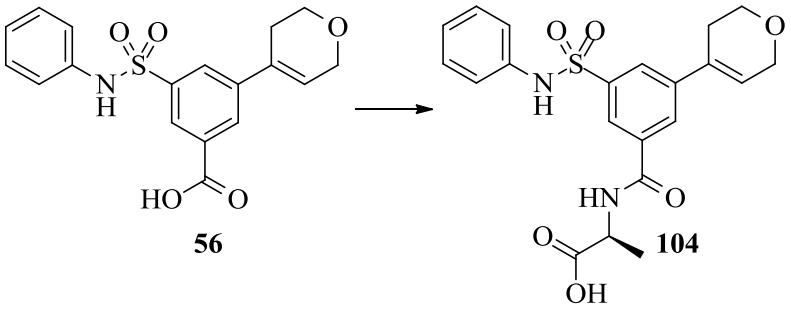
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (50 mg, 0.139 mmol) 50 % T_3P in ethyl acetate (0.1 mL, 0.168 mmol), DIPEA (0.097 mL, 0.556 mmol) and ethyl 2-aminoacetate hydrochloride (25 mg, 0.179 mmol) were dissolved in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 15 h and under a flow of nitrogen. To the residue, water (5 mL) was added and then extracted into ethyl acetate (2 x 5 mL). The organic layer was washed with aqueous lithium chloride (2 x 5 mL, 5 %), brine (5 mL) passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in THF (0.5 mL) and to this, aqueous lithium hydroxide (0.209 mL, 0.417 mmol, 2 M) was added and stirred overnight. The solvent was concentrated *in vacuo*. The product was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetic acid (23 mg, 39 %) as a cream solid. The reaction was repeated on 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (200 mg, 0.556 mmol) to give to give 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetic acid **102** (150 mg, 64 %) as a cream solid. M.P. 200 °C decomposition; LCMS (Formic acid) 97 %, R_t= 0.85, $[MH]^+$= 417; δ_H (400 MHz, DMSO-d_6) 8.92 (1H, t, *J* = 5.6 Hz), 8.18 – 8.16 (1H, m), 8.15 – 8.12 (1H, m), 7.87 – 7.84 (1H, m), 7.27 – 7.19 (2H, m), 7.14 – 7.08 (2H, m), 7.06 – 7.00 (1H, m), 6.42 – 6.36 (1H, m), 4.28 – 4.22 (2H, m), 3.87 – 3.81 (4H, m), 2.45 – 2.38 (2H, m). Sulfonamide N-H and carboxylic acid O-H not observed. δ_C (101 MHz, DMSO-d_6) 171.5, 165.0, 141.1, 140.8, 138.0, 135.7, 132.0, 129.6, 127.3, 126.3, 125.0, 124.7, 121.0, 119.5, 65.4, 63.8, 42.6, 26.7; ν_{max} (liquid film)/cm^{-1} 3221, 3075, 2887, 1723, 1644, 1597, 1540, 1493, 1419, 1340, 1303, 1220, 1150; *m/z* (ES) Found: $[MH]^+$ 417.1118, $C_{20}H_{21}N_2O_6S$ is $[MH]^+$ 417.1115.

(*R*)-2-(3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)propanoic acid 103



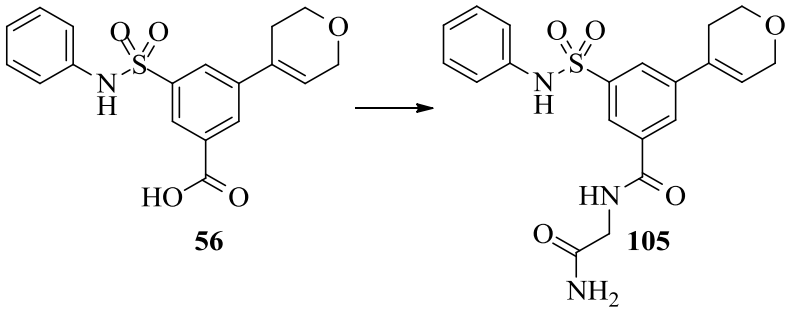
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (40 mg, 0.111 mmol), DIPEA (0.097 mL, 0.334 mmol) and HATU (42 mg, 0.111 mmol) were dissolved in DMF (0.5 mL)and stirred for 10 minutes. To this, (*R*)-methyl 2-aminopropanoate hydrochloride (14 mg, 0.134 mmol) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated *in vacuo*. The residue was dissolved in THF (0.25 mL) and water (0.25 mL). To this, 2 M aqueous lithium hydroxide (0.167 mL, 0.333 mmol) was added and stirred for 16 h. The solvent was concentrated *in vacuo*. The product was dissolved in DMSO (0.5 mL) and purified by MDAP on XBridge column using acetonitrile/water with a formic acid modifier (method B) to give (*R*)-2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)propanoic acid **103** (16.5 mg, 31 %) as a white solid. M.P. 205 °C decomposition; LCMS (Ammonium carbonate) 100 %, R_t= 0.60, $[MH]^-$= 429; δ_H (600 MHz, DMSO-d_6) 8.96 (1H, d, *J* = 7.1 Hz), 8.19 – 8.12 (1H, m), 8.18 – 8.13 (1H, m), 7.88 – 7.82 (1H, m), 7.28 – 7.20 (2H, m), 7.12 – 7.07 (2H, m), 7.05 – 6.99 (1H, m), 6.42 – 6.37 (1H, m), 4.45 – 4.38 (1H, m), 4.27 – 4.23 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 2.44 – 2.36 (2H, m), 1.39 (3H, d, *J* = 7.3 Hz). Sulfonamide N-H and carboxylic acid O-H not observed. δ_C (151 MHz, DMSO-d_6) 174.3, 164.9, 141.1, 140.6, 137.8, 135.4, 131.9, 129.7, 127.5, 126.3, 125.1, 124.9, 124.8, 120.9, 65.3, 63.8, 48.9, 26.6, 17.4; ν_{max} (liquid film)/cm^{-1} 3321, 3080, 2971, 1723, 1646, 1597, 1576, 1494, 1342, 1221, 1152; *m/z* (ES) Found: $[MH]^+$ 431.1276, $C_{21}H_{23}N_2O_6S$ is $[MH]^+$ 431.1271; $[\alpha]_D^{22}$ -28 (*c* 0.005, MeOH).

(*S*)-2-(3-(3,6-Dihydro-2H-pyran-4-yl)-5-(N-phenylsulfamoyl)benzamido)propanoic acid 104



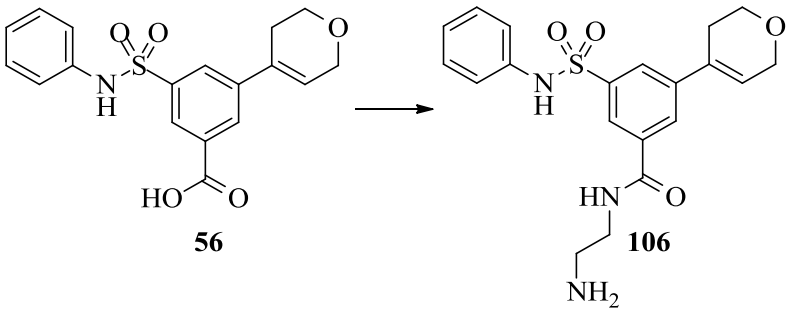
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (40 mg, 0.111 mmol), DIPEA (0.097 mL, 0.334 mmol) and HATU (42 mg, 0.111 mmol) were dissolved in DMF (0.5 mL) and stirred for 10 minutes. To this, (*S*)-methyl 2-aminopropanoate hydrochloride (14 mg, 0.134 mmol) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated *in vacuo*. The residue was dissolved in THF (0.25 mL) and water (0.25 mL). To this, 2 M aqueous lithium hydroxide (0.167 mL, 0.333 mmol) was added and stirred for 16 h. The solvent was concentrated *in vacuo*. The product was dissolved in DMSO (0.5 mL) and purified by MDAP on XBridge column using acetonitrile/water with a formic acid modifier (method B) to give (*S*)-2-(3-(3,6-dihydro-2H-pyran-4-yl)-5-(N-phenylsulfamoyl)benzamido)propanoic acid **104** (9.4 mg, 17 %) as a white solid. M.P. 210 °C decomposition; LCMS (Ammonium carbonate) 100 %, R_t= 0.60, $[MH]^-$= 429; δ_H (600 MHz, DMSO-d_6) 8.88 (1H, d, *J* = 6.9 Hz), 8.16 – 8.12 (2H, m), 7.86 – 7.83 (1H, m), 7.26 – 7.21 (2H, m), 7.11 – 7.07 (2H, m), 7.05 – 7.01 (1H, m), 6.40 – 6.37 (1H, m), 4.39 – 4.32 (1H, m), 4.27 – 4.24 (2H, m), 3.82 (2H, t, *J* = 5.3 Hz), 2.43 – 2.38 (2H, m), 1.40 – 1.34 (3H, d, *J* = 7.3 Hz). Sulfonamide N-H and carboxylic acid O-H not observed. δ_C (151 MHz, DMSO-d_6) 174.3, 164.7, 141.1, 140.6, 137.8, 135.6, 131.9, 129.6, 127.5, 126.3, 125.0, 124.9, 124.8, 121.0, 65.3, 63.8, 49.1, 26.6, 17.6; ν_{max} (liquid film)/cm^{-1} 3319, 3075, 2970, 1722, 1643, 1598, 1493, 1419, 1341, 1220, 1153; *m/z* (ES) Found: $[MH]^+$ 431.1276, $C_{21}H_{23}N_2O_6S$ is $[MH]^+$ 431.1270; $[\alpha]_D^{22}$ +28 (*c* 0.005, MeOH).

***N*-(2-Amino-2-oxoethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide 105**



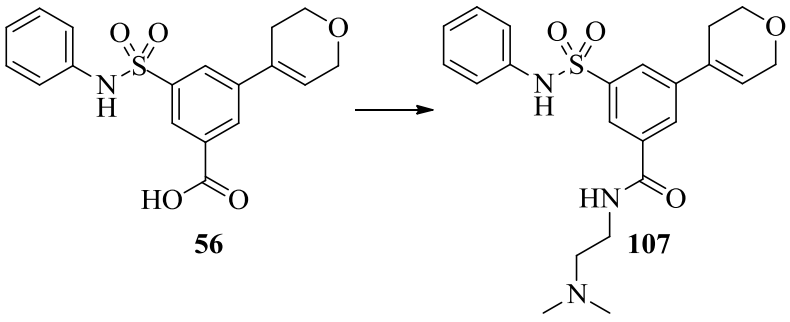
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (40 mg, 0.111 mmol), DIPEA (0.097 mL, 0.556 mmol), and HATU (42 mg, 0.111 mmol) were dissolved in DMF (0.5 mL) and stirred for 10 min. To this, 2-aminoacetamide (0.10 mg, 0.134 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated *in vacuo*. The sample was dissolved in DMSO (0.5 mL) and purified by MDAP XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give *N*-(2-amino-2-oxoethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide **105** (30 mg, 61 %) as a white solid. M.P. 169-171 °C; LCMS (Formic acid) 100 %, R_t= 0.66, $[MH]^+$= 416; δ_H (600 MHz, DMSO-d_6) 10.38 (1H, s), 9.07 – 9.03 (1H, m), 8.21 - 8.19 (1H, m), 8.18 – 8.16 (1H, m), 7.89 (1H, s), 7.43 (1H, s), 7.25 – 7.21 (2H, m), 7.13 – 7.10 (2H, m), 7.05 – 7.01 (2H, m), 6.43 – 6.41 (1H, m), 4.26 – 4.24 (2H, m), 4.13 – 4.09 (2H, m), 3.83 (2H, t, *J* = 5.8 Hz), 2.44 – 2.40 (2H, m); δ_C (151 MHz, DMSO-d_6) 171.2, 165.3, 141.0, 140.6, 137.8, 135.6, 132.0, 129.6, 127.6, 126.3, 125.0, 124.9, 124.8, 120.9, 65.4, 63.8, 42.9, 26.7; ν_{max} (liquid film)/cm^{-1} 3442, 3313, 3080, 2804, 1675, 1630, 1597, 1578, 1486, 1426, 1449, 1383, 1340, 1307, 1268, 1232, 1165, 1151, 1130, 1027, 1001; *m/z* (ES) Found: $[MH]^+$ 416.1285, $C_{20}H_{22}N_3O_5S$ is $[MH]^+$ 416.1275.

***N*-(2-Aminoethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide 106**



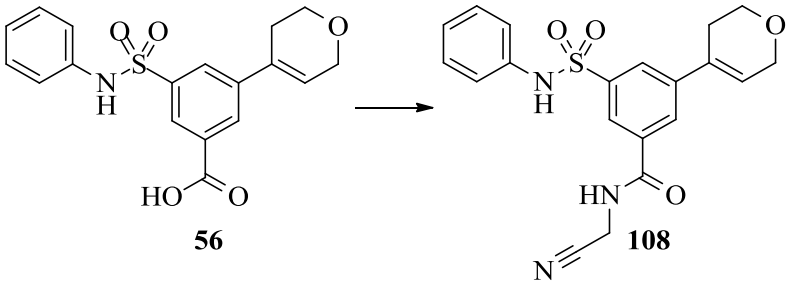
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (50 mg, 0.139 mmol), 50 % T_3P in ethyl acetate (0.099 mL, 0.167 mmol), DIPEA (0.097 mL, 0.556 mmol) and *tert*-butyl (2-aminoethyl)carbamate (0.031 mL, 0.278 mmol) were dissolved in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 15 h and concentrated under a flow of nitrogen. Water (5 mL) was added and then extracted with ethyl acetate (3 x 5 mL). The organic layer was washed with brine (5 mL) passed through a hydrophobic frit and concentrated *in vacuo.* The residue was dissolved in DCM (0.5 mL). To this, TFA (0.2 mL) was added and stirred for 1 h then neutralised with saturated aqueous sodium carbonate, diluted with water (5 mL) and extracted into ethyl acetate (2 x 5 mL). The organic layer was washed with brine (5 mL) passed through a hydrophobic frit and concentrated *in vacuo.* The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give *N*-(2-aminoethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **106** (35 mg, 62 %) as a yellow solid. M.P. 196-198 °C; LCMS (Formic acid) 100 %, R_t= 0.58, $[MH]^+$= 402; δ_H (400 MHz, DMSO-d_6) 8.88 – 8.79 (1H, m), 8.18 – 8.14 (1H, m), 8.12 – 8.08 (1H, m), 7.89 – 7.85 (1H, m), 7.22 – 7.14 (2H, m), 7.08 – 7.02 (2H, m), 6.97 – 6.90 (1H, m), 6.42 – 6.34 (1H, m), 4.30 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.39 – 3.32 (2H, m), 2.80 (2H, t, *J* = 6.3 Hz), 2.46 – 2.39 (2H, m). Sulfonamide N-H is not observed, NH_2 is not observed. δ_C (101 MHz, DMSO-d_6) 165.5, 141.8, 140.8, 139.6, 135.8, 132.1, 129.4, 127.0, 126.0, 124.9, 124.8, 123.7, 120.9, 65.4, 63.8, 41.2, 26.7. One carbon observed below DMSO peak (observed in HSQC). ν_{max} (liquid film)/cm^{-1} 3358, 3079, 2967, 2891, 1660, 1532, 1495, 1427, 1304, 1227, 1165.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-(2-(dimethylamino)ethyl)-5-(*N*-phenylsulfamoyl) benzamide 107



A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) 50 % T_3P in ethyl acetate (0.060 mL, 0.1 mmol), DIPEA (0.058 mL, 0.334 and *N*1,*N*1-dimethylethane-1,2-diamine (0.018 mL, 0.167 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 15 h and concentrated under a flow of nitrogen. The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) and then XBridge column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-(2-(dimethylamino)ethyl)-5-(*N*-phenylsulfamoyl) benzamide **107** (15 mg, 41 %) as a white solid. M.P. 127-129 °C; LCMS (Formic acid) 100 %, R_t= 0.59, $[MH]^+$= 430; δ_H (400 MHz, DMSO-d_6) 8.70 (1H, t, *J* = 5.6 Hz), 8.15 – 8.11 (1H, m), 8.11 – 8.08 (1H, m), 7.87 – 7.84 (1H, m), 7.27 – 7.20 (2H, m), 7.13 – 7.07 (2H, m), 7.06 – 7.01 (1H, m), 6.41 – 6.36 (1H, m), 4.28 – 4.23 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.40 – 3.33 (2H, m), 2.46 – 2.39 (4H, m), 2.19 (6H, s). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 164.9, 141.1, 140.7, 138.0, 136.0, 132.0, 129.6, 127.3, 126.3, 126.2, 124.8, 124.7, 120.9, 65.4, 63.8, 58.5, 45.6, 37.9, 26.7; ν_{max} (liquid film)/cm^{-1} 3358, 3078, 2965, 2894, 1660, 1600, 1532, 1495, 1340, 1165, 935; *m/z* (ES) Found: $[MH]^+$ 430.1807, $C_{22}H_{28}N_3O_4S$ is $[MH]^+$ 430.1795.

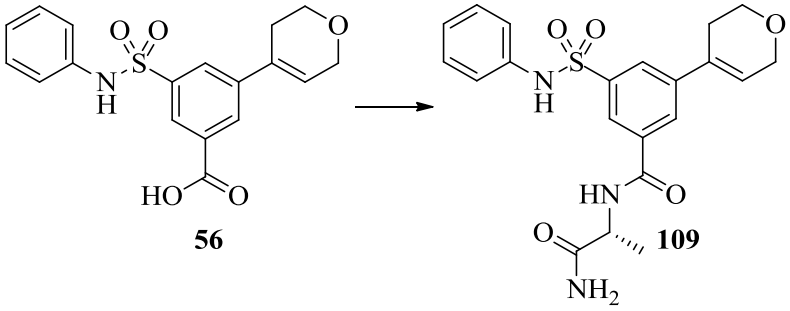
***N*-(Cyanomethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide 108**



A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (83 mg, 0.231 mmol), DIPEA (0.242 mL, 1.386 mmol) and HATU (105 mg, 0.277 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 minutes. To this, 2-aminoacetonitrile hydrochloride (25.6 mg, 0.277 mmol) was added. The resulting mixture was stirred at room temperature for 16 h and concentrated under a flow of nitrogen. The product was dissolved in 1:1 MeOH:DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) to give *N*-(cyanomethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **108** (47 mg, 51 %) as a colourless

oil. LCMS (Formic acid) 99 %, R_t= 0.92, $[MH]^+$= 398; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 9.48 (1H, t, *J* = 5.3 Hz), 8.21 – 8.09 (2H, m), 7.94 – 7.88 (1H, m), 7.29 – 7.19 (2H, m), 7.14 – 7.08 (2H, m), 7.08 – 7.01 (1H, m), 6.46 – 6.36 (1H, m), 4.35 (2H, d, *J* = 5.4 Hz), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 165.5, 141.3, 140.8, 137.7, 134.3, 131.8, 129.7, 127.5, 126.6, 125.6, 124.9, 124.8, 121.0, 117.8, 65.4, 63.8, 28.2, 26.6; v_{max} (liquid film)/cm^{-1} 3268, 3077, 2964, 2888, 2325, 1658, 1599, 1535, 1495, 1344, 1165.

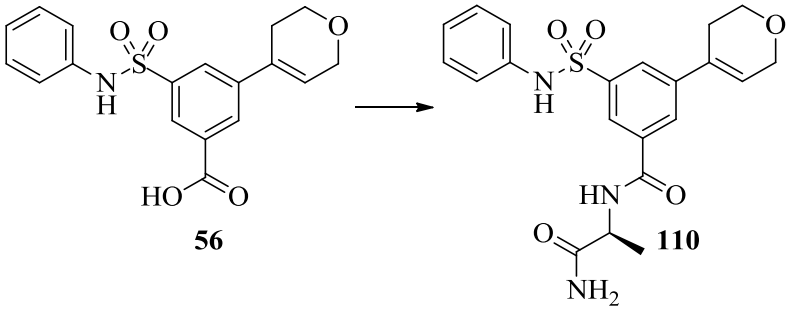
(*R*)-*N*-(1-Amino-1-oxopropan-2-yl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenyl sulfamoyl)benzamide 109



A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol), DIPEA (0.087 mL, 0.501 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, (*R*)-2-aminopropanamide hydrochloride (11 mg, 0.083 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The sample was dissolved in DMSO (0.6 mL) and purified by MDAP XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) to give (*R*)-*N*-(1-amino-1-oxopropan-2-yl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **109** (26 mg, 72 %) as a white solid. M.P. 154-156 °C; LCMS (Formic acid) 100 %, R_t= 0.81, $[MH]^+$= 430; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 8.77 (1H, d, *J* = 7.5 Hz), 8.20 – 8.14 (2H, m), 7.88 – 7.83 (1H, m), 7.40 (1H, s), 7.27 – 7.20 (2H, m), 7.14 – 7.07 (2H, m), 7.06 – 7.01 (1H, m), 6.98 (1H, s), 6.43 – 6.38 (1H, m), 4.43 (1H, p, *J* = 7.2 Hz), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.38 (2H, m), 1.34 (3H, d, *J* = 7.2 Hz); δ_C (101 MHz, DMSO-d_6) 174.5, 164.8, 141.0, 140.6, 138.0, 135.7, 132.1, 129.6, 127.8, 126.2, 125.0, 124.9, 124.7, 120.9, 65.4, 63.8, 49.4, 26.7, 18.3; v_{max} (liquid film)/cm^{-1} 3267, 3075, 2966, 1641, 1598, 1535,

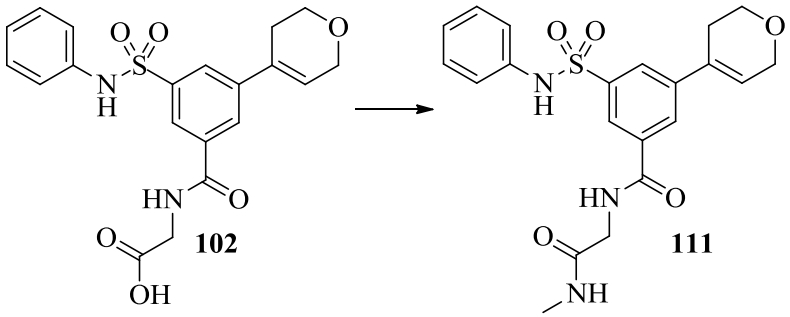
1493, 1420, 1335, 1304, 1153; *m/z* (ES) Found: $[MH]^+$ 430.1448, $C_{21}H_{24}N_3O_5S$ is $[MH]^+$ 430.1431. $[\alpha]_D^{22}$ -34 (*c* 0.005, MeOH).

(*S*)-*N*-(1-Amino-1-oxopropan-2-yl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenyl sulfamoyl)benzamide 110



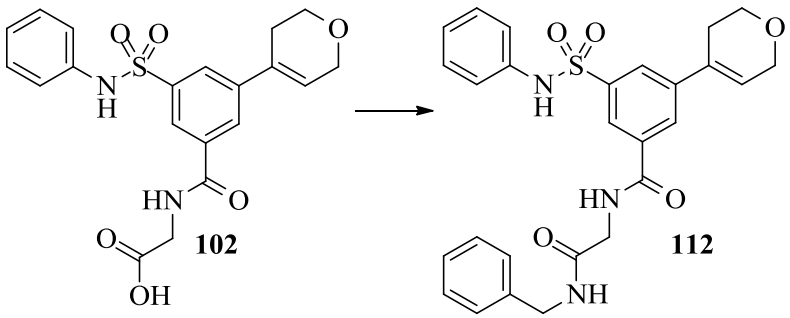
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) DIPEA (0.087 mL, 0.501 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, (*S*)-2-aminopropanamide hydrochloride (11 mg, 0.083 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a flow of nitrogen. The sample was dissolved in DMSO (0.6 mL) and purified by MDAP XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) to give (S)-*N*-(1-amino-1-oxopropan-2-yl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **110** (25 mg, 69 %) as a white solid. M.P. 148-150 °C; LCMS (Formic acid) 98 %, R_t= 0.81, $[MH]^+$= 430; δ_H (400 MHz, DMSO-d_6) 10.36 (1H, s), 8.77 (1H, d, *J* = 7.5 Hz), 8.20 – 8.15 (2H, m), 7.87 – 7.83 (1H, m), 7.41 (1H, s), 7.26 – 7.20 (2H, m), 7.12 – 7.07 (2H, m), 7.05 – 7.00 (1H, m), 6.98 (1H, s), 6.44 – 6.38 (1H, m), 4.43 (1H, p, *J* = 7.2 Hz), 4.30 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.38 (2H, m), 1.34 (3H, d, *J* = 7.2 Hz); δ_C (101 MHz, DMSO-d_6) 174.5, 164.9, 140.9, 140.7, 138.2, 135.7, 132.1, 129.6, 127.7, 126.2, 125.1, 124.9, 124.6, 120.9, 65.4, 63.8, 49.4, 26.7, 18.3; ν_{max} (liquid film)/cm^{-1} 3267, 3077, 2964, 2887, 1640, 1598, 1534, 1420, 1335, 1303, 1153; *m/z* (ES) Found: $[MH]^+$ 430.1440, $C_{21}H_{24}N_3O_5S$ is $[MH]^+$ 430.1431; $[\alpha]_D^{22}$ +24 (*c* 0.005, MeOH).

3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(*N*-phenylsulfamoyl) benzamide 111



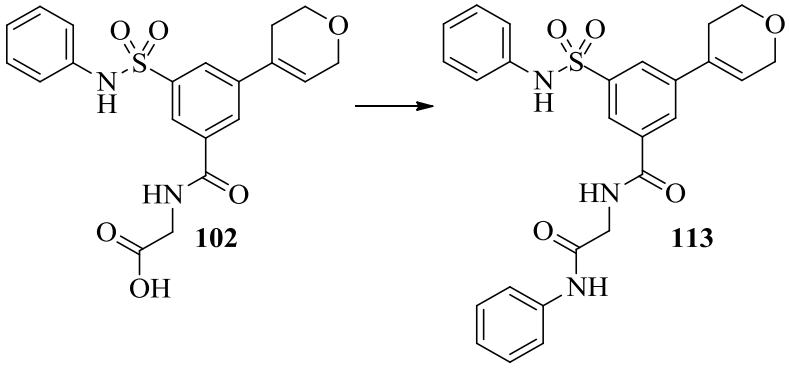
A mixture of 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetic acid **102** (50 mg, 0.12 mmol) DIPEA (0.084 mL, 0.48 mmol) 50 % T_3P in ethyl acetate (0.1 mL, 0.144 mmol) and methylamine (0.06 mL, 0.12 mmol, 2 M in THF) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h and concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-(2-(methylamino)-2-oxoethyl)-5-(*N*-phenylsulfamoyl) benzamide **111** (19 mg, 36 %) as a white solid. M.P. 159-161 °C; LCMS (Formic acid) 100 %, R_t= 0.82, $[MH]^+$= 430; δ_H (400 MHz, DMSO-d_6) 10.34 (1H, s), 9.05 (1H, t, *J* = 5.8 Hz), 8.20 – 8.16 (2H, m), 7.91 – 7.80 (2H, m), 7.27 – 7.18 (2H, m), 7.14 – 7.06 (2H, m), 7.06 – 7.00 (1H, m), 6.45 – 6.37 (1H, m), 4.29 – 4.23 (2H, m), 3.88 – 3.80 (4H, m), 2.60 (3H, d, *J* = 4.6 Hz), 2.45 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 169.4, 165.3, 141.0, 140.8, 138.1, 135.5, 132.0, 129.6, 127.6, 126.2, 125.0, 125.0, 124.7, 120.8, 65.4, 63.8, 43.2, 26.7, 25.9; ν_{max} (liquid film)/cm^{-1} 3275, 3079, 2966, 1656, 1539, 1494, 1320, 1270, 1250, 1148; *m/z* (ES) Found: $[MH]^+$ 430.1448, $C_{21}H_{24}N_3O_5S$ is $[MH]^+$ 430.1431.

***N*-(2-(Benzylamino)-2-oxoethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide 112**



A mixture of 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetic acid **102** (50 mg, 0.12 mmol), DIPEA (0.1 mL, 0.556 mmol), 50 % T_3P in ethyl acetate (0.1 mL, 0.167 mmol) and phenylmethanamine (0.015 mL, 0.139 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h and concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XSelect CSA column using acetonitrile/water with an ammonium carbonate modifier (method C) to give *N*-(2-(benzylamino)-2-oxoethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide **112** (24 mg, 34 %) as a yellow solid. M.P. 206-208 °C; LCMS (Formic acid) 100 %, R_t= 1.01, $[MH]^+$= 506; δ_H (400 MHz, DMSO-d_6) 9.11 (1H, t, *J* = 5.8 Hz), 8.47 (1H, t, *J* = 6.0 Hz), 8.22 – 8.19 (1H, m), 8.18 – 8.14 (1H, m), 7.90 – 7.86 (1H, m), 7.35 – 7.17 (7H, m), 7.13 – 7.05 (2H, m), 7.00 – 6.94 (1H, m), 6.42 – 6.36 (1H, m), 4.30 (2H, d, *J* = 6.0 Hz), 4.28 – 4.23 (2H, m), 3.94 (2H, d, *J* = 5.9 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.38 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 169.1, 165.5, 141.5, 140.9, 139.8, 139.0, 135.4, 132.1, 129.5, 128.6, 127.6, 127.3, 127.1, 126.1, 125.1, 125.0, 124.0, 120.9, 65.4, 63.8, 43.3, 42.4, 26.7; ν_{max} (liquid film)/cm^{-1} 3378, 3259, 3079, 2962, 1670, 1650, 1543, 1420, 1345, 1148, 1131; *m/z* (ES) Found: $[MH]^+$ 506.1744, $C_{27}H_{27}N_3O_5S$ is $[MH]^+$ 506.1744.

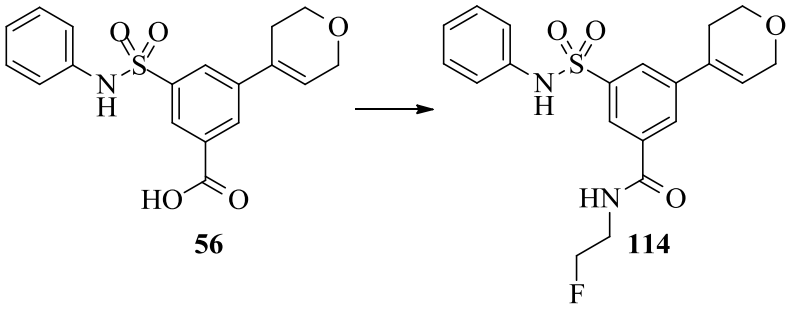
3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-(2-oxo-2-(phenylamino)ethyl)-5-(*N*-phenylsulfamoyl) benzamide 113



A mixture of 2-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamido)acetic acid **102** (50 mg, 0.12 mmol) DIPEA (0.097 mL, 0.556 mmol), 50 % T_3P in ethyl acetate (0.086 mL, 0.144 mmol) and aniline (0.011 mL, 0.12 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h and concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on

Sunfire column using acetonitrile/water with a formic acid modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-(2-oxo-2-(phenylamino)ethyl)-5-(*N*-phenylsulfamoyl) benzamide **113** (26 mg, 44 %) as a white solid. M.P. 225-227 °C; LCMS (Formic acid) 99 %, R_t= 1.03, $[MH]^+$= 492;δ_H (400 MHz, DMSO-d_6) 10.33 (1H, s), 10.06 (1H, s), 9.16 (1H, t, *J* = 5.5 Hz), 8.27 – 8.14 (2H, m), 7.93 – 7.84 (1H, m), 7.65 – 7.56 (2H, m), 7.35 – 7.28 (2H, m), 7.28 – 7.21 (2H, m), 7.14 – 7.09 (2H, m), 7.09 – 7.01 (2H, m), 6.43 – 6.40 (1H, m), 4.30 – 4.23 (2H, m), 4.09 (2H, d, *J* = 5.6 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.40 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.9, 165.5, 141.1, 140.7, 139.3, 137.9, 135.5, 132.0, 129.6, 129.1, 127.5, 126.3, 125.1, 124.9, 124.8, 123.7, 120.9, 119.6, 65.4, 63.8, 43.8, 26.7; ν_{max} (liquid film)/cm^{-1} 3387, 3266, 3137, 3078, 2962, 1672, 1650, 1600, 1540, 1492, 1478, 1344, 1147; *m/z* (ES) Found: $[MH]^+$ 492.1587, $C_{26}H_{26}N_3O_3S$ is $[MH]^+$ 492.1588.

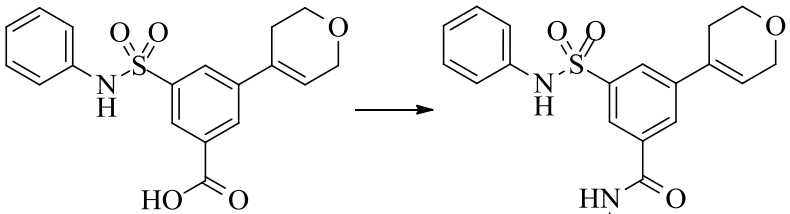
3-(3,6-Dihydro-2*H*-pyran-4-yl)-*N*-(2-fluoroethyl)-5-(*N*-phenylsulfamoyl)benzamide 114



A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol), DIPEA (0.087 mL, 0.501 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, 2-fluoroethanamine hydrochloride (9 mg, 0.09 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-*N*-(2-fluoroethyl)-5-(*N*-phenylsulfamoyl)benzamide **114** (14 mg, 41 %) as a white solid. M.P. 118-120 °C; LCMS (Formic acid) 98 %, R_t= 0.94, $[MH]^+$= 405; δ_H (400 MHz, DMSO-d_6) 8.98 (1H, t, *J* = 5.4 Hz), 8.18 – 8.15 (1H, m), 8.15 – 8.11 (1H, m), 7.88 – 7.83 (1H, m), 7.26 – 7.18 (2H, m), 7.12 – 7.05 (2H, m), 7.05 – 6.97 (1H, m), 6.41 – 6.36 (1H, m), 4.55 (2H, dt, *J* = 47.5, 5.1 Hz), 4.28 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.58 (2H, dq, *J* = 26.8, 5.2 Hz), 2.45 – 2.37 (2H, m). Sulfonamide N-H is not observed. δ_C (101 MHz,

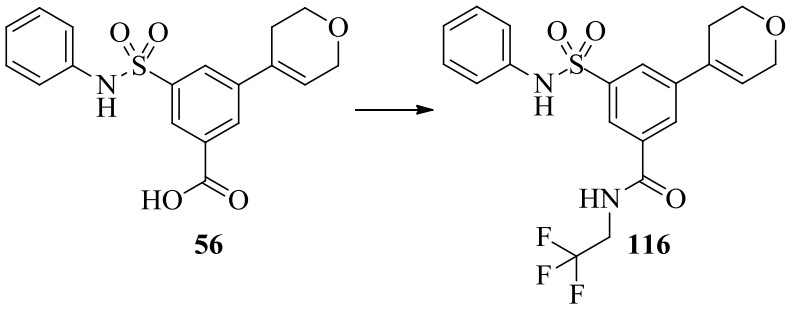
DMSO-d_6) 165.3, 141.1, 140.8, 138.0, 135.6, 132.0, 129.6, 127.3, 126.3, 125.0, 124.8, 124.7, 120.9, 82.55 (d, $^1J_{C\text{-}F}$ = 165.6 Hz), 65.4, 63.8, 26.7. One carbon hidden below DMSO peak (observed in HSQC). δ_F (376 MHz, , DMSO-d_6) -70.20 (d, *J* = 711.3 Hz); ν_{max} (liquid film)/cm^{-1} 3267, 3079, 2965, 2898, 1642, 1598, 1541, 1421, 1341, 1320, 1163, 1127; *m/z* (ES) Found: $[MH]^+$ 405.1289, $C_{20}H_{22}FN_2O_4S$ is $[MH]^+$ 405.1280

_N_-(2,2-Difluoroethyl)-3-(3,6-dihydro-2_H_-pyran-4-yl)-5-(_N_-phenylsulfamoyl)benzamide 115



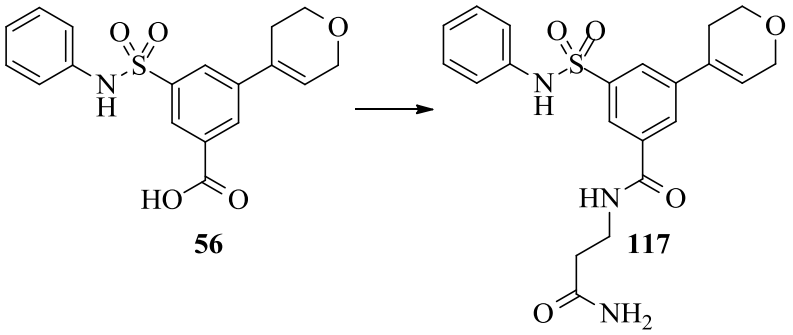
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol), DIPEA (0.058 mL, 0.334 mmol) and HATU (38 mg, 0.1 mmol)were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, 2,2-difluoroethanamine (7 mg, 0.083 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give *N*-(2,2-difluoroethyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **115** (18 mg, 51 %) as a white solid. M.P. 150-152 °C; LCMS (Formic acid) 100 %, R_t= 1.00, $[MH]^-$= 421;δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 9.14 (1H, t, *J* = 5.8 Hz), 8.18 – 8.11 (2H, m), 7.89 – 7.87 (1H, m), 7.27 – 7.21 (2H, m), 7.13 – 7.07 (2H, m), 7.07 – 7.01 (1H, m), 6.43 – 6.39 (1H, m), 6.13 (1H, tt, *J* = 55.9, 3.9 Hz), 4.30 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.70 (2H, tdd, *J* = 15.7, 5.7, 4.0 Hz), 2.46 – 2.38 (2H, m); δ_C (101 MHz, DMSO-d_6) 165.7, 141.2, 140.7, 137.8, 135.0, 131.9, 129.6, 127.5, 126.4, 125.3, 125.0, 124.9, 120.9, 114.9 (t, *J* = 239.9 Hz), 65.3, 63.8, 42.1 (t, *J* = 26.3 Hz), 26.6. δ_F (376 MHz, DMSO-d_6) -121.44 (dt, *J* = 55.9, 15.7 Hz); ν_{max} (liquid film)/cm^{-1} 3278, 3089, 2956, 2898, 1647, 1600, 1563, 1496, 1347, 1152, 1104; *m/z* (ES) Found: $[MH]^+$ 423.1203, $C_{20}H_{21}F_2N_2O_4S$ is $[MH]^+$ 423.1185.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)-*N*-(2,2,2-trifluoroethyl) benzamide 116



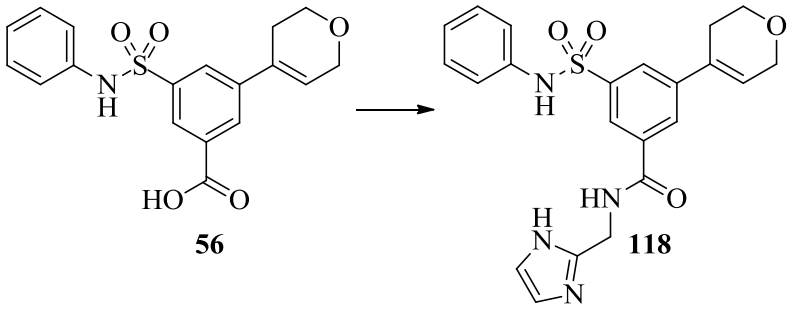
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) DIPEA (0.058 mL, 0.334 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, 2,2,2-trifluoroethanamine (10 mg, 0.101 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) then further purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)-*N*-(2,2,2-trifluoroethyl)benzamide **116** (19 mg, 51 %) as a cream solid. M.P. 116-118 °C; LCMS (Formic acid) 97 %, R_t= 1.06, $[MH]^+$= 441; δ_H (400 MHz, DMSO-d_6) 10.37 (1H, s), 9.37 (1H, t, *J* = 6.2 Hz), 8.19 – 8.12 (2H, m), 7.92 – 7.86 (1H, m), 7.26 – 7.20 (2H, m), 7.13 – 7.07 (2H, m), 7.07 – 7.00 (1H, m), 6.45 – 6.36 (1H, m), 4.30 – 4.20 (2H, m), 4.12 (2H, qd, *J* = 9.8, 6.3 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.37 (2H, m); δ_C (126 MHz, DMSO-d_6) 165.8, 141.3, 140.9, 138.1, 134.6, 131.9, 129.6, 127.5, 126.5, 126.3 – 123.8 (m), 125.5, 125.0, 124.7, 121.0, 65.4, 63.8, 41.20 – 40.36 (m), 26.6; δ_F (376 MHz, DMSO-d_6) -70.29 (t, *J* = 9.8 Hz); ν_{max} (liquid film)/cm^{-1} 3258, 3080, 3967, 1657, 1578, 1542, 1270, 1232, 1147; *m/z* (ES) Found: $[MH]^+$ 441.1105, $C_{20}H_{20}F_3N_2O_4S$ is $[MH]^+$ 441.1090.

***N*-(3-Amino-3-oxopropyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide 117**



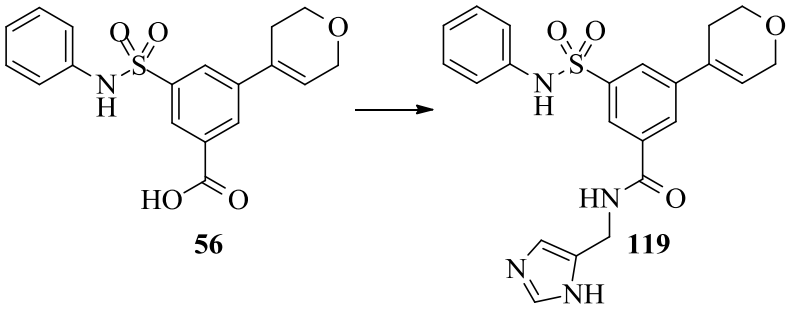
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (80 mg, 0.223 mmol) DIPEA (0.233 mL, 1.336 mmol) and HATU (102 mg, 0.267 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, 3-aminopropanamide hydrochloride (33 mg, 0.267 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a flow of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give *N*-(3-amino-3-oxopropyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **117** (45 mg, 47 %) as a white solid. M.P. 178-180 °C; LCMS (Formic acid) 100 %, R_t= 0.79, $[MH]^+$= 430; δ_H (400 MHz, DMSO-d_6) 10.30 (1H, s), 8.80 (1H, t, *J* = 5.5 Hz), 8.15 – 8.12 (1H, m), 8.12 – 8.09 (1H, m), 7.86 – 7.82 (1H, m), 7.33 (1H, s), 7.28 – 7.18 (2H, m), 7.13 – 7.07 (2H, m), 7.07 – 7.02 (1H, m), 6.81 (1H, s), 6.42 – 6.35 (1H, m), 4.26 (2H, d, *J* = 2.8 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 3.48 – 3.42 (2H, m), 2.44 – 2.39 (2H, m), 2.35 (2H, t, *J* = 7.1 Hz); δ_C (101 MHz, DMSO-d_6) 172.8, 165.0, 141.0, 140.6, 137.9, 132.0, 129.6, 127.3, 126.2, 124.8, 124.8, 124.4, 120.9, 65.4, 63.8, 36.6, 35.3, 26.7. One carbon not observed. ν_{max} (liquid film)/cm^{-1} 3423, 2066, 2925, 1642, 1598, 1534, 1494, 1319, 1163, 1147; *m/z* (ES) Found: $[MH]^+$ 430.1448, $C_{21}H_{24}N_3O_5S$ is $[MH]^+$ 430.1431.

***N*-((1*H*-Imidazol-2-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide 118**



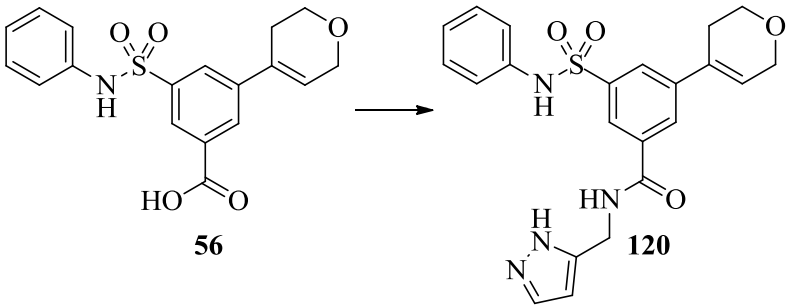
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol), DIPEA (0.087 mL, 0.501 mmol) HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, 1*H*-imidazol-2-yl)methanamine dihydrochloride (14.2 mg, 0.083 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give *N*-((1*H*-imidazol-2-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **118** (18 mg, 49 %) as a cream solid. M.P. 171-173 °C; LCMS (Formic acid) 98 %, R_t= 0.59, $[MH]^+$= 439; δ_H (400 MHz, DMSO-d_6) 9.26 (1H, t, *J* = 5.5 Hz), 8.22 – 8.19 (2H, m), 7.90 – 7.85 (1H, m), 7.27 – 7.19 (2H, m), 7.16 – 7.09 (2H, m), 7.07 – 7.00 (1H, m), 6.96 – 6.89 (2H, m), 6.44 – 6.36 (1H, m), 4.50 (2H, d, *J* = 5.6 Hz), 4.29 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.45 – 2.38 (2H, m). Sulfonamide N-H and imidazole N-H not observed. δ_C (101 MHz, DMSO-d_6) 165.1, 145.0, 141.0, 140.7, 137.9, 135.6, 132.0, 129.6, 127.5, 126.3, 125.0, 125.0, 124.8, 120.8, 119.5, 65.40, 63.84, 37.69, 26.72. One carbon not observed. ν_{max} (liquid film)/cm^{-1} 3242, 3072, 2926, 1643, 1537, 1494, 1425, 1319, 1147, 1163; *m/z* (ES) Found: $[MH]^+$ 439.1439, is $C_{22}H_{23}N_4O_4S$ $[MH]^+$ 439.1435.

***N*-((1*H*-Imidazol-5-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide 119**



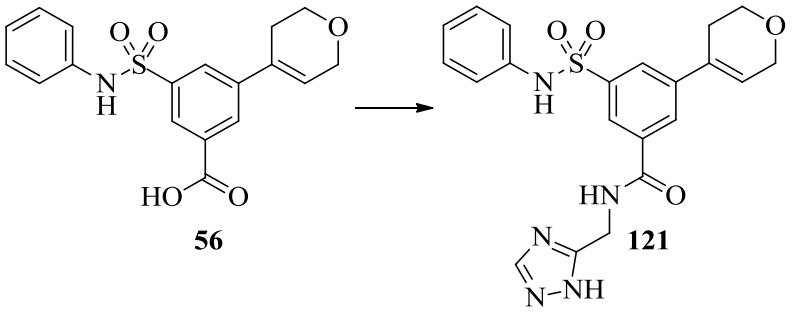
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol), DIPEA (0.087 mL, 0.501 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, (1*H*-imidazol-5-yl)methanamine dihydrochloride (15 mg, 0.088 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give *N*-((1*H*-imidazol-5-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **119** (11 mg, 30 %) as a cream solid. M.P. 163-165 °C; LCMS (Formic acid) 100 %, R_t= 0.60, $[MH]^+$= 439; δ_H (400 MHz, DMSO-d_6) 9.09 (1H, t, *J* = 5.4 Hz), 8.29 (1H, s), 8.19 – 8.16 (1H, m), 8.16 – 8.14 (1H, m), 7.87 – 7.83 (1H, m), 7.59 – 7.53 (1H, m), 7.26 – 7.18 (2H, m), 7.13 – 7.06 (2H, m), 7.06 – 6.98 (1H, m), 6.96 – 6.87 (1H, m), 6.43 – 6.36 (1H, m), 4.39 (2H, d, *J* = 5.4 Hz), 4.28 – 4.20 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 2.45 – 2.38 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 164.8, 141.0, 140.8, 135.9, 135.3, 132.0, 129.6, 127.3, 126.2, 125.0, 124.8, 124.6, 122.8, 120.9, 119.6, 119.5, 65.3, 63.8, 50.1, 26.7; ν_{max} (liquid film)/cm^{-1} 3242, 2067, 1642 1598, 1494, 1385, 1319, 1163, 1147; *m/z* (ES) Found: $[MH]^+$ 439.1450, $C_{22}H_{23}N_4O_4S$ is $[MH]^+$ 439.1435.

***N*-((1*H*-Pyrazol-5-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide 120**



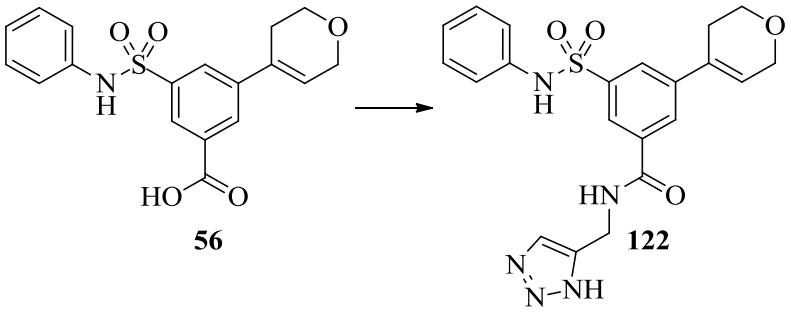
A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol), DIPEA (0.087 mL, 0.501 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, (1*H*-pyrazol-5-yl)methanamine (9 mg, 0.088 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) then further purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give *N*-((1*H*-pyrazol-5-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **120** (17 mg, 46 %) as a white solid. M.P. 139-141 °C; LCMS (Formic acid) 100 %, R_t= 0.85, $[MH]^+$= 439; δ_H (400 MHz, DMSO-d_6) 12.59 (1H, s), 10.30 (1H, s), 9.18 (1H, t, *J* = 5.4 Hz), 8.18 – 8.17 (1H, m), 8.17 – 8.15 (1H, m), 7.87 – 7.81 (1H, m), 7.69 – 7.53 (1H, m), 7.27 – 7.22 (2H, m), 7.14 – 7.07 (2H, m), 7.07 – 7.01 (1H, m), 6.42 – 6.36 (1H, m), 6.16 (1H, d, *J* = 2.0 Hz), 4.48 (2H, d, *J* = 5.2 Hz), 4.29 – 4.21 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 2.45 – 2.38 (2H, m); δ_C (101 MHz, DMSO-d_6) 164.9, 141.1, 140.6, 137.9, 135.8, 132.0, 129.6, 127.4, 126.3, 125.0, 124.9, 124.8, 120.9, 103.6, 65.4, 63.8, 26.7. Two carbons are not observed, one carbon observed below DMSO (observed in HSQC). ν_{max} (liquid film)/cm^{-1} 3240, 2924,6143, 1533, 1494, 1319, 1163, 1147; *m/z* (ES) Found: $[MH]^+$ 439.1430, $C_{22}H_{23}N_4O_4S$ is $[MH]^+$ 439.1435.

_N_-((1_H_-1,2,4-Triazol-5-yl)methyl)-3-(3,6-dihydro-2_H_-pyran-4-yl)-5-(_N_-phenyl sulfamoyl)benzamide 121



A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) DIPEA (0.087 mL, 0.501 mmol) and HATU (38 mg, 0.1 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, (1*H*-1,2,4-triazol-5-yl)methanamine dihydrochloride (14 mg, 0.083 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The product was dissolved in DMSO (0.6 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) then further purified by MDAP on XSelect CSH column using acetonitrile/water with an ammonium carbonate modifier (method B) to give *N*-((1*H*-imidazol-5-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzamide **121** (7 mg, 19 %) as a white solid. M.P. 231 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^+$= 440; δ_H (400 MHz, DMSO-d_6) 13.84 (1H, s), 10.36 (1H, s), 9.30 (1H, t, *J* = 5.7 Hz), 8.26 – 8.08 (3H, m), 7.88 – 7.85 (1H, m), 7.26 – 7.14 (2H, m), 7.14 – 7.03 (2H, m), 7.03 – 6.91 (1H, m), 6.42 – 6.36 (1H, m), 4.57 (2H, d, *J* = 5.7 Hz), 4.30 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.45 – 2.39 (2H, m); δ_C (126 MHz, DMSO-d_6) 165.9, 165.3, 150.2, 140.7, 140.0, 139.0, 135.3, 132.2, 129.4, 126.8, 125.9, 125.1, 125.0, 120.9, 65.4, 63.8, 36.7, 26.7. One carbon is not observed. ν_{max} (liquid film)/cm^{-1} 3194, 2968, 1677, 1625, 1492, 1403, 1304, 1156, 1129; *m/z* (ES) Found: $[MH]^+$ 440.1382, $C_{21}H_{22}O_4N_5S$ is $[MH]^+$ 440.1387.

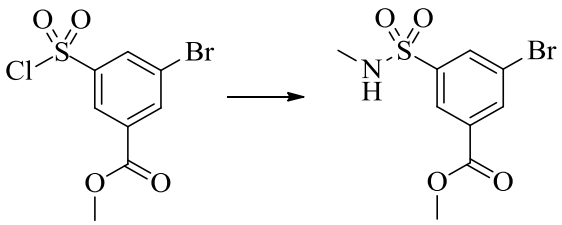
_N_-((1_H_-1,2,3-Triazol-4-yl)methyl)-3-(3,6-dihydro-2_H_-pyran-4-yl)-5-(_N_-phenyl sulfamoyl)benzamide 122



56 **122**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **56** (30 mg, 0.083 mmol) DIPEA (0.087 mL, 0.501 mmol) and HATU were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, (1*H*-1,2,3-triazol-4-yl)methanamine hydrochloride (11 mg, 0.083 mmol) was added. The resulting mixture was stirred for 16 h and then concentrated under a stream of nitrogen. The sample was dissolved in 1:1 methanol:DMSO (0.6 mL) and purified by MDAP Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give *N*-((1*H*-1,2,3-triazol-4-yl)methyl)-3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl) benzamide **122** (21 mg, 57 %) as a white solid. M.P. 240 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^+$= 440; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 9.33 (1H, t, *J* = 5.6 Hz), 8.28 (1H, s), 8.21 – 8.17 (2H, m), 7.90 – 7.85 (1H, m), 7.27 – 7.21 (2H, m), 7.14 – 7.08 (2H, m), 7.07 – 7.02 (1H, m), 6.43 – 6.37 (1H, m), 4.58 (2H, d, *J* = 5.6 Hz), 4.29 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.38 (2H, m). Triazole N-H not observed. δ_C (101 MHz, DMSO-d_6) 165.1, 141.1, 140.7, 137.8, 135.5, 132.0, 129.6, 127.5, 126.3, 125.0, 125.0, 124.8, 120.9, 65.40, 63.84, 36.68, 26.72. Two carbons not observed. ν_{max} (liquid film)/cm^{-1} 3138, 3075, 2888, 1648, 1598, 1536, 1493, 1338, 1164, 1149; *m/z* (ES) Found: $[MH]^+$440.1380, $C_{21}H_{22}O_4N_5S$ is $[MH]^+$ 440.1387.

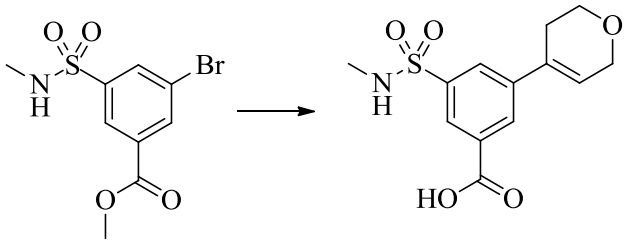
Methyl 3-bromo-5-(*N*-methylsulfamoyl)benzoate 123



53 **123**

Methyl 3-bromo-5-(chlorosulfonyl)benzoate **53** (500 mg, 1.595 mmol) and methanamine (1 mL, 2 mmol, 2 M in THF) were added to pyridine (1 mL). The resulting mixture was stirred at room temperature for 2 h and then diluted with water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with brine (2 x 25 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 – 60 % TBME/cyclohexane at 222 nm) provided methyl 3-bromo-5-(*N*-methylsulfamoylbenzoate **123** (347 mg, 70 %) as a white solid. LCMS (Formic acid) 97 %, R_t= 0.94, $[MH]^-$= 306, 308; δ_H (400 MHz, CD_3OD) 8.40 – 8.39 (1H, m), 8.39 – 8.37 (1H, m), 8.21 – 8.20 (1H, m), 3.99 (3H, s), 2.58 (3H, s). Sulfonamide N-H is not observed.

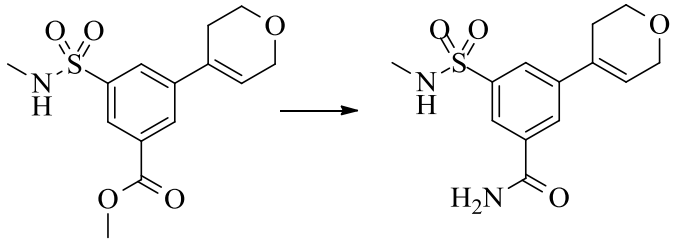
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-methylsulfamoyl)benzoic acid 126



123 **126**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (120 mg, 0.571 mmol), methyl 3-bromo-5-(*N*-methylsulfamoyl)benzoate **123** (160 mg, 0.519 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (13 mg, 0.023 mmol) and tripotassium phosphate (353 mg, 1.662 mmol) in 1,4-dioxane (1 mL) and water (0.2 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol, then with methanol/formic acid (99:1) and concentrated *in vacuo*. The residue was re-dissolved in THF (5 mL) and water (5 mL). To this, 2 M aqueous lithium hydroxide (0.26 mL, 0.519 mmol) was added and stirred for 5 h. The THF was removed *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid and filtered to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-methylsulfamoyl)benzoic acid **126** (151 mg, 98 %) as a black solid. LCMS (Formic acid) 98 %, R_t= 0.74, $[MH]^+$= 298; δ_H (400 MHz, DMSO-d_6) 13.50 (1H, s), 8.21 – 8.18 (2H, m), 8.03 – 8.01 (1H, m), 7.61 (1H, q, *J* = 5.0 Hz), 6.50 – 6.47 (1H, m), 4.29 – 4.25 (2H, m), 3.86 (2H, t, *J* = 5.4 Hz), 2.52 – 2.49 (2H, m), 2.43 (3H, d, *J* = 5.0 Hz).

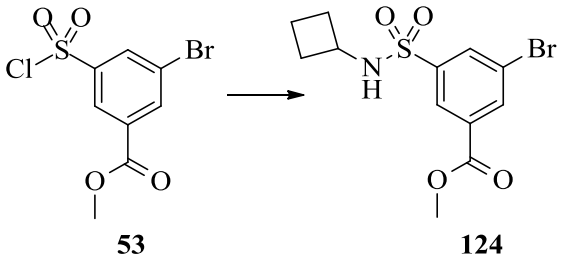
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-methylsulfamoyl)benzamide 128



126 **128**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-phenylsulfamoyl)benzoic acid **126** (100 mg, 0.336 mmol), DIPEA (0.294 mL, 1.682 mmol), and HATU (141 mg, 0.37 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, ammonia (3.36 mL, 1.682 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at room temperature for 16 h and then diluted with water (5 mL) and extracted with ethyl acetate (2 x 5 mL). The organic layer was washed with brine (5 mL), aqueous lithium chloride (2 x 5 mL, 9 %), dried through a hydrophobic frit and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-methylsulfamoyl) benzamide **128** (7.3 mg, 7 %) as a colourless gum. LCMS (Formic acid) 95 %, R_t= 0.64, $[MH]^+$= 297; δ_H (400 MHz, DMSO-d_6) 8.46 – 8.44 (1H, m), 8.42 – 8.40 (1H, m), 8.27 – 8.25 (1H, m), 6.67 – 6.63 (1H, m), 4.60 – 4.55 (2H, m), 4.20 (2H, t, *J* = 5.5 Hz), 2.85 – 2.81 (2H, m), 2.80 (3H, s). Sulfonamide N-H and amide NH_2 not observed. δ_C (126 MHz, DMSO-d_6) 166.9, 141.3, 140.4, 135.9, 132.2, 127.3, 126.1, 125.0, 65.4, 63.9, 29.1, 26.8. One carbon not observed. v_{max} (liquid film)/cm^{-1} 3365, 3211, 2870, 1673, 1624, 1601, 1384, 1356, 1291, 1115.

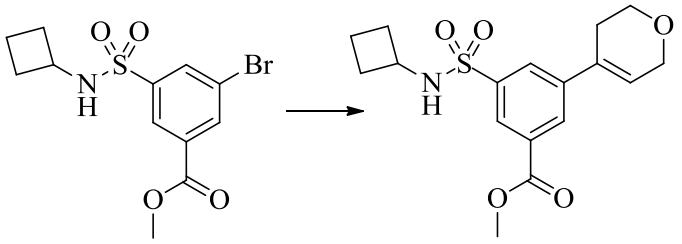
Methyl 3-bromo-5-(*N*-cyclobutylsulfamoyl)benzoate 124



53 **124**

Methyl 3-bromo-5-(chlorosulfonyl)benzoate **53** (512 mg, 1.633 mmol) and cyclobutylamine (0.154 mL, 1.758 mmol) were added to pyridine (2 mL). The resulting mixture was stirred at room temperature for 18 h and then diluted with water (100 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layer was washed with brine (100 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 40 % TBME/cyclohexane at 222 nm) provided methyl 3-bromo-5-(*N*-cyclobutylsulfamoyl) benzoate **124** (298 mg, 52 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 1.14, $[MH]^-$ = 346, 348; δ_H (400 MHz, $CDCl_3$) 8.45 – 8.43 (1H, m), 8.38 – 8.35 (1H, m), 8.21 – 8.18 (1H, m), 4.75 (1H, d, *J* = 8.7 Hz), 3.99 (3H, s), 3.94 – 3.82 (1H, m), 2.26 – 2.16 (2H, m), 1.89 – 1.77 (2H, m), 1.74 – 1.59 (2H, m).

Methyl 3-(*N*-cyclobutylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoate 125

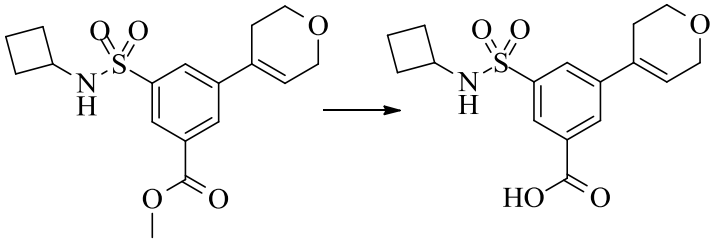


124 **125**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (100 mg, 0.474 mmol), methyl 3-bromo-5-(*N*-cyclobutylsulfamoyl)benzoate **124** (150 mg, 0.431 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (13 mg, 0.023 mmol) and tripotassium phosphate (293 mg, 1.378 mmol) in 1,4-dioxane (2 mL) and water (0.4 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min.

The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. Purification by flash chromatography (20 – 100 % TBME/cyclohexane at 222 nm) provided methyl 3-(*N*-cyclobutylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoate **125** (124 mg, 82 %) as a white solid. LCMS (Formic acid) 97 %, R_t= 1.05, $[MH]^+$= 352; δ_H (400 MHz, DMSO-d_6) 8.22 – 8.21 (1H, m), 8.18 – 8.17 (1H, m), 8.15 – 8.11 (1H, m), 8.08 – 8.06 (1H, m), 6.51 – 6.48 (1H, m), 4.30 – 4.25 (2H, m), 3.92 (3H, s), 3.86 (2H, t, *J* = 5.4 Hz), 3.71 – 3.61 (1H, m), 2.54 – 2.52 (2H, m) 1.94 – 1.85 (2H, m), 1.79 – 1.65 (2H, m), 1.54 – 1.42 (2H, m).

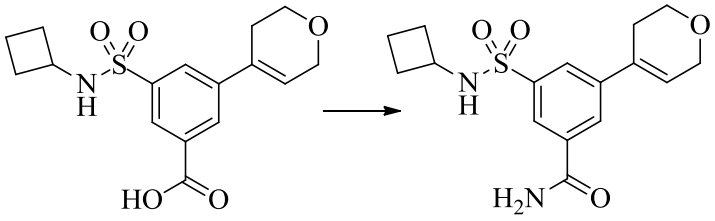
3-(*N*-Cyclobutylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoic acid 127



125 **127**

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-cyclobutylsulfamoyl)benzoate **125** (100 mg, 0.285 mmol) was dissolved in THF (1 mL) and water (1 mL). To this, aqueous lithium hydroxide (0.536 mL, 1.071 mmol, 2 M) was added and stirred for 16 h. The THF was removed *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid and extracted with ethyl acetate (5 mL). The organic layer was dried through a hydrophobic frit and concentrated *in vacuo* to give 3-(*N*-cyclobutylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoic acid **127** (80 mg, 83 %) as a white solid. LCMS (Formic acid) 94 %, R_t= 0.91, $[MH]^+$= 338; δ_H (400 MHz, DMSO-d_6) 13.49 (1H, s), 8.22 – 8.20 (1H, m), 8.17 – 8.16 (1H, m), 8.12 – 8.08 (1H, m), 8.04 – 8.03 (1H, m), 6.49– 6.46 (1H, m), 4.29 – 4.25 (2H, m), 3.86 (2H, t, *J* = 5.4 Hz), 3.73 – 3.61 (1H, m), 2.59 – 2.52 (2H, m), 1.95 – 1.84 (2H, m), 1.80 – 1.67 (2H, m), 1.55 – 1.42 (2H, m).

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-cyclobutylsulfamoyl)benzamide 129

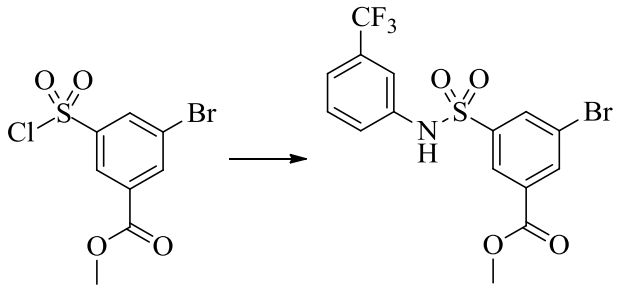


127 **129**

A mixture 3-(*N*-cyclobutylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoic acid **127** (50 mg, 0.278 mmol), DIPEA (0.129 mL, 0.741 mmol), and HATU (62 mg, 0.163 mmol) were dissolved in DMF (0.5 mL) and stirred for 30 min. To this, ammonia (1.48 mL, 0.741 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at room temperature for 16 h and then diluted with water (5 mL) and extracted with ethyl acetate (2 x 5 mL). The organic layer was washed with brine (2 x 5 mL), aqueous lithium chloride (2 x 5 mL, 9 %),

dried through a hydrophobic frit and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method A) to give 3-(*N*-cyclobutylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzamide **129** (21 mg, 42 %) as a white solid. M.P. 216-218 °C; LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^+$= 337; δ_H (400 MHz, DMSO-d_6) 8.27 (1H, s), 8.21 – 8.15 (2H, m), 8.09 – 8.00 (1H, m), 7.95 – 7.92 (1H, m), 7.60 (1H, s), 6.51 – 6.47 (1H, m), 4.30 – 4.25 (2H, m), 3.87 (2H, t, *J* = 5.4 Hz), 3.72 – 3.62 (1H, m), 2.51 – 2.50 (2H, m), 1.94 – 1.84 (2H, m), 1.79 – 1.66 (2H, m), 1.53 – 1.42 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.9, 142.8, 141.1, 135.7, 132.2, 127.2, 126.0, 124.9, 124.8, 65.4, 63.9, 48.0, 30.9, 26.8, 14.9; ν_{max} (liquid film)/cm^{-1} 3365, 3211, 2829, 1673, 1624, 1602, 1384, 1356, 1115; *m/z* (ES) Found: $[MH]^+$ 337.1219, $C_{16}H_{21}N_2O_4S$ is $[MH]^+$ 337.1217.

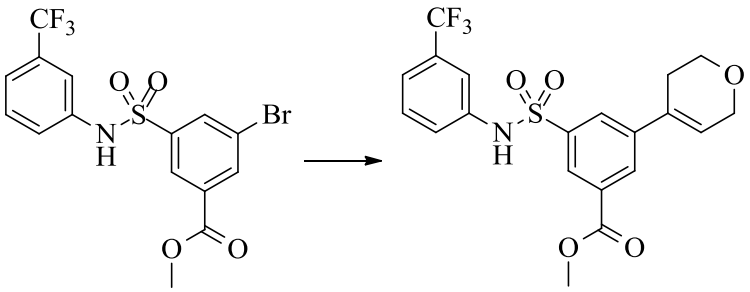
Methyl 3-bromo-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoate 130



53 **130**

Methyl 3-bromo-5-(chlorosulfonyl)benzoate **53** (500 mg, 1.59 mmol) and 3-(trifluoromethyl)aniline (0.22 mL, 1.755 mmol) were added to pyridine (5 mL). The resulting mixture was stirred at room temperature for 16 h and then concentrated *in vacuo.* Purification by flash chromatography (0 – 100 % ethyl acetate/hexane) provided methyl 3-bromo-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoate **130** (298 mg, 52 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 1.33, $[MH]^-$= 436, 438; δ_H (400 MHz, DMSO-d_6) 10.65 (1H, s), 8.33 – 8.28 (2H, m), 7.99 – 7.96 (1H, m), 7.53 – 7.65 (1H, m), 7.54 – 7.44 (3H, m), 3.94 (3H, s).

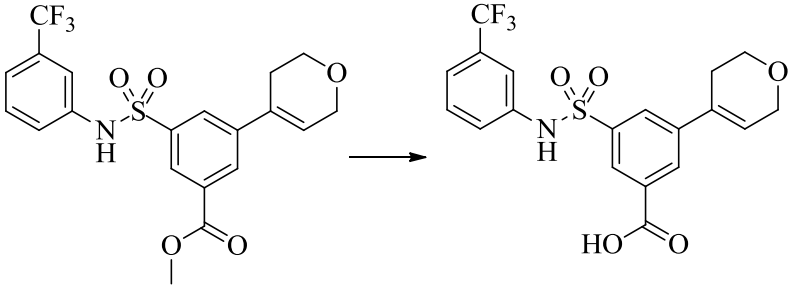
Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl) benzoate 133



130 **133**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (102 mg, 0.51 mmol), methyl 3-bromo-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoate **130** (150 mg, 0.42 mmol), $PdCl_2$(dppf) (34 mg, 0.041 mmol) and sodium carbonate (134 mg, 1.26 mmol) in 1,4-dioxane (2.4 mL) and water (0.6 mL) was heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (25 mL), and extracted with ethyl acetate (2 x 25 mL). The organic layer was passed through a hydrophobic frit and the solvent concentrated *in vacuo*. Purification by flash chromatography (0 – 100 % ethyl acetate/cyclohexane) provided methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoate **133** (136 mg, 73 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 1.25, $[MH]^-$= 372; δ_H (400 MHz, DMSO-d_6) 10.59 (1H, s), 8.31 – 8.26 (2H, m), 7.89 – 7.86 (1H, m), 7.53 – 7.62 (1H, m), 7.44 – 7.38 (3H, m), 6.48 – 6.37 (1H, m), 4.31 (2H, m), 3.95 (3H, s), 3.86 (2H, t, *J* = 5.4 Hz), 2.46 – 2.38 (2H, m).

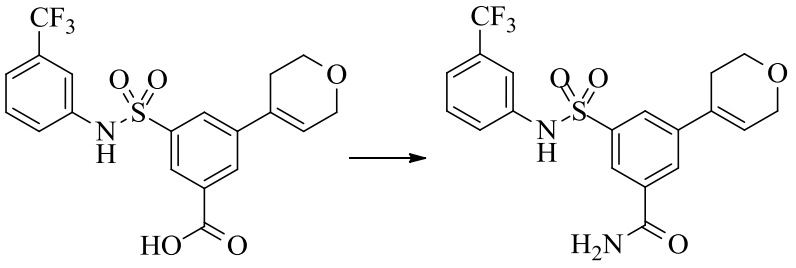
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoic acid 136



133 **136**

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoate **133** (136 mg, 0.31 mmol) was dissolved in THF (2 mL) and water (2 mL). To this, aqueous lithium hydroxide (0.31 mL, 0.62 mmol, 2 M) was added and stirred for 16 h. The THF was removed *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid and extracted with ethyl acetate (2 x 5 mL). The organic layer was dried through a hydrophobic frit and concentrated *in vacuo* to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoic acid **136** (118 mg, 89 %) as a white solid. LCMS LCMS (Formic acid) 100 %, R_t= 1.21, $[MH]^-$= 358; δ_H (400 MHz, DMSO-d_6) 13.71 (1H, s), 10.98 (1H, s), 8.25-8.23 (2H, m), 8.05-7.97 (1H, m), 7.53 – 7.62 (1H, m), 7.49 – 7.41 (3H, m), 6.50 – 6.48 (1H, m), 4.32 (2H, m), 3.86 (2H, t, *J* = 5.4 Hz), 2.46 – 2.38 (2H, m).

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzamide 139

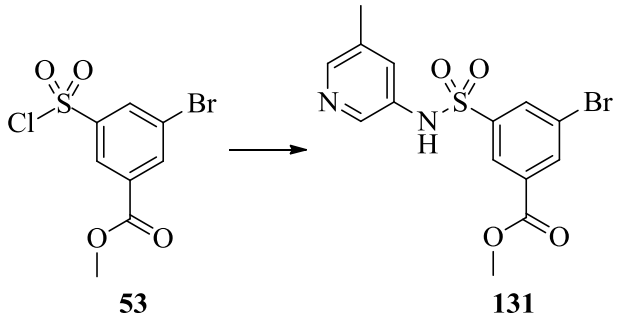


136 **139**

A mixture 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl) benzoic acid **136** (118 mg, 0.27 mmol), DIPEA (0.093 mL, 0.6 mmol), and HATU (106 mg, 0.27 mmol) were dissolved in DMF (2 mL) and stirred for 20 min. To this, ammonia (1.47 mL, 0.73 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at room temperature for 16 h and then concentrated *in vacuo*. The product was dissolved in DMF (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl) sulfamoyl)benzamide **139** (42 mg, 37 %) as an off-white solid. M.P. 212-214 °C; LCMS (Formic acid) 100 %, R_t= 1.00, $[MH]^-$= 425; δ_H (400 MHz, DMSO-d_6) 10.75 (1H, s), 8.25 (1H, s), 8.21 – 8.16 (2H, m), 7.89 – 7.84 (1H, m), 7.60 (1H, s), 7.53 – 7.45 (1H, m), 7.44 – 7.34 (3H, m), 6.44 – 6.36 (1H, m), 4.29 – 4.17 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 2.46 – 2.36 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.5, 141.2, 140.2, 139.1, 136.0 – 135.9 (m), 131.9, 131.1, 130.3 (q, *J* = 31.8 Hz), 127.9, 126.4, 125.5, 125.0, 124.1, 122.8, 125.1 – 124.7 (m), 116.4 (q, *J* = 3.9 Hz), 65.3, 63.8, 26.6; ν_{max} (liquid film)/cm^{-1} 3417, 3068, 2964, 2855, 1663,

1456, 1406, 1350, 1329, 1166, 1114; *m/z* (ES) Found: $[MH]^+$ 427.0941, $C_{19}H_{18}F_3N_2O_4S$ is $[MH]^+$ 427.0934.

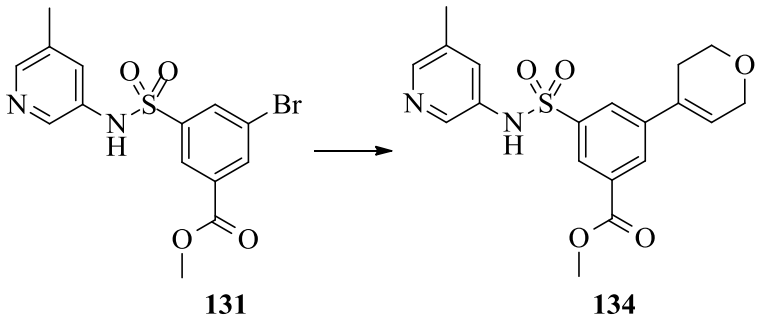
Methyl 3-bromo-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoate 131



53 **131**

Methyl 3-bromo-5-(chlorosulfonyl)benzoate **53** (500 mg, 1.59 mmol) and 5-methylpyridin-3-amine (190 mg, 1.755 mmol) were added to pyridine (5 mL). The resulting mixture was stirred at room temperature for 16 h and then concentrated *in vacuo.* Purification by flash chromatography (0 – 100 % ethyl acetate/hexane) provided methyl 3-bromo-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoate **131** (298 mg, 52 %) as a off-white solid. LCMS (Formic acid) 100 %, R_t= 1.01, $[MH]^+$= 384, 386; δ_H (400 MHz, DMSO-d_6) 8.28 – 8.23 (2H, m), 8.16 – 8.10 (2H, m), 7.98 – 7.96 (1H, m), 7.43 – 7.40 (1H, m), 3.96 (3H, s), 2.22 (3H, s). Sulfomamide N-H not observed.

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoate 134

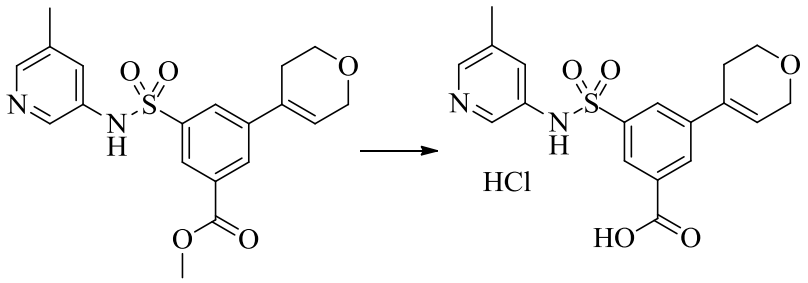


131 **134**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (94 mg, 0.467 mmol), methyl 3-bromo-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoate **131**

(150 mg, 0.389 mmol), $PdCl_2$(dppf) (32 mg, 0.039 mmol) and sodium carbonate (124 mg, 1.17 mmol) in 1,4-dioxane (2.4 mL) and water (0.6 mL) was heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (25 mL), and extracted with ethyl acetate (2 x 25 mL). The organic layer was passed through a hydrophobic frit and the solvent concentrated *in vacuo*. Purification by flash chromatography (0 – 100 % ethyl acetate/cyclohexane) provided methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl)benzoate **134** (70 mg, 46 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.87, $[MH]^+$= 389; δ_H (400 MHz, DMSO-d_6) 8.25 – 8.20 (2H, m), 8.11 – 8.07 (2H, m), 7.90 – 7.84 (1H, m), 7.33 – 7.30 (1H, m), 6.49 – 6.43 (1H, m), 4.29 – 4.24 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 3.95 (3H, s), 2.45 – 2.39 (2H, m), 2.23 (3H, s). Sulfomamide N-H not observed.

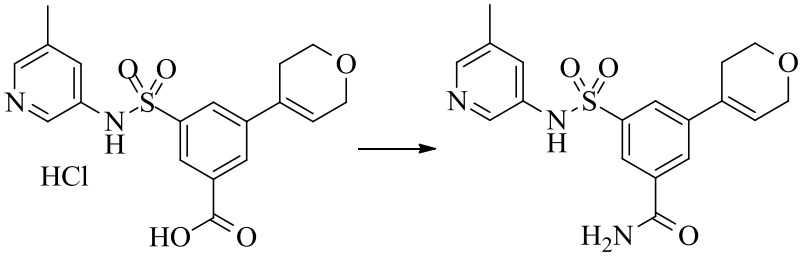
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoic acid 137



134 **137**

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoate **134** (70 mg, 0.18 mmol) was dissolved in THF (2 mL) and water (2 mL). To this, 2 M aqueous lithium hydroxide (0.18 mL, 0.36 mmol) was added and stirred for 16 h. The THF was removed *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid and filtered to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl) benzoic acid hydrochloride **137** (71 mg, 97 %) as an off-white solid. LCMS (Formic acid) 94 %, R_t= 0.91, $[MH]^+$= 338; LCMS (Formic acid) 100 %, R_t= 0.87, $[MH]^+$= 389; δ_H (400 MHz, DMSO-d_6) 8.29 – 8.23 (2H, m), 8.16 – 8.11 (2H, m), 7.98 – 7.93 (1H, m), 7.33 – 7.30 (1H, m), 6.48 – 6.43 (1H, m), 4.30 – 4.25 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 2.45 – 2.39 (2H, m), 2.22 (3H, s). Sulfonamide N-H and carboxylic acid O-H not observed.

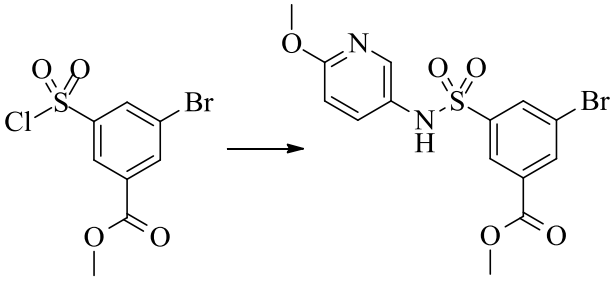
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzamide 140



137 **140**

A mixture 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzoic acid hydrochloride **137** (71 mg, 0.2 mmol), DIPEA (0.106 mL, 0.6 mmol), and HATU (77 mg, 0.2 mmol) were dissolved in DMF (2 mL) and stirred for 20 min. To this, ammonia (1.12 mL, 0.56 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at room temperature for 16 h and then concentrated *in vacuo*. The product was dissolved in DMF (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylpyridin-3-yl)sulfamoyl)benzamide **140** (17 mg, 27 %) as an off-white solid. M.P. 189-191 °C; LCMS (Formic acid) 100 %, R_t= 0.62, $[MH]^+$= 374; δ_H (400 MHz, DMSO-d_6) 8.26 (1H, s), 8.18 – 8.14 (2H, m), 8.09 – 8.02 (2H, m), 7.90 – 7.86 (1H, m), 7.59 (1H, s), 7.33 – 7.30 (1H, m), 6.45 – 6.39 (1H, m), 4.28 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.40 (2H, m), 2.21 (3H, s). Sulfonamide N-H not observed. δ_C (126 MHz, DMSO-d_6) 166.7, 145.5, 141.1, 139.7, 135.9, 135.8, 135.5, 133.7, 132.0, 128.3, 127.5, 126.2, 125.0, 124.9, 65.4, 63.8, 26.7, 18.2; ν_{max} (liquid film)/cm^{-1} 3365, 3076 2966, 2870, 1669, 1597, 1435, 1397, 1344, 1316, 1162, 1128; *m/z* (ES) Found: $[MH]^+$ 374.1181, $C_{18}H_{20}N_3O_4S$ is $[MH]^+$ 374.1169.

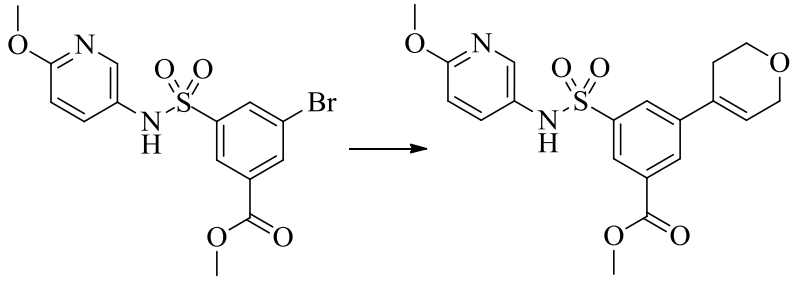
Methyl 3-bromo-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoate 132



53 **132**

Methyl 3-bromo-5-(chlorosulfonyl)benzoate **53** (500 mg, 1.59 mmol) and 6-methoxypyridine-3-amine (198 mg, 1.59 mmol) were added to pyridine (5 mL). The resulting mixture was stirred at room temperature for 16 h and then concentrated *in vacuo.* Purification by flash chromatography (0 – 100 % ethyl acetate/hexane) provided methyl 3-bromo-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoate **132** (460 mg, 72 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 1.17, $[MH]^+$= 401, 403; δ_H (400 MHz, DMSO-d_6) 10.31 (1H, s), 8.27 – 8.24 (1H, m), 8.16 – 8.14 (1H, m), 7.89 – 7.87 (1H, m), 7.49 – 7.43 (2H, m), 6.82 (1H, d, *J* = 8.8 Hz), 3.90 (3H, s).

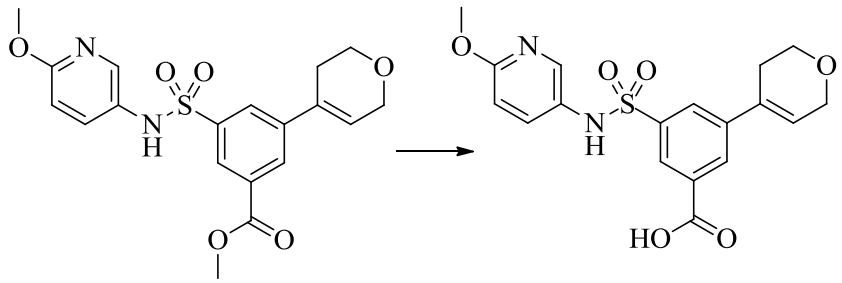
Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoate 135



132 **135**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (94 mg, 0.45 mmol), methyl 3-bromo-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoate **132** (150 mg, 0.37 mmol), $PdCl_2$(dppf) (30 mg, 0.036 mmol) and sodium carbonate (119 mg, 1.1 mmol) in 1,4-dioxane (2.4 mL) and water (0.6 mL) was heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction mixture was concentrated *in vacuo*. The residue was dissolved in water (25 mL), and extracted with ethyl acetate (2 x 25 mL). The organic layer was passed through a hydrophobic frit and the solvent concentrated *in vacuo*. Purification by flash chromatography (0 – 100 % ethyl acetate/cyclohexane) provided methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)phenyl)sulfamoyl)benzoate **135** (140 mg, 93 %) as an off-white solid. LCMS (Formic acid) 100 %, R_t= 1.06, $[MH]^+$= 405; δ_H (400 MHz, DMSO-d_6) 10.22 (1H, s), 8.19 – 8.15 (1H, m), 8.07 – 8.05 (1H, m), 7.86 – 7.83 (1H, m), 7.45 – 7.35 (2H, m), 6.79 (1H, d, *J* = 8.8 Hz), 6.43 – 6.36 (1H, m), 4.27 – 4.24 (2H, m), 3.86 (2H, t, *J* = 5.4 Hz), 3.87 (3H, s), 2.46 – 2.38 (2H, m).

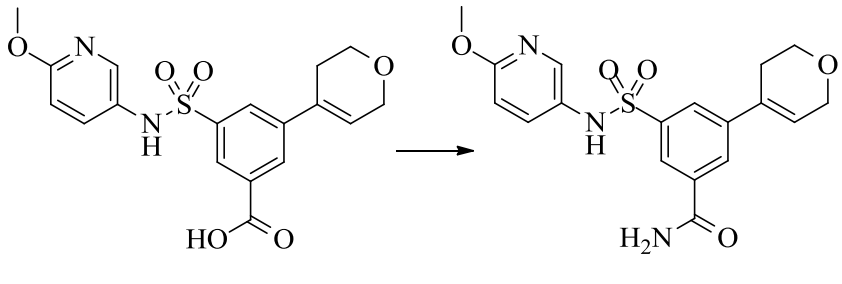
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoic acid 138



135 **138**

Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoate **135** (140 mg, 0.35 mmol) was dissolved in THF (2 mL) and water (2 mL). To this, 2 M aqueous lithium hydroxide (0.34 mL, 0.7 mmol) was added and stirred for 16 h. The THF was removed *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid and extracted with ethyl acetate (2 x 5 mL). The organic layer was dried through a hydrophobic frit and concentrated *in vacuo* to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzoic acid **138** (136 mg, 100 %) as an off-white solid. LCMS (Formic acid) 100 %, R_t= 1.00, $[MH]^+$= 391; δ_H (400 MHz, DMSO-d_6) 10.30 (1H, s), 8.22 – 8.20 (1H, m), 8.10 – 8.07 (1H, m), 7.87 – 7.84 (1H, m), 7.48 – 7.35 (2H, m), 6.76 (1H, d, *J* = 8.8 Hz), 6.45 – 6.37 (1H, m), 4.26 – 4.23 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 3.80 (3H, s), 2.45 – 2.39 (2H, m). Carboxylic acid O-H not observed.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzamide 141

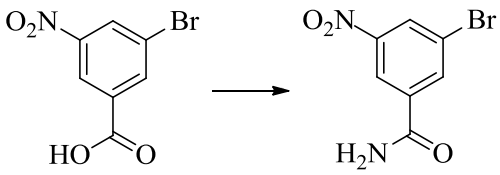


138 **141**

A mixture 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(trifluoromethyl)phenyl)sulfamoyl) benzoic acid **138** (136 mg, 0.35 mmol), DIPEA (0.183 mL, 1.05 mmol), and HATU (198 mg, 0.52 mmol) were dissolved in DMF (2 mL) and stirred for 20 min. To this, ammonia (1.9 mL, 0.96 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at

room temperature for 16 h and then concentrated *in vacuo*. The product was dissolved in DMF (1 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methoxypyridin-3-yl)sulfamoyl)benzamide **141** (37 mg, 27 %) as an off-white solid. M.P. 163-165 °C; LCMS (Formic acid) 100 %, R_t= 0.79, $[MH]^+$= 390; δ_H (400 MHz, DMSO-d_6) 10.19 (1H, s), 8.24 (1H, s), 8.19 – 8.17 (1H, m), 8.11 – 8.08 (1H, m), 7.82 – 7.79 (1H, m), 7.59 (1H, s), 7.45 – 7.32 (2H, m), 6.75 (1H, d, *J* = 8.8 Hz), 6.45 – 6.38 (1H, m), 4.29 – 4.24 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.77 (3H, s), 2.47 – 2.40 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.6, 161.6, 141.4, 141.2, 140.2, 135.8, 135.0, 132.0, 128.1, 127.7, 126.3, 125.1, 125.0, 111.2, 65.4, 63.8, 53.7, 26.7; ν_{max} (liquid film)/cm^{-1} 3251, 3157, 1693, 1604, 1489, 1423, 1391, 1327, 1281, 1163, 1121; *m/z* (ES) Found: $[MH]^+$ 390.1120, $C_{18}H_{20}N_3O_5S$ is $[MH]^+$ 390.1118.

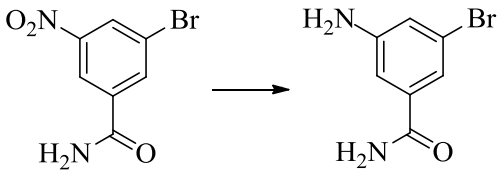
3-Bromo-5-nitrobenzamide 144



143 **144**

3-Bromo-5-nitrobenzoic acid **143** (5 g, 20.32 mmol) was dissolved in thionyl chloride (15 mL, 206 mmol) and stirred at 70 °C for 3 h. The thionyl chloride was concentrated *in vacuo*. The residue was diluted with THF (5 mL) and added dropwise to ammonium hydroxide (28.3 mL, 203 mmol, 28 %) at 0 °C. The THF was concentrated *in vacuo*. The residue was neutralised to pH 7 with 2 M aqueous hydrochloric acid and extracted into ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 3-bromo-5-nitrobenzamide **144** (4.91 g, 99 %) a white solid. The reaction was repeated on 3-bromo-5-nitrobenzoic acid **143** (15 g, 61 mmol) to give 3-bromo-5-nitrobenzamide **144** (14.62 g, 98 %) a white solid. The reaction was repeated on 3-bromo-5-nitrobenzoic acid **143** (25 g, 102 mmol) to give 3-bromo-5-nitrobenzamide **144** (23.32 g, 94 %) a white solid. LCMS (Formic acid) 98 %, R_t= 0.76, $[MH]^-$= 243, 245; δ_H (400 MHz, DMSO-d_6) 8.67 (1H, dd, *J* = 2.1, 1.5 Hz), 8.56 – 8.54 (1H, m), 8.50 – 8.48 (1H, m), 8.39 (1H, s), 7.83 (1H, s).

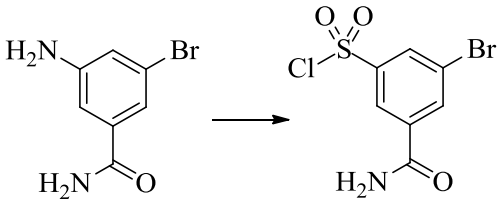
3-Amino-5-bromobenzamide 145



144 **145**

A mixture of 3-bromo-5-nitrobenzamide **144** (15.5 g, 59.2 mmol), ammonium chloride (1.583 g, 29.6 mmol) and iron (9.91 g, 60.1 mmol) was dissolved in ethanol (300 mL) and water (100 mL) and stirred at 80 °C for 17 h. The reaction was cooled and the solvent concentrated *in vacuo*. The residue was diluted with water (1 L) and ethyl acetate (2 L) and filtered through Celite, further washing the Celite with water (2 x 100 mL) and ethyl acetate (2 x 100 mL). The organic layer was separated, washed with brine (1 L), passed through a hydrophobic frit and the solvent concentrated *in vacuo* to give 3-amino-5-bromobenzoic acid **145** (12.61 g, 99 %) as a white solid. The reaction was repeated on 3-amino-5-bromobenzoic acid **144** (23 g, 94 mmol) to give 3-amino-5-bromobenzamide **145** (18.8 g, 93 %) a white solid. The reaction was repeated on 3-amino-5-bromobenzoic acid **144** (4.91 g, 20.04 mmol) to give 3-amino-5-bromobenzamide **145** (3.76 g, 87 %) a white solid. LCMS (Formic acid) 96 %, R_t= 0.55, $[MH]^+$= 215, 217; δ_H (400 MHz, DMSO-d_6) 7.82 (1H, s), 7.24 (1H, s), 7.13 – 7.10 (1H, m), 7.04 – 7.01 (1H, m), 6.88 – 6.85 (1H, m), 5.53 (2H, s).

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride 142

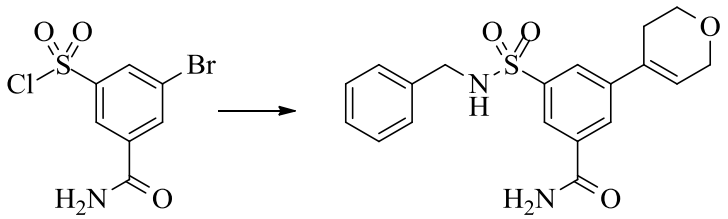


145 **142**

In a 2-necked flask, thionyl chloride (27.2 mL, 372 mmol) was added dropwise to water (25 mL) over 60 min and then stirred for 24 h. Copper (I) chloride (46 mg, 0.465 mmol) was added to the mixture and the resultant yellow green solution was cooled to 0 °C. In a separate 3-necked flask, 37.5 % aqueous hydrochloric acid (11.3 mL, 372 mmol) was added with vigorous stirring to 3-amino-5-bromobenzamide **145** (2 g, 9.3 mmol) and then cooled to

-5 °C. To this, sodium nitrite (1.283 g, 18.6 mmol) in water (25 mL) was added dropwise over 1 h at -5 °C, and then further stirred for 10 min. This mixture was added via cannula to the 2-necked flask over 30 min at -5 °C and further stirred for 1 h. The precipitated solid was collected via vacuum filtration and washed with aqueous hydrochloric acid (2 x 50 mL, 2 M) to give 3-bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (1.86 g, 67 %, 84 % purity via LCMS) of a cream solid. The reaction was repeated on 3-amino-5-bromobenzamide (5 g, 23.25 mmol to give 3-bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (3.76 g, 43 %, 71 % purity via LCMS) as an off white solid. The reaction was repeated on 3-amino-5-bromobenzamide (3.76 g, 17.48 mmol to give 3-bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (2.96 g, 56 %, 44 % purity via LCMS) as an orange solid.

3-(*N*-Benzylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzamide 147

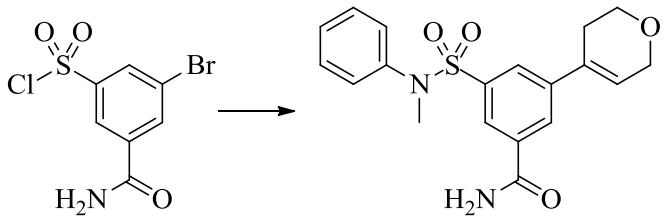


142 **147**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and *N*-benzylamine (0.036 mL, 0.335 mmol) were added to 1,4-dioxane (1 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(*N*-benzylsulfamoyl)-5-(3,6-dihydro-2*H*-pyran-4-yl)benzamide **147** (27 mg, 21 %) as a cream solid. M.P. 119-121 °C; LCMS (Formic acid) 100 %, R_t= 0.83, $[MH]^+$= 373; δ_H (400 MHz, DMSO-d_6) 8.27 – 8.20 (2H, m), 8.20 – 8.18

(1H, m), 8.16 – 8.14 (1H, m), 7.92 – 7.87 (1H, m), 7.57 (1H, s), 7.31 – 7.15 (5H, m), 6.47 – 6.42 (1H, m), 4.30 – 4.24 (2H, m), 4.02 (2H, d, *J* = 6.0 Hz), 3.86 (2H, t, *J* = 5.4 Hz), 2.50 – 2.46 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.9, 141.9, 141.2, 137.8, 135.7, 132.2, 128.6, 128.0, 127.5, 127.2, 126.0, 125.0, 124.8, 65.4, 63.9, 46.6, 26.8; ν_{max} (liquid film)/cm^{-1} 3199, 3924, 1662, 1575, 1385, 1314, 1160; *m/z* (ES) Found: $[MH]^+$ 373.1230, $C_{19}H_{21}N_2O_4S$ is $[MH]^+$ 373.1217.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-methyl-*N*-phenylsulfamoyl)benzamide 148

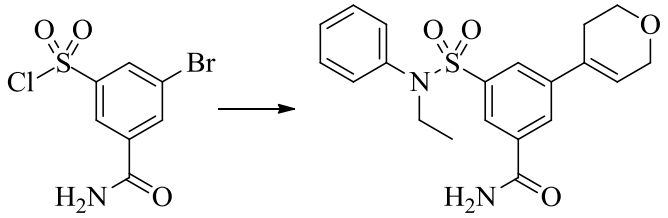


142 **148**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and *N*-methylaniline (0.036 mL, 0.335 mmol) were added to pyridine (0.5 mL). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (9 mg, 0.017 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-methyl-*N*-phenylsulfamoyl)benzamide **148** (31 mg, 24 %) as a yellow solid. M.P. 149-151 °C; LCMS (Formic acid) 100 %, R_t= 0.92, $[MH]^+$= 373; δ_H (400 MHz, DMSO-d_6) 8.29 (1H, s), 8.27 – 8.24 (1H, m), 7.99 – 7.96 (1H, m), 7.60 (1H, s), 7.43 – 7.39 (1H, m), 7.39 – 7.28 (3H, m), 7.16 – 7.08 (2H, m), 6.37 – 6.32 (1H, m), 4.28 – 4.20 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 3.16 (3H, s), 2.40 – 2.31 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.6, 141.4, 141.1, 137.0, 135.8, 131.9, 129.4, 128.1, 127.8, 126.6, 126.3, 125.6, 125.5, 65.4, 63.8, 38.4, 26.6; ν_{max} (liquid film)/cm^{-1} 3431, 3231, 3051, 1637,

1560, 1344, 1311, 1201, 1156; *m/z* (ES) Found: $[MH]^+$ 373.1213, $C_{19}H_{21}N_2O_4S$ is $[MH]^+$ 373.1217.

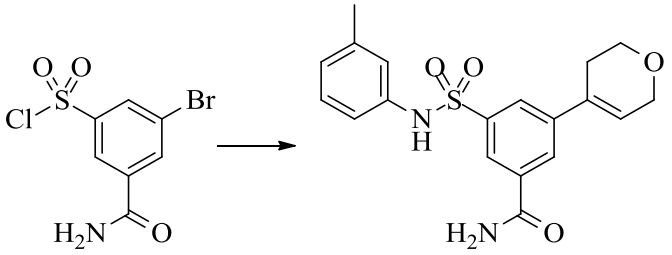
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-ethyl-*N*-phenylsulfamoyl)benzamide 149



142 **149**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and *N*-ethylaniline (0.026 mL, 0.335 mmol) were added to pyridine (0.5 mL). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (9 mg, 0.017 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method C) then purified by MDAP on XBridge C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-ethyl-*N*-phenylsulfamoyl)benzamide **149** (14.5 mg, 11 %) as a white solid. M.P. 170-172 °C; LCMS (Formic acid) 100 %, R_t= 0.98, $[MH]^+$= 387; δ_H (400 MHz, DMSO-d_6) 8.30 (1H, s), 8.26 – 8.24 (1H, m), 8.02 – 7.98 (1H, m), 7.62 (1H, s), 7.52 – 7.50 (1H, m), 7.42 – 7.33 (3H, m), 7.10 – 7.04 (2H, m), 6.42 – 6.36 (1H, m), 4.28 – 4.21 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.62 (2H, q, *J* = 7.1 Hz), 2.45 – 2.36 (2H, m), 0.99 (3H, t, *J* = 7.1 Hz); δ_C (126 MHz, DMSO-d_6) 166.6, 141.1, 138.8, 138.6, 135.8, 132.0, 129.5, 128.9, 128.4, 127.9, 126.3, 125.5, 125.3, 65.4, 63.8, 45.5, 26.6, 14.3; ν_{max} (liquid film)/cm^{-1} 3420, 3074, 2966, 1630, 1351, 1343, 1169, 1136; *m/z* (ES) Found: $[MH]^+$ 387.1385, $C_{20}H_{23}N_2O_4S$ is $[MH]^+$ 387.1373.

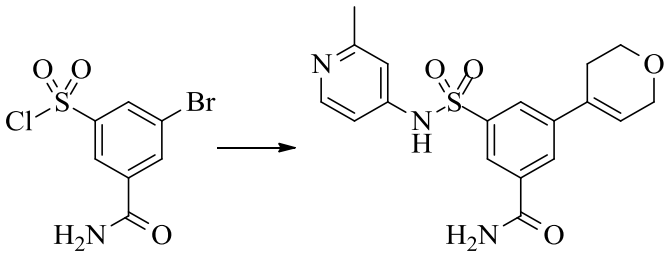
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(*m*-tolyl)sulfamoyl)benzamide 150



142 **150**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and *m*-toluidine (0.037 mL, 0.335 mmol) were added to pyridine (0.5 mL). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (9 mg, 0.017 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.2 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) then purified by MDAP on XBridge C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(*m*-tolyl)sulfamoyl)benzamide **150** (13 mg, 10 %) as a white solid. M.P. 185-187 °C; LCMS (Formic acid) 100 %, R_t= 0.91, $[MH]^+$= 373; δ_H (400 MHz, DMSO-d_6) 10.27 (1H, s), 8.25 (1H, s), 8.18 – 8.13 (2H, m), 7.88 – 7.85 (1H, s), 7.59 (1H, s), 7.11 (1H, t, *J* = 7.8 Hz), 6.94 – 6.87 (2H, m), 6.85 (1H, d, *J* = 7.5 Hz), 6.42 – 6.39 (1H, m), 4.29 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.39 (2H, m), 2.20 (3H, s); δ_C (126 MHz, DMSO-d_6) 166.7, 141.0, 140.7, 138.9, 138.0, 135.8, 132.0, 129.4, 127.6, 126.2, 125.4, 125.1, 125.0, 121.3, 117.8, 65.4, 63.8, 26.7, 21.4; ν_{max} (liquid film)/cm^{-1} 3250, 2971, 2901, 1663, 1608, 1385, 1333, 1156, 1126; *m/z* (ES) Found: $[MH]^+$ 373.1221, $C_{19}H_{21}N_2O_4S$ is $[MH]^+$ 373.1217.

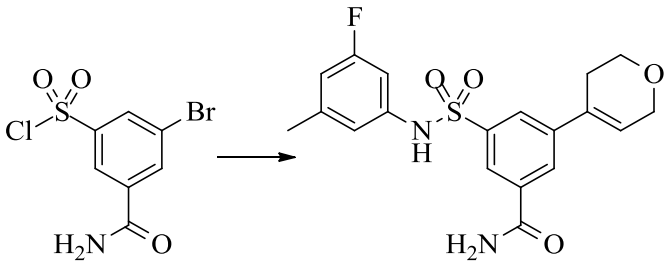
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(2-methylpyridin-4-yl)sulfamoyl)benzamide 151



142 **151**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 2-methylpyridin-4-amine (36 mg, 0.335 mmol) were added to THF (0.5 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (77 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(2-methylpyridin-4-yl)sulfamoyl)benzamide **151** (4.7 mg, 3 %) as a white solid. M.P. 215-217 °C; LCMS (Formic acid) 99 %, R_t= 0.47, $[MH]^+$= 374; δ_H (400 MHz, DMSO-d_6) 8.22 (1H, s), 8.19 – 8.18 (1H, m), 8.07 – 8.04 (1H, m), 7.93 – 7.91 (1H, m), 7.87 (1H, d, *J* = 7.0 Hz), 7.49 (1H, s), 6.83 (1H, dd, *J* = 7.0, 2.4 Hz), 6.77 (1H, d, *J* = 2.1 Hz), 6.45 – 6.36 (1H, m), 4.28 – 4.23 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 2.50 – 2.45 (2H, m), 2.32 (3H, s). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 167.2, 157.6, 140.6, 139.3, 138.9, 135.3, 132.5, 126.1, 125.5, 124.5, 124.4, 124.3, 114.7, 113.1, 65.4, 63.9, 26.8, 19.7; ν_{max} (liquid film)/cm^{-1} 3365, 3251, 3184, 2971, 2923, 1663, 1608, 1386, 1333, 1156, 1126; *m/z* (ES) Found: $[MH]^+$ 374.1180, $C_{18}H_{20}N_3O_4S$ is $[MH]^+$ 374.1169.

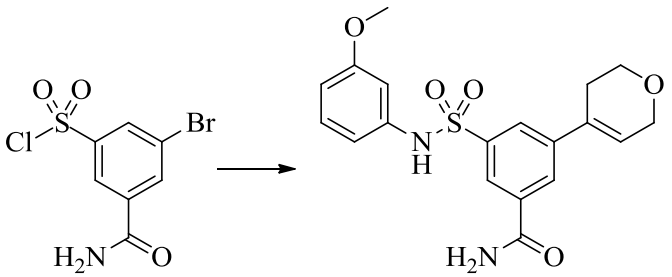
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-fluoro-5-methylphenyl)sulfamoyl)benzamide 152



142 **152**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3-fluoro-5-methylaniline (42 mg, 0.335 mmol) were added to THF (0.5 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-fluoro-5-methylphenyl)sulfamoyl)benzamide **152** (45 mg, 34 %) as a cream solid. M.P. 190-192 °C; LCMS (Formic acid) 100 %, R_t= 0.95, $[MH]^-$= 389; δ_H (400 MHz, DMSO-d_6) 10.56 (1H, s), 8.26 (1H, s), 8.21 – 8.14 (2H, m), 7.97 – 7.85 (1H, m), 7.59 (1H, s), 6.83 – 6.62 (3H, m), 6.48 – 6.37 (1H, m), 4.32 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.3 Hz), 2.47 – 2.41 (2H, m), 2.20 (3H, s); δ_C (101 MHz, DMSO-d_6) 166.6, 162.6 (d, *J* = 242.6 Hz), 141.6 (d, *J* = 9.4 Hz), 141.2, 140.5, 139.7 (d, *J* = 11.4 Hz), 135.9, 132.0, 127.8, 126.3, 124.9 (d, *J* = 8.8 Hz), 116.5, 116.5, 111.6 (d, *J* = 21.0 Hz), 104.1 (d, *J* = 25.5 Hz), 65.4, 63.8, 26.7, 21.4 (d, *J* = 1.9 Hz); δ_F (376 MHz, DMSO-d_6) -112.58 (t, *J* = 10.1 Hz); v_{max} (liquid film)/cm^{-1} 3440, 3343, 3289, 2903, 1659, 1604, 1399, 1332, 1168, 1132.

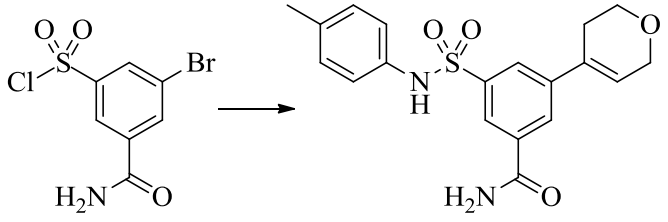
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-methoxyphenyl)sulfamoyl)benzamide 143



142 **153**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-methoxyaniline (42 mg, 0.341 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with an acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-methoxyphenyl)sulfamoyl)benzamide **153** (51 mg, 39 %) as a white solid. M.P. 193-195 °C; LCMS (Formic acid) 98 %, R_t= 0.84, $[MH]^+$= 389; δ_H (400 MHz, DMSO-d_6) 8.24 (1H, s), 8.20 – 8.16 (1H, m), 8.16 – 8.12 (1H, m), 7.91 – 7.86 (1H, m), 7.57 (1H, s), 7.12 (1H, t, *J* = 8.2 Hz), 6.70 – 6.65 (2H, m), 6.63 – 6.55 (1H, m), 6.44 – 6.36 (1H, m), 4.29 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.66 (3H, s), 2.47 – 2.40 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 160.1, 141.0, 140.9, 139.7, 135.8, 132.0, 130.4, 127.5, 126.2, 125.1, 125.0, 112.8, 109.5, 106.5, 65.4, 63.8, 55.4, 26.7. Sulfonamide N-H not observed. ν_{max} (liquid film)/cm^{-1} 3395, 3246, 3139, 1698, 1604, 1439, 1390, 1330, 1266, 1165; *m/z* (ES) Found: $[MH]^+$ 389.1170, $C_{19}H_{21}N_2O_5S$ is $[MH]^+$ 389.1166.

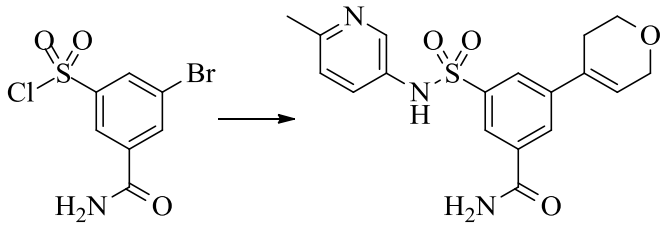
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(*p*-tolyl)sulfamoyl)benzamide 154



142 **154**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and *p*-toluidine (36 mg, 0.336 mmol) were added to pyridine (0.5 mL). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (9 mg, 0.017 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.2 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) then purified by MDAP on XBridge C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(*p*-tolyl)sulfamoyl)benzamide **154** (8.5 mg, 6 %) as a white solid. M.P. 204-206 °C; LCMS (Formic acid) 100 %, R_t= 0.89, $[MH]^+$= 373; δ_H (400 MHz, DMSO-d_6) 10.13 (1H, s), 8.24 (1H, s), 8.16 – 8.12 (2H, m), 7.84 – 7.82 (1H, m), 7.58 (1H, s), 7.07 – 7.01 (2H, m), 7.01 – 6.96 (2H, m), 6.42 – 6.37 (1H, m), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.38 (2H, m), 2.18 (3H, s); δ_C (126 MHz, DMSO-d_6) 166.7, 141.0, 140.6, 135.7, 135.2, 134.1, 132.0, 130.0, 127.5, 126.2, 125.1, 125.0, 121.4, 65.4, 63.8, 26.7, 20.7; ν_{max} (liquid film)/cm^{-1} 3393, 3258, 3145, 1694, 1508, 1432, 1389, 1333, 1161, 1124; *m/z* (ES) Found: $[MH]^+$ 373.1220, $C_{19}H_{21}N_2O_4S$ is $[MH]^+$ 373.1217.

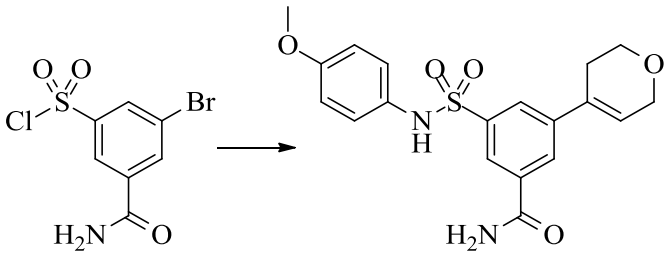
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methylpyridin-3-yl)sulfamoyl)benzamide 155



142 **155**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 6-methylpyridin-3-amine (36 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on XSelect column using acetonitrile/water with an ammonium carbonate modifier (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(6-methylpyridin-3-yl)sulfamoyl)benzamide **155** (33 mg, 26 %) as a white solid. M.P. 228-230 °C; LCMS (Formic acid) 100 %, R_t= 0.56, $[MH]^+$= 374; δ_H (400 MHz, DMSO-d_6) 10.39 (1H, s), 8.25 (1H, s), 8.19 – 8.16 (1H, m), 8.15 – 8.12 (2H, m), 7.88 – 7.80 (1H, m), 7.59 (1H, s), 7.40 (1H, dd, *J* = 8.4, 2.7 Hz), 7.15 (1H, d, *J* = 8.4 Hz), 6.45 – 6.38 (1H, m), 4.29 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.39 (2H, m), 2.35 (3H, s); δ_C (101 MHz, DMSO-d_6) 166.6, 154.6, 142.3, 141.2, 140.3, 135.9, 132.0, 131.9, 129.5, 127.8, 126.3, 125.0, 124.9, 123.7, 65.4, 63.8, 26.7, 23.7; ν_{max} (liquid film)/cm^{-1} 3408, 3257, 2969, 2857, 1645, 1497, 1380, 1160, 1143; *m/z* (ES) Found: $[MH]^+$ 374.1184, $C_{18}H_{20}N_3O_4S$ is $[MH]^+$ 374.1169.

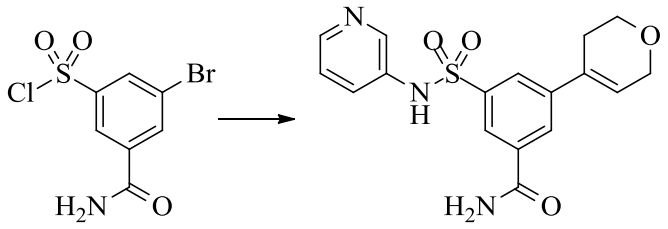
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-methoxyphenyl)sulfamoyl)benzamide 156



142 **156**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-methoxyaniline (42 mg, 0.341 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on XSelect column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-methoxyphenyl)sulfamoyl)benzamide **156** (50 mg, 38 %) as a cream solid. M.P. 219-221 °C; LCMS (Formic acid) 100 %, R_t= 0.82, $[MH]^+$= 389; δ_H (400 MHz, DMSO-d_6) 9.94 (1H, s), 8.23 (1H, s), 8.17 – 8.13 (1H, m), 8.13 – 8.09 (1H, m), 7.81 – 7.77 (1H, m), 7.57 (1H, s), 7.03 – 6.94 (2H, m), 6.86 – 6.77 (2H, m), 6.41 – 6.36 (1H, m), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.67 (3H, s), 2.46 – 2.37 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 157.2, 140.9, 140.5, 135.7, 132.0, 130.3, 127.4, 126.1, 125.1, 125.0, 124.1, 114.8, 65.4, 63.8, 55.6, 26.7; ν_{max} (liquid film)/cm^{-1} 3406, 3252, 3143, 1697, 1606, 1508, 1394, 1333, 1252, 1161; *m/z* (ES) Found: $[MH]^+$ 389.1166, $C_{19}H_{21}N_2O_5S$ is $[MH]^+$ 389.1166.

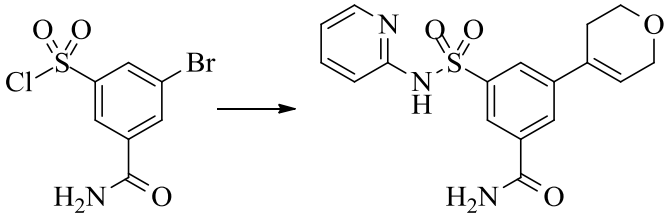
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(pyridin-3-yl)sulfamoyl)benzamide 157



142 **157**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and pyridin-3-amine (31 mg 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(pyridin-3-yl)sulfamoyl)benzamide **157** (9 mg, 7 %) as a cream solid. M.P. 190 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.67, $[MH]^+$= 360; δ_H (400 MHz, DMSO-d_6) 10.63 (1H, s), 8.33 – 8.25 (3H, m), 8.21 – 8.15 (2H, m), 7.91 – 7.87 (1H, m), 7.62 (1H, s), 7.55 – 7.51 (1H, m), 7.31 (1H, dd, *J* = 8.2, 4.7 Hz), 6.48 – 6.40 (1H, m), 4.28 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.46 – 2.41 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.6, 146.0, 142.5, 141.3, 140.2, 135.9, 134.6, 131.9, 128.3, 127.9, 126.4, 125.0, 124.9, 124.5, 65.4, 63.8, 26.6; ν_{max} (liquid film)/cm^{-1} 3377, 3246, 2931, 1668, 1387, 1359, 1313, 1160, 1127; *m/z* (ES) Found: $[MH]^+$ 360.1019, $C_{17}H_{18}N_3O_4S$ is $[MH]^+$ 360.1013.

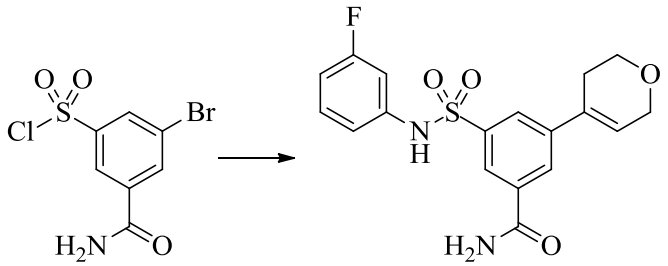
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(pyridin-2-yl)sulfamoyl)benzamide 158



142 **158**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and pyridin-2-amine (31 mg 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on XSelect column using acetonitrile/water with an ammonium carbonate modifier (method B) then purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(pyridin-2-yl)sulfamoyl)benzamide **158** (9 mg, 7 %) as a white solid. M.P. 240 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.57, $[MH]^+$= 360; δ_H (400 MHz, DMSO-d_6) 8.28 – 8.20 (2H, m), 8.16 – 8.09 (1H, m), 8.05 – 8.00 (1H, m), 7.99 (1H, s), 7.79 – 7.72 (1H, m), 7.56 (1H, s), 7.21 (1H, d, *J* = 8.6 Hz), 6.90 – 6.81 (1H, m), 6.48 – 6.41 (1H, m), 4.31 – 4.19 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 2.50 – 2.45 (2H, m). Sulfonamide N-H not observed. δ_C (126 MHz, DMSO-d_6) 167.0, 140.8, 135.6, 135.3, 135.2, 132.2, 131.7, 126.9, 125.9, 125.0, 124.9, 124.7, 65.4, 63.9, 26.8. Two carbons are not observed. ν_{max} (liquid film)/cm^{-1} 3378, 3246, 2931, 1668, 1596, 1387, 1359, 1160, 1127; *m/z* (ES) Found: $[MH]^+$ 360.1028, $C_{17}H_{18}N_3O_4S$ is $[MH]^+$ 360.1013.

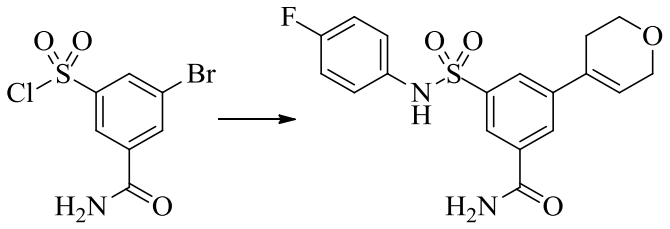
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-fluorophenyl)sulfamoyl)benzamide 159



142 **159**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3-fluoroaniline (37 mg 0.335 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-fluorophenyl)sulfamoyl)benzamide **159** (16 mg, 12 %) as a yellow solid. M.P. 163-165 °C; LCMS (Formic acid) 97 %, R_t= 0.88, $[MH]^+$= 377; δ_H (400 MHz, DMSO-d_6) 10.41 (1H, s), 8.24 (1H, s), 8.18 – 8.16 (1H, m), 8.16 – 8.13 (1H, m), 7.91 – 7.88 (1H, m), 7.57 (1H, s), 7.28 – 7.20 (1H, m), 6.93 – 6.85 (2H, m), 6.85 – 6.76 (1H, m), 6.44 – 6.39 (1H, m), 4.28 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.42 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 162.7 (d, *J* = 243.1 Hz), 141.2, 141.1, 139.1, 135.8, 132.0, 131.2 (d, *J* = 9.7 Hz), 131.3 – 131.2 (m), 130.9, 127.5, 126.2, 124.9 (d, *J* = 10.5 Hz), 116.2 (d, *J* = 2.5 Hz), 107.0 (d, *J* = 24.8 Hz), 65.4, 63.8, 26.7; δ_F (376 MHz, DMSO-d_6) -111.74 – -111.94 (m); ν_{max} (liquid film)/cm^{-1} 3423, 3245, 3126, 2927, 1664, 1603, 1505, 1389, 1331, 1169, 1122.

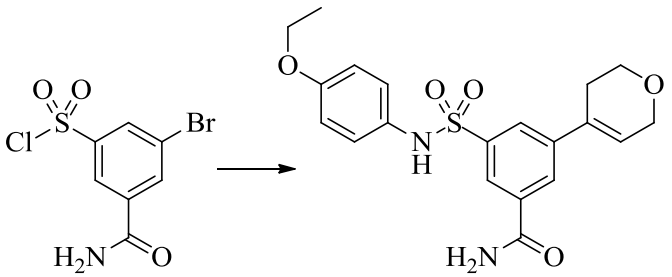
3-(3,6-dihydro-2H-pyran-4-yl)-5-(N-(4-fluorophenyl)sulfamoyl)benzamide160



142 **160**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-fluoroaniline (0.032 mL, 0.335 mmol) were added to 1,4-dioxane (1 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (9 mg, 0.017 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.5 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) then purified by MDAP on XBridge C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2H-pyran-4-yl)-5-(N-(4-fluorophenyl)sulfamoyl)benzamide **160** (27 mg, 21 %) as a white solid. M.P. 138-140 °C; LCMS (Formic acid) 100 %, R_t= 0.87, $[MH]^+$= 377; δ_H (400 MHz, DMSO-d_6) 10.24 (1H, s), 8.24 (1H, s), 8.21 – 8.16 (1H, m), 8.16 – 8.11 (1H, m), 7.88 – 7.83 (1H, m), 7.57 (1H, s), 7.28 – 7.09 (4H, m), 6.44 – 6.38 (1H, m), 4.30 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 2.47 – 2.39 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.7, 159.6 (d, *J* = 241.3 Hz), 141.1, 140.5, 135.8, 134.3, 132.0, 127.6, 126.2, 125.1, 124.9, 123.6 (d, *J* = 8.3 Hz), 116.3 (d, *J* = 22.6 Hz), 65.4, 63.8, 26.7; δ_F (376 MHz, DMSO-d_6) -118.22 – -118.31 (m); ν_{max} (liquid film)/cm^{-1} 3245, 2928, 1664, 1505, 1388, 1333, 1163, 1144.

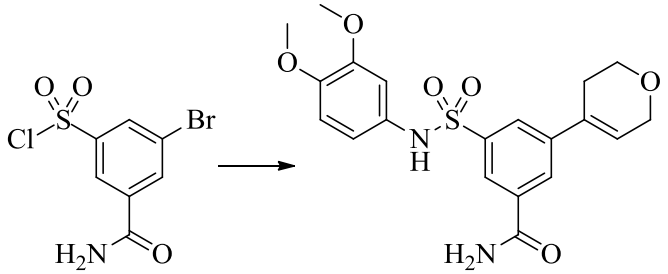
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-ethoxyphenyl)sulfamoyl)benzamide 161



142 **161**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-ethoxyaniline (0.044 mL, 0.342 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-ethoxyphenyl)sulfamoyl)benzamide **161** (56 mg, 41 %) as a white solid. M.P. 232-234 °C; LCMS (Formic acid) 100 %, R_t= 0.91, $[MH]^+$= 403; δ_H (400 MHz, DMSO-d_6) 9.96 (1H, s), 8.22 (1H, s), 8.16 – 8.12 (1H, m), 8.12 – 8.09 (1H, m), 7.79 – 7.75 (1H, m), 7.56 (1H, s), 7.00 – 6.92 (2H, m), 6.82 – 6.75 (2H, m), 6.42 – 6.35 (1H, m), 4.25 (2H, d, *J* = 2.7 Hz), 3.91 (2H, q, *J* = 7.0 Hz), 3.83 (2H, t, *J* = 5.4 Hz), 2.44 – 2.38 (2H, m), 1.26 (3H, t, *J* = 7.0 Hz); δ_C (101 MHz, DMSO-d_6) 166.7, 156.4, 140.9, 140.6, 135.7, 132.0, 130.3, 127.4, 126.1, 125.1, 124.1, 115.3, 65.4, 63.8, 63.5, 26.7, 15.0. One carbon not observed. ν_{max} (liquid film)/cm^{-1} 3403, 3247, 3157, 1697, 1509, 1393, 1333, 1163, 1124; *m/z* (ES) Found: $[MH]^+$ 403.1339, $C_{20}H_{23}N_2O_5S$ is $[MH]^+$ 403.1322.

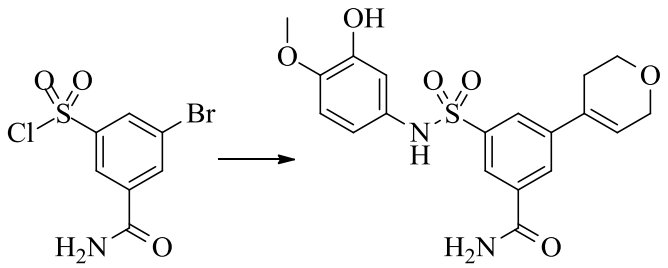
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3,4-dimethoxyphenyl)sulfamoyl)benzamide 162



142 **162**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3,4-dimethoxyaniline (51 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3,4-dimethoxyphenyl)sulfamoyl)benzamide **162** (40 mg, 28 %) as a cream solid. M.P. 205-207 °C; LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^+$= 418; δ_H (400 MHz, DMSO-d_6) 9.97 (1H, s), 8.23 (1H, s), 8.17 – 8.11 (2H, m), 7.85 – 7.79 (1H, m), 7.57 (1H, s), 6.80 (1H, d, *J* = 8.7 Hz), 6.69 (1H, d, *J* = 2.4 Hz), 6.57 (1H, dd, *J* = 8.6, 2.5 Hz), 6.42 – 6.37 (1H, m), 4.28 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.66 (3H, s), 3.63 (3H, s), 2.46 – 2.38 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 149.2, 146.7, 140.9, 140.6, 135.7, 132.1, 130.9, 127.4, 126.1, 125.2, 125.1, 114.4, 112.6, 107.3, 65.4, 63.8, 56.0, 55.8, 26.7; ν_{max} (liquid film)/cm^{-1} 3408, 3303, 3237, 2810, 1659, 1515, 1341, 1316, 1234, 1162, 1126.

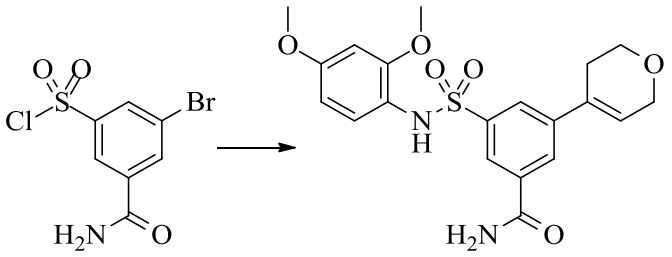
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-hydroxy-4-methoxyphenyl)sulfamoyl) benzamide 163



142 **163**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 5-amino-2-methoxyphenol (46 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.187 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-hydroxy-4-methoxyphenyl)sulfamoyl)benzamide **163** (8 mg, 5 %) as a cream solid. M.P. 140 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.72, $[MH]^+$= 405; δ_H (400 MHz, DMSO-d_6) 9.86 (1H, s), 9.13 (1H, s), 8.23 (1H, s), 8.15 – 8.13 (1H, m), 8.13 – 8.11 (1H, m), 7.83 – 7.79 (1H, m), 7.56 (1H, s), 6.75 (1H, d, *J* = 8.7 Hz), 6.59 (1H, d, *J* = 2.6 Hz), 6.46 (1H, dd, *J* = 8.6, 2.6 Hz), 6.43 – 6.36 (1H, m), 4.28 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.66 (3H, s), 2.46 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.8, 147.2, 145.5, 140.9, 140.8, 135.7, 132.1, 131.0, 127.3, 126.0, 125.1, 113.0, 112.8, 110.4, 65.4, 63.8, 56.2, 26.7. One carbon not observed. ν_{max} (liquid film)/cm^{-1} 3377, 3250, 3157, 1693, 1511, 1398, 1307, 1273, 1237, 1143, 1119, 1026.

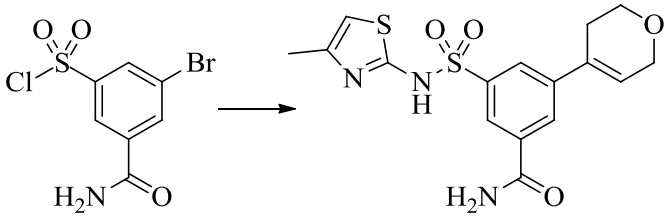
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(2,4-dimethoxyphenyl)sulfamoyl)benzamide 164



142 **164**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 2,4-dimethoxyaniline (0.048 mL, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(2,4-dimethoxyphenyl)sulfamoyl)benzamide **164** (58 mg, 41 %) as a yellow solid. M.P. 188-190 °C; LCMS (Formic acid) 98 %, R_t= 0.78, $[MH]^+$= 418; δ_H (400 MHz, DMSO-d_6) 9.36 (1H, s), 8.21 (1H, s), 8.16 – 8.12 (1H, m), 8.11 – 8.06 (1H, m), 7.76 – 7.72 (1H, m), 7.54 (1H, s), 7.10 (1H, d, *J* = 8.7 Hz), 6.47 (1H, dd, *J* = 8.7, 2.7 Hz), 6.42 (1H, d, *J* = 2.7 Hz), 6.39 – 6.34 (1H, m), 4.29 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.71 (3H, s), 3.33 (3H, s), 2.46 – 2.38 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.9, 159.4, 155.0, 141.8, 140.6, 135.3, 132.3, 129.0, 127.1, 125.8, 125.3, 125.1, 117.8, 105.1, 99.3, 65.4, 63.8, 55.8, 55.7, 26.8; ν_{max} (liquid film)/cm^{-1} 3384, 3259, 3148, 2839, 1698, 1668, 1607, 1508, 1392, 1333, 1207, 1159, 1124.

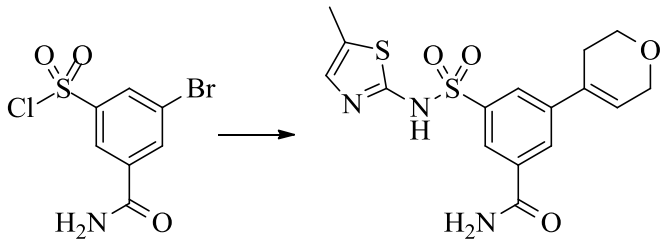
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-methylthiazol-2-yl)sulfamoyl)benzamide 165



142 **165**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-methylthiazol-2-amine (38 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by on XSelect column using acetonitrile/water with an ammonium carbonate modifier MDAP (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-methylthiazol-2-yl)sulfamoyl)benzamide **165** (7 mg, 5 %) as a white solid. M.P. 210 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.65, $[MH]^+$= 380; δ_H (400 MHz, DMSO-d_6) 12.69 (1H, s), 8.25 (1H, s), 8.20 – 8.17 (1H, m), 8.14 – 8.10 (1H, m), 7.94 – 7.89 (1H, m), 7.55 (1H, s), 6.43 (1H, s), 6.38 (1H, s), 4.31 – 4.23 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 2.51 – 2.47 (2H, m), 2.08 (3H, s); δ_C (126 MHz, DMSO-d_6) 169.3, 167.0, 143.7, 140.7, 135.5, 132.3, 126.7, 126.5, 125.8, 124.3, 124.2, 102.9, 65.4, 63.9, 26.8, 14.0; ν_{max} (liquid film)/cm^{-1} 3412, 3079, 2968, 2915, 1674, 1520, 1389, 1162, 1130; *m/z* (ES) Found: $[MH]^+$ 380.0740, $C_{16}H_{18}N_3O_4S_2$ is $[MH]^+$ 380.0733.

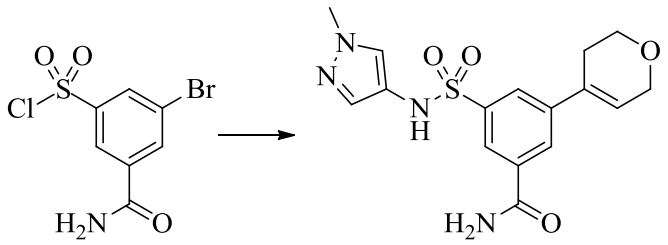
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylthiazol-2-yl)sulfamoyl)benzamide 166



142 **166**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 5-methylthiazol-2-amine (38 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.1 mL, 0.717 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-methylthiazol-2-yl)sulfamoyl)benzamide **166** (13 mg, 10 %) as a cream solid. M.P. 198 °C decomposition; LCMS (Formic acid) 96 %, R_t= 0.67, $[MH]^+$= 380; δ_H (400 MHz, DMSO-d_6) 12.46 (1H, s), 8.24 (1H, s), 8.18 – 8.16 (1H, m), 8.14 – 8.11 (1H, m), 7.92 – 7.89 (1H, m), 7.54 (1H, s), 6.97 (1H, d, *J* = 1.4 Hz), 6.46 – 6.40 (1H, m), 4.31 – 4.21 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 2.50 – 2.46 (2H, m), 2.19 (3H, d, *J* = 1.4 Hz); δ_C (126 MHz, DMSO-d_6) 168.5, 166.9, 143.6, 140.9, 135.6, 132.3, 126.8, 125.9, 124.2, 124.1, 121.1, 120.7, 65.4, 63.9, 26.8, 12.5; ν_{max} (liquid film)/cm^{-1} 3409, 3104, 2919, 1672, 1592, 1522, 1388, 1311, 1279, 1129; *m/z* (ES) Found: $[MH]^+$ 380.0738, $C_{16}H_{18}N_3O_4S_2$ is $[MH]^+$ 380.0733.

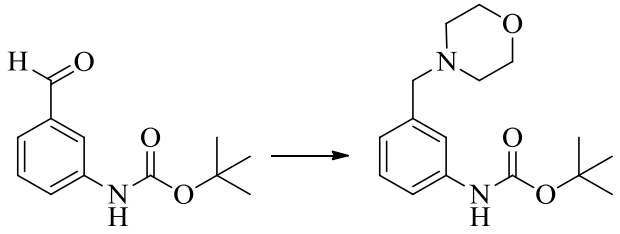
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(1-methyl-1*H*-pyrazol-4-yl)sulfamoyl)benzamide 167



142 **167**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 1-methyl-1*H*-pyrazol-4-amine hydrochloride (45 mg 0.335 mmol) were added to THF (1 mL) and triethylamine (0.15 mL, 1.076 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(1-methyl-1*H*-pyrazol-4-yl)sulfamoyl)benzamide **167** (20 mg, 16 %) as a yellow solid. M.P. 195-197 °C; LCMS (Formic acid) 100 %, R_t= 0.64, $[MH]^+$= 363; δ_H (400 MHz, DMSO-d_6) 9.80 (1H, s), 8.26 (1H, s), 8.18 – 8.16 (1H, m), 8.12 – 8.10 (1H, m), 7.85 – 7.80 (1H, m), 7.59 (1H, s), 7.48 – 7.42 (1H, m), 7.07 – 7.01 (1H, m), 6.46 – 6.39 (1H, m), 4.30 – 4.24 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 3.70 (3H, s), 2.49 – 2.43 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.8, 141.0, 140.6, 135.7, 133.7, 132.1, 127.4, 126.1, 125.3, 125.1, 125.0, 119.4, 65.4, 63.9, 39.2, 26.7; ν_{max} (liquid film)/cm^{-1} 3363, 3273, 3188, 2919, 1674, 1621, 1386, 1353, 1321, 1168, 1134; *m/z* (ES) Found: $[MH]^+$ 363.1132, $C_{16}H_{19}N_4O_4S$ is $[MH]^+$ 363.1122.

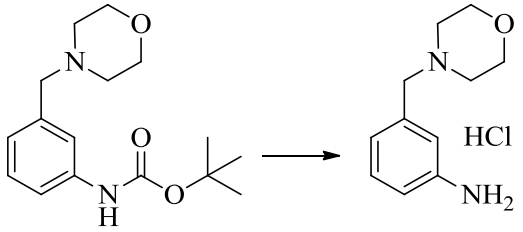
***Tert*-butyl (3-(morpholinomethyl)phenyl)carbamate 190**



189 **190**

Tert-butyl (3-formylphenyl)carbamate **189** (400 mg, 1.808 mmol) and morpholine (0.161 mL, 1.808 mmol) were dissolved in THF (10 mL) and stirred for 5 min. To this sodium triacetoxyborohydride (766 mg, 3.62 mmol) was added and stirred for 16 h. To this, saturated ammonium chloride (10 mL) was added and then concentrated *in vacuo*. The residue was dissolved in water (20 mL) and then extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in 5 % acetonitrile in water and purified by reverse phase chromatography, eluting with 5-30 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give *tert*-butyl (3-(morpholinomethyl)phenyl)carbamate **190** (420 mg, 79 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.52, $[MH]^+$= 293; δ_H (400 MHz, $CDCl_3$) 7.36 (1H, s), 7.28 – 7.23 (2H, m), 7.05 – 7.01 (1H, m), 6.50 – 6.46 (1H, m), 3.79 – 3.70 (4H, m), 3.52 (2H, s), 2.57 – 2.45 (4H, m), 1.54 (9H, s).

3-(Morpholinomethyl)aniline hydrochloride 191

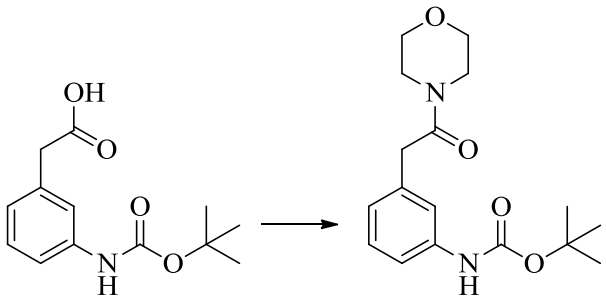


190 **191**

Tert-butyl (3-(morpholinomethyl)phenyl)carbamate **190** (350 mg, 1.197 mmol) was dissolved in hydrochloric acid (2 mL, 8 mmol, 4 M in 1,4-dioxane) and stirred for 16 h, then filtered, washing the precipitate with diethyl ether (2 x 5 mL) to give 3-(morpholinomethyl)aniline hydrochloride **191** (174 mg, 89 %) as a white solid. LCMS (Ammonium carbonate) 100 %, R_t= 0.63, $[MH]^+$= 193; δ_H (400 MHz, DMSO-d_6) 11.70 (1H,

s), 9.71 (2H, s), 7.66 – 7.59 (1H, m), 7.53 – 7.47 (2H, m), 7.40 – 7.35 (1H, m), 4.36 (2H, s), 4.02 – 3.77 (4H, m), 3.27 – 2.99 (4H, m). The product was used as a crude component in further reactions.

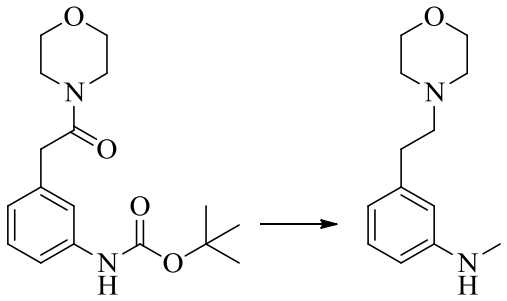
***Tert*-butyl (3-(2-morpholino-2-oxoethyl)phenyl)carbamate 193**



192 **193**

A mixture of 2-(3-((*tert*-butoxycarbonyl)amino)phenyl)acetic acid **192** (350 mg, 1.393 mmol) DIPEA (0.973 mL, 5.57 mmol), 50 % T_3P in ethyl acetate (0.995 mL, 1.671 mmol)and morpholine (0.121 mL, 1.393 mmol) were combined in DMF (2 mL). The reaction mixture was diluted with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (75 - 100 % TBME/cyclohexane) provided *tert*-butyl (3-(2-morpholino-2-oxoethyl)phenyl)carbamate **193** (300 mg, 67 %) as a yellow solid. LCMS (Formic acid) 99 %, R_t= 0.91, $[MH]^+$= 321; δ_H (400 MHz, DMSO-d_6) 9.28 (1H, s), 7.39 – 7.36 (1H, m), 7.29 – 7.25 (1H, m), 7.17 (1H, t, *J* = 7.8 Hz), 6.83 – 6.79 (1H, m), 3.66 (2H, s), 3.57 – 3.43 (8H, m), 1.48 (9H, s).

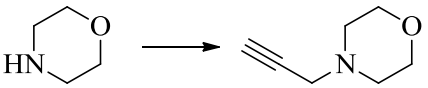
***N*-Methyl-3-(2-morpholinoethyl)aniline 195**



193 **195**

Tert-butyl (3-(2-morpholino-2-oxoethyl)phenyl)carbamate **193** (250 mg, 0.78 mmol) was dissolved in THF (5 mL) and cooled to 0 °C under nitrogen. To this, borane (1.56 mL, 1.561 mmol, 1 M in THF) was added dropwise and stirred for 16 h and then heated to 60 °C and stirred for 4 h. Saturated aqueous ammonium chloride (5 mL) was added and then concentrated *in vacuo*. The residue was dissolved in water (30 mL) and then extracted into ethyl acetate (2 x 30 mL). The organic layer was washed with brine (30 mL), dried over magnesium sulfate, passed through a hydrophobic frit and concentrated *in vacuo* to give *N*-methyl-3-(2-morpholinoethyl)aniline **195** (167 mg, 97 %) as a colourless oil. LCMS (Formic acid) 78 %, R_t= 0.80, $[MH]^+$= 221; δ_H (400 MHz, $CDCl_3$) 7.16 – 7.10 (1H, m), 6.60 – 6.56 (1H, m), 6.51 – 6.46 (2H, m), 3.80 – 3.75 (4H, m), 2.85 (3H, s), 2.80 – 2.73 (2H, m), 2.66 – 2.59 (2H, m), 2.58 – 2.53 (4H, m). Aniline N-H is not observed. The product was used as a crude component in further reactions.

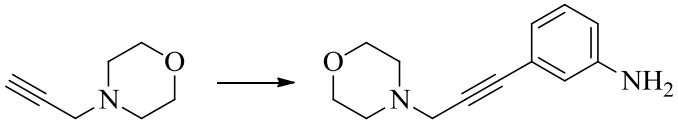
4-(Prop-2-yn-1-yl)morpholine 196



196

A mixture of morpholine (2.97 mL, 34.4 mmol) and cesium carbonate (11.22 g, 34.4 mmol) were dissolved in acetone (100 mL). 80 % by weight 3-bromoprop-1-yne in toluene (3.84 mL, 34.4 mmol) was added and stirred for 16 h. The solvent was concentrated *in vacuo*. The residue was diluted with water (15 mL) and extracted with DCM (2 x 20 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give (4-(prop-2-yn-1-yl)morpholine **196** (3.76 g, 87 %) as a yellow oil. δ_H (400 MHz, $CDCl_3$) 3.79 – 3.74 (4H, m), 3.31 (2H, d, *J* = 2.5 Hz), 2.62 – 2.55 (4H, m), 2.28 (1H, t, *J* = 2.5 Hz).

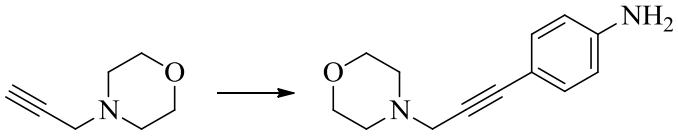
3-(3-Morpholinoprop-1-yn-1-yl)aniline 197



196 **197**

A mixture of 3-iodoaniline (0.240 mL, 1.997 mmol), triethylamine (0.835 mL, 5.99 mmol), 4-(prop-2-yn-1-yl)morpholine **196** (250 mg, 1.997 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (70 mg, 0.1 mmol) and copper(I) iodide (38 mg, 0.2 mmol) were combined in THF (2 mL). The resulting mixture was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was filtered through Celite, washing with ethyl acetate (2 x 20 mL) and concentrated *in vacuo*. The residue was diluted with water (20 mL) and ammonium hydroxide (5 mL, 28 %) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-55 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give 3-(3-morpholinoprop-1-yn-1-yl)aniline **197** (150 mg, 34 %) as a cream solid. LCMS (Ammonium carbonate) 100 %, R_t= 0.76, $[MH]^+$= 217; δ_H (400 MHz, $CDCl_3$) 7.10 (1H, t, *J* = 7.8 Hz), 6.88 – 6.84 (1H, m), 6.80 – 6.76 (1H, m), 6.65 (1H, ddd, *J* = 8.1, 2.3, 0.8 Hz), 3.83 – 3.74 (4H, m), 3.67 (2H, s), 3.52 (2H, s), 2.70 – 2.61 (4H, m). The product was used as a crude component in further reactions.

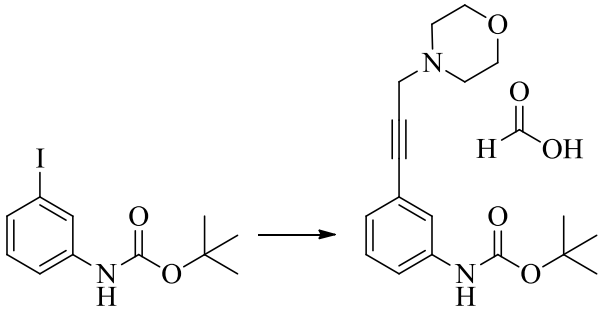
4-(3-Morpholinoprop-1-yn-1-yl)aniline 198



196 **198**

A mixture of 4-iodoaniline (437 mg, 1.997 mmol) triethylamine (0.835 mL, 5.99 mmol) 4-(prop-2-yn-1-yl)morpholine **196** (250 mg, 1.997 mmol) bis-(triphenylphosphine)-palladium(II)dichloride (70 mg, 0.1 mmol) and copper(I) iodide (38 mg, 0.2 mmol) were combined in THF (2 mL). The resulting mixture was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was filtered through Celite, washing with ethyl acetate (2 x 20 mL) and concentrated *in vacuo*. The residue was diluted with water (20 mL) and ammonium hydroxide (5 mL, 28 %) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (30 – 100 % ethyl acetate/cyclohexane) provided 4-(3-morpholinoprop-1-yn-1-yl)aniline **198** (190 mg, 44 %) as a yellow solid. LCMS (Ammonium carbonate) 88 %, R_t= 0.74, $[MH]^+$= 217; δ_H (400 MHz, $CDCl_3$) 7.28 – 7.22 (2H, m), 6.63 – 6.58 (2H, m), 3.82 – 3.74 (6H, m), 3.50 (2H, s), 2.70 – 2.60 (4H, m). The product was used as a crude component in further reactions.

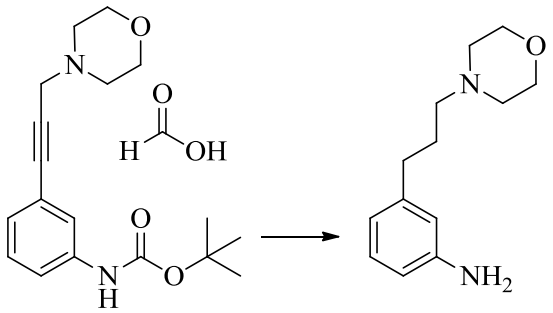
***Tert*-butyl (3-(3-morpholinoprop-1-yn-1-yl)phenyl)carbamate formate 201**



199 **201**

A mixture of *tert*-butyl (4-iodophenyl)carbamate **199** (2.1 g, 6.58 mmol), triethylamine (2.73 mL, 19.58 mmol), 4-(prop-2-yn-1-yl)morpholine **196** (1 g, 6.53 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (230 mg, 0.328 mmol) and copper(I) iodide (0.124 g, 0.653 mmol) were combined in THF (8 mL). The resulting mixture was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was diluted with water (20 mL) and ammonium hydroxide (5 mL, 28 %) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-70 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give *tert*-butyl (3-(3-((2S,6R)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **201** (181 mg, 71 %) as a brown gum. LCMS (Formic acid) 90 %, R_t= 0.68, $[MH]^+$= 317; δ_H (400 MHz, $CDCl_3$) 8.27 (1H, s), 7.53 – 7.48 (1H, m), 7.34 – 7.27 (1H, m), 7.25 – 7.19 (1H, m), 7.11 (1H, dt, *J* = 7.6, 1.3 Hz), 6.62 (1H, s), 3.83 – 3.75 (4H, m), 3.54 (2H, s), 2.73 – 2.66 (4H, m), 1.53 (9H, s).

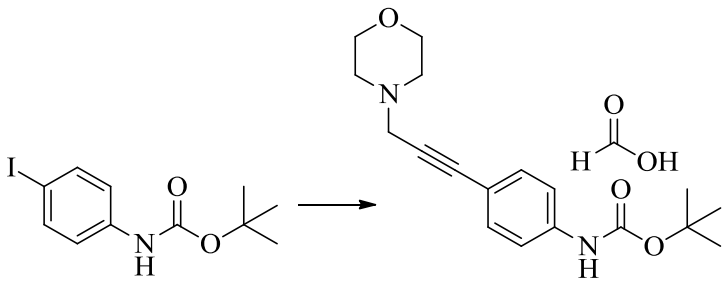
3-(3-Morpholinopropyl)aniline 203



201 **203**

Palladium on carbon (33 mg, 0.316 mmol, 10 %) was degassed under alternating nitrogen and vacuum purges and to this, *tert*-butyl (3-(3-morpholinoprop-1-yn-1-yl)phenyl)carbamate formate **201** (100 mg, 0.316 mmol)n ethanol (2 mL) was added and then placed under an atmosphere of hydrogen for 16 h. The reaction mixture was passed through Celite and concentrated *in vacuo*. The residue was dissolved in hydrochloric acid (1 mL, 4 mmol, 4 M in 1,4-dioxane) and stirred for 1 h under nitrogen, then neutralized with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 3-(3-morpholinopropyl)aniline **203** (41 mg, 58 %) as a yellow oil. LCMS (Ammonium carbonate) 65 %, R_t= 0.75, $[MH]^+$= 221; δ_H (400 MHz, $CDCl_3$) 7.11 – 7.03 (1H, m), 6.61 (1H, d, *J* = 7.8 Hz), 6.55 – 6.49 (2H, m), 3.76 – 3.70 (4H, m), 3.62 (2H, s), 2.62 – 2.52 (2H, m), 2.48 – 2.40 (4H, m), 2.41 – 2.34 (2H, m), 1.86 – 1.76 (2H, m). The product was used as a crude component in further reactions.

***Tert*-butyl (4-(3-morpholinoprop-1-yn-1-yl)phenyl)carbamate formate 202**

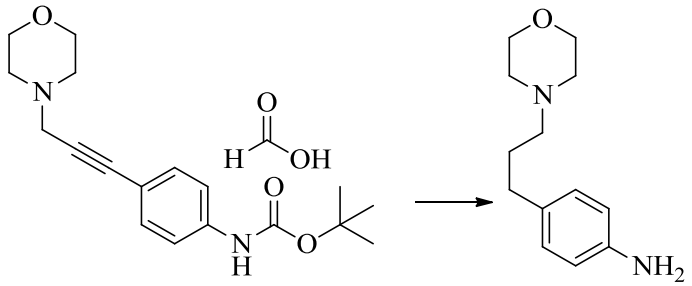


200 **202**

A mixture of *tert*-butyl (4-iodophenyl)carbamate **200** (637 mg, 1.997 mmol), triethylamine (0.835 mL, 5.99 mmol), 4-(prop-2-yn-1-yl)morpholine **196** (250 mg, 1.997 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (70 mg, 0.1 mmol) and copper(I) iodide (38 mg, 0.2. mmol) were combined in THF (2 mL). The resulting mixture was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was diluted with water (20 mL) and ammonium hydroxide (5 mL, 28 %) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-55 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give *tert*-butyl (4-(3-morpholinoprop-1-yn-1-yl)phenyl)carbamate formate **202** (605 mg, 96 %) as a brown gum. LCMS (Formic acid) 100

%, R_t= 0.65, $[MH]^+$= 317; δ_H (400 MHz, $CDCl_3$) 8.17 (1H, s), 7.41 – 7.36 (2H, m), 7.34-7.31 (2H, m), 6.56 (1H, s), 3.87 – 3.78 (4H, m), 3.59 (2H, s), 2.81 – 2.71 (4H, m), 1.54 (9H, s).

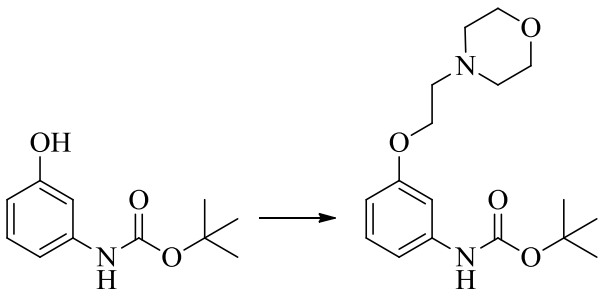
4-(3-Morpholinopropyl)aniline 204



202 **204**

Palladium on carbon (84 mg, 0.789 mmol, 10 %) was degassed under alternating nitrogen and vacuum purges and to this, *tert*-butyl (4-(3-morpholinoprop-1-yn-1-yl)phenyl)carbamate formate **202** (200 mg, 0.632 mmol)in ethanol (2 mL) was added and then placed under an atmosphere of hydrogen for 16 h. The reaction mixture was passed through Celite and concentrated *in vacuo*. The residue was dissolved in hydrochloric acid (1 mL, 4 mmol, 4 M in 1,4-dioxane) and stirred for 1 h under nitrogen, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(3-morpholinopropyl)aniline **204** (60 mg, 83 %) as a yellow oil. LCMS (Ammonium carbonate) 97 %, R_t= 1.03, $[MH]^+$= no ionisation; δ_H (400 MHz, $CDCl_3$) 7.02 – 6.96 (2H, m), 6.67 – 6.61 (2H, m), 3.78 – 3.70 (4H, m), 3.69 – 3.25 (2H, m), 2.59 – 2.50 (2H, m), 2.51 – 2.40 (4H, m), 2.40 – 2.33 (2H, m), 1.85 – 1.71 (2H, m). The product was used as a crude component in further reactions.

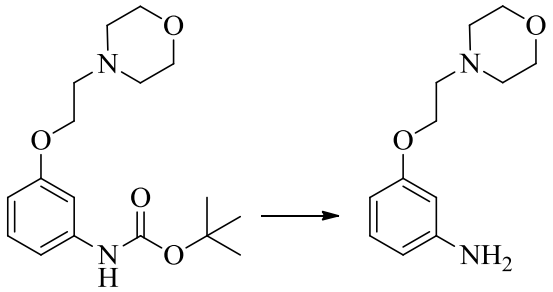
***Tert*-butyl (3-(2-morpholino)ethoxy)phenyl)carbamate 207**



205 **207**

A mixture of 4-(2-chloroethyl)morpholine hydrochloride (0.889 g, 4.78 mmol), potassium carbonate (2.432 g, 17.6 mmol), *tert*-butyl (3-hydroxyphenyl)carbamate **205** (1 g, 4.78 mmol) were dissolved in acetonitrile (9.5 mL) and stirred at 80 °C for 6 h. The reaction mixture was filtered and then concentrated *in vacuo* to give *tert*-butyl (3-(2-morpholino)ethoxy)phenyl)carbamate **207** (1.4 g, 91 %) as a cream solid. LCMS (Formic acid) 100 %, R_t= 0.63, $[MH]^+$= 323; δ_H (400 MHz, $CDCl_3$) 7.21 – 7.15 (2H, m), 6.84 – 6.79 (1H, m), 6.61 (1H, dd, *J* = 8.3, 1.7 Hz), 6.45 (1H, s), 4.13 (2H, t, *J* = 5.7 Hz), 3.78 – 3.71 (4H, m), 2.81 (2H, t, *J* = 5.7 Hz), 2.63 – 2.56 (4H, m), 1.54 (9H, s). The product was used as a crude component in further reactions.

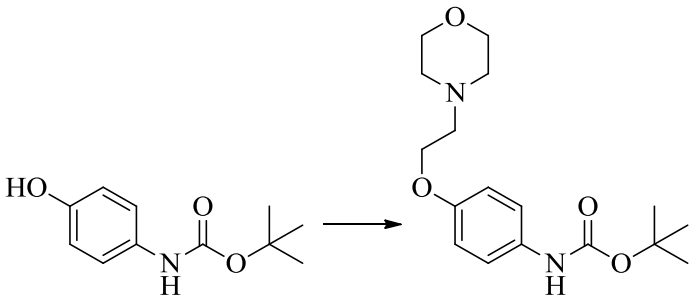
3-(2-Morpholino)ethoxy)aniline 209



207 **209**

Tert-butyl (3-(2-morpholinoethoxy)phenyl)carbamate **207** (0.5 g, 1.551 mmol) was dissolved in hydrochloric acid (1.9 mL, 7.75 mmol, 4 M in 1,4-dioxane) and stirred for stirred for 1 h and then concentrated *in vacuo*. The gum was dissolved in water (50 mL), basified to pH 9 with saturated aqueous sodium carbonate and extracted into ethyl acetate (3 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(2-morpholinoethoxy)aniline **209** (328 mg, 95 %) as a black gum. LCMS (Ammonium carbonate) 97 %, R_t= 0.66, $[MH]^+$= 223; δ_H (400 MHz, $CDCl_3$) 7.07 (1H, t, *J* = 8.0 Hz), 6.37 – 6.29 (2H, m), 6.29 – 6.26 (1H, m), 4.10 (2H, t, *J* = 5.8 Hz), 3.78 – 3.72 (4H, m), 3.70 – 3.62 (2H, m), 2.80 (2H, t, *J* = 5.8 Hz), 2.63 – 2.55 (4H, m). The product was used as a crude component in further reactions.

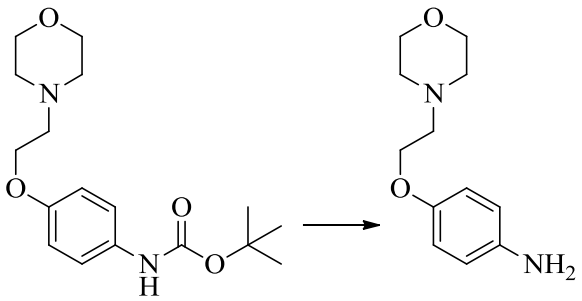
***Tert*-butyl (4-(2-morpholino)ethoxy)phenyl)carbamate 208**



206 **208**

A mixture of 4-(2-chloroethyl)morpholine hydrochloride (0.978 g, 5.26 mmol), potassium carbonate (2.432 g, 17.6 mmol) *tert*-butyl (4-hydroxyphenyl)carbamate **206** (1 g, 4.78 mmol) were dissolved in acetonitrile (9.5 mL) and stirred at 80 °C for 6 h. The reaction mixture was filtered and then concentrated *in vacuo* to give *tert*-butyl (4-(2-morpholino)ethoxy)phenyl)carbamate **208** (1.45 g, 94 %) as a cream solid. LCMS (Formic acid) 100 %, R_t= 0.53, $[MH]^+$= 323; δ_H (400 MHz, $CDCl_3$) 7.29-7.24 (2H, m), 6.89 – 6.83 (2H, m), 6.35 (1H, s), 4.10 (2H, t, *J* = 5.8 Hz), 3.78 – 3.73 (4H, m), 2.80 (2H, t, *J* = 5.8 Hz), 2.62 – 2.56 (4H, m), 1.53 (9H, s).

4-(2-Morpholinoethoxy)aniline 210

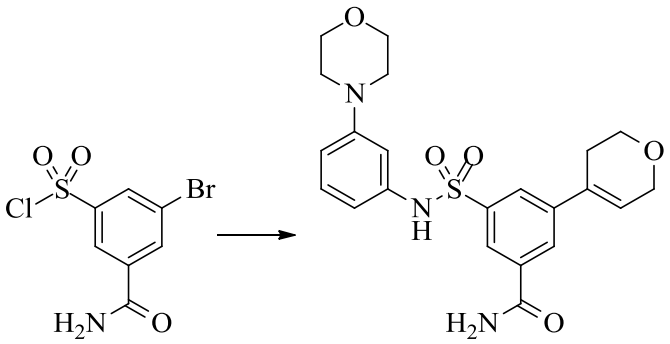


208 **210**

Tert-butyl (4-(2-morpholinoethoxy)phenyl)carbamate **208** (1.45 g, 4.5 mmol) was dissolved in hydrochloric acid (11 mL, 44 mmol, 4 M in 1,4-dioxane), stirred for 1 h and then concentrated *in vacuo*. The gum was dissolved in water (50 mL), basified to pH 9 with saturated aqueous sodium carbonate and extracted into ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(2-morpholinoethoxy)aniline **210** (1.05 g, 90 %) as a brown oil. LCMS (Ammonium carbonate) 94 %, R_t= 0.60, $[MH]^+$= 223; δ_H (400 MHz, DMSO-d_6)

6.68 – 6.62 (2H, m), 6.53 – 6.47 (2H, m), 4.62 (2H, s), 3.93 (2H, t, *J* = 5.8 Hz), 3.61 – 3.55 (4H, m), 2.63 (2H, t, *J* = 5.9 Hz), 2.48 – 2.39 (4H, m). The product was used as a crude component in further reactions.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-morpholinophenyl)sulfamoyl) benzamide 177

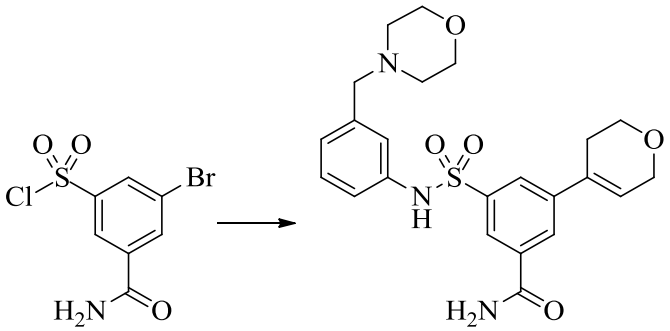


142 **177**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3-morpholinoaniline (59 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-morpholinophenyl)sulfamoyl) benzamide **177** (86 mg, 57 %) as a white solid. M.P. 135-137 °C; LCMS (Formic acid) 96 %, R_t= 0.92, $[MH]^+$= 444; δ_H (400 MHz, DMSO-d_6) 10.13 (1H, s), 8.24 (1H, s), 8.20 – 8.18 (1H, m), 8.18 – 8.14 (1H, m), 7.89 – 7.85 (1H, m), 7.59 (1H, s), 7.06 (1H, t, *J* = 8.1 Hz), 6.67 – 6.63 (1H, m), 6.63 – 6.59 (1H, m), 6.57 – 6.51 (1H, m), 6.43 – 6.37 (1H, m), 4.29 – 4.21 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.72 – 3.65 (4H, m), 3.01 – 2.94 (4H, m), 2.46 – 2.39 (2H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 152.0,

141.0, 140.6, 138.7, 135.8, 132.0, 130.0, 127.6, 126.2, 125.2, 125.1, 111.6, 111.5, 107.1, 66.4, 65.4, 63.8, 48.6, 26.7; ν_{max} (liquid film)/cm^{-1} 3185, 2960, 2828, 1664, 1602, 1449, 1381, 1335, 1160, 1115; *m/z* (ES) Found: $[MH]^+$ 444.1599, $C_{22}H_{26}N_3O_5S$ is $[MH]^+$ 444.1588.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(morpholinomethyl)phenyl)sulfamoyl) benzamide 178

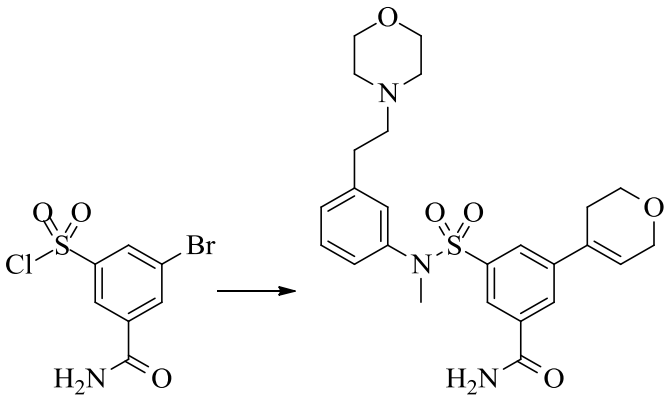


142 **178**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3-morpholinoaniline **191** (59 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) then purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(morpholinomethyl)phenyl)sulfamoyl) benzamide **178** (43 mg, 28 %) as a orange solid. M.P. 143-145 °C; LCMS (Formic acid) 100 %, R_t= 0.47, $[MH]^+$= 458; δ_H (400 MHz, DMSO-d_6) 8.22 (1H, s), 8.15 – 8.14 (1H, m), 8.13 – 8.11 (1H, m), 7.84 – 7.80 (1H, m), 7.56 (1H, s),

7.24 – 7.16 (1H, m), 7.04 – 6.98 (2H, m), 6.98 – 6.94 (1H, m), 6.42 – 6.37 (1H, m), 4.27 – 4.23 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.50 – 3.46 (4H, m), 3.33 (2H, s), 2.45 – 2.40 (2H, s), 2.20 – 2.15 (4H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 166.5, 141.0, 140.4, 139.3, 137.8, 135.7, 131.9, 129.4, 127.5, 126.1, 125.6, 125.1, 125.0, 121.5, 120.2, 66.5, 65.4, 63.8, 62.4, 53.3, 26.7; ν_{max} (liquid film)/cm^{-1} 3196, 2967, 2856, 1665, 1602, 1449, 1381, 1335, 1160, 1115; *m/z* (ES) Found: $[MH]^+$ 458.1736, $C_{23}H_{28}N_3O_5S$ is $[MH]^+$ 458.1744.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-methyl-*N*-(3-(2-morpholinoethyl)phenyl) sulfamoyl)benzamide 179

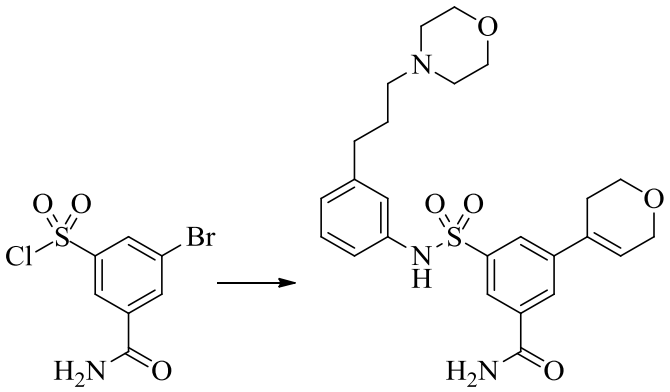


142 **179**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and *N*-methyl-3-(2-morpholinoethyl)aniline **195** (78 mg, 0.354 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-methyl-*N*-(3-(2-

morpholinoethyl)phenyl)sulfamoyl)benzamide **179** (48 mg, 29 %) as a cream solid. M.P. 161-163 °C; LCMS (Formic acid) 96 %, R_t= 0.55, $[MH]^+$= 486; δ_H (400 MHz, DMSO-d_6) 8.29 (1H, s), 8.28 – 8.25 (1H, m), 7.98 – 7.94 (1H, m), 7.61 (1H, s), 7.42 – 7.39 (1H, m), 7.27 (1H, t, *J* = 7.8 Hz), 7.20 – 7.14 (1H, m), 7.02 – 6.97 (1H, m), 6.91 – 6.88 (1H, m), 6.37 – 6.32 (1H, m), 4.26 – 4.19 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 3.56 – 3.51 (4H, m), 3.14 (3H, s), 2.68 – 2.60 (2H, m), 2.43 – 2.28 (8H, m); δ_C (101 MHz, DMSO-d_6) 166.5, 141.8, 141.3, 140.9, 136.9, 135.7, 131.9, 129.1, 128.2, 128.0, 126.5, 126.2, 125.6, 125.5, 124.7, 66.6, 65.4, 63.8, 60.1, 53.6, 38.5, 32.4, 26.6; ν_{max} (liquid film)/cm^{-1} 3206, 2965, 2924, 2855, 1664, 1603, 1383, 1344, 1163, 1115; *m/z* (ES) Found: $[MH]^+$ 486.2047, $C_{25}H_{32}N_3O_5S$ is $[MH]^+$ 486.2057.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-morpholinopropyl)phenyl)sulfamoyl) benzamide 180

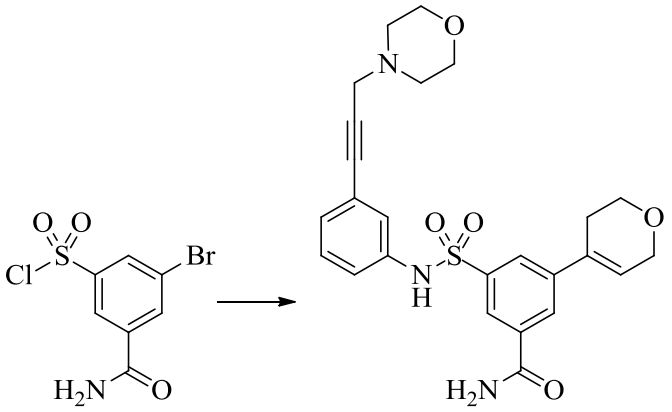


142 **180**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (40 mg, 0.134 mmol) and 3-(3-morpholinopropyl)aniline **203** (41 mg, 0.149 mmol) were added to THF (1 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (31 mg, 0.147 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (91 mg, 0.429 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The

product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method A) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-morpholinopropyl)phenyl)sulfamoyl) benzamide **180** (15 mg, 23 %) as a orange solid. M.P. 114-116 °C; LCMS (Formic acid) 96 %, R_t= 0.55, $[MH]^+$= 486; δ_H (400 MHz, DMSO-d_6) 10.20 (1H, s), 8.23 (1H, s), 8.19 – 8.12 (2H, m), 7.84 – 7.80 (1H, m), 7.58 (1H, s), 7.13 (1H, t, *J* = 7.7 Hz), 6.94 – 6.84 (3H, m), 6.41 – 6.35 (1H, m), 4.29 – 4.22 (2H, m), 3.83 (2H, t, *J* = 5.3 Hz), 3.60 – 3.50 (4H, m), 2.47 (2H, t, *J* = 7.5 Hz), 2.44 – 2.37 (2H, m), 2.28 – 2.22 (4H, m), 2.13 (2H, t, *J* = 7.1 Hz), 1.61 – 1.51 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.6, 143.5, 140.9, 140.8, 138.2, 135.7, 132.0, 129.4, 127.4, 126.1, 125.1, 125.0, 124.7, 120.9, 118.6, 66.6, 65.4, 63.8, 57.6, 53.7, 33.0, 28.0, 26.7; ν_{max} (liquid film)/cm^{-1} 3398, 3305, 3204, 2967, 2858, 2828, 1663, 1608, 1383, 1264, 1158, 1117; *m/z* (ES) Found: $[MH]^+$ 486.2051, $C_{25}H_{32}N_3O_5S$ is $[MH]^+$ 486.2057.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-morpholinoprop-1-yn-1-yl)phenyl)sulfamoyl) benzamide 181

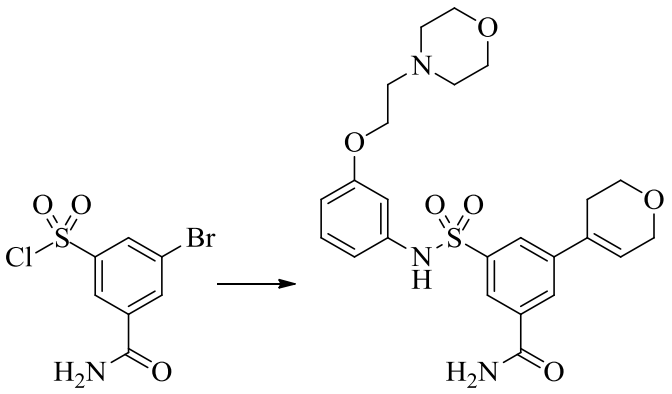


142 **181**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3-(3-morpholinoprop-1-yn-1-yl)aniline **197** (72 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.187 mL, 1.34 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-

tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-morpholinoprop-1-yn-1-yl)phenyl)sulfamoyl) benzamide **181** (26 mg, 16 %) as a white solid. M.P. 150 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.56, $[MH]^+$= 482; δ_H (400 MHz, DMSO-$d_{6=}$) 10.46 (1H, s), 8.26 (1H, s), 8.20 – 8.14 (2H, m), 7.90 – 7.84 (1H, m), 7.60 (1H, s), 7.27 – 7.24 (1H, m), 7.16 – 7.06 (3H, m), 6.45 – 6.39 (1H, m), 4.30 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.63 – 3.58 (4H, m), 3.48 (2H, s), 2.50 – 2.46 (4H, m), 2.45 – 2.40 (2H, m);δ_C (126 MHz, DMSO-d_6) 166.6, 141.1, 140.6, 138.6, 135.9, 132.0, 130.1, 127.7, 127.4, 126.3, 125.0, 124.9, 123.6, 123.1, 120.7, 86.2, 84.7, 66.5, 65.4, 63.8, 52.2, 47.3, 26.7; ν_{max} (liquid film)/cm^{-1} 2353, 3172, 2964, 2925, 2859, 1668, 1598, 1386, 1333, 1279, 1156, 1111; *m/z* (ES) Found: $[MH]^+$ 482.1735, $C_{25}H_{28}N_3O_5S$ is $[MH]^+$ 482.1744.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(2-morpholinoethoxy)phenyl)sulfamoyl) benzamide 182

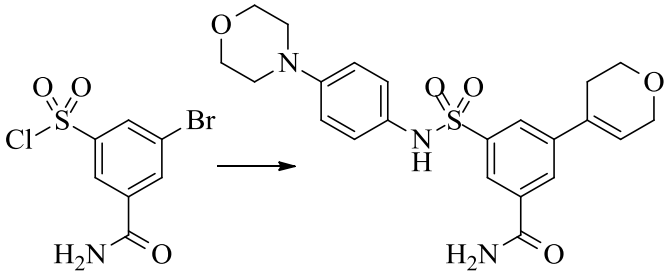


142 **182**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 3-(2-morpholinoethoxy)aniline **209** (45 mg, 0.202 mmol) were added to THF (1 mL) and triethylamine (0.14 mL, 1.038 mmol). The resulting mixture was stirred at room temperature

for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (50 mg, 0.238 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 0.643 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(2,4-dimethoxyphenyl)sulfamoyl)benzamide **182** (31 mg, 31 %) as a white solid. M.P. 130-132 °C; LCMS (Formic acid) 100 %, R_t= 0.54, $[MH]^+$= 488; δ_H (400 MHz, DMSO-d_6) 10.32 (1H, s), 8.23 (1H, s), 8.18 – 8.16 (1H, m), 8.16 – 8.14 (1H, m), 7.89 – 7.86 (1H, m), 7.58 (1H, s), 7.12 (1H, t, *J* = 8.1 Hz), 6.70 – 6.66 (1H, m), 6.66 – 6.64 (1H, m), 6.64 – 6.59 (1H, m), 6.44 – 6.37 (1H, m), 4.27 – 4.23 (2H, m), 3.96 (2H, t, *J* = 5.7 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 3.59 – 3.53 (4H, m), 2.62 (2H, t, *J* = 5.7 Hz), 2.45 – 2.38 (6H, m); δ_C (101 MHz, DMSO-d_6) 166.6, 159.3, 141.0, 140.7, 135.8, 132.0, 131.6, 130.5, 126.2, 125.1, 125.0, 117.3, 116.3, 112.9, 107.0, 66.6, 65.7, 65.4, 63.8, 57.2, 54.0, 26.7; ν_{max} (liquid film)/cm^{-1} 3184, 2924, 2822, 1667, 1595, 1335 ,1279, 1141, 1112; *m/z* (ES) Found: $[MH]^+$ 488.1852, $C_{24}H_{30}N_3O_6S$ is $[MH]^+$ 488.1850.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-morpholinophenyl)sulfamoyl)benzamide 183

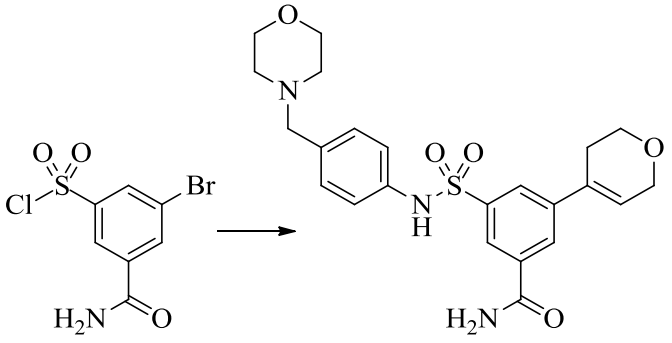


142 **183**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-morpholinoaniline (62 mg, 0.348 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The

reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) then purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(morpholinomethyl)phenyl)sulfamoyl)benzamide **183** (14 mg, 9 %) as a white solid. M.P. 250 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.79, $[MH]^+$= 444; δ_H (400 MHz, DMSO-d_6) 9.90 (1H, s), 8.23 (1H, s), 8.19 – 8.09 (2H, m), 7.78 – 7.71 (1H, m), 7.57 (1H, s), 6.92 (2H, d, *J* = 8.8 Hz), 6.81 (2H, d, *J* = 8.8 Hz), 6.41 – 6.32 (1H, m), 4.29 – 4.22 (2H, m), 3.83 (2H, t, *J* = 5.3 Hz), 3.73 – 3.65 (4H, m), 3.03 – 2.95 (4H, m), 2.44 – 2.36 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.8, 148.8, 140.9, 140.8, 135.6, 132.1, 129.4, 127.2, 126.0, 125.1, 125.0, 123.8, 115.9, 66.4, 65.4, 63.8, 48.9, 26.7; ν_{max} (liquid film)/cm^{-1} 3398, 3254, 3158, 2957, 2860, 2826, 1664, 1603, 1390, 1333, 1163, 1124; *m/z* (ES) Found: $[MH]^+$ 444.1599, $C_{22}H_{26}N_3O_5S$ is $[MH]^+$ 444.1588.

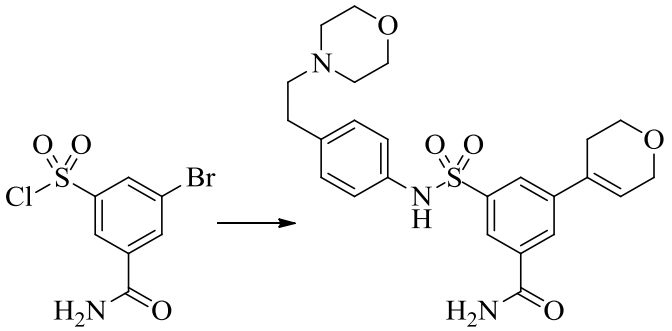
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(morpholinomethyl)phenyl)sulfamoyl) benzamide 184



142 **184**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-(morpholinomethyl)aniline (64 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(morpholinomethyl)phenyl)sulfamoyl) benzamide **184** (38 mg, 24 %) as a white solid. M.P. 209 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.49, $[MH]^+$= 458; δ_H (400 MHz, DMSO-d_6) 10.20 (1H, s), 8.22 (1H, s), 8.17 – 8.13 (1H, m), 8.14 – 8.11 (1H, m), 7.85 – 7.79 (1H, m), 7.56 (1H, s), 7.16 (2H, d, *J* = 8.5 Hz), 7.04 (2H, d, *J* = 8.5 Hz), 6.43 – 6.35 (1H, m), 4.29 – 4.20 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.57 – 3.47 (4H, m), 3.33 (2H, s), 2.47 – 2.36 (2H, m), 2.30 – 2.19 (4H, m); δ_C (101 MHz, DMSO-d_6) 166.6, 141.0, 140.6, 136.7, 135.7, 134.5, 132.0, 130.1, 127.5, 126.2, 125.0, 124.9, 121.1, 66.6, 65.4, 63.8, 62.2, 53.5, 26.7; ν_{max} (liquid film)/cm^{-1} 3186, 2965, 2858, 1664, 1603, 1387, 1335, 1160, 1115; *m/z* (ES) Found: $[MH]^+$ 458.1745, $C_{23}H_{28}N_3O_5S$ is $[MH]^+$ 458.1744.

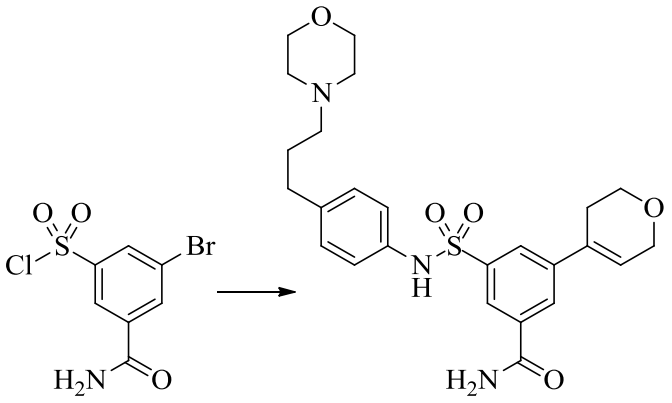
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(2-morpholinoethyl)phenyl)sulfamoyl) benzamide 185



142 **185**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-(morpholinoethyl)aniline (69 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.187 mL, 1.34 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(morpholinoethyl)phenyl)sulfamoyl) benzamide **185** (54 mg, 34 %) as a white solid. M.P. 157-159 °C; LCMS (Formic acid) 100 %, R_t= 0.48, $[MH]^+$= 472; δ_H (400 MHz, DMSO-d_6) 10.12 (1H, s), 8.23 (1H, s), 8.16 – 8.12 (2H, m), 7.81 – 7.76 (1H, m), 7.57 (1H, s), 7.12 – 7.07 (2H, m), 7.03 – 6.95 (2H, m), 6.42 – 6.34 (1H, m), 4.26 – 4.22 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.57 – 3.50 (4H, m), 2.65 – 2.58 (2H, m), 2.44 – 2.32 (8H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 141.0, 140.6, 137.1, 135.8, 135.6, 132.0, 129.8, 127.5, 126.2, 125.1, 125.0, 121.3, 66.5, 65.4, 63.8, 60.3, 53.6, 32.1, 26.6; ν_{max} (liquid film)/cm^{-1} 3399, 3309, 3206, 2967, 1663, 1603, 1383, 1330, 1264, 1158, 1117.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(morpholinopropyl)phenyl)sulfamoyl) benzamide 186

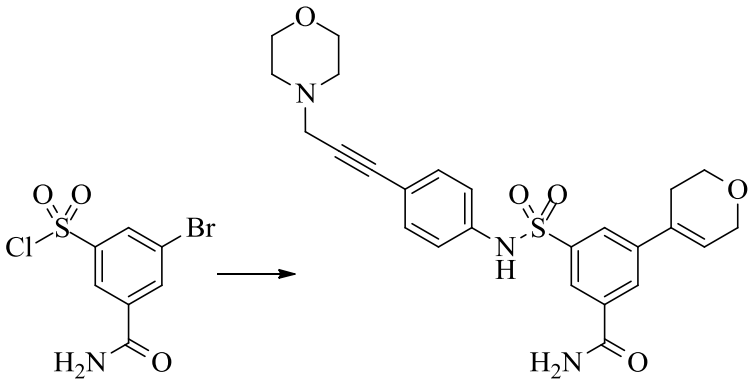


142 **186**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 4-(morpholinopropyl)aniline **204** (45 mg, 0.204 mmol) were added to THF (1 mL) and triethylamine (0.087 mL, 0.623 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (50 mg, 0.238 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 0.643 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(morpholinopropyl)phenyl) sulfamoyl) benzamide **186** (24 mg, 24 %) as a yellow solid. M.P. 220 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.51, $[MH]^+$= 486; δ_H (400 MHz, DMSO-d_6) 10.15 (1H, s), 8.24 (1H, s), 8.18 – 8.12 (2H, m), 7.83 – 7.76 (1H, m), 7.58 (1H, s), 7.07 (2H, d, *J* = 8.3 Hz), 6.99 (2H, d, *J* = 8.1 Hz), 6.43 – 6.34 (1H, m), 4.28 – 4.22 (2H, m), 3.83 (2H, t, *J* = 5.3 Hz), 3.58 – 3.50 (4H, m), 2.47 (2H, t, *J* = 7.6 Hz), 2.43 – 2.37 (2H, m), 2.34 – 2.22 (4H, m), 2.18 (2H, t, *J* = 7.2 Hz), 1.67 – 1.58 (2H, m); δ_C (126 MHz, DMSO-d_6) 166.7, 140.9, 140.7, 138.7, 135.7, 135.6, 132.0, 129.4, 127.5, 126.1, 125.1, 125.0, 121.4, 66.6, 65.4, 63.8, 57.9, 53.7,

32.5, 28.1, 26.6; v_{max} (liquid film)/cm^{-1} 3396, 3268, 2957, 2927, 2857, 2823, 1664, 1602, 1508, 1391, 1334, 1162, 1110; *m/z* (ES) Found: $[MH]^+$ 486.2051, $C_{25}H_{32}N_3O_5S$ is $[MH]^+$ 486.2057.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(3-morpholinoprop-1-yn-1-yl)phenyl)sulfamoyl) benzamide 187

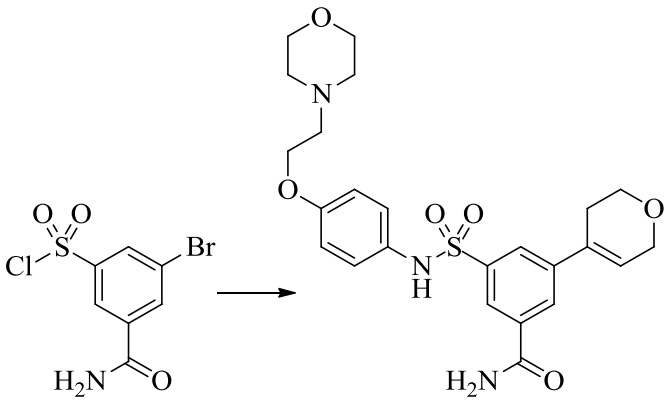


142 **187**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 3-(3-morpholinoprop-1-yn-1-yl)aniline **198** (72 mg, 0.335 mmol) were added to THF (1 mL) and triethylamine (0.187 mL, 1.34 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(3-morpholinoprop-1-yn-1-yl)phenyl)sulfamoyl) benzamide **187** (19 mg, 11 %) as a off-white solid. M.P. 165 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.55, $[MH]^+$= 482; δ_H (400 MHz, DMSO-d_6) 10.56 (1H, s), 8.25 (1H, s), 8.19 – 8.17 (1H, m), 8.17 – 8.15 (1H, m), 7.88 – 7.86 (1H, m), 7.60 (1H, s), 7.31

(2H, d, *J* = 8.4 Hz), 7.10 (2H, d, *J* = 8.4 Hz), 6.45 – 6.39 (1H, m), 4.28 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.64 – 3.53 (4H, m), 3.45 (2H, s), 2.49 – 2.40 (6H, m); δ_C (126 MHz, DMSO-d_6) 166.6, 141.1, 140.6, 138.4, 135.9, 132.9, 132.0, 127.7, 126.3, 125.0, 124.9, 120.3, 118.2, 85.3, 84.8, 66.5, 65.4, 63.8, 52.2, 47.4, 26.6; ν_{max} (liquid film)/cm^{-1} 3361, 3169, 2964, 2920, 1667, 1599, 1386, 1332, 1150, 1110; *m/z* (ES) Found: $[MH]^+$ 482.1729, $C_{25}H_{28}N_3O_5S$ is $[MH]^+$ 482.1744.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(2-morpholinoethoxy)phenyl)sulfamoyl) benzamide 188

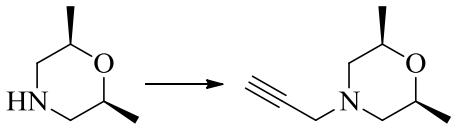


142 **188**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (100 mg, 0.335 mmol) and 4-(2-morpholinoethoxy)aniline **210** (0.76 mL, 0.342 mmol) were added to THF (1 mL) and triethylamine (0.145 mL, 1.038 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (228 mg, 1.072 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) then purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP

(method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(2-morpholinoethoxy)phenyl) sulfamoyl)benzamide **188** (85 mg, 52 %) as a yellow solid. M.P. 218-220 °C; LCMS (Formic acid) 100 %, R_t= 0.47, $[MH]^+$= 488; δ_H (400 MHz, DMSO-d_6) 9.93 (1H, s), 8.17 (1H, s), 8.15 – 8.13 (1H, m), 8.12 – 8.09 (1H, m), 7.80 – 7.75 (1H, m), 7.56 (1H, s), 7.01 – 6.94 (2H, m), 6.85 – 6.80 (2H, m), 6.42 – 6.36 (1H, m), 4.28 – 4.22 (2H, m), 3.98 (2H, t, *J* = 5.8 Hz), 3.83 (2H, t, *J* = 5.4 Hz), 3.57 – 3.52 (4H, m), 2.62 (2H, t, *J* = 5.7 Hz,), 2.45 – 2.38 (6H, m); δ_C (101 MHz, DMSO-d_6) 166.7, 163.5, 156.3, 140.9, 140.5, 135.7, 132.0, 130.3, 127.4, 126.1, 125.1, 124.1, 115.4, 66.5, 65.8, 65.4, 63.8, 57.4, 54.0, 26.7; ν_{max} (liquid film)/cm^{-1} 3187, 2966, 2924, 2863, 1668, 1596, 1385, 1335, 1279, 1154, 1112; *m/z* (ES) Found: $[MH]^+$ 488.1851, $C_{24}H_{30}N_3O_6S$ is $[MH]^+$ 488.1850.

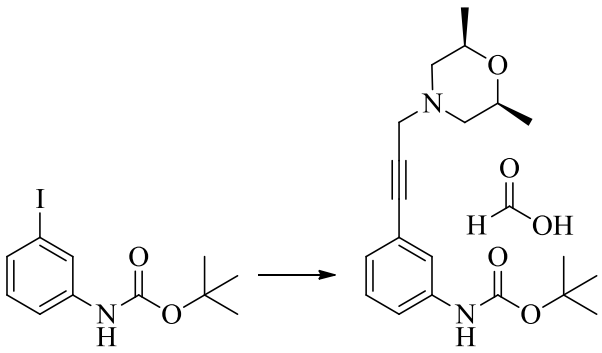
(2*S*,6*R*)-2,6-Dimethyl-4-(prop-2-yn-1-yl)morpholine 218



217 **218**

A mixture of (2S,6R)-2,6-dimethylmorpholine **217** (2 g, 17.37 mmol) and cesium carbonate (5.66 g, 17.37 mmol) were dissolved in acetone (50 mL). 80 % by weight 3-bromoprop-1-yne in toluene (1.934 mL, 17.37 mmol) was added and stirred for 16 h. The solvent was concentrated *in vacuo*. The residue was diluted with water (15 mL) and extracted with DCM (2 x 20 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give (2S,6R)-2,6-dimethyl-4-(prop-2-yn-1-yl)morpholine **218** (2.45 g, 92 %) as a yellow oil. δ_H (400 MHz, $CDCl_3$) 3.77 – 3.68 (2H, m), 3.31 (2H, d, *J* = 2.4 Hz), 2.76 – 2.70 (2H, m), 2.28 (1H, t, *J* = 2.4 Hz), 2.00 (2H, dd, *J* = 11.3, 10.3 Hz), 1.20 (6H d, *J* = 6.3 Hz).

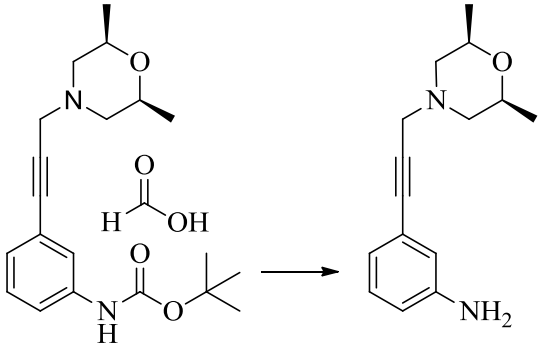
***Tert*-butyl (3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate 219**



199 **219**

A mixture of *tert*-butyl (3-iodophenyl)carbamate **199** (0.521 g, 1.632 mmol), triethylamine (0.682 mL, 4.89 mmol), (2*S*,6*R*)-2,6-dimethyl-4-(prop-2-yn-1-yl)morpholine **218** (0.25 g, 1.632 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (0.115 g, 0.163 mmol) and copper(I) iodide (0.031 g, 0.163 mmol) were combined in THF (8 mL). The resulting mixture was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was diluted with water (20 mL) and ammonium hydroxide (5 mL, 28 %) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-70 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give *tert*-butyl (3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **219** (407 mg, 63 %) as a brown gum. LCMS (Formic acid) 98 %, R_t= 0.76, $[MH]^+$= 345; δ_H (400 MHz, $CDCl_3$) 8.22 (1H, s), 7.52 (1H, s), 7.35 – 7.30 (1H, m), 7.24 (1H, t, *J* = 7.9 Hz), 7.16 – 7.11 (1H, m), 6.54 (1H, s), 3.86 – 3.75 (2H, m), 3.61 (2H, s), 2.91 (2H, d, *J* = 10.4 Hz), 2.23 – 2.16 (2H, m), 1.54 (9H, s), 1.23 (6H, d, *J* = 6.3 Hz).

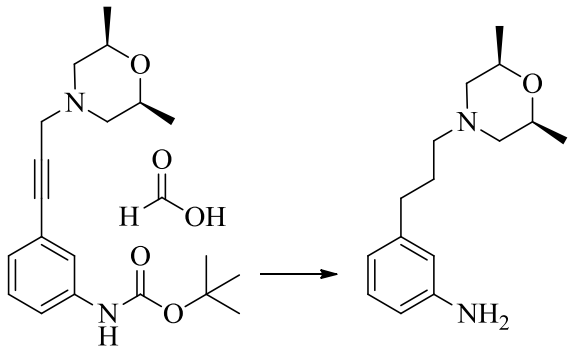
3-(3-((2*S*,6*R*)-2,6-Dimethylmorpholino)prop-1-yn-1-yl)aniline 221



219 **221**

Tert-butyl (3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **219** (150 mg, 0.435 mmol) was dissolved in DCM (1 mL). To this, TFA (0.366 mL, 4.35 mmol) was added and stirred for 1 h under nitrogen, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)aniline **221** (84 mg, 79 %) as a orange oil. LCMS (Formic acid) 91 %, R_t= 0.37, $[MH]^+$= 245; δ_H (400 MHz, DMSO-d_6) 6.99 (1H, t, *J* = 7.8 Hz), 6.64 – 6.61 (1H, m), 6.57 – 6.53 (2H, m), 5.16 (2H, s), 3.66 – 3.51 (2H, m), 3.47 (2H, s), 2.77 – 2.65 (2H, m), 1.95 – 1.83 (2H, m), 1.07 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

3-(3-((2*S*,6*R*)-2,6-Dimethylmorpholino)propyl)aniline 223

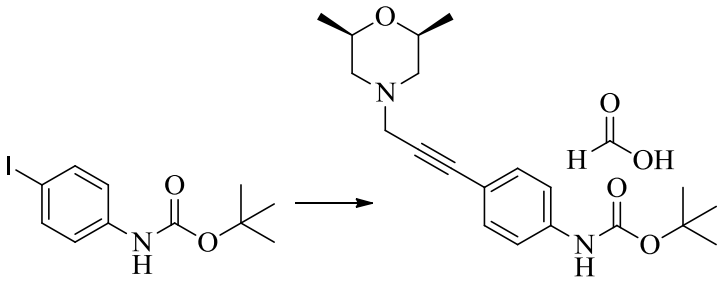


219 **223**

Palladium on carbon (31 mg, 0.29 mmol, 10 %) was degassed under alternating nitrogen and vacuum purges and to this, *tert*-butyl (3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **219** (100 mg, 0.29 mmol)in ethanol (2 mL) was added and then

placed under an atmosphere of hydrogen for 16 h. The reaction mixture was passed through Celite and concentrated *in vacuo.* The residue was dissolved in hydrochloric acid (1 mL, 4 mmol, 4 M in 1,4-dioxane) and stirred for 1 h under nitrogen, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)propyl)aniline **223** (60 mg, 83 %) as a yellow oil. LCMS (Formic acid) 98 %, R_t= 0.92, $[MH]^+$= 249; δ_H (400 MHz, $CDCl_3$) 7.12 – 7.05 (1H, m), 6.64 – 6.57 (1H, m), 6.56 – 6.51 (2H, m), 3.79 – 3.68 (2H, m), 3.62 (2H, s), 2.80 - 2.75 (2H, m), 2.62 – 2.53 (2H, m), 2.43 – 2.30 (2H, m), 1.89 – 1.78 (2H, m), 1.78 – 1.67 (2H, m), 1.18 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

***Tert*-butyl (4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate 220**

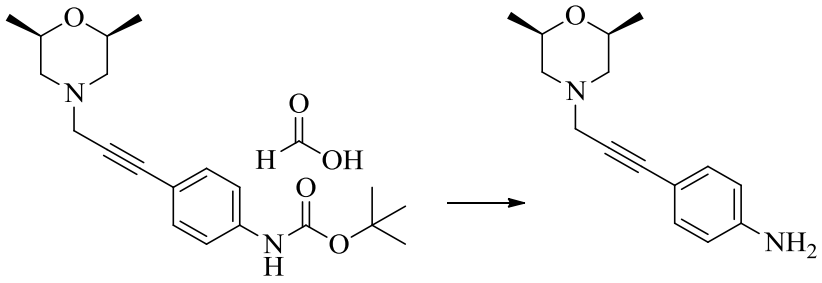


200 **220**

A mixture of *tert*-butyl (3-iodophenyl)carbamate **200** (2.1 g, 6.53 mmol) triethylamine (2.73 mL, 19.58 mmol), (2*S*,6*R*)-2,6-dimethyl-4-(prop-2-yn-1-yl)morpholine **218** (1 g, 6.53 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (0.23 g, 0.328 mmol) and copper(I) iodide (0.124 g, 0.653 mmol) were combined in THF (8 mL). The resulting mixture was stirred at room temperature for 72 h and concentrated *in vacuo*. The residue was diluted with water (20 mL) and ammonium hydroxide (5 mL, 28 %) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), passed through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-70 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give *tert*-butyl (4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **220** (1.61 g, 71 %) as a brown gum. LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^+$= 345; δ_H (400 MHz, $CDCl_3$) 8.17

(1H, s), 7.42 – 7.36 (2H, m), 7.36 – 7.31 (2H, m), 6.54 (1H, s), 3.85 – 3.74 (2H, m), 3.58 (2H, s), 2.92 – 2.85 (2H, m), 2.19 – 2.10 (2H, m), 1.55 (9H, s), 1.23 (6H, t, *J* = 6.7 Hz).

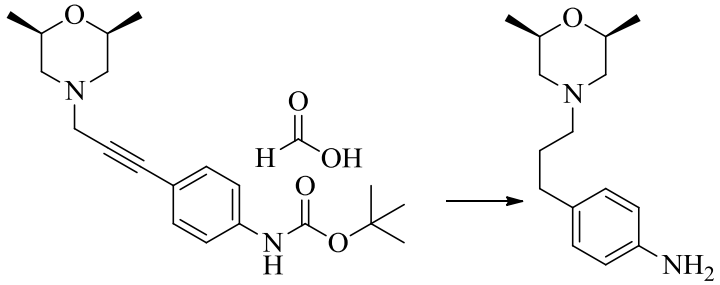
4-(3-((2*S*,6*R*)-2,6-Dimethylmorpholino)prop-1-yn-1-yl)aniline 222



220 **222**

Tert-butyl (4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **220** (100 mg, 0.29 mmol) was dissolved in THF (0.5 mL) and to this TBAF (1.5 mL, 1.5 mmol, 1 M in THF) was added and stirred at 70 °C for 24 h. The reaction was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)aniline **222** (70 mg, 99 %) as a colourless oil. LCMS (Formic acid) 90 %, R_t= 0.37, $[MH]^+$= 245; δ_H (400 MHz, $CDCl_3$) 7.27 – 7.24 (2H, m), 6.64 – 6.59 (2H, m), 3.83 – 3.71 (4H, m), 3.48 (2H, s), 2.85 – 2.79 (2H, m), 2.07 – 1.99 (2H, m), 1.21 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

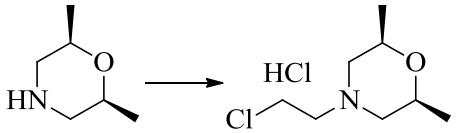
4-(3-((2*S*,6*R*)-2,6-Dimethylmorpholino)propyl)aniline 224



220 **224**

Palladium on carbon (31 mg, 0.29 mmol, 10 %) was degassed under alternating nitrogen and vacuum purges and to this, *tert*-butyl (4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)carbamate formate **220** (100 mg, 0.29 mmol) in ethanol (2 mL) was added and then placed under an atmosphere of hydrogen for 16 h. The reaction mixture was passed through Celite and concentrated *in vacuo*. The residue was dissolved in hydrochloric acid (1 mL, 4 mmol, 4 M in 1,4-dioxane) and stirred for 1 h under nitrogen, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)propyl)aniline **224** (60 mg, 83 %) as a yellow oil. LCMS (Formic acid) 87 %, R_t= 0.90, $[MH]^+$= 249; δ_H (400 MHz, $CDCl_3$) 7.01 – 6.96 (2H, m), 6.67 – 6.60 (2H, m), 3.75 – 3.63 (2H, m), 3.64 – 3.09 (2H, s), 2.79 – 2.70 (2H, m), 2.58 – 2.49 (2H, m), 2.38 – 2.29 (2H, m), 1.85 – 1.74 (2H, m), 1.74 – 1.64 (2H, m), 1.17 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

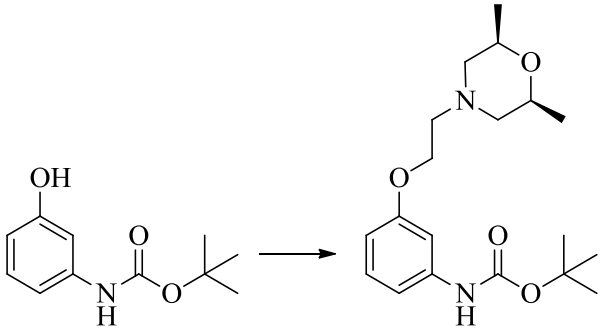
(2*S*,6*R*)-4-(2-Chloroethyl)-2,6-dimethylmorpholine hydrochloride 226



217 **226**

A mixture of (2*S*,6*R*)-2,6-dimethylmorpholine **217** (1 g, 8.68 mmol), potassium carbonate (3 g, 21.71 mmol) and 2-bromoethanol (1.085 g, 8.68 mmol) were dissolved in acetonitrile (10 mL) and stirred at 80 °C for 6 h. The reaction mixture was filtered and then concentrated *in vacuo*. The residue was suspended in thionyl chloride (5 mL, 68.5 mmol) and stirred at 70 °C for 9 h. The reaction mixture was concentrated *in vacuo*, triturating the solid with toluene (2 x 10 mL) to give (2*S*,6*R*)-4-(2-chloroethyl)-2,6-dimethylmorpholine hydrochloride **226** (1.56 g, 84 %) as a white solid. δ_H (400 MHz, D_2O) 4.10 – 3.98 (2H, m), 3.98 – 3.90 (2H, m), 3.65 – 3.54 (2H, m), 2.85 (2H, t, *J* = 11.7 Hz), 1.24 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

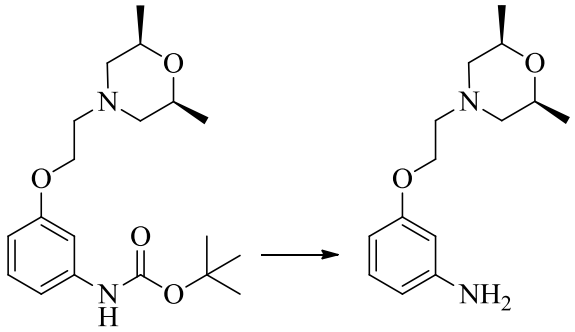
***Tert*-butyl (3-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)phenyl)carbamate 227**



205 **227**

(2*R*,6*S*)-4-(2-Chloroethyl)-2,6-dimethylmorpholine **226** (289 mg, 1.627 mmol), potassium carbonate (198 mg, 1.434 mmol) and *tert*-butyl (3-hydroxyphenyl)carbamate **205** (350 mg, 1.434 mmol) were dissolved in acetonitrile (5 mL) and stirred at 80 °C for 6 h. The reaction mixture was filtered and then concentrated *in vacuo*. The residue was dissolved in aqueous sodium hydroxide (25 mL, 2 M) and then extracted into ethyl acetate (2 x 25 mL). The organic layer was washed with brine (25 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (0 – 20 % methanol/TBME) provided *tert*-butyl (3-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)phenyl) carbamate **227** (550 mg, 94 %) as a cream solid. LCMS (Formic acid) 92 %, R_t= 0.69, $[MH]^+$= 351; δ_H (400 MHz, $CDCl_3$) 7.22 – 7.14 (2H, m), 6.82 (1H, dd, *J* = 8.0, 1.9 Hz), 6.61 (1H, dd, *J* = 8.3, 2.4 Hz), 6.46 (1H, s), 4.13 (2H, t, *J* = 5.6 Hz), 3.83 – 3.65 (2H, m), 2.88 – 2.82 (2H, m), 2.83 – 2.75 (2H, m), 1.97 – 1.84 (2H, m), 1.54 (9H, s), 1.19 (6H, d, *J* = 6.3 Hz).

3-(2-((2*S*,6*R*)-2,6-Dimethylmorpholino)ethoxy)aniline 229

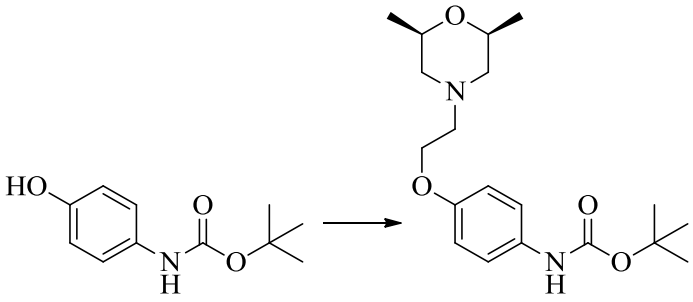


227 **229**

Tert-butyl (3-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)phenyl)carbamate **227** (275 mg, 0.785 mmol) was dissolved in hydrochloric acid (2 mL, 8 mmol, 4 M in 1,4-dioxane) and stirred for 16 h, then neutralised with saturated aqueous sodium carbonate (20 mL) and

extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)aniline **229** (174 mg, 89 %) as a brown oil. LCMS (Ammonium carbonate) 95 %, R_t= 0.82, $[MH]^+$= 251; δ_H (400 MHz, $CDCl_3$) 7.07 (1H, t, *J* = 8.0 Hz), 6.37 – 6.29 (2H, m), 6.29 – 6.26 (1H, m), 4.09 (2H, t, *J* = 5.8 Hz), 3.77 – 3.69 (2H, m), 3.66 (2H, s), 2.87 – 2.81 (2H, m), 2.78 (2H, t, *J* = 5.8 Hz), 1.94 – 1.85 (2H, m), 1.18 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

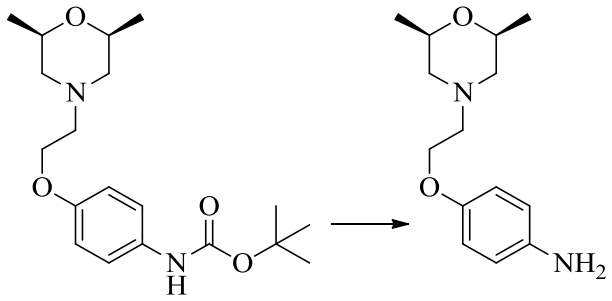
***Tert*-butyl (4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)phenyl)carbamate 228**



206 **228**

A mixture of (2*R*,6*S*)-4-(2-chloroethyl)-2,6-dimethylmorpholine **226** (289 mg, 1.627 mmol)potassium carbonate (198 mg, 1.434 mmol)and *tert*-butyl (4-hydroxyphenyl)carbamate **206** (300 mg, 1.434 mmol) were dissolved in acetonitrile (3 mL) and stirred at 80 °C for 6 h. The reaction mixture was filtered and then concentrated *in vacuo*. The residue was dissolved in aqueous sodium hydroxide (25 mL, 2 M) and then extracted into ethyl acetate (2 x 25 mL). The organic layer was washed with brine (25 mL), passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (50 – 100 % TBME/cyclohexane) provided *tert*-butyl (4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy) phenyl)carbamate **228** (310 mg, 57 %) as a white solid. LCMS (Formic acid) 88 %, R_t= 0.69, $[MH]^+$= 351; δ_H (400 MHz, $CDCl_3$) 7.31 – 7.25 (2H, m), 6.89 – 6.84 (2H, m), 6.34 (1H, s), 4.09 (2H, t, *J* = 5.8 Hz), 3.78 – 3.64 (2H, m), 2.86 – 2.80 (2H, m), 2.78 (2H, t, *J* = 5.9 Hz), 1.92 – 1.86 (2H, m), 1.53 (9H, s), 1.18 (6H, d, *J* = 6.3 Hz).

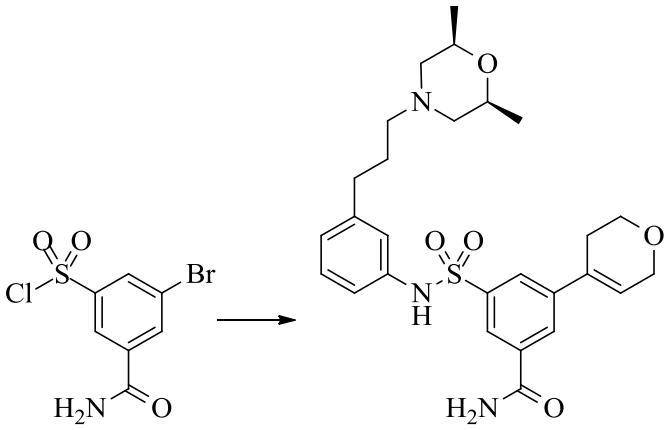
4-(2-((2*S*,6*R*)-2,6-Dimethylmorpholino)ethoxy)aniline 230



228 **230**

Tert-butyl (4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)phenyl)carbamate **228** (110 mg, 0.314 mmol) was dissolved in hydrochloric acid (1 mL, 4 mmol, 4 M in 1,4-dioxane) and stirred for 16 h, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)aniline **230** (62 mg, 79 %) as a orange oil. LCMS (Ammonium carbonate) 91 %, R_t= 0.37, $[MH]^+$= 245; δ_H (400 MHz, $CDCl_3$) 6.80 – 6.75 (2H, m), 6.68 – 6.63 (2H, m), 4.05 (2H, t, *J* = 5.8 Hz), 3.80 – 3.67 (2H, m), 2.88 – 2.80 (2H, m), 2.76 (2H, t, *J* = 5.8 Hz), 1.88 (2H, dd, *J* = 11.5, 10.3 Hz), 1.18 (6H, d, *J* = 6.3 Hz). Aniline NH_2 is not observed. The product was used as a crude component in further reactions.

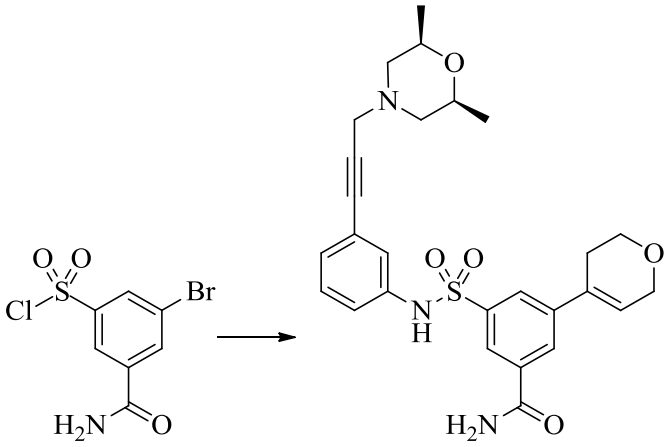
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)propyl) phenyl)sulfamoyl)benzamide 211



142 **211**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (40 mg, 0.134 mmol) and 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)propyl)aniline **233** (43 mg, 0.147 mmol) were added to THF (1 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (31 mg, 0.147 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (91 mg, 0.429 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)propyl)phenyl) sulfamoyl)benzamide **211** (24 mg, 34 %) as a white solid. M.P. 123-125 °C; LCMS (Formic acid) 100 %, R_t= 0.59, $[MH]^+$= 514; δ_H (400 MHz, DMSO-d_6) 8.23 (1H, s), 8.19 – 8.13 (2H, m), 7.82 – 7.79 (1H, m), 7.56 (1H, s), 7.17 – 7.10 (1H, m), 6.94 – 6.85 (3H, m), 6.40 – 6.35 (1H, m), 4.26 – 4.21 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.59 – 3.46 (2H, m), 2.62 (2H, d, *J* = 10.2 Hz), 2.47 (2H, t, *J* = 7.5 Hz), 2.43 – 2.36 (2H, m), 2.12 (2H, t, *J* = 7.1 Hz), 1.63 – 1.54 (2H, m), 1.54 – 1.46 (2H, m), 1.03 (6H, d, *J* = 6.3 Hz). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 166.6, 143.6, 141.0, 140.6, 137.8, 135.8, 132.0, 129.4, 127.5, 126.1, 125.1, 125.0, 121.0, 118.5, 71.4, 65.4, 63.8, 59.5, 57.1, 33.0, 28.0, 26.7, 19.4. One carbon is not observed. ν_{max} (liquid film)/cm^{-1} 3172, 2972, 2933, 2870, 1670, 1590 ,1381, 1334, 1158, 1141; *m/z* (ES) Found: $[MH]^+$ 514.2364, $C_{27}H_{36}N_3O_5S$ is $[MH]^+$ 514.2370.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)sulfamoyl)benzamide 212

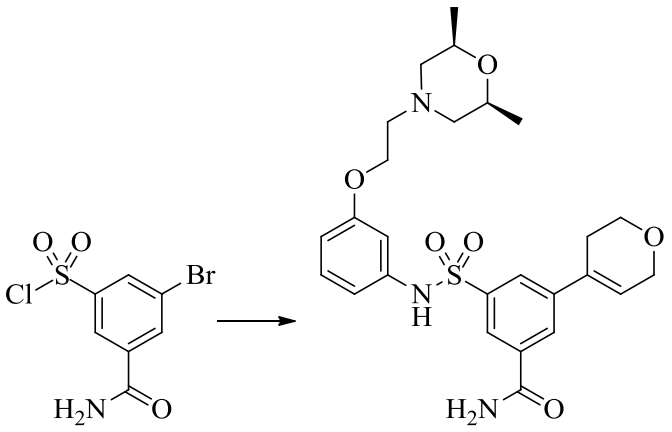


142 **212**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (70 mg, 0.234 mmol) and 3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl) aniline **221** (65 mg, 0.266 mmol) were added to THF (1 mL) and triethylamine (0.163 mL, 1.172 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (50 mg, 0.238 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (154 mg, 0.727 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(3-((2*S*,6*R*)-2,6-dimethylmorpholino) prop-1-yn-1-yl)phenyl)sulfamoyl)benzamide **212** (19 mg, 15 %) as a white solid. M.P. 135-137 °C; LCMS (Formic acid) 100 %, R_t= 0.63, $[MH]^+$= 510; δ_H (400 MHz, DMSO-d_6) 10.43 (1H, s), 8.24 (1H, s), 8.18 – 8.13 (2H, m), 7.87 – 7.83 (1H, m), 7.58 (1H, s), 7.26 – 7.21 (1H, m), 7.15 – 7.05 (3H, m), 6.44 – 6.38 (1H, m), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.62 – 3.53 (2H, m), 3.48 (2H, s), 2.74 – 2.66 (2H, m), 2.47 – 2.40 (2H, m), 1.91 – 1.80 (2H, m), 1.06 (6H, d, *J* = 6.3 Hz); δ_C (126 MHz,

DMSO-d_6) 166.6, 141.2, 140.4, 138.3, 135.9, 131.9, 130.2, 127.8, 127.7, 126.3, 125.0, 124.9, 123.6, 123.1, 120.6, 86.2, 84.6, 71.3, 65.4, 63.8, 57.9, 46.9, 26.7, 19.4; ν_{max} (liquid film)/cm^{-1} 3193, 2970, 2926, 2860, 1669, 1603, 1578, 1383, 1335, 1161, 1140; *m/z* (ES) Found: $[MH]^+$ 510.2050, $C_{27}H_{32}N_3O_5S$ is $[MH]^+$ 510.2057.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy) phenyl)sulfamoyl)benzamide 213

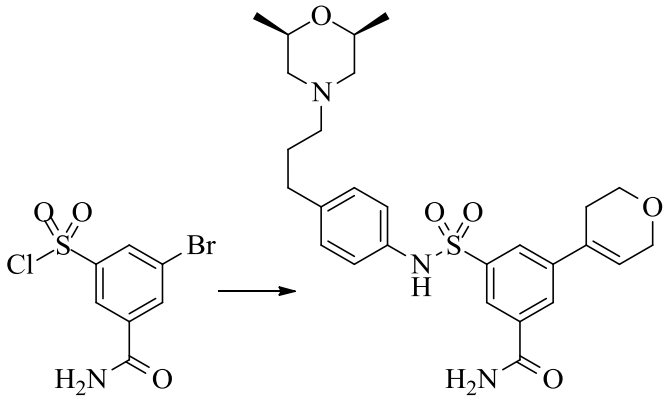


142 **213**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (50 mg, 0.167 mmol) and 3-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)aniline **229** (55 mg, 0.176 mmol) were added to THF (1 mL) and triethylamine (0.117 mL, 0.623 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (50 mg, 0.238 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 0.643 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3-(2-((2*S*,6*R*)-2,6-dimethyl

morpholino)ethoxy)phenyl)sulfamoyl)benzamide **213** (13 mg, 15 %) as a white solid. M.P. 119-121 °C; LCMS (Formic acid) 100 %, R_t= 0.56, $[MH]^+$= 516; δ_H (400 MHz, DMSO-d_6) 10.34 (1H, s), 8.25 (1H, s), 8.20 – 8.14 (2H, m), 7.90 – 7.86 (1H, m), 7.59 (1H, s), 7.12 (1H, t, *J* = 8.1 Hz), 6.71 – 6.63 (2H, m), 6.62 – 6.58 (1H, m), 6.44 – 6.39 (1H, m), 4.29 – 4.21 (2H, m), 3.96 (2H, t, *J* = 5.7 Hz), 3.84 (2H, t, *J* = 5.4 Hz), 3.58 – 3.49 (2H, m), 2.80 – 2.73 (2H, m),), 2.60 (2H, t, *J* = 5.7 Hz), 2.48 – 2.39 (2H, m), 1.68 (2H, t, *J* = 10.7 Hz), 1.03 (6H, d, *J* = 6.3 Hz); δ_C (126 MHz, DMSO-d_6) 166.6, 159.3, 141.0, 140.8, 139.5, 135.8, 132.0, 130.5, 127.5, 126.2, 125.1, 124.9, 112.8, 110.3, 107.1, 71.3, 65.6, 65.4, 63.8, 59.7, 56.8, 26.7, 19.4; v_{max} (liquid film)/cm^{-1} 3174, 2925, 2822, 1667, 1595, 1154, 1385, 1335, 1279, 1141; *m/z* (ES) Found: $[MH]^+$ 516.2153, $C_{26}H_{34}N_3O_6S$ is $[MH]^+$ 516.2163.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)propyl) phenyl)sulfamoyl)benzamide 214

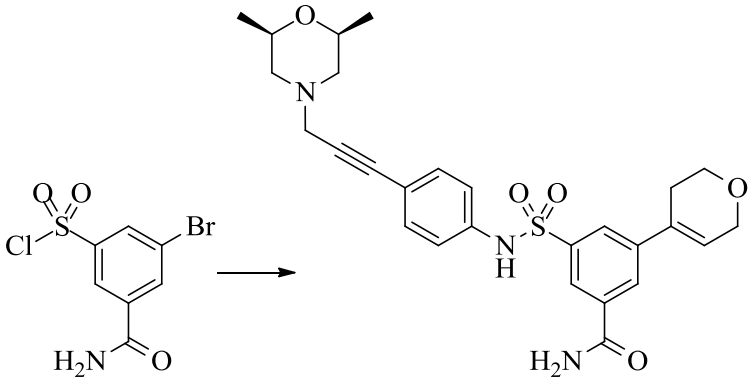


142 **214**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 4-(3-((2*R*,6*S*)-2,6-dimethylmorpholino)propyl)aniline **224** (60 mg, 0.242 mmol) were added to THF (1 mL) and triethylamine (0.140 mL, 1.005 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (46 mg, 0.219 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 1.005 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was

passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) then purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino) propyl)phenyl)sulfamoyl)benzamide **214** (36 mg, 34 %) as a white solid. M.P. 133-135 °C; LCMS (Formic acid) 100 %, R_t= 0.57, $[MH]^+$= 514; δ_H (400 MHz, DMSO-d_6) 10.98 (1H, s), 8.23 (1H, s), 8.17 – 8.12 (2H, m), 7.80 – 7.77 (1H, m), 7.56 (1H, s), 7.07 (2H, d, *J* = 8.5 Hz), 6.99 (2H, d, *J* = 8.5 Hz), 6.40 – 6.35 (1H, m), 4.27 – 4.22 (2H, m), 3.83 (2H, t, *J* = 5.4 Hz), 3.56 – 3.47 (2H, m), 2.67 (2H, d, *J* = 10.3 Hz), 2.49 – 2.42 (2H, m), 2.42 – 2.36 (2H, m), 2.23 – 2.14 (2H, m), 1.69 – 1.57 (2H, m), 1.52 (2H, t, *J* = 10.7 Hz), 1.02 (6H, d, *J* = 6.3 Hz); δ_C (101 MHz, DMSO-d_6) 166.7, 141.0, 140.6, 138.8, 135.8, 135.4, 132.0, 129.4, 127.5, 126.1, 125.1, 125.0, 121.4, 71.3, 65.4, 63.8, 59.5, 57.5, 32.5, 28.1, 26.7, 19.4; ν_{max} (liquid film)/cm^{-1} 3252, 3187, 2966, 2924, 2863, 1668, 1596, 1385, 1335, 1279, 1154, 1142, 1112; *m/z* (ES) Found: $[MH]^+$ 514.2348, $C_{27}H_{36}N_3O_5S$ is $[MH]^+$ 514.2370.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)phenyl)sulfamoyl)benzamide 215

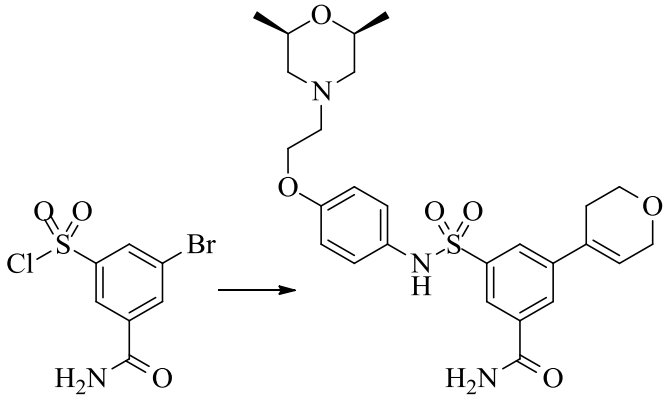


142 **215**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl) aniline **220** (70 mg, 0.244 mmol) were added to THF (1 mL) and triethylamine (0.140 mL, 1.005 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl

acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (50 mg, 0.238 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (154 mg, 0.727 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(3-((2*S*,6*R*)-2,6-dimethylmorpholino) prop-1-yn-1-yl)phenyl)sulfamoyl)benzamide **215** (9 mg, 8 %) as a yellow solid. M.P. 142-144 °C; LCMS (Formic acid) 100 %, R_t= 0.61, $[MH]^+$= 510; δ_H (400 MHz, DMSO-d_6) 10.58 (1H, s), 8.25 (1H, s), 8.19 – 8.12 (2H, m), 7.90 – 7.83 (1H, m), 7.60 (1H, s), 7.30 (2H, d, *J* = 8.4 Hz), 7.09 (2H, d, *J* = 8.4 Hz), 6.45 – 6.36 (1H, m), 4.29 – 4.22 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.59 – 3.51 (2H, m), 3.45 (2H, s), 2.72 – 2.66 (2H, m), 2.46 – 2.41 (2H, m), 1.89 – 1.79 (2H, m), 1.05 (6H, d, *J* = 6.3 Hz); δ_C (126 MHz, DMSO-d_6) 166.6, 141.1, 140.5, 138.4, 135.9, 132.9, 132.0, 127.6, 126.3, 125.0, 124.9, 120.3, 117.9, 85.2, 84.8, 71.3, 65.4, 63.8, 57.9, 47.0, 26.7, 19.4; ν_{max} (liquid film)/cm^{-1} 3181, 2971, 2929, 2862, 1668, 1605, 1508, 1336, 1163, 1140, 1081; *m/z* (ES) Found: $[MH]^+$ 510.2051, $C_{27}H_{32}N_3O_5S$ is $[MH]^+$ 510.2057.

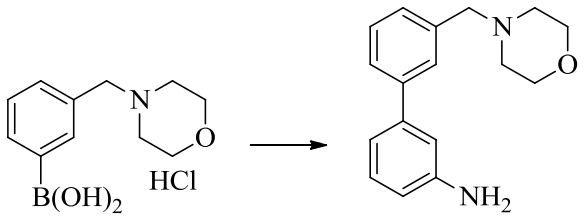
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino) ethoxy) phenyl)sulfamoyl)benzamide 216



142 **216**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino)ethoxy)aniline **230** (62 mg, 0.211 mmol) were added to THF (1 mL) and triethylamine (0.140 mL, 1.005 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (46 mg, 0.221 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 0.643 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method A) then purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4-(2-((2*S*,6*R*)-2,6-dimethylmorpholino) ethoxy)phenyl)sulfamoyl)benzamide **216** (28 mg, 27 %) as a white solid. M.P. 127-129 °C; LCMS (Formic acid) 100 %, R_t= 0.55, $[MH]^+$= 516; δ_H (400 MHz, DMSO-d_6) 9.95 (1H, s), 8.22 (1H, s), 8.16 – 8.13 (1H, m), 8.12 – 8.09 (1H, m), 7.79 – 7.74 (1H, m), 7.56 (1H, s), 7.01 – 6.93 (2H, m), 6.87 – 6.76 (2H, m), 6.42 – 6.35 (1H, m), 4.29 – 4.20 (2H, m), 3.97 (2H, t, *J* = 5.8 Hz), 3.83 (2H, t, *J* = 5.4 Hz), 3.53 (2H, dqd, *J* = 12.5, 6.2, 2.0 Hz), 2.82 – 2.73 (2H, m), 2.60 (2H, t, *J* = 5.8 Hz), 2.45 – 2.36 (2H, m), 1.75 – 1.62 (2H, m), 1.02 (6H, d, *J* = 6.3 Hz); δ_C (101 MHz, DMSO-d_6) 166.7, 156.3, 140.9, 140.6, 135.7, 132.0, 130.4, 127.4, 126.1, 125.1, 124.0, 115.4, 71.3, 65.8, 65.4, 63.8, 59.8, 57.0, 26.7, 19.4. One carbon not observed. ν_{max} (liquid film)/cm^{-1} 3361, 3252, 2964, 2924, 2862, 2829, 1668, 1596, 1386, 1334, 1279, 1155, 1112; *m/z* (ES) Found: $[MH]^+$ 516.2153, $C_{26}H_{34}N_3O_6S$ is $[MH]^+$ 516.2163.

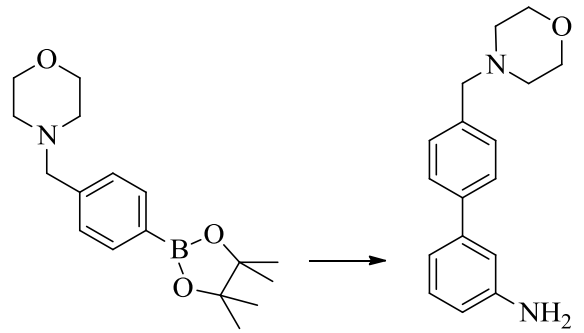
3'-(Morpholinomethyl)-[1,1'-biphenyl]-3-amine 235



237 **235**

(3-(Morpholinomethyl)phenyl)boronic acid hydrochloride **237** (176 mg, 0.685 mmol), 3-iodoaniline (150 mg, 0.685 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (19 mg, 0.034 mmol) and tripotassium phosphate (581 mg, 2.74 mmol) in 1,4-dioxane (3 mL) and water (0.5 mL) were heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 25-80 % acetonitrile in water with a formic acid modifier on a 30 g C_{18} column to give 3'-(morpholinomethyl)-[1,1'-biphenyl]-3-amine **235** (140 mg, 76 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.96, $[MH]^+$= 269;δ_H (400 MHz, $CDCl_3$) 7.55 (1H, m), 7.48 (1H, m), 7.39 (1H, t, *J* = 7.6 Hz), 7.32 (1H, m), 7.25 (1H, t, *J* = 7.8 Hz), 7.02 (1H, m), 6.94 (1H, m), 6.70 (1H, ddd, *J* = 7.9, 2.3, 0.8 Hz), 3.74 (6H, m), 3.58 (2H, s), 2.50 (4H, m).

4'-(Morpholinomethyl)-[1,1'-biphenyl]-3-amine 236

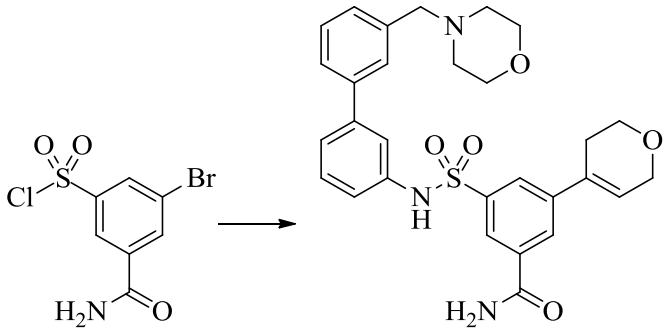


238 **236**

4-(4-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzyl)morpholine **238** (208 mg, 0.685 mmol), 3-iodoaniline (150 mg, 0.685 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (20 mg, 0.036 mmol) and tripotassium phosphate

(581 mg, 2.74 mmol) in 1,4-dioxane (3 mL) and water (0.5 mL) were heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 25-80 % acetonitrile in water with a formic acid modifier on a 30 g C_{18} column to give 4'-(morpholinomethyl)-[1,1'-biphenyl]-3-amine **236** (159 mg, 87 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.95, $[MH]^+$= 269; δ_H (400 MHz, $CDCl_3$) 7.56 – 7.51 (2H, m), 7.42 – 7.36 (2H, m), 7.24 (1H, t, *J* = 7.8 Hz), 7.03 – 6.98 (1H, m), 6.92 (1H, t, *J* = 2.0 Hz), 6.69 (1H, ddd, *J* = 7.9, 2.3, 0.8 Hz), 3.89 – 3.62 (6H, m), 3.56 (2H, s), 2.54 – 2.45 (4H, m).

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(3'-(morpholinomethyl)-[1,1'-biphenyl]-3-yl)sulfamoyl) benzamide 231

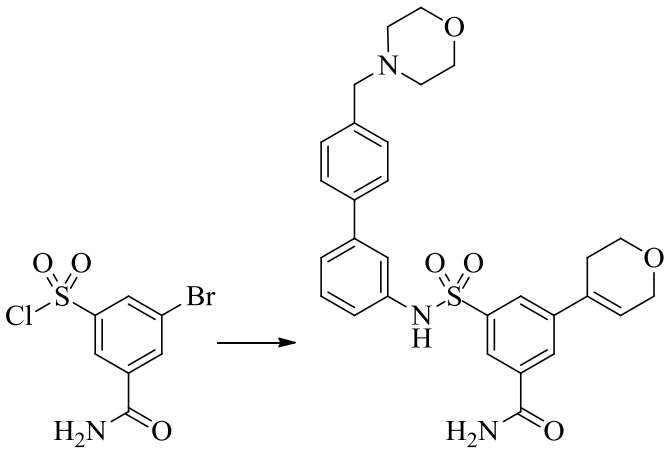


142 **231**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (40 mg, 0.134 mmol) and 3'-(morpholinomethyl)-[1,1'-biphenyl]-3-amine **235** (45 mg, 0.134 mmol) were added to THF (1 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (31 mg, 0.147 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (91 mg, 0.429 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column

using acetonitrile/water with an ammonium carbonate modifier MDAP (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(3'-(morpholinomethyl)-[1,1'-biphenyl]-3-yl)sulfamoyl) benzamide **231** (25 mg, 35 %) as a white solid. M.P. 151-153 °C; LCMS (Formic acid) 100 %, R_t= 0.88, $[MH]^+$= 534; δ_H (400 MHz, DMSO-d_6) 10.42 (1H, s), 8.26 (1H, s), 8.23 – 8.20 (1H, m), 8.18 – 8.16 (1H, m), 7.91 – 7.87 (1H, m), 7.60 (1H, s), 7.44 – 7.36 (3H, m), 7.36 – 7.30 (4H, m), 7.14 – 7.07 (1H, m), 6.41 – 6.36 (1H, m), 4.26 – 4.20 (2H, m), 3.80 (2H, t, *J* = 5.3 Hz), 3.61 – 3.56 (4H, m), 3.52 (2H, s), 2.45 – 2.32 (6H, m); δ_C (126 MHz, DMSO-d_6) 166.6, 141.6, 141.1, 140.5, 139.9, 139.1, 138.5, 135.9, 132.0, 130.3, 129.3, 128.8, 127.7, 127.4, 126.3, 125.7, 125.1, 125.1, 123.3, 119.9, 119.0, 66.6, 65.4, 63.8, 62.7, 53.6, 26.7; ν_{max} (liquid film)/cm^{-1} 3365, 3177, 2971, 2926, 2869, 1669, 1592, 1383, 1333, 1160, 1141; *m/z* (ES) Found: $[MH]^+$ 534.2046, $C_{29}H_{32}N_3O_5S$ is $[MH]^+$ 534.2057

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(4'-(morpholinomethyl)-[1,1'-biphenyl]-3-yl)sulfamoyl) benzamide 232

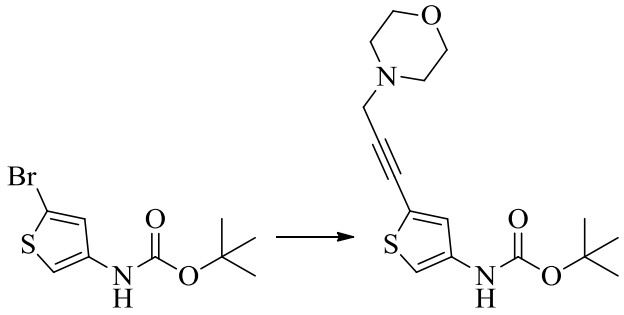


142 **232**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (40 mg, 0.134 mmol) and 4'-(morpholinomethyl)-[1,1'-biphenyl]-3-amine **236** (45 mg, 0.134 mmol) were added to THF (1 mL) and triethylamine (0.093 mL, 0.67 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (31 mg, 0.147 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and

tripotassium phosphate (91 mg, 0.429 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(4'-(morpholinomethyl)-[1,1'-biphenyl]-3-yl)sulfamoyl) benzamide **232** (22 mg, 30 %) as a white solid. M.P. 144-146 °C; LCMS (Formic acid) 100 %, R_t= 0.88, $[MH]^+$= 534; δ_H (400 MHz, DMSO-d_6) 10.44 (1H, s), 8.25 (1H, s), 8.24 – 8.21 (1H, m), 8.16 – 8.13 (1H, m), 7.92 – 7.88 (1H, m), 7.60 (1H, s), 7.45 (2H, d, *J* = 8.0 Hz), 7.38 (2H, d, *J* = 8.0 Hz), 7.35 – 7.26 (3H, m), 7.08 – 7.04 (1H, m), 6.41 – 6.37 (1H, m), 4.27 – 4.20 (2H, m), 3.80 (2H, t, *J* = 5.4 Hz), 3.63 – 3.55 (4H, m), 3.49 (2H, s), 2.45 – 2.32 (6H, m); δ_C (126 MHz, DMSO-d_6) 166.7, 141.4, 141.0, 140.9, 139.1, 138.8, 137.9, 135.8, 132.0, 130.2, 130.0, 127.5, 126.8, 126.2, 125.2, 125.1, 122.7, 119.7, 118.7, 66.6, 65.4, 63.8, 62.4, 53.6, 26.7; ν_{max} (liquid film)/cm^{-1} 2176, 2970, 2866, 1669, 1604, 1384, 1333, 1161, 1110; *m/z* (ES) Found: $[MH]^+$ 534.2034, $C_{29}H_{32}N_3O_5S$ is $[MH]^+$ 534.2057.

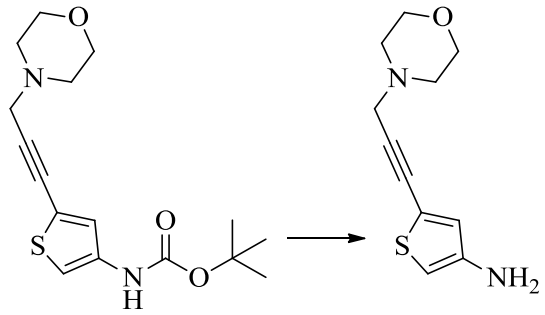
***Tert*-butyl (5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-yl)carbamate 242**



A mixture of tri-*tert*-butylphosphonium tetrafluoroborate (31 mg, 0.108 mmol), *tert*-butyl (5-bromothiophen-3-yl)carbamate **241** (300 mg, 1.078 mmol), 4-(prop-2-yn-1-yl)morpholine **196** (162 mg, 1.294 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (76 mg, 0.108 mmol) and cesium carbonate (703 mg, 2.157 mmol) in DMF (0.5 mL) and DBU (0.033 mL, 0.216 mmol) were heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-60 % acetonitrile in water with a formic acid

modifier on a 60 g C_{18} column to give *tert*-butyl (5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-yl)carbamate **242** (175 mg, 50 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.71, $[MH]^+$= 323;δ_H (400 MHz, $CDCl_3$) 7.15 – 7.06 (1H, m), 7.02 (1H, s), 6.65 – 6.51 (1H, m), 3.84 – 3.75 (4H, m), 3.56 – 3.50 (2H, m), 2.73 – 2.60 (4H, m), 1.53 (9H, s).

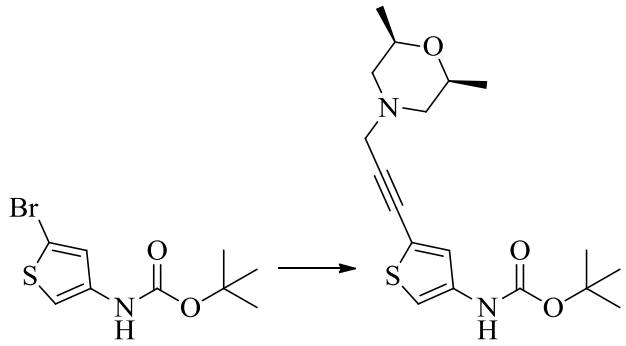
5-(3-Morpholinoprop-1-yn-1-yl)thiophen-3-amine 244



242 **244**

Tert-butyl (5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-yl)carbamate **242** (150 mg, 0.465 mmol) was dissolved in DCM (1 mL). To this, TFA (0.44 mL, 4.65 mmol) was added and stirred for 1 h under nitrogen, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-amine **244** (83 mg, 80 %) as a yellow solid. LCMS (Formic acid) 81 %, R_t= 0.70, $[MH]^+$= 223; δ_H (400 MHz, $CDCl_3$) 6.75 (1H, d, *J* = 1.7 Hz), 6.09 (1H, d, *J* = 1.7 Hz), 3.81 – 3.74 (4H, m), 3.64 – 3.47 (4H, m), 2.68 – 2.60 (4H, m). The product was used as a crude component in further reactions.

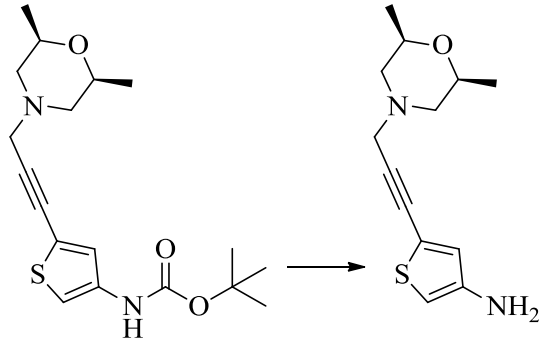
***Tert*-butyl (5-(3-((2*R*,6*S*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-yl) carbamate 243**



241 **243**

A mixture of tri-*tert*-butylphosphonium tetrafluoroborate (31 mg, 0.108 mmol), *tert*-butyl (5-bromothiophen-3-yl)carbamate **241** (300 mg, 1.078 mmol), (2*S*,6*R*)-2,6-dimethyl-4-(prop-2-yn-1-yl)morpholine **218** (198 mg, 1.294 mmol), bis-(triphenylphosphine)-palladium(II)dichloride (76 mg, 0.108 mmol) and cesium carbonate (703 mg, 2.157 mmol)n DMF (0.5 mL) and DBU (0.033 mL, 0.216 mmol) were heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-60 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column. The acetonitrile was concentrated *in vacuo*. The solution was neutralised to pH 7 with 2 M sodium hydroxide and extracted with ethyl acetate (7 x 50 mL). The organic layer was washed with brine (2 x 50 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give *tert*-butyl (5-(3-((2*R*,6*S*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-yl)carbamate **243** (298 mg, 79 %) as a white solid. LCMS (Formic acid) 98 %, R_t= 0.80, $[MH]^+$= 351; δ_H (400 MHz, $CDCl_3$) 7.14 – 7.06 (1H, m), 7.03 (1H, s), 6.64 – 6.57 (1H, m), 3.75 (2H, dqd, *J* = 12.6, 6.3, 2.1 Hz), 3.54 (2H, s), 2.84 – 2.78 (2H, m), 2.20 – 2.02 (2H, m), 1.53 (9H, s), 1.22 (6H, d, *J* = 6.3 Hz).

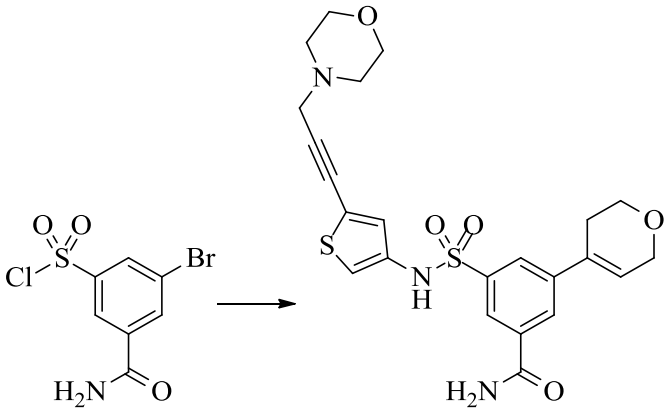
5-(3-((2*S*,6*R*)-2,6-Dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-amine 245



243 **245**

Tert-butyl (5-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-yl)carbamate **243** (200 mg, 0.571 mmol) was dissolved in DCM (1 mL). To this, TFA (0.44 mL, 5.71 mmol) was added and stirred for 1 h under nitrogen, then neutralised with saturated aqueous sodium carbonate (20 mL) and extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL) passed through a hydrophobic frit and concentrated *in vacuo* to give 5-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-amine **245** (120 mg, 84 %) as a colourless oil. LCMS (Formic acid) 85 %, R_t= 0.86, $[MH]^+$= 251; δ_H (400 MHz, $CDCl_3$) 6.75 (1H, d, *J* = 1.7 Hz), 6.10 (1H, d, *J* = 1.7 Hz), 3.79 – 3.69 (4H, m), 3.52 (2H, s), 2.84 – 2.76 (4H, m), 2.08 – 1.99 (2H, m), 1.21 (6H, d, *J* = 6.3 Hz). The product was used as a crude component in further reactions.

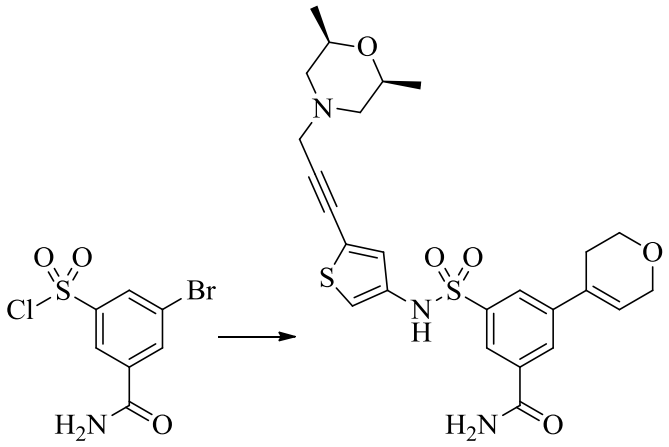
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-yl) sulfamoyl)benzamide 239



142 **239**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-amine **244** (60 mg, 0.216 mmol) were added to THF (1 mL) and triethylamine (0.140 mL, 1.005 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (46 mg, 0.219 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 1.005 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-(3-morpholinoprop-1-yn-1-yl)thiophen-3-yl)sulfamoyl) benzamide **239** (16 mg, 16 %) as a white solid. M.P. 155-157 °C; LCMS (Formic acid) 100 %, R_t= 0.60, $[MH]^+$= 488; δ_H (400 MHz, DMSO-d_6) 10.56 (1H, s), 8.27 (1H, s), 8.21 – 8.14 (2H, m), 7.87 – 7.84 (1H, m), 7.61 (1H, s), 6.96 – 6.90 (2H, m), 6.47 – 6.41 (1H, m), 4.31 – 4.19 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 3.63 – 3.56 (4H, m), 3.51 (2H, s), 2.49 – 2.39 (6H, m); δ_C (126 MHz, DMSO-d_6) 166.6, 141.1, 140.4, 135.8, 135.3, 132.0, 127.7, 127.0, 126.3, 125.0, 124.9, 122.1, 113.3, 90.5, 78.1, 66.4, 65.4, 63.8, 52.1, 47.5, 26.7; ν_{max} (liquid film)/cm^{-1} 3109, 2856, 1667, 1614, 1551, 1361, 1315, 1167, 1110; *m/z* (ES) Found: $[MH]^+$ 488.1291, $C_{23}H_{26}N_3O_5S_2$ is $[MH]^+$ 488.1308.

3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-yl)sulfamoyl)benzamide 240

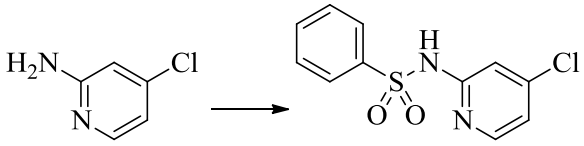


142 **240**

3-Bromo-5-carbamoylbenzene-1-sulfonyl chloride **142** (60 mg, 0.201 mmol) and 5-(3-((2*S*,6*R*)-2,6-dimethylmorpholino)prop-1-yn-1-yl)thiophen-3-amine **245** (70 mg, 0.224 mmol) were added to THF (1 mL) and triethylamine (0.14 mL, 1.005 mmol). The resulting mixture was stirred at room temperature for 30 min. The reaction was passed through a 1 g silica cartridge, eluting with 9:1 ethyl acetate:methanol (pre-conditioned with 9:1 ethyl acetate:methanol) and concentrated *in vacuo*. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (46 mg, 0.219 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) and tripotassium phosphate (137 mg, 1.005 mmol) in 1,4-dioxane (1 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 0.5 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The product was dissolved in 1:1 methanol:DMSO (2 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(*N*-(5-(3-((2*S*,6*R*)-2,6-dimethylmorpholino) prop-1-yn-1-yl)thiophen-3-yl)sulfamoyl)benzamide **240** (8 mg, 7 %) as a white solid. M.P. 140-142 °C; LCMS (Formic acid) 100 %, R_t= 0.66, $[MH]^+$= 516; δ_H (400 MHz, DMSO-d_6) 8.25 (1H, s), 8.18 – 8.14 (2H, m), 7.88 – 7.84 (1H, m), 7.58 (1H, s), 6.93 – 6.89 (1H, m), 6.87 – 6.82 (1H, m), 6.46 – 6.41 (1H, m), 4.30 – 4.24 (2H, m), 3.85 (2H, t, *J* = 5.3 Hz), 3.62 – 3.52 (2H, m), 3.50 (2H, s), 2.71 – 2.64 (2H, m), 2.48 – 2.42 (2H, m), 1.82 (2H, t, *J* = 10.6 Hz), 1.05 (6H, d, *J* = 6.2 Hz). Sulfonamide N-H not observed. δ_C (126 MHz, DMSO-d_6) 166.8, 141.0, 140.5, 135.7, 135.3, 132.1, 127.4, 127.3, 126.1, 125.0,

124.9, 121.7, 113.2, 90.3, 78.2, 71.3, 65.4, 63.9, 57.9, 47.0, 26.7, 19.4; ν_{max} (liquid film)/cm^{-1} 3194, 2922, 2857, 1666, 1615, 1551, 1361, 1316, 1167, 1110; *m/z* (ES) Found: $[MH]^+$ 516.1625, $C_{25}H_{3}N_3O_5S_2$ is $[MH]^+$ 516.1621.

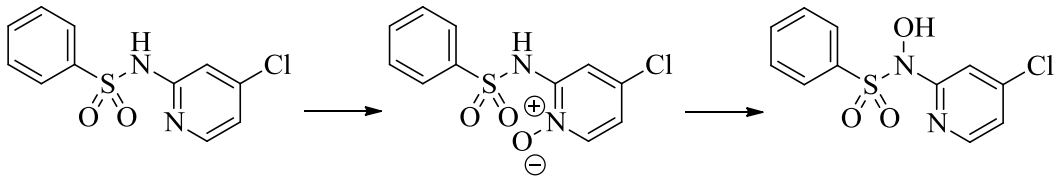
_N_-(4-Chloropyridin-2-yl)benzenesulfonamide 251



250 **251**

A mixture of 4-chloropyridin-2-amine **250** (2.5 g, 19.45 mmol) and benzenesulfonyl chloride (2.55 mL, 21.22 mmol) were combined in pyridine (10 mL). The resulting mixture was stirred at room temperature for 5 h and then diluted with water (200 mL) and extracted with ethyl acetate (2 x 200 mL). The organic layer was washed with brine (10 mL), aqueous hydrochloric acid (2 x 100 mL, 2 M), dried through a hydrophobic frit and concentrated *in vacuo*. Recrystalisation from boiling ethanol provided *N*-(4-chloropyridin-2-yl)benzenesulfonamide **251** (3.715 g, 71.1 %) as a white solid. LCMS (Ammonium carbonate) 100 %, R_t= 0.64, $[MH]^+$= 269, 271; δ_H (400 MHz, DMSO-d_6) 11.92 (1H, s) 8.08 (1H, d, *J* = 5.8 Hz) 7.88 - 7.94 (2H, m) 7.54 - 7.66 (4H, m) 7.15 (1H, d, *J* = 1.8 Hz) 7.04 (1H, dd, *J*=5.8, 1.8 Hz), δ_C (101 MHz, DMSO-d_6) 153.7, 147.0, 145.8, 141.4, 133.1, 129.5, 127.1, 117.4, 112.7.

_N_-(4-Chloropyridin-2-yl)-_N_-hydroxybenzenesulfonamide 254

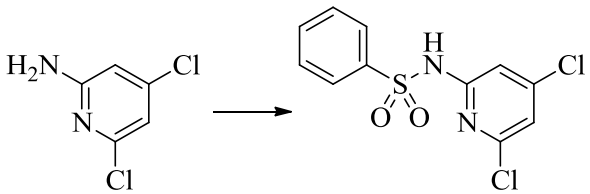


251 **252** **254**

N-(4-Chloropyridin-2-yl)benzenesulfonamide **251** (250 mg, 0.93 mmol) was dissolved in acetic acid (2 mL) and to this hydrogen peroxide (0.4 mL, 3.92 mmol, 30 % in water) was added and stirred at 85 °C for 2 h. The reaction mixture was quenched with aqueous

saturated sodium sulfite (1 mL), diluted with water (5 mL) and then extracted into ethyl acetate (2 x 10 mL). The organic layer was washed with brine (5 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give crude 4-chloro-2-(phenylsulfonamido)pyridine 1-oxide **252**. LCMS (Formic acid) 73 %, R_t= 0.29, $[MH]^-$= 283. The crude mixture was dissolved in acetic anhydride (2 mL, 21.2 mmol) and heated to 120 °C for 3 h. The solvent was concentrated *in vacuo*. The product was dissolved in 1:1 DMSO:methanol (0.6 mL) and purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give *N*-(4-chloropyridin-2-yl)-*N*-hydroxybenzenesulfonamide **254** (76 mg, 28 %) as a cream solid. LCMS (Formic acid) 100 %, R_t= 0.83, $[MH]^+$= 285; δ_C (600 MHz, DMSO-d_6) 7.94 (1H, d, *J* = 5.7 Hz), 7.81 – 7.74 (2H, m), 7.49 – 7.43 (3H, m), 7.03 (1H, d, *J* = 2.6 Hz), 6.59 – 6.53 (1H, m); δ_C (151 MHz, DMSO-d_6) 153.5, 145.1, 139.0, 131.0, 130.5, 128.9, 126.5, 112.9, 112.3.

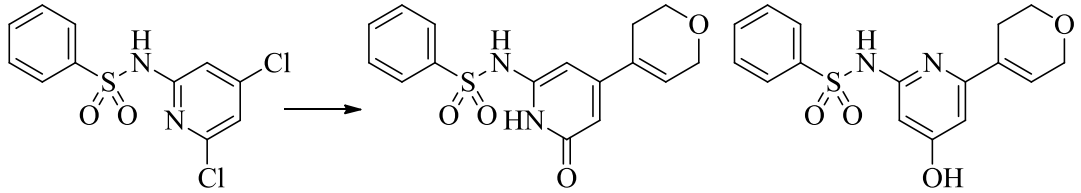
_N_-(4,6-Dichloropyridin-2-yl)benzenesulfonamide 256



255 **256**

A mixture of 4,6-dichloropyridin-2-amine **255** (1 g, 6.13 mmol) and benzenesulfonyl chloride (0.774 mL, 6.44 mmol) were combined in pyridine (2 mL). The resulting mixture was stirred at room temperature for 5 h and then diluted with water (100 mL) and extracted with ethyl acetate (2 x 100 mL). The organic layer was washed with aqueous hydrochloric acid (2 x 50 mL, 2 M), brine (50 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 – 60 % TBME/cyclohexane at 222 nm) provided *N*-(4,6-dichloropyridin-2-yl)benzenesulfonamide **256** (1.536 g, 83 %) as a white solid. LCMS (Formic acid) 85 %, R_t= 1.16, $[MH]^+$= 303, 305, 307; δ_H (400 MHz, $CDCl_3$) 8.01 – 7.92 (2H, m), 7.67 – 7.62 (1H, m), 7.60 – 7.52 (2H, m), 7.36 (1H, s), 7.35 (1H, d, *J* = 1.4 Hz), 7.04 (1H, d, *J* = 1.4 Hz).

***N*-(4-(3,6-Dihydro-2*H*-pyran-4-yl)-6-oxo-1,6-dihydropyridin-2-yl)benzenesulfonamide 246** and ***N*-(6-(3,6-dihydro-2*H*-pyran-4-yl)-4-hydroxypyridin-2-yl)benzenesulfonamide 259**



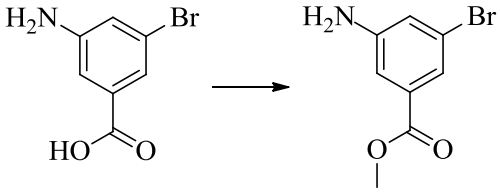
255 **246** **259**

A mixture of *N*-(4,6-dichloropyridin-2-yl)benzenesulfonamide **255** (200 mg, 0.66 mmol), dicyclohexyl(2',4',6'-triisopropyl-3,6-dimethoxy-[1,1'-biphenyl]-2-yl)phosphine (35 mg, 0.066 mmol), $Pd_2(dba)_3$ (48 mg, 0.053 mmol) and sodium *tert*-butoxide (317 mg, 3.3 mmol) were dissolved in THF (3 mL) and water (0.03 mL, 1.665 mmol) and stirred at 100 °C for 16 h. The reaction mixture was filtered through Celite, eluting with methanol and then concentrated *in vacuo.* The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15-70 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column. The residue was added to a mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (116 mg, 0.553 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (30 mg, 0.054 mmol) and tripotassium phosphate (358 mg, 1.686 mmol) in 1,4-dioxane (2 mL)/water (0.4 mL) and was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The product was dissolved in methanol (1 mL) and purified by XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier MDAP (method A) to give *N*-(4-(3,6-dihydro-2*H*-pyran-4-yl)-6-oxo-1,6-dihydropyridin-2-yl)benzenesulfonamide **246** (18 mg, 10 %) as a off-white solid. M.P. 200 °C decomposition; LCMS (Formic acid) 98 %, R_t= 0.78, $[MH]^+$= 333; δ_H (400 MHz, DMSO-d_6) 10.61 (1H, s), 7.92 – 7.81 (2H, m), 7.57 – 7.45 (3H, m), 7.11 (1H, s), 6.24 – 6.16 (2H, m), 5.93 – 5.87 (1H, m), 4.21 – 4.16 (2H, m), 3.75 (2H, t, *J* = 5.4 Hz), 2.30 – 2.18 (2H, m); δ_C (101 MHz, DMSO-d_6) 163.5, 152.4, 151.2, 142.9, 132.4, 132.2, 129.1, 127.1, 126.2, 99.5, 95.0, 65.3, 63.8, 26.3; ν_{max} (liquid film)/cm^{-1} 2965, 2926, 2811, 1638, 1589, 1460, 1396, 1266, 1161, 1128; *m/z* (ES) Found: $[MH]^+$ 333.0895, $C_{16}H_{17}N_2O_4S$ is $[MH]^+$ 333.0904.

The second product eluted was dissolved in DMSO (1 mL) and further purified by Sunfire C_{18} column using acetonitrile/water with a formic acid modifier MDAP (method B) to give *N*-(6-(3,6-dihydro-2*H*-pyran-4-yl)-4-hydroxypyridin-2-yl)benzenesulfonamide **259** (20 mg,

11 %) as a white solid. M.P. 218 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.73, $[MH]^+$= 333; δ_H (400 MHz, DMSO-d_6) 11.04 (2H, s), 7.93 – 7.77 (2H, m), 7.62 – 7.46 (3H, m), 6.62 – 6.53 (1H, m), 6.48 – 6.38 (1H, m), 6.37 – 6.25 (1H, m), 4.23 – 4.15 (2H, m), 3.74 (2H, t, *J* = 5.4 Hz), 2.34 – 2.26 (2H, m); δ_C (101 MHz, DMSO-d_6) 132.24, 129.23, 129.12, 128.30, 126.92, 102.83, 97.29, 65.24, 63.79, 25.58. Four carbons are not observed. ν_{max} (liquid film)/cm^{-1} 3180, 3134, 1599, 1412, 1247, 1228, 1128; *m/z* (ES) Found: $[MH]^+$ 333.0888, $C_{16}H_{17}N_2O_4S$ is $[MH]^+$ 333.0904.

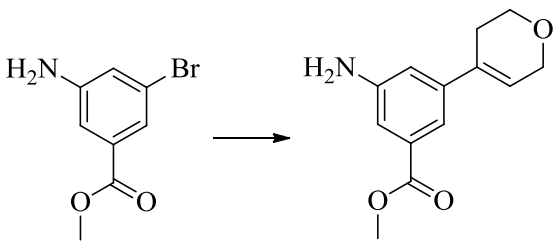
Methyl 3-amino-5-bromobenzoate 261



260 **261**

3-Amino-5-bromobenzoic acid **260** (500 mg, 2.314 mmol) was dissolved in methanol (5 mL) and to this concentrated sulfuric acid (2 mL, 37.5 mmol) was added. The resulting mixture was stirred at room temperature for 24 h and then concentrated *in vacuo*. The residue was poured onto saturated aqueous sodium bicarbonate (20 mL) and ice (20 g), and extracted with DCM (3 x 20 mL). The organic layer was passed through a hydrophobic frit and concentrated *in vacuo* to give methyl 3-amino-5-bromobenzoate **261** (479 mg, 9 0 %) as an off-white solid. The reaction was repeated on 3-amino-5-bromobenzoic acid **260** (10 g, 48.6 mmol) to give methyl 3-amino-5-bromobenzoate **261** (10.4 g, 93 %) as an off-white solid. LCMS (Formic acid) 100 %, R_t= 0.91, $[MH]^+$= 230, 232; δ_H (400 MHz, $CDCl_3$) 7.57 – 7.53 (1H, m), 7.27 (1H, dd, *J* = 2.2, 1.4 Hz), 7.02 – 7.00 (1H, m), 4.0 – 3.75 (2H, s), 3.91 (3H, s).

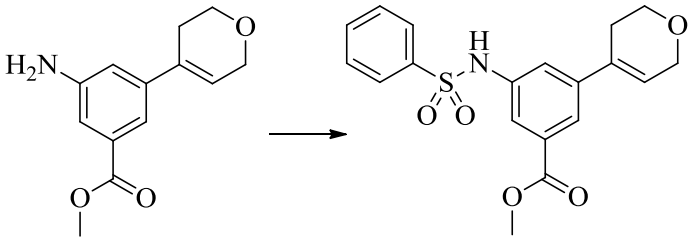
Methyl 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoate 262



261 **262**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (402 mg, 1.913 mmol), 3-amino-5-bromobenzoate **261** (400 mg, 1.739 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (97 mg, 0.174 mmol) and tripotassium phosphate (1181 mg, 5.56 mmol) and sodium carbonate (189 mg, 1.783 mmol) in 1,4-dioxane (4 mL) and water (0.8 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 60 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. Purification by flash chromatography (10 – 100 % TBME/cyclohexane at 222 nm) provided methyl 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzoate **262** (396 mg, 98 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.71, $[MH]^+$= 234; δ_H (400 MHz, $CDCl_3$) 7.51 – 7.49 (1H, m), 7.30 – 7.26 (1H, m), 6.91 – 6.87 (1H, m), 6.19 – 6.15 (1H, m), 4.38 – 4.30 (2H, m), 3.94 (2H, t, *J* = 5.5 Hz), 3.92 (3H, s), 2.57 – 2.48 (2H, m). Aniline NH_2 not observed.

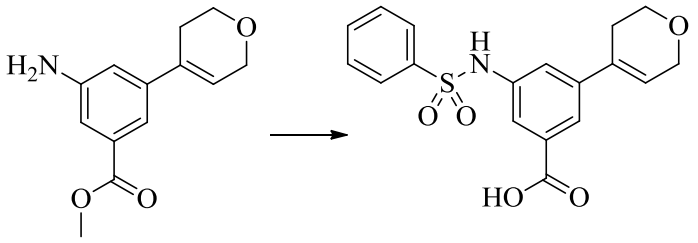
Methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzoate 263a



262 **263a**

A mixture of methyl 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoate **262** (60 mg, 0.257 mmol) and benzenesulfonyl chloride (0.031 mL, 0.257 mmol) were combined in pyridine (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with aqueous hydrochloric acid (10 mL, 2 M), brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give methyl 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzoate **263** (83 mg, 86 %) as a clear oil. LCMS (Formic acid) 91 %, R_t= 0.71, $[MH]^-$= 372; δ_H (400 MHz, $CDCl_3$) 7.86 – 7.83 (1H, m), 7.83 – 7.78 (2H, m), 7.62 – 7.55 (1H, m), 7.55 – 7.53 (1H, m), 7.51 – 7.45 (2H, m), 7.43 – 7.40 (1H, m), 6.69 (1H, s), 6.22 – 6.18 (1H, m), 4.36 – 4.31 (2H, m), 3.97 – 3.92 (2H, m), 3.91 (3H, s), 2.53 – 2.44 (2H, m).

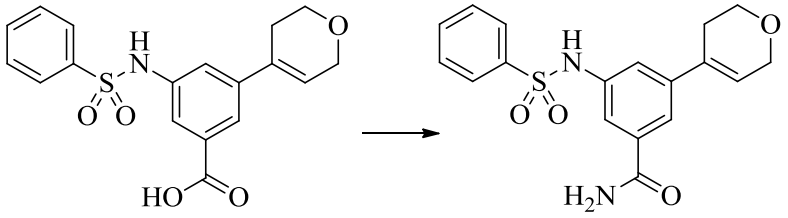
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzoic acid 249



262 **249**

A mixture of methyl 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)benzoate **262** (178 mg, 0.763 mmol) and benzenesulfonyl chloride (0.101 mL, 0.839 mmol) were combined in pyridine (1 mL). The resulting mixture was stirred at rt for 3 h and then diluted with water (20 mL) and extracted with ethyl acetate (2 x 15 mL). The organic layer was washed with aqueous hydrochloric acid (10 mL, 2 M), brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in methanol (2 mL) and to this aqueous sodium hydroxide (1 mL, 2 M) was added and stirred for 30 min. The solvent was concentrated *in vacuo*. The residue was dissolved in water (5 mL), acidified to pH 5 with 2 M aqueous hydrochloric acid and extracted into ethyl acetate (2 x 5 mL). The organic layer was dried through a hydrophobic frit and concentrated *in vacuo* to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzoic acid (195 mg, 71 %) as a crude colourless oil. At this point, 130 mg were used in further reactions. The remaining 65 mg was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzoic acid **249** (42 mg) as a white solid. M.P. 188-190 °C; LCMS (Formic acid) 100 %, R_t= 0.92, $[MH]^-$= 358; δ_H (400 MHz, DMSO-d_6) 13.04 (1H, s), 10.44 (1H, s), 7.81 – 7.76 (2H, m), 7.67 – 7.64 (1H, m), 7.63 – 7.53 (4H, m), 7.42 – 7.38 (1H, m), 6.22 – 6.17 (1H, m), 4.25 – 4.18 (2H, m), 3.80 (2H, t, *J* = 5.4 Hz), 2.40 – 2.30 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.1, 141.3, 139.7, 138.7, 133.5, 132.5, 132.5, 129.7, 127.1, 124.9, 121.2, 120.2, 119.7, 65.4, 63.9, 29.4, 26.8; ν_{max} (liquid film)/cm^{-1} 3186, 2930, 1720, 1691, 1595, 1449, 1320, 1258, 1154; *m/z* (ES) Found: $[MH]^+$ 360.0893, $C_{18}H_{18}NO_5S$ is $[MH]^+$ 360.0900.

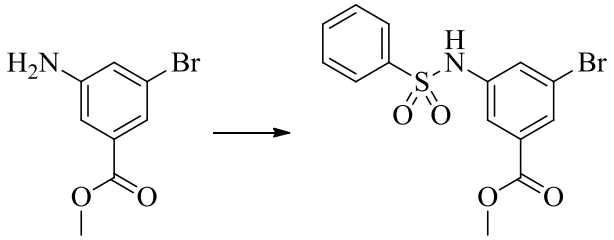
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzamide 247



249 **247**

A mixture of 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(phenylsulfonamido)benzoic acid **249** (60 mg, 0.167 mmol), DIPEA (0.087 mL, 0.501 mmol), and HATU (69 mg, 0.184 mmol) were dissolved in DMF (1 mL) and stirred for 30 min. To this, ammonia (1.669 mL, 0.835 mmol, 0.5 M in 1,4-dioxane) was added. The resulting mixture was stirred at rt for 72 h and then diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), aqueous lithium chloride (2 x 10 mL, 5 %), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(*N*-phenylsulfamoyl)benzamide **247** (25 mg, 41 %) as a white solid. M.P. 161-163 °C; LCMS (Formic acid) 100 %, R_t= 0.82, $[MH]^-$= 357; δ_H (400 MHz, DMSO-d_6) 10.42 (1H, s), 7.94 (1H, s), 7.81 – 7.74 (2H, m), 7.64 – 7.58 (2H, m), 7.58 – 7.52 (2H, m), 7.52 – 7.48 (1H, m), 7.30 (1H, s), 7.29 – 7.26 (1H, m), 6.23 – 6.19 (1H, m), 4.24 – 4.20 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 2.40 – 2.31 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.8, 140.9, 140.1, 138.8, 135.9, 133.3, 133.2, 132.9, 129.7, 127.1, 124.5, 119.3, 118.9, 65.4, 63.9, 26.8; ν_{max} (liquid film)/cm^{-1} 3464, 3355, 3152, 1670, 1587, 1382, 1330, 1163; *m/z* (ES) Found: $[MH]^+$ 359.1055, $C_{18}H_{19}N_2O_4S$ is $[MH]^+$ 359.1060.

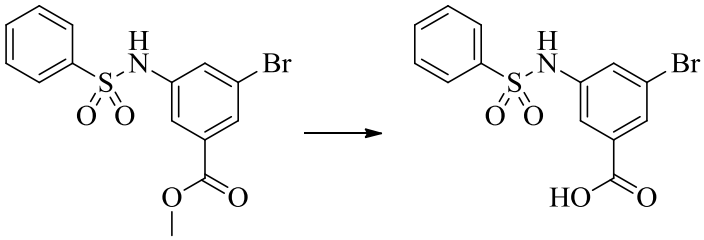
Methyl 3-bromo-5-(phenylsulfonamido)benzoate 264



261 **264**

A mixture of methyl 3-amino-5-bromobenzoate **261** (5 g, 21.73 mmol) and benzenesulfonyl chloride (2.74 mL, 22.82 mmol) were combined in pyridine (15 mL). The resulting mixture was stirred at room temperature for 3 h and then diluted with water (200 mL) and extracted with ethyl acetate (2 x 200 mL). The organic layer was washed with brine (100 mL), aqueous hydrochloric acid (2 x 100 mL, 2 M), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 –75 % ethyl acetate/cyclohexane) provided a purple solid which was recrystallized from boiling ethanol to give methyl 3-bromo-5-(phenylsulfonamido)benzoate **264** (5.65 g, 70 %) as white crystals. LCMS (Formic acid) 100 %, R_t= 1.15, $[MH]^-$= 368, 370; δ_H (400 MHz, $CDCl_3$) 7.94 – 7.91 (1H, m), 7.85 – 7.81 (2H, m), 7.64 – 7.61 (1H, m), 7.61 – 7.56 (2H, m), 7.54 – 7.47 (2H, m), 6.83 (1H, s), 3.92 (3H, s).

3-Bromo-5-(phenylsulfonamido)benzoic acid 265

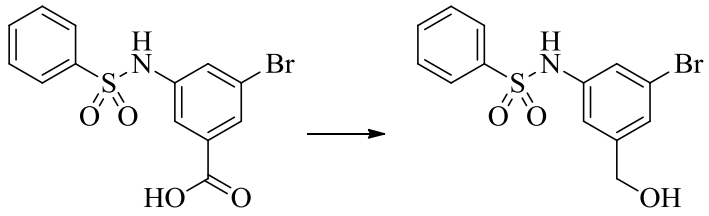


264 **265**

Methyl 3-bromo-5-(phenylsulfonamido)benzoate **264** (5.5 g, 14.86 mmol) was dissolved in THF (100 mL) and water (100 mL). To this, aqueous lithium hydroxide (22.3 mL, 44.6 mmol, 2 M) was added and stirred for 16 h. The THF was concentrated *in vacuo*. The solution was acidified to pH 5 with 2 M aqueous hydrochloric acid , filtered and washed with further water (5 mL) to give 3-bromo-5-(phenylsulfonamido)benzoic acid **265** (5.26 g, 99 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 1.00, $[MH]^-$= 354, 356; δ_H (400 MHz,

DMSO-d_6) 13.40 (1H, s), 10.81 (1H, s), 7.82 – 7.78 (2H, m), 7.71 – 7.69 (1H, m), 7.69 – 7.66 (1H, m), 7.66 – 7.63 (1H, m), 7.62 – 7.56 (2H, m), 7.50 – 7.48 (1H, m).

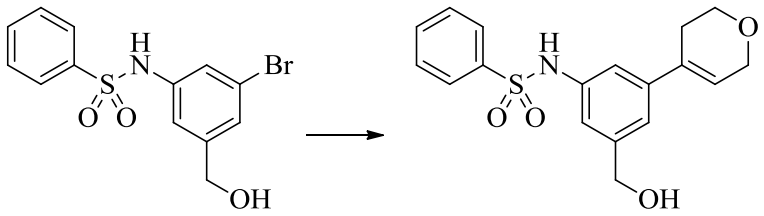
***N*-(3-Bromo-5-(hydroxymethyl)phenyl)benzenesulfonamide 266**



265 **266**

Bromo-5-(phenylsulfonamido)benzoic acid **265** (250 mg, 0.702 mmol) was dissolved in THF (2 mL) and cooled to 0 °C under nitrogen. To this, borane (1.4 mL, 1.4 mmol, 1 M in THF) was added dropwise and stirred for 16 h and then heated to 70 °C and stirred for 3 h. Aqueous hydrochloric acid (5 mL, 2 M) was added and the solvent concentrated *in vacuo*. The residue was dissolved in water (30 mL) and then extracted into ethyl acetate (2 x 30 mL). The organic layer was washed with brine (30 mL), dried over magnesium sulfate, passed through a hydrophobic frit and concentrated *in vacuo* to give *N*-(3-bromo-5-(hydroxymethyl)phenyl)benzenesulfonamide **266** (195 mg, 81 %) as a white solid. LCMS (Formic acid) 99 %, R_t= 0.97, $[MH]^+$= no ionization; δ_H (400 MHz, $CDCl_3$) 7.84 – 7.79 (2H, m), 7.62 – 7.57 (1H, m), 7.54 – 7.47 (2H, m), 7.31 – 7.28 (1H, m), 7.20 – 7.16 (1H, m), 7.05 – 7.02 (1H, m), 6.55 (1H, s), 4.63 (2H, d, *J* = 5.0 Hz). Alcohol O-H not observed.

***N*-(3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(hydroxymethyl)phenyl)benzenesulfonamide 248**

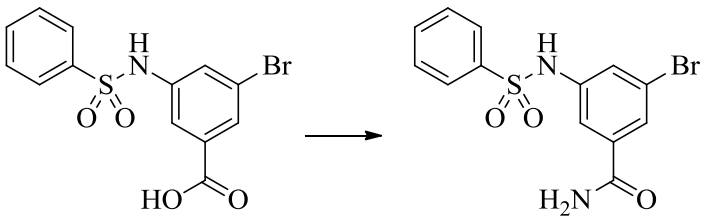


266 **248**

A mixture of *N*-(3-bromo-5-(hydroxymethyl)phenyl)benzenesulfonamide (75 mg, 0.219 mmol), 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (46 mg,

0.219 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (6 mg, 0.174 mmol) and tripotassium phosphate (140 mg, 0.657 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method A) to give *N*-(3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(hydroxymethyl) phenyl) benzenesulfonamide (37 mg, 49 %) as a white solid. M.P. 148-150 °C; LCMS (Formic acid) 100 %, R_t= 0.84, $[MH\text{-}H_2O]^+$= 328; δ_H (400 MHz, DMSO-d_6) 10.18 (1H, s), 7.82 – 7.76 (2H, m), 7.63 – 7.51 (3H, m), 7.06 – 7.01 (2H, m), 7.01 – 6.99 (1H, m), 6.14 – 6.04 (1H, m), 4.39 (2H, s), 4.23 – 4.16 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 2.36 – 2.27 (2H, m). Alcohol O-H not observed. δ_C (101 MHz, DMSO-d_6) 144.1, 140.6, 140.4, 138.7, 133.3, 133.1, 129.6, 127.1, 123.5, 118.4, 117.4, 114.8, 65.4, 64.0, 63.1, 26.9; ν_{max} (liquid film)/cm^{-1} 3268, 3085, 2880, 1450, 1328, 1155; *m/z* (ES) Found: $[MH]^+$ 346.1101, $C_{18}H_{20}NO_4S$ is $[MH]^+$ 346.1108.

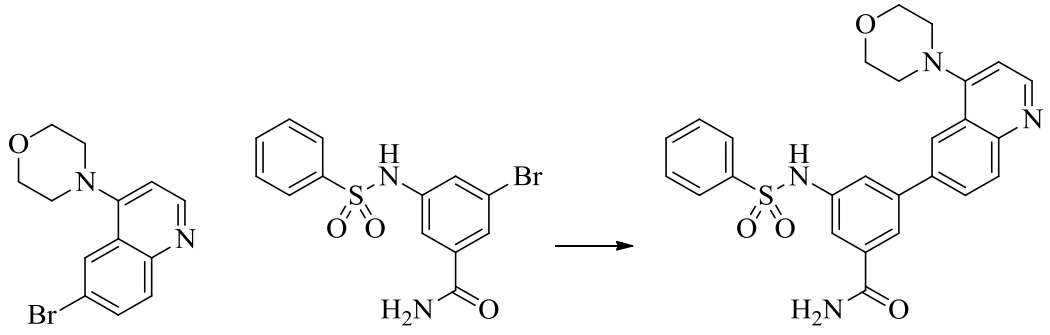
3-Bromo-5-(phenylsulfonamido)benzamide 269



265 **269**

3-Bromo-5-(phenylsulfonamido)benzoic acid **265** (1 g, 2.81 mmol) was dissolved in thionyl chloride (3 mL, 41.1 mmol) and stirred at 70 °C for 3 h. The thionyl chloride was concentrated *in vacuo*. The residue was diluted with THF and added dropwise to ammonium hydroxide (15 mL, 108 mmol, 28 %). The THF was concentrated *in vacuo*. The residue was neutralised to pH 7 with 2 M aqueous hydrochloric acid and extracted into ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 3-bromo-5-(phenylsulfonamido)benzamide **269** (985 mg, 99 %) a white solid. LCMS (Formic acid) 100 %, R_t= 0.85, $[MH]^-$= 353, 355; δ_H (400 MHz, CD_3OD) 8.54 – 8.43 (2H, m), 8.37 – 8.31 (1H, m), 8.30 – 8.14 (4H, m), 8.14 – 8.07 (1H, m). Sulfonamide N-H and amide NH_2 are not observed.

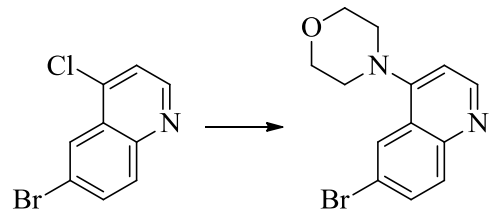
3-(4-Morpholinoquinolin-6-yl)-5-(phenylsulfonamido) benzamide 270



272 **269** **270**

A mixture of 3-bromo-5-(phenylsulfonamido)benzamide **269** (100 mg, 0.281 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (78 mg, 0.309 mmol), potassium acetate (84 mg, 0.86 mmol) and $PdCl_2$(dppf) (10 mg, 0.012 mmol) in 1,4-dioxane (1 mL) was heated and stirred at 80 °C under nitrogen for 6 h. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. To the residue was added a mixture of 4-(6-bromoquinolin-4-yl)morpholine **272** (76 mg, 0.26 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (8 mg, 0.014 mmol) and tripotassium phosphate (186 mg, 0.875 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) were heated and stirred in a Biotage microwave reactorat 80 °C for 60 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method A) to give 3-(4-morpholinoquinolin-6-yl)-5-(phenylsulfonamido) benzamide **270** (75 mg, 56 %) as a white solid. M.P. 210 °C decomposition; LCMS (Formic acid) 100 %, R_t= 0.54, $[MH]^+$= 489; δ_H (400 MHz, DMSO-d_6) 8.73 (1H, d, *J* = 5.0 Hz), 8.17 (1H, d, *J* = 1.7 Hz), 8.12 (1H, s), 8.07 (1H, d, *J* = 8.8 Hz), 8.02 – 7.95 (2H, m), 7.86 – 7.81 (2H, m), 7.72 – 7.66 (2H, m), 7.64 – 7.52 (3H, m), 7.43 (1H, s), 7.05 (1H, d, *J* = 5.0 Hz), 3.96 – 3.90 (4H, m), 3.26 – 3.18 (4H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 167.7, 156.6, 151.7, 149.2, 140.8, 140.1, 139.3, 136.8, 136.1, 133.4, 130.9, 129.8, 128.3, 127.0, 123.2, 121.7, 121.5, 121.0, 119.3, 110.0, 66.5, 52.7; v_{max} (liquid film)/cm^{-1} 3169, 2965, 2854, 1664, 1571, 1447, 1367, 1328, 1157; *m/z* (ES) Found: $[MH]^+$ 489.1570, $C_{26}H_{25}N_4O_4S$ is $[MH]^+$ 489.1591.

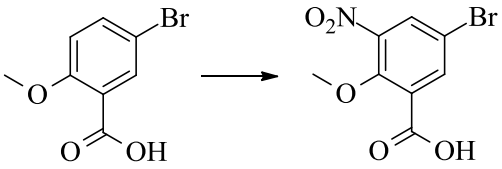
4-(6-Bromoquinolin-4-yl)morpholine 272



271 **272**

A mixture 6-bromo-4-chloroquinoline **271** (1g, 4.12 mmol) and morpholine (1.437 mL, 16.49 mmol) were dissolved in isopropanol (20 mL) and stirred at 85 °C for 2 h. The reaction was cooled to room temperature and the solvent concentrated *in vacuo*. The residue was dissolved in water (25 mL) and extracted with ethyl acetate (2 x 25 mL). The organic layer was washed with brine (25 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (50 – 100 % ethyl acetate/cyclohexane) provided 4-(6-bromoquinolin-4-yl)morpholine **272** (1.15 g, 95 %) as an off-white solid. LCMS (Formic acid) 100 %, R_t= 0.46, $[MH]^+$= 293, 295; δ_H (400 MHz, $CDCl_3$) 8.78 (1H, d, *J* = 5.0 Hz), 8.19 (1H, d, *J* = 2.2 Hz), 7.96 (1H, d, *J* = 9.0 Hz), 7.76 (1H, dd, *J* = 8.9, 2.2 Hz), 6.91 (1H, d, *J* = 5.0 Hz), 4.05 – 4.00 (4H, m), 3.28 – 3.19 (4H, m).

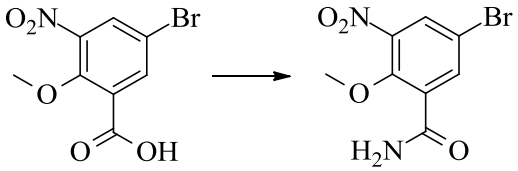
5-Bromo-2-methoxy-3-nitrobenzoic acid 276



275 **276**

A mixture of 5-bromo-2-methoxybenzoic acid **275** (1 g, 4.33 mmol) in concentrated sulfuric acid (8 mL, 15 mmol) was added dropwise to a solution of concentrated nitric acid (0.387 mL, 8.66 mmol) and concentrated sulfuric acid (0.508 mL, 9.52 mmol) at 0 °C and stirred for 30 min. The reaction mixture was poured onto ice and filtered, washing the precipitate with cold water (10 mL) to give 5-bromo-2-methoxy-3-nitrobenzoic acid **276** (886 mg, 74 %) as a yellow solid. The reaction was repeated on 5-bromo-2-methoxybenzoic acid (3 g, 12.98 mmol) to give 5-bromo-2-methoxy-3-nitrobenzoic acid (2.2 g, 61 %) a yellow solid. LCMS (Formic acid) 92 %, R_t= 1.01, $[MH]^-$= 274, 276; δ_H (400 MHz, $CDCl_3$) 8.38 (1H, d, *J* = 2.6 Hz), 8.16 (1H, d, *J* = 2.6 Hz), 4.09 (3H, s). Carboxylic acid O-H not observed.

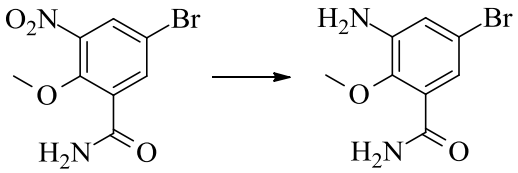
5-Bromo-2-methoxy-3-nitrobenzamide 277



276 **277**

5-Bromo-2-methoxy-3-nitrobenzoic acid **276** (800 mg, 2.9 mmol) was dissolved in toluene (4 mL) and thionyl chloride (1.058 mL, 14.49 mmol) and stirred at 70 °C for 3 h. The solvent was concentrated *in vacuo*. The residue was diluted with THF (4 mL) and added dropwise to ammonium hydroxide (5 mL, 36 mmol, 28 %) at 0 °C. The THF was concentrated *in vacuo*. The residue was neutralized to pH 7 with 2 M aqueous hydrochloric acid and extracted into ethyl acetate (3 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and concentrated *in vacuo* to give 5-bromo-2-methoxy-3-nitrobenzamide **277** (777 mg, 97 %) as a yellow solid. The reaction was repeated on 5-bromo-2-methoxy-3-nitrobenzoic acid **276** (2.21 g, 8.01 mmol) to give 5-bromo-2-methoxy-3-nitrobenzamide **277** (1.94 g, 88 %) as a yellow solid. LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^-$= 275, 277; δ_H (400 MHz, $CDCl_3$) 8.45 (1H, d, *J* = 2.6 Hz), 8.10 (1H, d, *J* = 2.6 Hz), 7.30 (2H, m), 6.05 (1H, s), 4.03 (3H, s).

3-Amino-5-bromo-2-methoxybenzamide 278

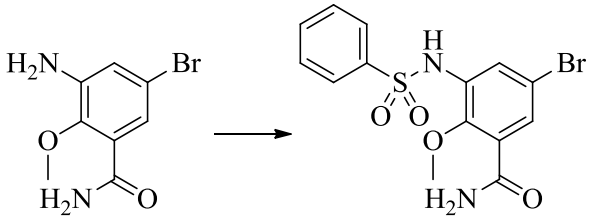


277 **278**

A mixture of 5-bromo-2-methoxy-3-nitrobenzamide **277** (750 mg, 2.73 mmol), ammonium chloride (74 mg, 1.383 mmol) and iron (457 mg, 8.18 mmol) was dissolved in ethanol (10 mL) and water (3 mL) and stirred at 80 °C for 17 h. The reaction was cooled and the solvent concentrated *in vacuo*. The residue was diluted with water (100 mL) and ethyl acetate (200 mL) and filtered through Celite, further washing with water (2 x 100 mL) and ethyl acetate (2 x 100 mL). The organic layer was separated, washed with brine (100 mL), passed through a hydrophobic frit and the solvent concentrated *in vacuo* to give 3-amino-5-bromo-2-methoxybenzamide **278** (645 mg, 97 %) as a yellow solid. The reaction was repeated on 5-bromo-2-methoxy-3-nitrobenzamide **277** (2.1 g, 7.63 mmol) to give 3-amino-5-

bromobenzamide **278** (1.54 g, 82 %) as a yellow solid. LCMS (Formic acid) 96 %, R_t= 0.61, $[MH]^-$= 245, 247; δ_H (400 MHz, DMSO-d_6) 7.65 (1H, s), 7.48 (1H, s), 6.93 (1H, d, *J* = 2.5 Hz), 6.80 (1H, d, *J* = 2.5 Hz), 5.39 (2H, s), 3.67 (3H, s).

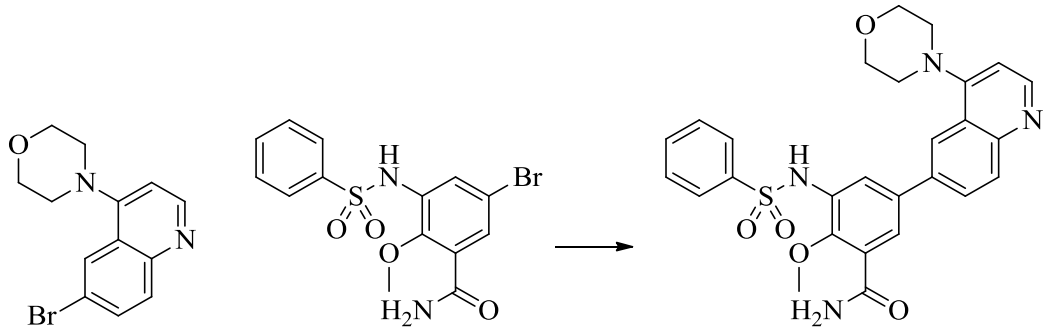
5-Bromo-2-methoxy-3-(phenylsulfonamido)benzamide 279



278 **279**

A mixture of 3-amino-5-bromo-2-methoxybenzamide **278** (300 mg, 1.224 mmol) and benzenesulfonyl chloride (0.154 mL, 1.285 mmol) were combined in pyridine (2 mL). The resulting mixture was stirred at room temperature for 5 h and then diluted with water (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL), aqueous hydrochloric acid (2 x 10 mL, 2 M), dried through a hydrophobic frit and concentrated *in vacuo*. Recrystallization from boiling ethanol gave 5-bromo-2-methoxy-3-(phenylsulfonamido)benzamide **279** (197 mg, 41 %) as white crystals. LCMS (Formic acid) 100 %, R_t= 0.89, $[MH]^+$= 385, 387; δ_H (400 MHz, DMSO-d_6) 10.08 (1H, s), 7.88 – 7.78 (2H, m), 7.74 (1H, s), 7.71 – 7.58 (3H, m), 7.57 (1H, s), 7.48 (1H, d, *J* = 2.5 Hz), 7.33 (1H, d, *J* = 2.5 Hz), 3.45 (3H, s).

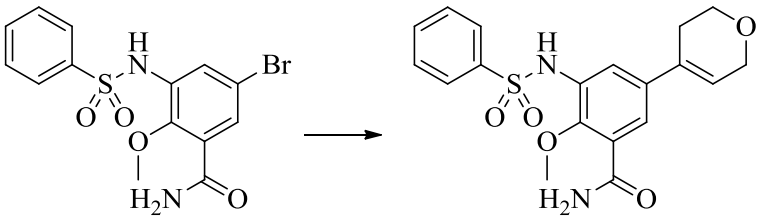
2-Methoxy-5-(4-morpholinoquinolin-6-yl)-3-(phenylsulfonamido)benzamide 273



272 **279** **273**

A mixture of 4-(6-bromoquinolin-4-yl)morpholine **272** (60 mg, 0.205 mmol), 4,4,4',4',5,5,5',5'-octamethyl-2,2'-bi(1,3,2-dioxaborolane) (58 mg, 228 mmol), potassium acetate (44 mg, 0.448 mmol) and $PdCl_2$(dppf) (15 mg, 0.021 mmol) in 1,4-dioxane (1 mL) was heated and stirred at 80 °C under nitrogen for 90 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. To the residue was added a mixture of 5-bromo-2-methoxy-3-(phenylsulfonamido)benzamide **279** (50 mg, 0.13 mmol), 3-(phenylsulfonamido)-5-(4,4,5,5-tetramethyl-1,3,2-dioxaborolan-2-yl)benzamide (110 mg, 0.273 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (7 mg, 0.014 mmol) and tripotassium phosphate (85 mg, 0.402 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) were heated and stirred in a Biotage microwave reactorat 80 °C for 60 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method A) to give 2-methoxy-5-(4-morpholinoquinolin-6-yl)-3-(phenylsulfonamido)benzamide **273** (49 mg, 72 %) as a white solid. M.P. 198-200 °C; LCMS (Formic acid) 100 %, R_t= 0.54, $[MH]^+$= 519; δ_H (400 MHz, DMSO-d_6) 8.72 (1H, d, *J* = 4.9 Hz), 8.17 (1H, s), 8.15 (1H, d, *J* = 1.9 Hz), 8.05 (1H, d, *J* = 8.7 Hz), 7.94 – 7.89 (1H, m), 7.90 – 7.85 (2H, m), 7.82 – 7.79 (2H, m), 7.67 – 7.56 (4H, m), 7.54 (1H, s), 7.05 (1H, d, *J* = 5.0 Hz), 3.98 – 3.86 (4H, m), 3.53 (3H, s), 3.27 – 3.19 (4H, m); δ_C (101 MHz, DMSO-d_6) 167.9, 156.6, 151.5, 149.7, 148.9, 140.9, 135.9, 135.0, 133.4, 131.6, 131.1, 130.9, 129.7, 128.0, 127.0, 124.2, 123.2, 122.9, 121.2, 110.0, 66.5, 61.8, 52.7; ν_{max} (liquid film)/cm^{-1} 3179, 2968, 2854, 1664, 1571, 1447, 1368, 1329, 1159, 1091; *m/z* (ES) Found: $[MH]^+$ 519.1679, $C_{27}H_{27}N_4O_5S$ is $[MH]^+$ 519.1697.

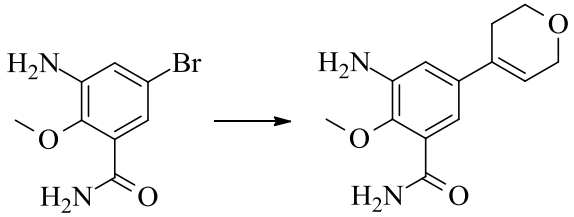
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(phenylsulfonamido)benzamide 274



A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (30 mg, 0.143 mmol), 5-bromo-2-methoxy-3-(phenylsulfonamido)benzamide **279** (50 mg, 0.13

mmol), tripotassium phosphate (83 mg, 0.389 mmol) and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (14 mg, 0.174 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(phenylsulfonamido) benzamide **274** (26 mg, 51 %) as a white solid. M.P. 150-152 °C; LCMS (Formic acid) 100 %, R_t= 0.82, $[MH]^+$= 388; δ_H (400 MHz, DMSO-d_6) 7.85 – 7.79 (2H, m), 7.65 (1H, s), 7.63 – 7.53 (3H, m), 7.43 (1H, s), 7.36 (1H, d, *J* = 2.3 Hz), 7.26 (1H, d, *J* = 2.2 Hz), 6.09 – 6.06 (1H, m), 4.24 – 4.13 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 3.50 (3H, s), 2.37 – 2.23 (2H, m). Sulfonamide N-H is not observed. δ_C (101 MHz, DMSO-d_6) 167.9, 149.4, 141.5, 135.1, 132.9, 132.5, 132.1, 130.0, 129.5, 127.1, 123.3, 121.1, 120.7, 65.4, 63.9, 61.6, 26.8; ν_{max} (liquid film)/cm^{-1} 3170, 2969, 2854, 1665, 1572, 1447, 1369, 1330, 1159, 1113, 1091; *m/z* (ES) Found: $[MH]^+$ 389.1155, $C_{19}H_{21}N_2O_5S$ is $[MH]^+$ 389.1166.

3-Amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide 280

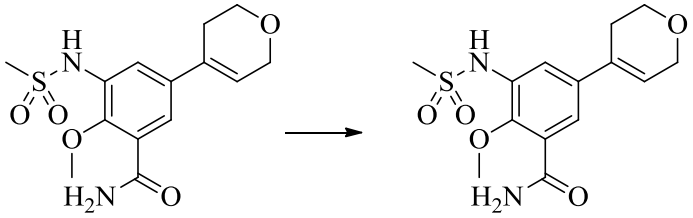


278 **280**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (471 mg, 2.244 mmol), 3-amino-5-bromo-2-methoxybenzamide **278** (500 mg, 2.04 mmol), 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (57 mg, 0.102 mmol) and tripotassium phosphate (1.299 g, 6.12 mmol) in 1,4-dioxane (10 mL) and water (2 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. Purification by flash chromatography (0 – 30 % methanol/TBME) provided 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (500 mg, 99 %) as a brown solid. The reaction was repeated on 3-amino-5-bromo-2-methoxybenzamide **278** (100 mg, 0.408 mmol) to give 3-amino-5-bromobenzamide **280** (98 mg, 98 %) as a brown solid. M.P. 160-162 °C; LCMS (Formic acid) 94 %, R_t= 0.52, $[MH]^+$= 249; δ_H (400 MHz, $CDCl_3$) 7.60 (1H,

s), 7.52 (1H, d, *J* = 2.3 Hz), 6.95 (1H, d, *J* = 2.3), 6.14 – 6.11 (1H, m), 5.71 (1H, s), 4.33 – 4.31 (2H, m), 3.95 – 3.88 (4H, m), 3.85 (3H, s), 2.54 – 2.49 (2H, m); δ_C (101 MHz, DMSO-d_6) 168.2, 143.7, 142.0, 135.9, 133.4, 128.9, 122.2, 113.5, 113.4, 65.4, 64.1, 60.4, 27.1; ν_{max} (liquid film)/cm^{-1} 3168, 2968, 2854, 1666, 1572, 1447, 1368, 1330, 1158, 113, 1091; *m/z* (ES) Found: $[MH]^+$ 249.1230, $C_{13}H_{17}N_2O_3$ is $[MH]^+$ 249.1234.

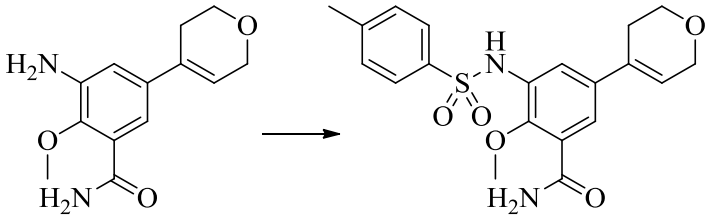
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(methylsulfonamido)benzamide 281



280 **281**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (60 mg, 0.242 mmol) and methanesulfonyl chloride (0.02 mL, 0.254 mmol) were combined in pyridine (1 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(methylsulfonamido)benzamide **281** (25 mg, 31 %) as a white solid. M.P. 144-146 °C; LCMS (Formic acid) 100 %, R_t= 0.60, $[MH]^+$= 327; δ_H (400 MHz, DMSO-d_6) 9.22 (1H, s), 7.73 (1H, s), 7.54 (1H, s), 7.48 (1H, d, *J* = 2.4 Hz), 7.37 (1H, d, *J* = 2.4 Hz), 6.24 – 6.13 (1H, m), 4.26 – 4.17 (2H, m), 3.82 (2H, t, *J* = 5.5 Hz), 3.79 (3H, s), 3.08 (3H, s), 2.45 – 2.37 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.9, 149.8, 135.5, 132.5, 131.5, 130.4, 123.7, 122.2, 122.2, 65.4, 64.0, 62.1, 41.0, 26.9; ν_{max} (liquid film)/cm^{-1} 3174, 2969, 2954, 1660, 1576, 1446, 1368, 1328, 1159; *m/z* (ES) Found: $[MH]^+$ 327.1002, $C_{14}H_{19}N_2O_5S$ is $[MH]^+$ 327.1009.

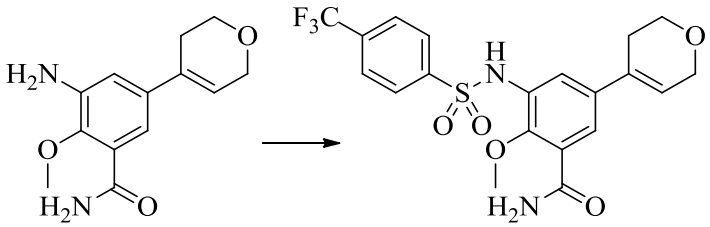
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(4-methylphenylsulfonamido)benzamide 282



280 **282**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (50 mg, 0.201) and 4-methylbenzene-1-sulfonyl chloride (43 mg, 0.226 mmol) were combined in pyridine (0.5 mL) and DCM (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(3-(trifluoromethyl)phenylsulfonamido)benzamide **282** (20 mg, 24 %) as a white solid. M.P. 165-167 °C; LCMS (Formic acid) 100 %, R_t= 0.89, $[MH]^+$= 403; δ_H (400 MHz, DMSO-d_6) 7.73 – 7.68 (2H, m), 7.66 (1H, s), 7.46 (1H, s), 7.39 – 7.34 (3H, m), 7.25 (1H, d, *J* = 2.3 Hz), 6.11 – 6.08 (1H, d, *J* = 1.3 Hz), 4.24 – 4.16 (2H, m), 3.80 (2H, t, *J* = 5.4 Hz), 3.50 (3H, s), 2.35 (3H, s), 2.34 – 2.27 (2H, m). Sulfonamide N-H not observed. δ_C (101 MHz, DMSO-d_6) 167.9, 149.1, 143.4, 138.3, 135.1, 132.5, 131.8, 130.1, 130.0, 127.2, 123.4, 121.1, 120.3, 65.4, 63.9, 61.7, 26.8, 21.4; ν_{max} (liquid film)/cm^{-1} 3167, 2966, 2857, 1671, 1571, 1447, 1368, 1329, 1163; *m/z* (ES) Found: $[MH]^+$ 403.1314, $C_{20}H_{23}N_2O_5S$ is $[MH]^+$ 403.1322.

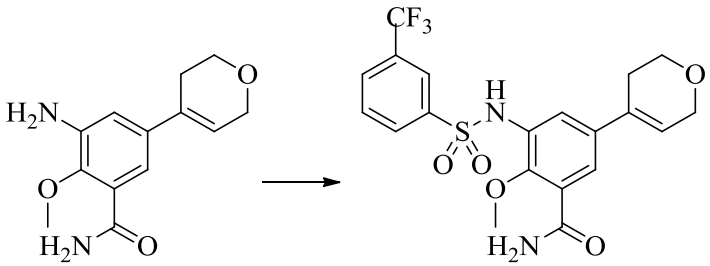
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(4-(trifluoromethyl)phenylsulfonamido) benzamide 283



280 **283**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (40 mg, 0.201)and 4-(trifluoromethyl)benzene-1-sulfonyl chloride (43 mg, 0.213 mmol) were combined in pyridine (0.5 mL) and DCM (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(3-(trifluoromethyl)phenylsulfonamido) benzamide (39 mg, 67 %) as a white solid. M.P. 158-160 °C; LCMS (Formic acid) 100 %, R_t= 0.99, $[MH]^+$= 457; δ_H (400 MHz, DMSO-d_6) 10.15 (1H, s), 8.05 – 7.95 (4H, m), 7.68 (1H, s), 7.49 (1H, s), 7.32 (1H, d, *J* = 2.3 Hz), 7.30 (1H, d, *J* = 2.3 Hz), 6.12 – 6.07 (1H, m), 4.24 – 4.15 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 3.49 (3H, s), 2.36 – 2.23 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.9, 150.0, 145.3, 135.1, 132.7 (q, *J* = 32.3 Hz), 131.2, 130.2, 128.1, 126.8, 125.2, 123.5, 122.5, 122.1, 121.8, 65.4, 63.9, 61.6, 26.7; δ_F (376 MHz, DMSO-d_6) -61.64 (s); ν_{max} (liquid film)/cm^{-1} 3149, 2971, 2855, 1681, 1579, 1319, 1155; *m/z* (ES) Found: $[MH]^+$ 457.1024, $C_{20}H_{20}F_3N_2O_5S$ is $[MH]^+$ 457.1040.

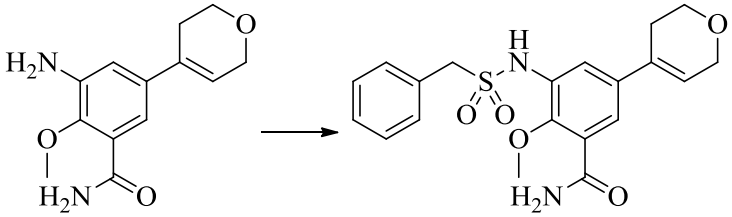
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(3-(trifluoromethyl)phenylsulfonamido) benzamide 284



280 **284**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (50 mg, 0.201) and 3-(trifluoromethyl)benzene-1-sulfonyl chloride (52 mg, 0.213 mmol) were combined in pyridine (0.5 mL) and DCM (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method C) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(3-(trifluoromethyl)phenylsulfonamido)benzamide **284** (62 mg, 67 %) as a white solid. M.P. 177-179 °C; LCMS (Formic acid) 99 %, R_t= 0.98, $[MH]^+$= 457; δ_H (400 MHz, $CDCl_3$) 10.11 (1H, s), 8.19 – 8.13 (1H, m), 8.13 – 8.03 (2H, m), 7.86 (1H, t, *J* = 7.9 Hz), 7.66 (1H, s), 7.51 (1H, s), 7.34 (1H, d, *J* = 2.4 Hz), 7.31 (1H, d, *J* = 2.4 Hz), 6.15 – 6.09 (1H, m), 4.23-4.19 (2H, m), 3.79 (2H, t, *J* = 5.4 Hz), 3.48 (3H, s), 2.33 - 2.28 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.8, 149.8, 142.0, 135.2, 132.2, 131.1, 130.5, 130.4, 130.3 (q, *J* = 32.7 Hz), 130.0, 123.9, 123.8, 123.8 (m), 123.7, 122.7, 121.8, 65.4, 63.9, 61.7, 26.7; δ_F (376 MHz, DMSO-d_6) -61.56 (s); ν_{max} (liquid film)/cm^{-1} 3179, 2969, 2855, 1663, 1572, 1446, 1368, 1327, 1160, 1090; *m/z* (ES) Found: $[MH]^+$ 457.1022, $C_{20}H_{20}F_3N_2O_5S$ is $[MH]^+$ 457.1040.

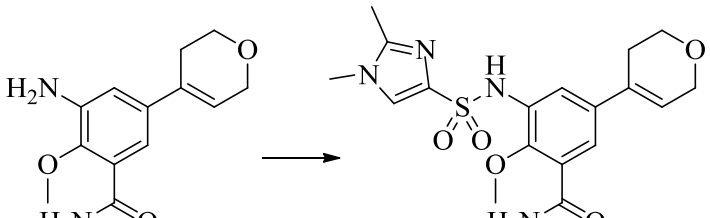
5-(3,6-Dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(phenylmethylsulfonamido)benzamide 285



280 **285**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (50 mg, 0.201)and phenylmethanesulfonyl chloride (42 mg, 0.222 mmol) were combined in pyridine (0.5 mL) and DCM (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxy-3-(phenylmethylsulfonamido)benzamide **285** (57 mg, 70 %) as a white solid. M.P. 140-142 °C; LCMS (Formic acid) 100 %, R_t= 0.84, $[MH]^+$= 403; δ_H (400 MHz, DMSO-d_6) 9.26 (1H, s), 7.73 (1H, s), 7.55 (1H, s), 7.43 – 7.34 (5H, m), 7.32 (1H, d, *J* = 1.6 Hz), 7.14 (1H, d, *J* = 2.3 Hz), 6.11 – 6.05 (1H, m), 4.55 (2H, s), 4.25 – 4.18 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 3.78 (3H, s), 2.36 – 2.29 (2H, m); δ_C (101 MHz, DMSO-d_6) 167.9, 149.2, 135.4, 132.4, 131.6, 131.5, 130.2, 130.1, 128.8, 128.6, 123.5, 121.6, 121.5, 65.4, 64.0, 62.0, 59.1, 26.8; ν_{max} (liquid film)/cm^{-1} 3167, 2967, 2851, 1670, 1571, 1447, 1375, 1332, 1157; *m/z* (ES) Found: $[MH]^+$ 403.1317, $C_{20}H_{23}N_2O_5S$ is $[MH]^+$ 403.1322.

5-(3,6-Dihydro-2*H*-pyran-4-yl)-3-(1,2-dimethyl-1*H*-imidazole-4-sulfonamido)-2-methoxybenzamide 286

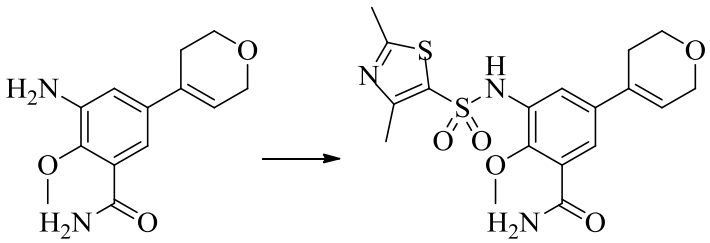


280 **286**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (50 mg,

0.201) and 1,2-dimethyl-1*H*-imidazole-4-sulfononyl chloride (44 mg, 0.226 mmol) were combined in pyridine (0.5 mL) and DCM (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated under a flow of nitrogen. The residue was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-3-(1,2-dimethyl-1*H*-imidazole-4-sulfonamido)-2-methoxybenzamide **286** (51 mg, 62 %) as a white solid. M.P. 180-182 °C; LCMS (Formic acid) 100 %, R_t= 0.62, $[MH]^+$= 407; δ_H (400 MHz, DMSO-d_6) 9.42 (1H, s), 7.74 (1H, s), 7.68 (1H, s), 7.63 (1H, d, *J* = 2.3 Hz), 7.49 (1H, s), 7.30 (1H, d, *J* = 2.3 Hz), 6.18 – 6.12 (1H, m), 4.26 – 4.19 (2H, m), 3.82 (2H, t, *J* = 5.4 Hz), 3.70 (3H, s), 3.57 (3H, s), 2.38 – 2.32 (2H, m), 2.28 (3H, s); δ_C (101 MHz, DMSO-d_6) 167.8, 148.8, 147.1, 137.1, 135.1, 132.6, 131.8, 129.9, 126.2, 123.3, 120.9, 120.5, 65.4, 64.0, 61.9, 33.3, 26.8, 12.9; ν_{max} (liquid film)/cm^{-1} 3186, 2970, 2829, 1668, 1581, 1447, 1336, 1159; *m/z* (ES) Found: $[MH]^+$ 407.1381, $C_{18}H_{23}N_4O_5S$ is $[MH]^+$ 407.1384.

5-(3,6-Dihydro-2*H*-pyran-4-yl)-3-(2,4-dimethylthiazole-5-sulfonamido)-2-methoxy benzamide 287

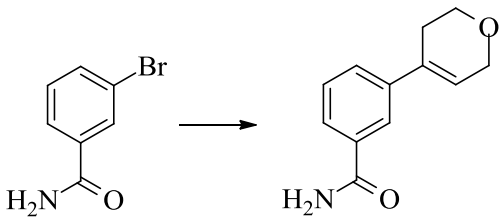


280 **287**

A mixture of 3-amino-5-(3,6-dihydro-2*H*-pyran-4-yl)-2-methoxybenzamide **280** (50 mg, 0.201) and 2,4-dimethylthiazole-5-sulfonyl chloride (47 mg, 0.222 mmol) were combined in pyridine (0.5 mL) and DCM (0.5 mL). The resulting mixture was stirred at room temperature for 3 h and then concentrated *in vacuo*. The residue was diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 5-(3,6-dihydro-2*H*-pyran-4-yl)-3-(2,4-dimethylthiazole-5-sulfonamido)-2-methoxy benzamide **287** (47 mg, 55 %) as a white solid.

M.P. 149-151 °C; LCMS (Formic acid) 100 %, R_t= 0.78, $[MH]^+$= 424; δ_H (400 MHz, DMSO-d_6) 10.25 (1H, s), 7.70 (1H, s), 7.52 (1H, s), 7.39 – 7.36 (2H, m), 6.19 – 6.10 (1H, m), 4.25 – 4.18 (2H, m), 3.81 (2H, t, *J* = 5.4 Hz), 3.58 (3H, s), 2.60 (3H, s), 2.40 (3H, s), 2.38 – 2.32 (2H, m); δ_C (101 MHz, DMSO-d_6) 168.8, 167.9, 155.4, 150.4, 135.1, 132.34, 130.4, 130.2, 123.6, 122.7, 122.57, 65.4, 63.9, 61.8, 26.8, 19.3, 16.3. One carbon is not observed. v_{max} (liquid film)/cm^{-1} 3167, 2970, 2851, 1662, 1578, 1447, 1369, 1336, 1163; *m/z* (ES) Found: $[MH]^+$ 424.0985, $C_{18}H_{22}N_3O_5S_2$ is $[MH]^+$ 424.0995.

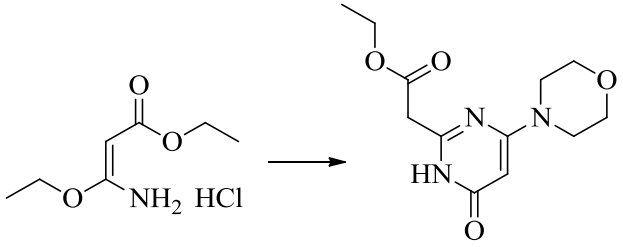
3-(3,6-Dihydro-2*H*-pyran-4-yl)benzamide 289



288 **289**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (55 mg, 0.262 mmol), 3-bromobenzamide **288** (50 mg, 0.25 mmol), tripotassium phosphate (170 mg, 0.8 mmol) and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (7 mg, 0.012 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)benzamide **289** (39 mg, 77 %) as a white solid. M.P. 130-132 °C; LCMS (Formic acid) 100 %, R_t= 0.67, $[MH]^+$= 204; δ_H (400 MHz, DMSO-d_6) 8.01 (1H, s), 7.96 – 7.92 (1H, m), 7.81 – 7.74 (1H, m), 7.63 – 7.54 (1H, m), 7.43 (1H, t, *J* = 7.7 Hz), 7.35 (1H, s), 6.38 – 6.30 (1H, m), 4.30 – 4.20 (2H, m), 3.84 (2H, t, *J* = 5.5 Hz), 2.51 – 2.46 (2H, m); δ_C (101 MHz, DMSO-d_6) 168.3, 140.0, 134.8, 133.2, 128.8, 127.5, 126.7, 124.0, 123.9, 65.5, 64.0, 26.9; v_{max} (liquid film)/cm^{-1} 3433, 3300, 3150, 1682, 1448, 1387, 1333, 1170, 1090; *m/z* (ES) Found: $[MH]^+$ 204.1014, $C_{12}H_{14}NO_2$ is $[MH]^+$ 204.1019.

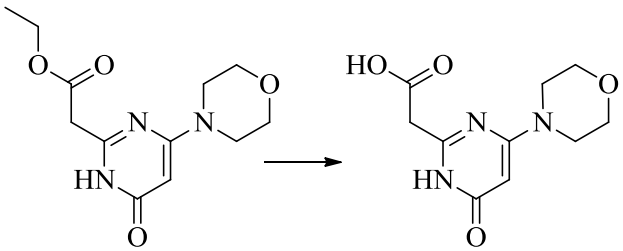
Ethyl 2-(4-morpholino-6-oxo-1,6-dihydropyrimidin-2-yl)acetate 294



293 **294**

Morpholine (0.369 mL, 4.22 mmol) was diluted with ethanol (9 mL) and heated to 95 °C. To this, ethyl 3-ethoxy-3-iminopropanoate hydrochloride **293** (2.5 g, 12.78 mmol) and DIPEA (2.343 mL and 13.42 mmol) was added and stirred for 30 h. The reaction was cooled to room temperature, filtered, washing the solid with ethanol (2 x 5 mL), diethyl ether (5 mL) to give ethyl 2-(4-morpholino-6-oxo-1,6-dihydropyrimidin-2-yl)acetate (260 mg, 7 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.56, $[MH]^+$= 268; δ_H (400 MHz, $CDCl_3$) 12.61 (1H, s), 5.31 (1H, s), 4.21 (2H, q, *J* = 7.1 Hz), 3.79 – 3.75 (4H, m), 3.55 (2H, s), 3.56 – 3.51 (4H, m), 1.25 (3H, t, *J* = 7.1 Hz).

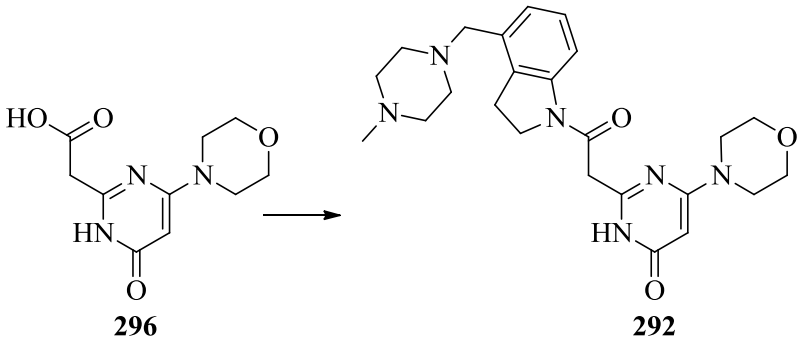
2-(4-Morpholino-6-oxo-1,6-dihydropyrimidin-2-yl)acetic acid 295



294 **295**

Ethyl 2-(4-morpholino-6-oxo-1,6-dihydropyrimidin-2-yl)acetate **294** (250mg, 0.935 mmol) was dissolved in THF (3 mL) and methanol (2 mL) and to this 1 M aqueous lithium hydroxide (2.5 mL, 2.5 mmol)was added and stirred for 3 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with water (10 mL), neutralised with 2M aqueous hydrochloric acid and filtered to give 5-bromo-2-methyl-3-(*N*-phenylsulfamoyl)benzoic acid **295** as a white solid (200 mg, 89 %). LCMS (Formic acid or ammonium carbonate) Elutes before solvent front; δ_H (400 MHz, DMSO-d_6) 11.58 (1H, s), 5.16 (1H, s), 3.63 (4H, m), 3.42 – 3.39 (4H, m), 3.25 (2H, s).

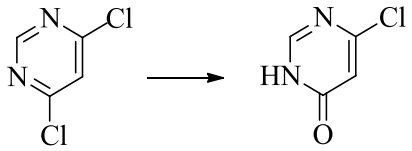
2-(2-(4-((4-Methylpiperazin-1-yl)methyl)indolin-1-yl)-2-oxoethyl)-6-morpholino pyrimidin-4(3*H*)-one 292



296 **292**

A mixture of 2-(4-morpholino-6-oxo-1,6-dihydropyrimidin-2-yl)acetic acid **296** (200 mg, 0.836 mmol) DIPEA (0.438 mL, 2.508 mmol) 50 % T_3P in ethyl acetate (0.493 mL, 1.672 mmol) and 4-((4-methylpiperazin-1-yl)methyl)indoline **324** (232 mg, 1.003 mmol) were combined in THF (10 mL). The resulting mixture was stirred at room temperature for 16 h and concentrated *in vacuo*. The residue was diluted with water (50 mL) and extracted with 5 % methanol in DCM (2 x 50 mL). The organic layer was washed with brine (30 mL), dried over sodium sulfate, passed through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (10 % methanol/DCM) provided 2-(2-(4-((4-methylpiperazin-1-yl)methyl)indolin-1-yl)-2-oxoethyl)-6-morpholinopyrimidin-4(3*H*)-one **292** (33 mg, 8 %) as an off-white solid. LCMS (Formic acid) 100 %, R_t= 0.42, $[MH]^+$= 453; δ_H (400 MHz, DMSO-d_6) 11.62 (1H, s), 7.94 (1H, d, *J* = 7.9 Hz), 7.13 (1H, t, *J* = 7.7 Hz), 6.95 (1H, d, *J* = 7.5 Hz), 5.21 (1H, s), 4.15 (2H, t, *J* = 8.4 Hz), 3.76 (2H, s), 3.64 – 3.58 (4H, m), 3.47 – 3.36 (6H, m), 2.46 – 2.26 (8H, m), 2.20 (3H, s).

6-Chloropyrimidin-4(3*H*)-one 297

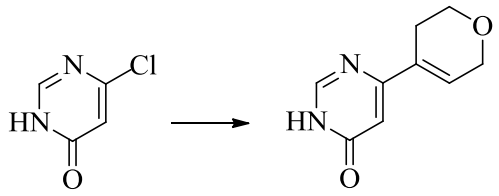


296 **297**

4,6-Dichloropyrimidine **296** (1.2 g, 8.05 mmol) was dissolved in 1,4-dioxane (5.75 mL), water (5.75 mL) and 37.5 % aqueous hydrochloric acid (6 mL) and stirred at 70 °C for 5 h. The reaction was cooled and concentrated *in vacuo* to give 6-chloropyrimidin-4(3*H*)-one **297**

(927 mg, 88 %) as a white solid. LCMS (Formic acid) 100 %, R_t= 0.38, $[MH]^+$= 131, 133; δ_H (400 MHz, DMSO-d_6) 12.94 (1H, s), 8.20 (1H, s), 6.51 (1H, s).

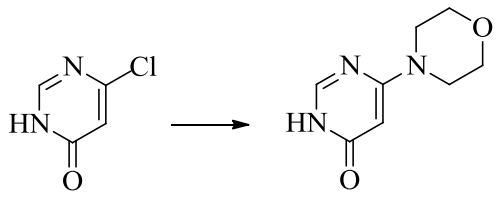
6-(3,6-Dihydro-2*H*-pyran-4-yl)pyrimidin-4(3*H*)-one 298



297 **298**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (84 mg, 0.402 mmol), 6-chloropyrimidin-4(3*H*)-one **297** (50 mg, 0.383 mmol), tripotassium phosphate (260 mg, 1.226 mmol) and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) in 1,4-dioxane (2 mL) and water (0.4 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (3 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method A) to give 6-(3,6-dihydro-2*H*-pyran-4-yl)pyrimidin-4(3*H*)-one **298** (31 mg, 45 %) as a white solid. M.P. 232 °C decomposition; LCMS (Ammonium carbonate) 100 %, R_t= 0.45, $[MH]^+$= 179; δ_H (400 MHz, DMSO-d_6) 8.11 (1H, s), 7.02 – 6.96 (1H, m), 6.20 (1H, s), 4.30 – 4.23 (2H, m), 3.78 (2H, t, *J* = 5.5 Hz), 2.37 – 2.31 (2H, m). Pyrimidone N-H is not observed. δ_C (101 MHz, DMSO-d_6) 162.6, 160.1, 149.3, 131.6, 130.9, 108.4, 65.6, 63.7, 24.9; ν_{max} (liquid film)/cm^{-1} 2963, 2929, 2864, 2825, 1634, 1605, 1428, 1240, 1184, 1125; *m/z* (ES) Found: $[MH]^+$ 179.0814, $C_9H_{11}N_2O_2$ is $[MH]^+$ 179.0815.

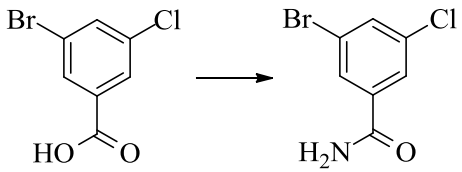
6-Morpholinopyrimidin-4(3*H*)-one 299



297 **299**

6-Chloropyrimidin-4(3*H*)-one **297** (50 mg, 0.383 mmol) was dissolved in 1,4-dioxane (1 mL), morpholine (0.066 mL, 0.766 mmol) and triethylamine (0.107 mL, 0.766 mmol) and stirred at 80 °C for 16 h. The reaction was cooled, diluted with water (10 mL) and filtered. The solid was dried in a vacuum oven for 3 h (1 mbar, 40 °C) to give 6-morpholinopyrimidin-4(3*H*)-one **299** (36 mg, 51 %) as a white solid. M.P. 245-247 °C; LCMS (Ammonium carbonate) 100 %, R_t= 0.41, $[MH]^+$= 182; δ_H (400 MHz, DMSO-d_6) 11.62 (1H, s), 7.90 (1H, s), 5.27 (1H, s), 3.66 – 3.59 (4H, m), 3.47 – 3.40 (4H, m); δ_C (101 MHz, DMSO-d_6) 162.7, 162.5, 149.2, 86.7, 66.1, 44.8; ν_{max} (liquid film)/cm^{-1} 2965, 2914, 2953, 1627, 1609, 1477, 1426, 1237, 1201, 1110; *m/z* (ES) Found: $[MH]^+$ 182.0918, $C_8H_{12}N_3O_2$ is $[MH]^+$ 182.0924.

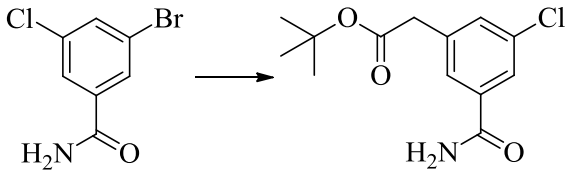
3-Bromo-5-chlorobenzamide 315



314 **315**

3-Bromo-5-chlorobenzoic acid **314** (5 g, 21.23 mmol) was dissolved in thionyl chloride (10 mL, 206 mmol) and stirred at 70 °C for 3 h. The thionyl chloride was concentrated *in vacuo*. The residue was diluted with THF (5 mL) and added dropwise to ammonium hydroxide (29.5 mL, 212 mmol, 28 %) at 0 °C. The THF was concentrated *in vacuo*. The mixture was filtered, drying the solid in a vacuum oven (1 mbar, 40 °C) for 3 h to give 3-bromo-5-chlorobenzamide **315** (4.429 g, 89 %) a white solid. LCMS (Formic) 100 %, R_t= 0.86, $[MH]^+$= 182; δ_H (400 MHz, DMSO-d_6) 8.16 (1H, s), 8.04 – 8.00 (1H, m), 7.95 – 7.89 (2H, m), 7.65 (1H, s).

***Tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate 316**



315 **316**

Method A

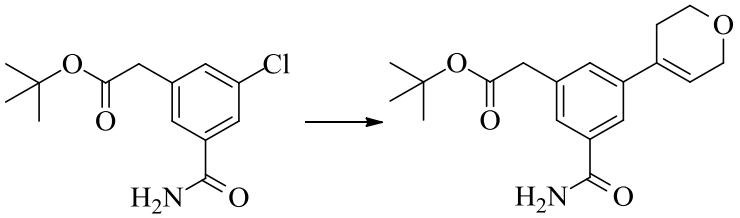
In a dried 2-necked flask, zinc (840 mg, 12.85 mmol) was suspended in diethyl ether (6 mL) under nitrogen. To this, TMS-Cl (1.64 mL, 12.83 mmol) was added and stirred for 15 min. The reaction mixture was heated to 40 °C and at reflux, *tert*-butyl 2-bromoacetate (1.9 mL, 12.87 mmol) was added and further stirred for 1 h. The reaction was cooled to room temperature and to this QPhos (91 mg, 0.128 mmol), $Pd_2(dba)_3$ (70 mg, 0.077 mmol) and 3-bromo-5-chlorobenzamide **315** (600 mg, 2.56 mmol) in THF (5.00 mL) were added and stirred for 16 h. The reaction mixture was passed through a plug of Celite eluting with methanol and the solvent concentrated *in vacuo*. The residue was dissolved in water (50 mL) and extracted with ethyl acetate (2 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and the solvent concentrated *in vacuo*. Purification by flash chromatography (25 – 100 % ethyl acetate/cyclohexane) provided *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (443 mg, 73 %) as a white solid. LCMS (Ammonium carbonate) 88 %, R_t= 0.87, $[MH]^-$= 287, 289; δ_H (400 MHz, DMSO-d_6) 8.04 (1H, s), 7.83 – 7.80 (1H, m), 7.74 – 7.71 (1H, m), 7.51 – 7.45 (2H, m), 3.66 (2H, s), 1.41 (9H, s).

Method B.

A mixture of QPhos (76 mg, 0.107 mmol), $Pd_2(dba)_3$ (48 mg, 0.053 mmol) and 3-bromo-5-chlorobenzamide **315** (500 mg, 2.132 mmol) in THF (4 mL) were combined in a microwave vial degassed five times. To this, (2-(*tert*-butoxy)-2-oxoethyl)zinc(II) chloride **317** (9 mL, 4.5 mmol, 0.5 M in diethyl ether) was added and the reaction mixture was degassed five times, placed under nitrogen and heated in a Biotage microwave reactorat 60 °C for 1 h. The reaction mixture was passed through a plug of Celite eluting with methanol and the solvent concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 15 - 70 % acetonitrile in water with a formic acid modifier on a 60 g C_{18} column to give *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316**

(494 mg, 86 %) as a white solid. The reaction was repeated on 3-bromo-5-chlorobenzamide **315** (443 mg, 1.886 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (201 mg, 39 %) as a white solid. The reaction was repeated on 3-bromo-5-chlorobenzamide **315** (280 mg, 1.194 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (186 mg, 57 %) as a white solid. The reaction was repeated on 3-bromo-5-chlorobenzamide **315** (300 mg, 1.279 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (228 mg, 66 %) as a white solid.

Tert-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate 319

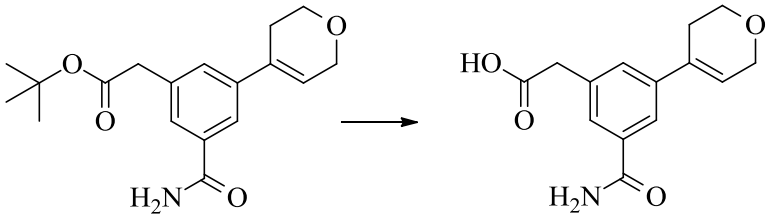


316 **319**

A mixture of 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (386 mg, 1.835 mmol), *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (450 mg, 1.668 mmol), tripotassium phosphate (1.062 g, 5.01 mmol) and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (94 mg, 0.167 mmol) in 1,4-dioxane (8 mL) and water (2 mL) was heated and stirred in a Biotage microwave reactorat 100 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The residue was dissolved in water (50 mL) and then extracted into ethyl acetate (2 x 50 mL). The organic layer was washed with brine (50 mL), passed through a hydrophobic frit and the solvent concentrated *in vacuo*. Purification by flash chromatography (40 – 100 % ethyl acetate/cyclohexane) provided *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (406 mg, 77 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (228 mg, 0.847 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (201 mg, 74 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (229 mg, 0.851 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (210 mg, 78 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (210 mg, 0.78 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-

yl)phenyl)acetate **319** (173 mg, 74 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-chlorophenyl)acetate **316** (161 mg, 0.598 mmol) to give *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (171 mg, 74 %) as a white solid. LCMS (Ammonium carbonate) 91 %, R_t= 0.94, $[MH\text{-}t\text{Bu}]^+$= 262; δ_H (400 MHz, $CDCl_3$) 7.79 – 7.77 (1H, m), 7.61 – 7.58 (1H, m), 7.50 – 7.47 (1H, m), 6.24 – 6.21 (1H, m), 4.38 – 4.33 (2H, m), 3.96 (2H, t, *J* = 5.5 Hz), 3.60 (2H, s), 2.60 – 2.53 (2H, m), 1.47 (9H, s). Amide NH_2 not observed.

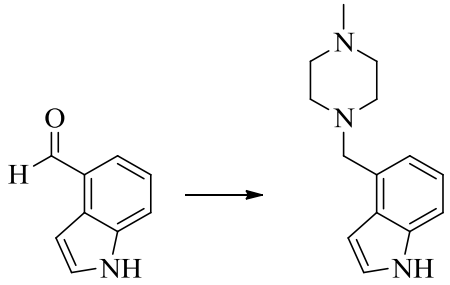
2-(3-Carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid 320



319 **320**

Tert-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (380 mg, 1.197 mmol) was dissolved in DCM (1 mL) and to this, hydrochloric acid (6 mL, 24 mmol, 4 M in 1,4-dioxane) was added and stirred for 6 h. The reaction mixture was diluted with water (25 mL) and extracted with ethyl acetate (3 x 25 mL). The organic layer was washed with brine (25 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (228 mg, 72 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (174 mg, 0.548 mmol) to give 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (118 mg, 82 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (210 mg, 0.662 mmol) to give 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (142 mg, 73 %) as a white solid. The reaction was repeated on *tert*-butyl 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetate **319** (173 mg, 0.545 mmol) to give 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (120 mg, 84 %) as a white solid. LCMS (Ammonium carbonate) 84 %, R_t= 0.43, $[MH]^+$= 279; δ_H (400 MHz, DMSO-d_6) 12.17 (1H, s), 7.99 (1H, s), 7.84 – 7.81 (1H, m), 7.69 – 7.65 (1H, m), 7.51 – 7.47 (1H, m), 7.32 (1H, s), 6.35 – 6.30 (1H, m), 4.26 – 4.23 (2H, m), 3.84 (2H, t, *J* = 5.4 Hz), 3.64 (2H, s), 2.49 – 2.44 (2H, m).

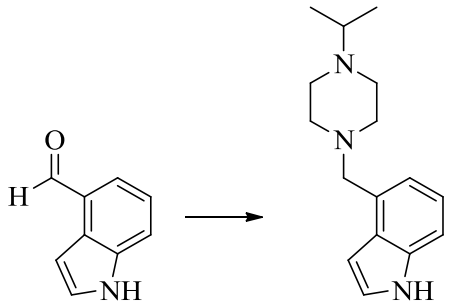
4-((4-Methylpiperazin-1-yl)methyl)-1*H*-indole 321



321

A mixture of 1*H*-indole-4-carbaldehyde (5 g, 34.4 mmol) and 1-methylpiperazine (4.14 g, 41.3 mmol) **321** was dissolved in methanol (100 mL) at 0 °C and to this, sodium cyanoborohydride (2.165 g, 34.4 mmol) was added and stirred for 16 h. The reaction mixture was concentrated *in vacuo*. The residue was basified to pH 9 with saturated sodium hydrogen carbonate (100 mL) and extracted with 10 % methanol in DCM (2 x 300 mL). The organic layer was washed with brine (200 mL), dried through a hydrophobic frit and concentrated *in vacuo*. Purification by flash chromatography (5 % methanol in DCM) provided 4-((4-methylpiperazin-1-yl)methyl)-1*H*-indole **321** (2.8 g, 35 %) as brown solid. LCMS (Ammonium carbonate) 85 %, R_t= 0.81, $[MH]^+$= 258; δ_H (400 MHz, $CDCl_3$) 11.00 (1H, s), 7.28 – 7.24 (2H, m), 7.05 – 7.00 (1H, m), 6.92 – 7.87 (1H, m), 6.60 – 6.54 (1H, m), 3.65 (2H, s), 2.46 – 2.24 (8H, m), 2.19 (3H, s).

4-((4-Isopropylpiperazin-1-yl)methyl)-1*H*-indole 322

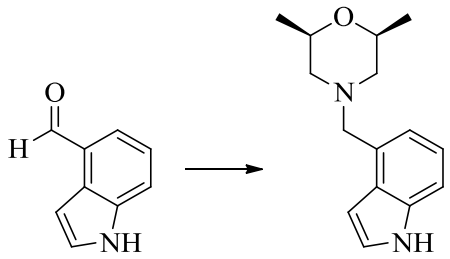


322

A mixture of 1*H*-indole-4-carbaldehyde (500 mg, 3.44 mmol) and isopropylpiperazine (442 mg, 3.44 mmol) was dissolved in THF (10 mL) at 0 °C and to this, sodium triacetoxyborohydride (1.46 g, 6.8 mmol) was added and stirred for 16 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with water (10 mL), basified to pH 9 with sodium hydroxide (20 mL) and extracted with ethyl acetate (2 x 20 mL). The

organic layer was washed with brine (20 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give (2*R*,6*S*)-4-((1*H*-indol-4-yl)methyl)-2,6-dimethylmorpholine **322** (790 mg, 89 %) as brown solid. LCMS (Ammonium carbonate) 90 %, R_t= 0.89, $[MH]^+$= 258; δ_H (400 MHz, $CDCl_3$) 8.19 (1H, s), 7.35 – 7.30 (1H, m), 7.25 – 7.20 (1H, m), 7.19 – 7.14 (1H, m), 7.14 – 7.10 (1H, m), 6.78 – 6.73 (1H, m), 3.83 (2H, s), 2.68 – 2.63 (1H, m), 2.62 – 2.46 (8H, m), 1.06 (6H, d, *J* = 6.5 Hz).

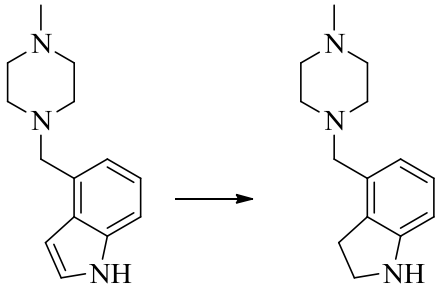
(2*R*,6*S*)-4-((1*H*-Indol-4-yl)methyl)-2,6-dimethylmorpholine 323



323

A mixture of 1*H*-indole-4-carbaldehyde (500 mg, 3.44 mmol) and (2*R*,6*S*)-2,6-dimethylmorpholine (397 mg, 3.44 mmol) was dissolved in THF (10 mL) at 0 °C and to this, sodium triacetoxyborohydride (1.46 g, 6.8 mmol) was added and stirred for 16 h. The reaction mixture was concentrated *in vacuo*. The residue was diluted with water (10 mL), basified to pH 9 with sodium hydroxide (20 mL) and extracted with ethyl acetate (2 x 20 mL). The organic layer was washed with brine (20 mL), dried through a hydrophobic frit and concentrated *in vacuo* to give (2*R*,6*S*)-4-((1*H*-indol-4-yl)methyl)-2,6-dimethylmorpholine **323** (650 mg, 77 %) as brown solid. LCMS (Formic acid) 97 %, R_t= 0.43, $[MH]^+$= 245; δ_H (400 MHz, $CDCl_3$) 8.20 (1H, s), 7.37 – 7.33 (1H, m), 7.24 (1H, dd, *J* = 3.1, 2.5 Hz), 7.20 – 7.16 (1H, m), 7.11 (1H, dd, *J* = 7.1, 0.8 Hz), 6.78 – 6.75 (1H, m), 3.79 (2H, s), 3.78 – 3.67 (2H, m), 2.83 – 2.75 (2H, m), 1.84 (2H, dd, *J* = 11.5, 10.2 Hz), 1.15 (6H, d, *J* = 6.3 Hz).

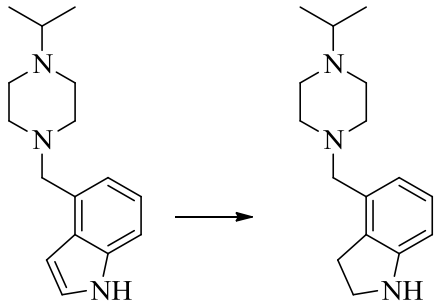
4-((4-Methylpiperazin-1-yl)methyl)indoline 324



321 **324**

4-((4-Isopropylpiperazin-1-yl)methyl)-1*H*-indole **321** (1.8 g, 7.85 mmol) was dissolved in acetic acid (35 mL) and to this, sodium cyanoborohydride (493 mg, 7.85 mmol) was added and stirred for 2 h. The reaction mixture was basified to pH 14 with ammonium hydroxide (20 mL, 28 %) and extracted with DCM (2 x 250 mL). The organic layer was washed with brine (2 x 100 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 10 – 75 % acetonitrile in water with an ammonium carbonate modifier on a 80 g C_{18} column to give 4-((4-isopropylpiperazin-1-yl)methyl)indoline **324** (1.08 mg, 59 %) as an off-white solid. LCMS (Ammonium carbonate) 90 %, R_t= 0.76, $[MH]^+$= 232; δ_H (400 MHz, $CDCl_3$) 7.23 (1H, s), 6.95 (1H, t, *J* = 7.6 Hz), 6.68 – 6.63 (1H, m), 6.59 – 6.52 (1H, m), 3.54 (2H, t, *J* = 8.4 Hz), 3.41 (2H, s), 3.03 (2H, t, *J* = 8.4 Hz), 2.61 – 2.16 (8H, m), 2.13 (3H, s).

4-((4-Isopropylpiperazin-1-yl)methyl)indoline 325

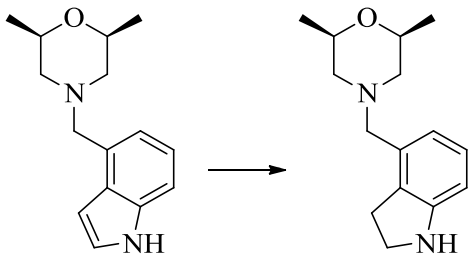


322 **325**

4-((4-Isopropylpiperazin-1-yl)methyl)-1*H*-indole **322** (200 mg, 0.777 mmol) was dissolved in acetic acid (5 mL) and to this, sodium cyanoborohydride (98 mg, 1.554 mmol) was added and stirred for 16 h. The reaction mixture was basified to pH 14 with aqueous sodium

hydroxide (20 mL, 2 M) and extracted with ethyl acetate (2 x 3 mL). The organic layer was washed with brine (2 x 10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 10 – 65 % acetonitrile in water with an ammonium carbonate modifier on a 30 g C_{18} column to give 4-((4-isopropylpiperazin-1-yl)methyl)indoline **325** (111 mg, 55 %) as a white solid. LCMS (Ammonium carbonate) 100 %, R_t= 0.85, $[MH]^+$= 260; δ_H (400 MHz, $CDCl_3$) 6.99 (1H, t, *J* = 7.6 Hz), 6.73 – 6.69 (1H, m), 6.60 – 6.55 (1H, m), 3.57 (2H, t, *J* = 8.4 Hz), 3.45 (2H, s), 3.06 (2H, t, *J* = 8.4 Hz), 2.72 – 2.64 (1H, m), 2.62 – 2.43 (8H, m), 1.08 (6H, d, *J* = 6.5 Hz). Aniline N-H not observed.

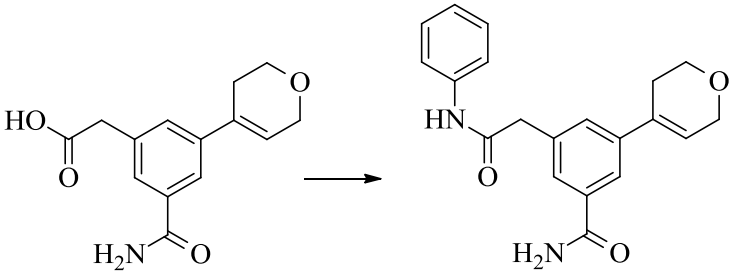
(2*S*,6*R*)-4-(Indolin-4-ylmethyl)-2,6-dimethylmorpholine 326



323 **326**

4-((4-Isopropylpiperazin-1-yl)methyl)-1*H*-indole **323** (200 mg, 0.819 mmol) was dissolved in acetic acid (5 mL) and to this, sodium cyanoborohydride (103 mg, 1.667 mmol) was added and stirred for 16 h. The reaction mixture was basified to pH 14 with aqueous sodium hydroxide (20 mL, 2 M) and extracted with ethyl acetate (2 x 3 mL). The organic layer was washed with brine (2 x 10 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The residue was dissolved in acetonitrile and was purified by reverse phase chromatography, eluting with 10 – 65 % acetonitrile in water with an ammonium carbonate modifier on a 30 g C_{18} column to give 4-((4-isopropylpiperazin-1-yl)methyl)indoline **326** (149 mg, 73 %) as a white solid. LCMS (Ammonium carbonate) 100 %, R_t= 0.94, $[MH]^+$= 247; δ_H (400 MHz, $CDCl_3$) 7.04 – 6.98 (1H, m), 6.75 – 6.67 (1H, m), 6.62 – 6.57 (1H, m), 3.77 – 3.62 (2H, m), 3.58 (2H, t, *J* = 8.4 Hz), 3.42 (2H, s), 3.08 (2H, t, *J* = 8.4 Hz), 2.80 – 2.65 (2H, m), 1.89 – 1.69 (2H, m), 1.16 (6H, d, *J* = 6.3 Hz). Aniline N-H not observe
d.

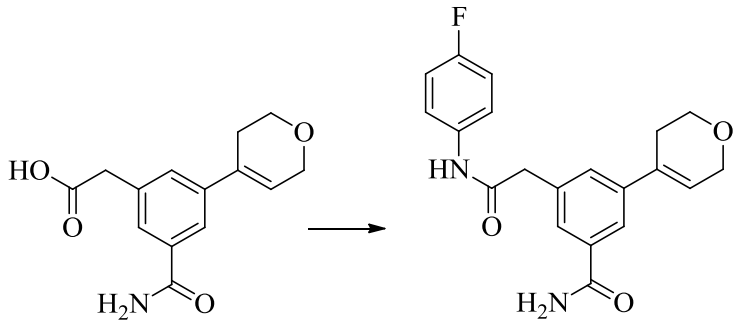
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-oxo-2-(phenylamino)ethyl)benzamide 302



320 **302**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (53 mg, 0.203 mmol), DIPEA (0.058 mL, 0.335 mmol), 50 % T_3P in ethyl acetate (0.133 mL, 0.223 mmol) and aniline (0.011 mL, 0.123 mmol) were combined in THF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h. To this further DIPEA (0.058 mL, 0.335 mmol) 50 % T_3P in ethyl acetate (0.133 mL, 0.223 mmol) was added. The resulting mixture was stirred at room temperature for 1 h, then diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with aqueous hydrochloric acid (5 mL, 2 M), brine (5 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-oxo-2-(phenylamino)ethyl)benzamide **302** (10 mg, 14 %) as a white solid. M.P. 160-162 °C; LCMS (Ammonium carbonate) 96 %, R_t= 0.82, $[MH]^+$= 337; δ_H (400 MHz, DMSO-d_6) 10.15 (1H, s), 7.99 (1H, s), 7.84 – 7.81 (1H, m), 7.76 – 7.73 (1H, m), 7.63 – 7.53 (3H, m), 7.34 – 7.25 (3H, m), 7.08 – 7.00 (1H, m), 6.35 – 6.30 (1H, m), 4.28 – 4.23 (2H, m), 3.85 (2H, t, *J* = 5.5 Hz), 3.71 (2H, s), 2.50 – 2.46 (2H, m); δ_C (101 MHz, DMSO-d_6) 169.2, 168.2, 157.1, 140.1, 139.6, 136.6, 134.9, 133.3, 129.1, 128.5, 127.8, 124.0, 122.2, 119.6, 65.5, 64.0, 43.6, 27.0; ν_{max} (liquid film)/cm^{-1} 3342, 3188, 3136, 3085, 1655, 1599, 1547, 1443, 1412, 1353; *m/z* (ES) Found: $[MH]^+$ 337.1544, $C_{20}H_{21}N_2O_3$ is $[MH]^+$ 337.1547.

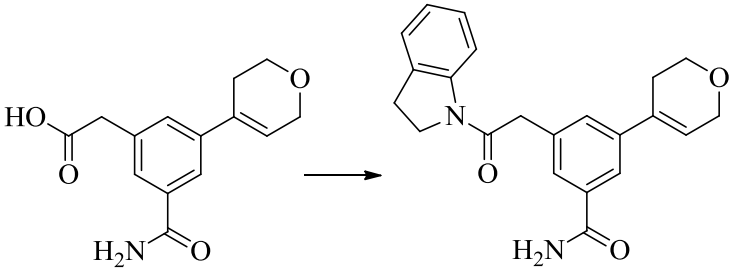
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-((4-fluorophenyl)amino)-2-oxoethyl)benzamide 303



320 **303**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (35 mg, 0.134 mmol), DIPEA (0.07 mL, 0.402 mmol), 50 % T_3P in ethyl acetate (0.159 mL, 0.268 mmol) and 4-fluoroaniline (0.014 mL, 0.153 mmol) were combined in THF (0.75 mL). The resulting mixture was stirred at room temperature for 16 h. To this further DIPEA (0.07 mL, 0.402 mmol)50 % T_3P in ethyl acetate (0.159 mL, 0.268 mmol) was added. The resulting mixture was stirred at 60 °C for 16 h, cooled, then diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with aqueous hydrochloric acid (5 mL, 2 M), brine (5 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-((4-fluorophenyl)amino)-2-oxoethyl)benzamide **303** (20 mg, 42 %) as a white solid. M.P. 154-156 °C; LCMS (Ammonium carbonate) 98 %, R_t= 0.84, $[MH]^+$= 355; δ_H (400 MHz, DMSO-d_6) 10.20 (1H, s), 7.99 (1H, s), 7.86 – 7.80 (1H, m), 7.76 – 7.72 (1H, m), 7.65 – 7.58 (2H, m), 7.58 – 7.54 (1H, m), 7.30 (1H, s), 7.20 – 7.09 (2H, m), 6.36 – 6.30 (1H, m), 4.29 – 4.22 (2H, m), 3.85 (2H, t, *J* = 5.4 Hz), 3.70 (2H, s, *J* = 7.6 Hz), 2.50 – 2.45 (2H, m); δ_C (101 MHz, DMSO-d_6) 169.1, 168.2, 158.4 (d, $^1J_{C\text{-}F}$ = 239.9 Hz), 140.1, 136.5, 135.9 (d, $^4J_{C\text{-}F}$ = 2.5 Hz), 134.9, 133.3, 128.5, 127.8, 124.0, 122.2, 121.3 (d, $^3J_{C\text{-}F}$ = 7.8 Hz), 115.7 (d, $^2J_{C\text{-}F}$ = 22.2 Hz), 65.5, 64.0, 43.5, 27.0; δ_F (376 MHz, DMSO-d_6) -119.33 – -119.42 (m); ν_{max} (liquid film)/cm^{-1} 3339, 3179, 3082, 1656, 1656, 1556, 1508, 1407, 1359, 1226, 1132; *m/z* (ES) Found: $[MH]^+$ 355.1441, $C_{20}H_{20}FN_2O_3$ is $[MH]^+$ 355.1453.

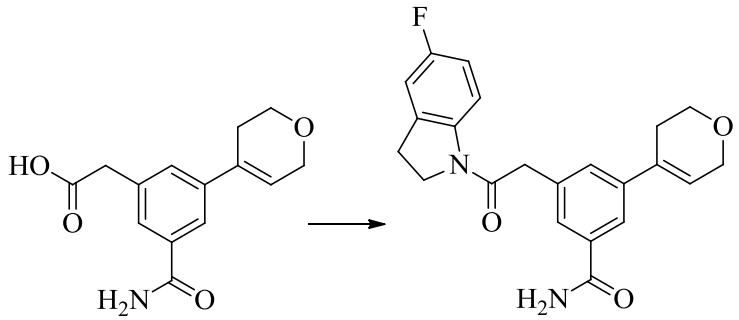
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-(indolin-1-yl)-2-oxoethyl)benzamide 304



320 **304**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (36 mg, 0.138 mmol), DIPEA (0.072 mL, 0.413 mmol), 50 % T_3P in ethyl acetate (0.164 mL, 0.276 mmol) and indoline (0.017 mL, 0.152 mmol) were combined in THF (0.5 mL). The resulting mixture was stirred at room temperature for overnight, diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with aqueous hydrochloric acid (5 mL, 2 M), brine (5 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-(indolin-1-yl)-2-oxoethyl)benzamide **304** (14 mg, 49 %) as a white solid. M.P. 169-171 °C; LCMS (Ammonium carbonate) 97 %, R_t= 0.90, $[MH]^+$= 363; δ_H (400 MHz, DMSO-d_6) 8.09 – 8.02 (1H, m), 7.98 (1H, s), 7.86 – 7.82 (1H, m), 7.72 – 7.68 (1H, m), 7.54 – 7.49 (1H, m), 7.30 (1H, s), 7.27 – 7.22 (1H, m), 7.18 – 7.11 (1H, m), 7.02 – 6.96 (1H, m), 6.36 – 6.30 (1H, m), 4.27 – 4.23 (2H, m), 4.20 (2H, t, *J* = 8.6 Hz), 3.91 (2H, s), 3.85 (2H, t, *J* = 5.5 Hz), 3.21 – 3.14 (2H, m), 2.49 – 2.46 (2H, m); δ_C (126 MHz, DMSO-d_6) 169.3, 168.3, 143.4, 140.0, 135.9, 134.8, 133.3, 132.2, 129.0, 128.3, 127.4, 125.2, 124.0, 123.7, 122.1, 116.4, 65.5, 64.0, 48.1, 42.3, 27.9, 27.0; ν_{max} (liquid film)/cm^{-1} 3337, 3180, 3082, 1656, 1616, 1555, 1508, 1407, 1358, 1226, 1131; *m/z* (ES) Found: $[MH]^+$ 363.1693, $C_{22}H_{23}N_2O_3$ is $[MH]^+$ 363.1703.

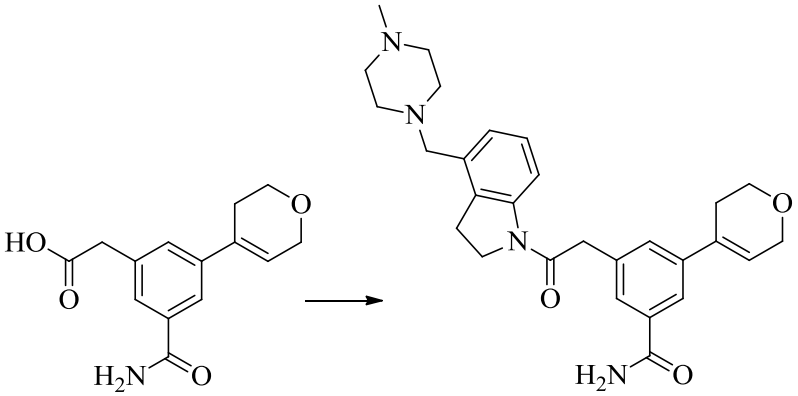
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-(5-fluoroindolin-1-yl)-2-oxoethyl)benzamide 305



320 **305**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (40 mg, 0.153 mmol), DIPEA (0.08 mL, 0.459 mmol), 50 % T_3P in ethyl acetate (0.182 mL, 0.306 mmol) and 5-fluoroindoline (23 mg, 0.168 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h, diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed brine (10 mL), aqueous lithium chloride (10 mL, 5 %), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-(5-fluoroindolin-1-yl)-2-oxoethyl) benzamide **305** (21 mg, 36 %) as a cream solid. M.P. 144-146 °C; LCMS (Formic) 100 %, R_t= 0.93, $[MH]^+$= 381; δ_H (400 MHz, DMSO-d_6) 8.04 (1H, dd, *J* = 8.8, 5.1 Hz), 8.00 (1H, s), 7.85 – 7.82 (1H, m), 7.72 – 7.67 (1H, m), 7.53 – 7.49 (1H, m), 7.32 (1H, s), 7.11 (1H, dd, *J* = 8.6, 2.5 Hz), 6.96 (1H, td, *J* = 9.2, 2.8 Hz), 6.35 – 6.30 (1H, m), 4.27 – 4.20 (4H, m), 3.90 (2H, s), 3.84 (2H, t, *J* = 5.4 Hz), 3.19 (2H, t, *J* = 8.4 Hz), 2.50 – 2.46 (2H, m); δ_C (101 MHz, $CDCl_3$) 169.1, 168.3, 164.2 (d, *J* = 236.8 Hz), 140.0, 135.8 (m), 134.8, 133.3, 129.0, 128.3, 124.0, 122.2, 117.1, 113.5 (d, *J* = 18.5 Hz), 113.3 (d, *J* = 0.5 Hz), 65.5, 64.0, 48.5, 42.0, 27.9, 27.0. Two carbons are not observed. δ_F (376 MHz, DMSO-d_6) -119.81 – -119.90 (m); ν_{max} (liquid film)/cm^{-1} 3339, 3179, 3082, 1656, 1656, 1556, 1508, 1407, 1359, 1226, 1132; *m/z* (ES) Found: $[MH]^+$ 381.1596, $C_{22}H_{22}FN_2O_3$ is $[MH]^+$ 381.1609.

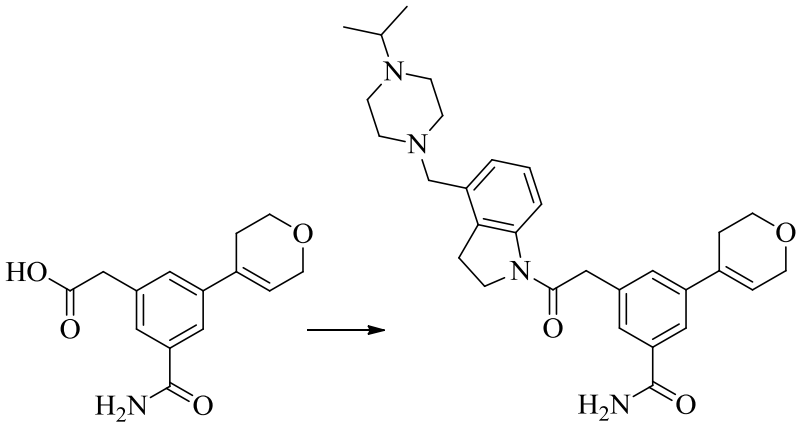
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-(4-((4-methylpiperazin-1-yl)methyl)indolin-1-yl)-2-oxoethyl)benzamide 306



320 **306**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (35 mg, 0.134 mmol), DIPEA (0.07 mL, 0.402 mmol), 50 % T_3P in ethyl acetate (0.159 mL, 0.268 mmol) and 4-((4-methylpiperazin-1-yl)methyl)indoline **324** (35 mg, 0.151 mmol) were combined in THF (0.75 mL). The resulting mixture was stirred at room temperature for 16 h. To this further DIPEA (0.07 mL, 0.402 mmol)50 % T_3P in ethyl acetate (0.159 mL, 0.268 mmol) was added. The resulting mixture was at room temperature for 1 h, diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed with brine (5 mL), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method B) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-(4-((4-methylpiperazin-1-yl)methyl)indolin-1-yl)-2-oxoethyl) benzamide **306** (25 mg, 39 %) as a white solid. M.P. 191-193 °C; LCMS (Ammonium carbonate) 100 %, R_t= 0.83, $[MH]^+$= 475;δ_H (400 MHz, DMSO-d_6) 8.03 – 7.92 (2H, m), 7.85 – 7.82 (1H, m), 7.72 – 7.67 (1H, m), 7.53 – 7.49 (1H, m), 7.29 (1H, s), 7.14 – 7.07 (1H, m), 6.96 – 6.90 (1H, m), 6.34 – 6.31 (1H, m), 4.27 – 4.24 (2H, m), 4.21 (2H, t, *J* = 8.3 Hz), 3.90 (2H, s), 3.85 (2H, t, *J* = 5.5 Hz), 3.40 (2H, s), 3.18 (2H, t, *J* = 8.5 Hz), 2.49 – 2.46 (2H, m), 2.42 – 2.25 (8H, m), 2.15 (3H, s); δ_C (101 MHz, DMSO-d_6) 169.1, 168.3, 143.4, 140.0, 135.9, 135.0, 134.8, 133.3, 131.6, 129.0, 128.3, 127.3, 124.5, 124.0, 122.2, 115.1, 65.5, 64.0, 60.1, 55.2, 53.1, 46.2, 44.5, 42.3, 27.0, 26.7; ν_{max} (liquid film)/cm^{-1} 3342, 3181, 3084, 2923, 1655, 1617, 1557, 1509, 1407, 1359, 1234, 1132; *m/z* (ES) Found: $[MH]^+$ 475.2696, $C_{28}H_{35}N_4O_3$ is $[MH]^+$ 475.2704.

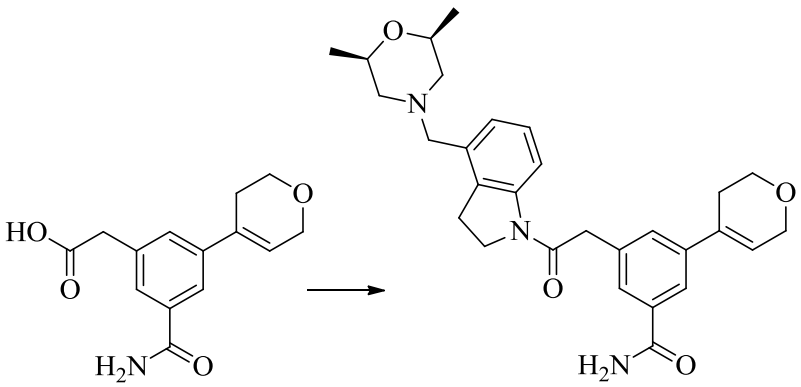
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-(4-((4-isopropylpiperazin-1-yl)methyl)indolin-1-yl)-2-oxoethyl)benzamide 307



320 **307**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (45 mg, 0.172 mmol), DIPEA (0.09 mL, 0.517 mmol), 50 % T_3P in ethyl acetate (0.205 mL, 0.344 mmol) and 4-((4-isopropylpiperazin-1-yl)methyl)indoline **325** (50 mg, 0.193 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h, diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed brine (10 mL), aqueous lithium chloride (10 mL, 5 %), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-(4-((4-isopropylpiperazin-1-yl)methyl)indolin-1-yl)-2-oxoethyl)benzamide **307** (21 mg, 24 %) as a white solid. M.P. 161-163 °C; LCMS (Formic) 100 %, R_t= 0.53, $[MH]^+$= 503; δ_H (400 MHz, DMSO-d_6) 8.05 – 7.92 (2H, m), 7.86 – 7.82 (1H, m), 7.73 – 7.67 (1H, m), 7.54 – 7.49 (1H, m), 7.31 (1H, s), 7.10 (1H, t, *J* = 7.8 Hz), 6.93 (1H, d, *J* = 7.5 Hz), 6.35 – 6.30 (1H, m), 4.29 – 4.24 (2H, m), 4.21 (2H, t, *J* = 8.4 Hz), 3.90 (2H, s), 3.84 (2H, t, *J* = 5.4 Hz), 3.38 (2H, s), 3.17 (2H, t, *J* = 8.3 Hz), 2.63 – 2.54 (1H, m), 2.49 – 2.45 (2H, m), 2.45 – 2.30 (8H, m), 0.95 (6H, d, *J* = 6.5 Hz); δ_C (101 MHz, DMSO-d_6) 169.2, 168.3, 143.5, 140.0, 135.9, 135.1, 134.8, 133.3, 131.6, 129.0, 128.3, 127.3, 124.4, 124.0, 122.2, 115.1, 65.5, 64.0, 60.2, 54.0, 53.6, 48.5, 48.3, 42.3, 27.0, 26.7, 18.7; ν_{max} (liquid film)/cm^{-1} 3339, 3179, 3082, 1656, 1656, 1556, 1508, 1407, 1359, 1226, 1132; *m/z* (ES) Found: $[MH]^+$ 503.3009, $C_{30}H_{39}N_4O_3$ is $[MH]^+$ 503.3017.

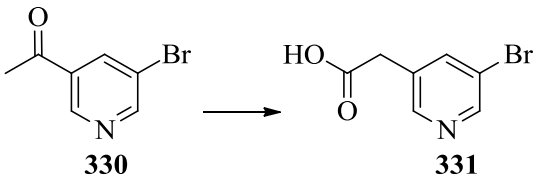
3-(3,6-Dihydro-2*H*-pyran-4-yl)-5-(2-(4-(((2*S*,6*R*)-2,6-dimethylmorpholino)methyl) indolin-1-yl)-2-oxoethyl)benzamide 308



320 **308**

A mixture of 2-(3-carbamoyl-5-(3,6-dihydro-2*H*-pyran-4-yl)phenyl)acetic acid **320** (45 mg, 0.172 mmol), DIPEA (0.09 mL, 0.517 mmol), 50 % T_3P in ethyl acetate (0.205 mL, 0.344 mmol) and (2*S*,6*R*)-4-(indolin-4-ylmethyl)-2,6-dimethylmorpholine **326** (50 mg, 0.178 mmol) were combined in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h, diluted with water (10 mL) and extracted with ethyl acetate (2 x 10 mL). The organic layer was washed brine (10 mL), aqueous lithium chloride (10 mL, 5 %), dried through a hydrophobic frit and concentrated *in vacuo*. The sample was dissolved in 1:1 MeOH:DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method A) to give 3-(3,6-dihydro-2*H*-pyran-4-yl)-5-(2-(4-(((2*S*,6*R*)-2,6-dimethylmorpholino)methyl)indolin-1-yl)-2-oxoethyl)benzamide **308** (21 mg, 24 %) as a cream solid. M.P. 159-161 °C; LCMS (Ammonium carbonate) 100 %, R_t= 0.58, $[MH]^+$= 490; δ_H (400 MHz, DMSO-d_6) 8.03 – 7.93 (2H, m), 7.86 – 7.81 (1H, m), 7.72 – 7.67 (1H, m), 7.54 – 7.49 (1H, m), 7.32 (1H, s), 7.11 (1H, t, *J* = 7.8 Hz), 6.93 (1H, d, *J* = 7.5 Hz), 6.36 – 6.30 (1H, m), 4.27 – 4.24 (2H, m), 4.21 (2H, t, *J* = 8.6 Hz), 3.90 (2H, s), 3.84 (2H, t, *J* = 5.4 Hz), 3.58 – 3.49 (2H, m), 3.38 (2H, s), 3.18 (2H, t, *J* = 8.3 Hz), 2.67 – 2.60 (2H, m), 2.50 – 2.45 (2H, m), 1.71 – 1.60 (2H, m), 1.02 (6H, d, *J* = 6.3 Hz); δ_C (101 MHz, DMSO-d_6) 169.2, 168.3, 143.5, 140.0, 135.9, 134.8, 134.6, 133.3, 131.8, 129.0, 128.3, 127.3, 124.6, 124.0, 122.1, 115.2, 71.5, 65.5, 64.0, 60.1, 59.5, 48.2, 42.3, 27.0, 26.7, 19.4; v_{max} (liquid film)/cm^{-1} 3343, 2192, 2971, 2928, 1654, 1588, 1459, 1391, 1359, 1129, 1079; *m/z* (ES) Found: $[MH]^+$ 490.2686, $C_{29}H_{36}N_3O_4$ is $[MH]^+$ 490.2700.

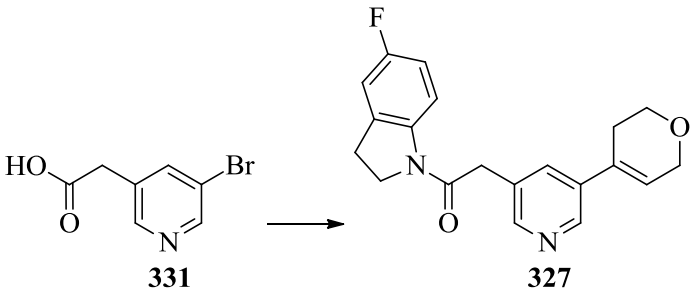
2-(5-Bromopyridin-3-yl)acetic acid 331



330 **331**

A mixture of 1-(5-bromopyridin-3-yl)ethanone **330** (100 mg, 0.5 mmol) and sulfur (27 mg, 0.85 mmol) was suspended in morpholine (0.131 mL, 1.5 mmol) and stirred at 130 °C for 16 h. The reaction was cooled to 100 °C and to this aqueous sodium hydroxide (2.5 mL, 2 M) was added and stirred for 1 h. The reaction mixture was cooled to room temperature and filtered through Celite, washing with water (10 mL). The aqueous layer was washed with ethyl acetate (10 mL), neutralised to pH 7 and then concentrated *in vacuo*. The residue was dissolved in 1:1 water: methanol (2 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method B) to give 2-(5-bromopyridin-3-yl)acetic acid **331** (44 mg, 40 %) as an off-white solid. LCMS (Formic acid) 97 %, R_t= 0.59, $[MH]^+$= 218; δ_H (400 MHz, DMSO-d_6) 12.58 (1H, s), 8.60 (1H, d, *J* = 2.3 Hz), 8.47 (1H, d, *J* = 1.7 Hz), 8.04 – 7.94 (1H, m), 3.69 (2H, s).

2-(5-(3,6-Dihydro-2*H*-pyran-4-yl)pyridin-3-yl)-1-(5-fluoroindolin-1-yl)ethanone 327

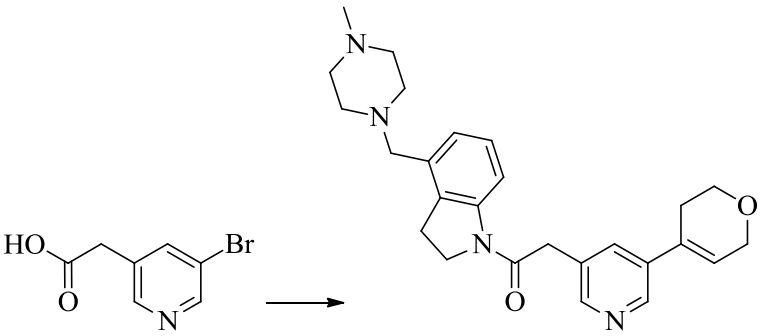


331 **327**

A mixture of 2-(5-bromopyridin-3-yl)acetic acid **331** (50 mg, 0.231 mmol), DIPEA (0.162 mL, 0.926 mmol), 50 % T_3P in ethyl acetate (0.207 mL, 0.347 mmol) and 5-fluoroindoline (35 mg, 0.255 mmol) were dissolved *in* DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h, diluted with water (20 mL) and then extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL), aqueous lithium chloride (2 x 10 mL, 5 %) passed through a hydrophobic frit and concentrated *in vacuo.* The residue was combined with 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (54 mg, 0.256 mmol) tripotassium phosphate (148 mg, 0.698 mmol) and2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (13 mg, 0.023 mmol) in 1,4-

dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 2-(5-(3,6-dihydro-2*H*-pyran-4-yl)pyridin-3-yl)-1-(5-fluoroindolin-1-yl)ethanone **327** (46 mg, 58 %) as a white solid. M.P. 130-132 °C; LCMS (Formic acid) 100 %, R_t= 0.74, $[MH]^+$= 339; δ_H (400 MHz, DMSO-d_6) 8.57 (1H, d, *J* = 2.2 Hz), 8.38 (1H, d, *J* = 1.8 Hz), 8.02 (1H, dd, *J* = 8.8, 5.1 Hz), 7.76 – 7.71 (1H, m), 7.12 (1H, dd, *J* = 8.5, 2.5 Hz), 6.96 (1H, td, *J* = 9.1, 2.7 Hz), 6.38 – 6.33 (1H, m), 4.31 – 4.22 (4H, m), 3.91 (2H, s), 3.84 (2H, t, *J* = 5.5 Hz), 3.21 (2H, t, *J* = 8.4 Hz), 2.50 – 2.44 (2H, m); δ_C (101 MHz, DMSO-d_6) 168.7, 158.8 (d, *J* = 239.2 Hz), 149.8, 144.5, 139.8, 134.9 – 134.8 (m), 134.7, 133.7, 131.2, 131.1, 124.9, 117.0 (d, *J* = 8.3 Hz), 113.5 (d, *J* = 22.7 Hz), 112.5 (d, *J* = 24.0 Hz), 65.5, 63.9, 48.4, 39.0, 27.9, 26.6. δ_F (376 MHz, DMSO-d_6) -119.76 – -119.84 (m); ν_{max} (liquid film)/cm^{-1} 2968, 2927, 1654, 1481, 1395, 1242, 1128; *m/z* (ES) Found: $[MH]^+$ 339.1497, $C_{20}H_{20}FN_2O_2$ is $[MH]^+$ 339.1503.

2-(5-(3,6-Dihydro-2*H*-pyran-4-yl)pyridin-3-yl)-1-(4-((4-methylpiperazin-1-yl)methyl) indolin-1-yl)ethanone 328

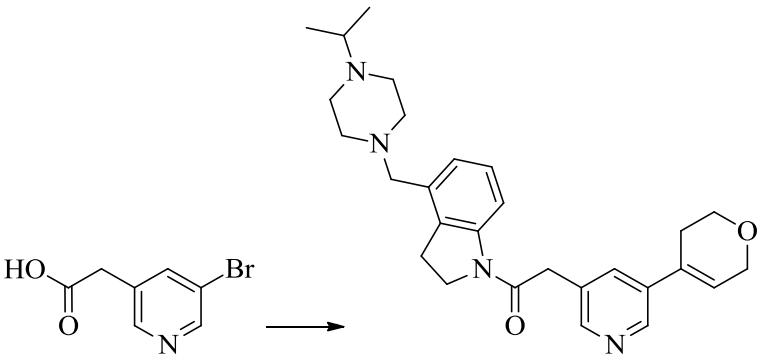


331 **328**

A mixture of 2-(5-bromopyridin-3-yl)acetic acid **331** (30 mg, 0.139 mmol), DIPEA (0.097 mL, 0.555 mmol), 50 % T_3P in ethyl acetate (0.124 mL, 0.208 mmol)and 4-((4-methylpiperazin-1-yl)methyl)indoline **324** (33 mg, 0.143 mmol) were dissolved *in* DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h, diluted with water (20 mL), and then extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL). aqueous lithium chloride (2 x 10 mL, 5 %) passed through a hydrophobic frit and concentrated *in vacuo.* The residue was combined with 2-(3,6-dihydro-2*H*-pyran-4-yl)-

4,4,5,5-tetramethyl-1,3,2-dioxaborolane (30 mg, 0.143 mmol) tripotassium phosphate (82 mg, 0.384 mmol) and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (8 mg, 0.014 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on Sunfire C_{18} column using acetonitrile/water with a formic acid modifier (method A) to give 2-(5-(3,6-dihydro-2*H*-pyran-4-yl)pyridin-3-yl)-1-(4-((4-methylpiperazin-1-yl)methyl)indolin-1-yl)ethanone **328** (29 mg, 48 %) as a white solid. M.P. 127-129 °C; LCMS (Formic acid) 100 %, R_t= 0.45, $[MH]^+$= 433; δ_H (400 MHz, DMSO-d_6) 8.57 (1H, d, *J* = 2.0 Hz), 8.38 (1H, d, *J* = 1.4 Hz), 7.96 (1H, d, *J* = 7.9 Hz,), 7.76 – 7.72 (1H, m), 7.10 (1H, t, *J* = 7.8 Hz), 6.93 (1H, d, *J* = 7.5 Hz), 6.38 – 6.30 (1H, m), 4.29 – 4.19 (4H, m), 3.91 (2H, s), 3.84 (2H, t, *J* = 5.5 Hz), 3.41 (2H, s), 3.20 (2H, t, *J* = 8.3 Hz), 2.49 – 2.44 (2H, m), 2.43 – 2.24 (8H, m), 2.16 (3H, s); δ_C (101 MHz, DMSO-d_6) 168.9, 149.8, 144.5, 143.4, 135.0, 134.9, 133.7, 131.7, 131.3, 131.2, 127.3, 124.9, 124.5, 115.1, 65.5, 63.9, 60.1, 55.2, 53.0, 48.2, 46.1, 39.2, 26.6, 24.9; v_{max} (liquid film)/cm^{-1} 2927, 2815, 1651, 1607, 1482, 1395, 1346, 1248, 1128; *m/z* (ES) Found: $[MH]^+$ 433.2601, $C_{26}H_{33}N_4O_2$ is $[MH]^+$ 433.2598.

2-(5-(3,6-Dihydro-2*H*-pyran-4-yl)pyridin-3-yl)-1-(4-((4-isopropylpiperazin-1-yl)methyl) indolin-1-yl)ethanone 329



331 **329**

A mixture of 2-(5-bromopyridin-3-yl)acetic acid **331** (40 mg, 0.185 mmol), DIPEA (0.129 mL, 0.741 mmol), 50 % T_3P in ethyl acetate (0.165 mL, 0.278 mmol) and 4-((4-isopropylpiperazin-1-yl)methyl)indoline **325** (50 mg, 0.193 mmol) were dissolved in DMF (0.5 mL). The resulting mixture was stirred at room temperature for 16 h and diluted with

water (20 mL) and then extracted into ethyl acetate (2 x 20 mL). The organic layer was washed with brine (10 mL), aqueous lithium chloride (2 x 10 mL, 5 %) passed through a hydrophobic frit and concentrated *in vacuo*. The residue was combined with 2-(3,6-dihydro-2*H*-pyran-4-yl)-4,4,5,5-tetramethyl-1,3,2-dioxaborolane (43 mg, 0.204 mmol), tripotassium phosphate (118 mg, 0.557 mmol) and 2'-(dimethylamino)-2-biphenylpalladium(II) chloride dinorbornylphosphine complex (10 mg, 0.018 mmol) in 1,4-dioxane (0.5 mL) and water (0.1 mL) was heated and stirred in a Biotage microwave reactorat 80 °C for 30 min. The reaction was passed through a 1 g C_{18} cartridge, eluting with methanol and concentrated *in vacuo*. The sample was dissolved in DMSO (1 mL) and purified by MDAP on XSelect C_{18} column using acetonitrile/water with an ammonium carbonate modifier (method C) to give 2-(5-(3,6-dihydro-2*H*-pyran-4-yl)pyridin-3-yl)-1-(4-((4-isopropylpiperazin-1-yl)methyl)indolin-1-yl) ethanone **329** (45 mg, 52 %) as a white solid. M.P. 140-142 °C; LCMS (Formic acid) 98 %, R_t= 0.46, $[MH]^+$= 461; δ_H (400 MHz, DMSO-d_6) 8.57 (1H, d, *J* = 2.2 Hz), 8.37 (1H, d, *J* = 1.9 Hz), 7.95 (1H, d, *J* = 8.0 Hz), 7.77 – 7.71 (1H, m), 7.10 (1H, t, *J* = 7.8 Hz), 6.93 (1H, d, *J* = 7.4 Hz), 6.42 – 6.30 (1H, m), 4.35 – 4.15 (4H, m), 3.91 (2H, s), 3.84 (2H, t, *J* = 5.5 Hz), 3.39 (2H, s), 3.20 (2H, t, *J* = 8.4 Hz), 2.64 – 2.55 (1H, m), 2.49 – 2.44 (2H, m), 2.44 – 2.30 (8H, m), 0.95 (6H, d, *J* = 6.5 Hz); δ_C (101 MHz, $CDCl_3$) ^{13}C NMR (101 MHz, DMSO-d_6) 168.8, 149.8, 144.5, 143.4, 135.1, 134.9, 133.7, 131.6, 131.2, 131.2, 127.3, 124.9, 124.5, 115.0, 65.5, 63.9, 60.2, 54.0, 53.6, 48.5, 48.2, 39.2, 26.7, 26.6, 18.7. ν_{max} (liquid film)/cm^{-1} 2953, 2935, 2811, 1647, 1459, 1395, 1299, 1273, 1128; *m/z* (ES) Found: $[MH]^+$ 461.2908, $C_{28}H_{37}N_4O_2$ is $[MH]^+$ 461.2911.

Biochemical Assay Protocol

The binding of compounds to PI3K-alpha/beta/delta/gamma is determined by homogeneous time resolved fluorescence (HTRF) assays as follows;

Briefly, solid compound is dissolved in 100 % DMSO at a concentration of 2 mM. Dilutions are prepared in 100 % DMSO using a 1 in 4 serial step dilution. The dilutions are transferred to black low volume Greiner assay plates ensuring that the DMSO concentration is constant across the plate at 1 % (0.1 μL/well).

PI3K Reaction Buffer (contains 50 mM HEPES pH 7.0 (NaOH), 150 mM NaCl, 10 mM $MgCl_2$, 2.3 mM sodium cholate, 10 μM CHAPS made up in milliQ water). Fresh DTT is added at a final concentration of 1 mM on the day of use. Wortmannin at a concentration sufficient to produce 100 % inhibition (8.33e-6 M) is added to column 18 of compound plates.

Enzyme solutions: 1X PI3K assay Buffer containing:

• 550 pM PI3K-Alpha enzyme (275 pM final assay concentration)

• 800 pM PI3K-Beta enzyme (400 pM final assay concentration)

• 3 nM PI3K-Delta enzyme (1.5 nM final assay concentration)

• 10 nM PI3K-Gamma enzyme (5 nM final assay concentration)

These concentrations are optimal to achieve a signal:background of between 1.5-4.5. The enzyme solution is added to columns 1-24 (3 μL/well) and plates are incubated for 15 min at 5 room temperature.

Substrate solution: 1 x PI3K assay buffer containing:

• PI3K-Alpha: 500 μM ATP, 20 μM PIP_2 and 120 nM biotin-PIP_3. (Final assay concentrations are 250 μM ATP, 10 μM PIP_2 (both at K_m) and 40 nM biotin-PIP_3).

• PI3K-Beta: 800 μM ATP, 20 μM PIP_2 and 120 nM biotin-PIP3. (Final assay concentrations are 400 μM ATP, 10 μM PIP_2 (both at K_m) and 40 nM biotin-PIP_3).

• PI3K-Delta: 160 μM ATP, 20 μM PIP_2 and 120 nM biotin-PIP_3. (Final assay concentrations are 80 μM ATP, 10 μM PIP_2 (both at K_m) and 40 nM biotin-PIP3).

• PI3K-Gamma: 30 μM ATP, 20 μM PIP_2 and 120 nM biotin-PIP_3. (Final assay concentrations are 15 μM ATP, 10 μM PIP_2 (both at K_m) and 40 nM biotin-PIP_3).

This is added to all wells and plates are incubated for 1 h at room temperature. Detection solution: PI3K Detection Buffer (contains 50 mM HEPES pH 7.0 (hydrochloric acid), 150 mM NaCl, 2.3 mM sodium cholate, 10 μM CHAPS, 240 mM KF) containing 2 mM DTT (2 x final concentration), 90 nM GRP-1 PH domain, 300 nM Streptavidin-APC and 24 nM Europium-anti-GST (6 x final concentrations).

This is mixed and left at room temperature (protected from light).

STOP solution: PI3K STOP Buffer (contains 50 mM HEPES pH 7.0 (hydrochloric acid), 150 mM NaCl, 2.3 mM sodium cholate, 10 μM CHAPS, 150 mM EDTA).

Detection solution is diluted 1:1 with STOP solution and added to all wells (3 μL/well). Plates are covered and incubated on the bench for 45-60 min.

Plates are read on a PerkinElmer Envision, measuring TR-FRET between the complex formed between the GST-tagged PH domain and biotinylated PIP_3 which both recruit fluorophores (Europium-labelled anti-GST & Strep-APC respectively). In the presence of an inhibitor, this complex is disrupted by the competitive action of non-biotinylated PIP3 (formed in the assay by the phosphorylation of PIP2 by the kinase & ATP). From this, the ratio of acceptor/donor was calculated and used for data analysis.

Whole Blood Preparation:

The media used for diluting the cytostim (500 mL RPMI low endotoxin RPMI 1640 + 1 % Glutamax + 1 % Pen/Strep) was pre-warmed in a 37 °C water bath for at least 20 min prior to use. The compound plates were prepared before commencing whole blood preparation – two replicates of each master plate are required to account for donor variability. Solid compounds are dissolved in 100 % DMSO at a concentration of 10 mM. The compounds are serially diluted 1:4 in 100 % DMSO and 500 nL are dispensed into the assay plates.

Sufficient volumes of blood from two healthy volunteers were drawn by qualified GSK staff in the Blood Donation Unit, and heparinised with 10 µL of 1000 units/mL endotoxin-free heparin per 1 mLof blood (final concentration was 10 units/mL). In the CL2 biosafety cabinet, the blood was transferred into a sterile trough and 100 µL was added to each well of a 96- well plate using a multi-channel pipette, with each donor being processed separately. The plates were then incubated at 37 °C, 5 % CO_2 for 1 hour. After 1 hr, 25 µl of Cytostim solution (Miltenyi Biotech: 1:300 dilution of stock reagent in low endotoxin RPMI 1640+1 % glutamax + 1 % pen/strep) was added to each well using a Multidrop Combi (Thermo). The filled plates were then lidded and placed in the humidified primary cell incubator for 18-24 h at 37 °C, 5 % CO_2.

The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents.

Detection of IFNg Using Mesoscale Discovery Technology (electrochemiluminescence):

Whole blood plates were shaken vigorously on a plate shaker for 5-10 minutes until all wells were thoroughly mixed. 50 µL of physiological saline (0.85 % NaCl) was added to each well in the biosafety cabinet using a multidrop combi then the plates were centrifuged at 1300 rpm for 10 min. 50 µL of plasma supernatant was transferred to a 96-well MSD plate, pre-coated with anti-human IFNg capture antibody using the Biomek FX. The plates were then sealed and placed on a shaker at 600 rpm at room temperature for 2 h. After 2 h, the plates were washed three times with 150 µL of PBS/Tween 20 (0.05 % v/v = 250 µL/500 mL), and tapped on to blue roll to remove any residual liquid. Detection antibody solution was prepared by diluting stock anti-human IFNg antibody (50X, MSD kit) 1:50 in Diluent 100 (MSD) to generate a working solution of 1 µg/mL SULFO-TAG™ IFNγ, and 40 µL of the working antibody solution was then added to each well of the MSD plates using a Multidrop Combi. The plates were then re-sealed and returned to the shaker for 1 hour at 600rpm at room temperature. The plates were then washed three times with PBS, ensuring there was no liquid remaining in the plate after washing. 150 µL of 2X MSD Read Buffer T (stock 4X MSD Read Buffer T was diluted 50:50 with de-ionised water) was then added to each well using a Multidrop Combi and the plates were then read on the MSD Sector Imager 6000. The resulting data was used for data analysis.

Chapter 10 References

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