University of Strathclyde

Department of Pure and Applied Chemistry

Structure Activity Studies Of Aminoindole Anti-Malarial Agents To Enhance Physicochemical Properties And Safety Profile

Thesis submitted to the University of Strathclyde in fulfilment of the requirements for the degree of Doctor of Philosophy

by

Elena Sandoval Izquierdo

2018

Declaration of Copyright

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.

Signed: **Date:**

"All animal studies were ethically reviewed and carried out in accordance with European Directive 2010/63/EU and the GSK Policy on the Care, Welfare and Treatment of Animals."

ACKNOWLEDGEMENT

First of all, I would like to thank my industrial supervisor Nacho (Dr. José Ignacio Martín Hernando) for his continous support, for helping me at any moment during these four years, for sharing his big scientific knowledge, for his unconditional listening, for his smiling and laughing, for our trips to Scotland. I could have not done this Thesis without you, Nacho, it would be a Thesis…but not this one!

Secondly, I have to thank Billy (Prof. William Kerr) for all the times he has corrected this piece of work, I have no doubt that he has done an extra effort with a non-English speaker placed in Spain with lots of "Spanglish" sentences (probably he has laughed in certain moments!). I want to thank you also for the last big effort you did to make my personal life easier, I really appreciate it!

I would also thank Harry Kelly for making this possible along with Billy. You are great partners in crime!

Four years and some months ago I decided that I wanted to do a Thesis and I went to speak with my manager then, Dr. Esther Fernández about it. She inmediately did everything she could for me to get what I wanted and I started Strathclyde program 2 months after that. Thanks Esther, you gave me a great opportunity!

I also would like to mention Félix Calderón, because of all the motivation that he has always transmitted, the self-confidence that you made me feel and the scientist that I am now. Thanks Félix and, please,never change!

I also want to thank Mariola, my project leader during the last four years, because of the great job she has done in the project, and also because she has always understood that I had to make an independent work within the project team that allows me to write this Thesis book. I really want to have you back, so get better very soon!

Marga, Charly, Merce, Jorge.F., Nacho (my project mates): thanks for being such great peers, we were a really good team!

To my laboratory peers: thanks to be always so happy and helpful!

I couldn't forget Juan, Jesús, Tere, and Ana, I want to thank all the scientific support regarding the different techniques including in this piece of work. You are fantastic and DDW couldn't exist without you!

To my dear Mikko: thank for being so patient, so supportive, for coming to live in Madrid with me, to make our family possible. You know how much I love you!

Quiero dar las gracias a mi familia porque siempre me ha empujado a ser mejor en mi trabajo, porque "haz lo que te haga feliz y te guste, pero hazlo lo mejor que sepas" ha sido una máxima en mi casa, toda la vida. Porque hasta mi abuela de más de 90 años me animaba a seguir estudiando y se sentía tremendamente orgullosa de su nieta mayor. Gracias a mi madre que siempre me ha enseñado a pelear, a mi hermano porque me hace reir como nadie, a mi tía por enseñarme a amar la química, a mi primi por ser tan genial siempre, a mi tío por enseñarme que los pollos tienen dos patas, a mi cuñada por ser como una hermana más, a Sergio por su cariñosa sonrisa, y a Carlotis y Amelia por hacernos a todos tan felices siempre. Os quiero muchísimo a todos!

Por supuesto a todos mis amigos, porque sin los amigos la vida no es igual!

1. ABSTRACT

This thesis describes a programme of study relating to both the optimisation of Lead compounds and the investigation of further new entities focused on aminoindoles as potential new antimalarials.

This thesis communicates endeavours to identify molecules with enhanced properties over the Lead compound GSK3539992A (**3.23**) using three different strategies, in order to improve solubility, lipophilicity, and safety liabilities.

At the end of the first round of the Lead Optimisation program, four compounds with various improved properties were identified. Having this knowledge, a second generation of compounds was designed and synthesised in order to ameliorate the initial four molecules. From this effort, a tetrahydroindole core was identified in order to replace the initial indole core, reducing the number of aromatic rings and the planarity of the overall structure, and therefore improving the physicochemical properties.

From the study of the different substitutions in the 2-position of the molecule from *in vitro* activity and metabolic stability points of view, one of the linkers was found to be favoured: Amides type II. Based on this finding, a library of amides was computationally designed, and the most promising target molecules were synthesised. From this library of amides, one compound bearing a quinuclidine stood out due to its balanced profile. This was then introduced in the scaffold of the precandidate GSK3531659A (**5.53**).

Due to different issues, the progression of the precandidate was put on hold, and a back-up strategy with two different approaches to improve solubility and decrease the predicted human dose were carried out. This work has allowed the identification of 5,5'-difluorotetrahydroindole as a possible replacement for the original indole core. This unit, which has one aromatic ring less and is more flexible than the indole core gives a promising opportunity to work within optimised chemical space.

1. ABBREVIATIONS AND ACRONYMS

AAALAC	Association for Assessment and Accreditation of Laboratory Animal Care
ACN	Acetonitrile
ACTs	Artemisinin combination therapies
ADME	Absortion, distribution, metabolism, and excretion
ADMET	Absortion, distribution, metabolism, excretion, and toxicity
ATP	Adenosine triphosphate
AUC	Area under the curve
Å	Ångström (10^{-10} metres)
BINAP	2,2'-Bis(diphenylphosphino)-1,1'-binaphthalene
Boc	*tert*-Butyloxycarbonyl
Brettphos	2-(Dicyclohexylphosphino)-3,6-dimethoxy-2′,4′,6′-triisopropyl-1,1′-biphenyl
tBuBrettphos	2-(Di-*tert*-butylphosphino)-2′,4′,6′- triisopropyl-3,6-dimethoxy-1,1′-biphenyl
CAD	Charged aerosol detector
carl	Cyclic amine resistance locus
CDI	1,1'-Carbonyldiimidazole
ChromlogD$_{7.4}$	Chromatographic distribution coefficient at buffer pH 7.4
CIB	Centro de Investigación Básica (Centre of Basic Research)
Cl	Clearance
Cy	Cyclohexane
CLND	Chemiluminescent nitrogen detection
clogP	Calculated logarithm of the partition coefficient of a compound between *n*-octanol and water log($c_{octanol}/c_{water}$)
cmr	Calculated molar refractivity
DABCO	1,4-Diazabicyclo[2,2,2]octane
Dba	Dibenzylideneacetone
DBPR	Dutch Biomedical Primate Research Centre
DCC	*N,N'*-Dicyclohexylcarbodiimide
DCE	Dichloroethane

DCM	Dichloromethane
DDW	Diseases of the Developing World
DHFR	Dihydrofolate reductase
DHODH	Dihydroorotate dehydrogenase
DIBAL-H	Diisobutylaluminium hydride
DIPEA	Diisopropyl ethyl amine
DMAD	Dimethyl acetylenedicarboxylate
DME	Dimethoxyethane
DMF	Dimethylformamide
DMPK	Drug metabolism and pharmacokinetics
DMSO	Dimethylsulfoxide
DNA	Deoxyribonucleic acid
DPPA	Diphenylphosphoryl azide
DPU	Discovery performance unit
DSC	Differencial scanning calorimetry
ECG	Electrocardiogram
ED_{90}	Dose of compound that eradicates 90% of the pathogen
EDCI	1-Ethyl-3-(3-dimethylaminopropyl)carbodiimide
eLogD	Estimated octanol buffer distribution coefficient.
EMA	European Medicines Agency
EPR	Electron paramagnetic resonance
ES-MS	Electrospray mass spectroscopy
EtOAc	Ethyl acetate
EtOH	Ethanol
eXP	Enhanced cross screen panel
FACS	Fluorescence activated cell sorting
FaSSIF	Fasted state simulated intestinal fluid
FeSSIF	Fed state simulated intestinal fluid
%F	Oral bioavailability
GC	Gametocytes
GNF	Genomics Institute of the Novartis Research Foundation
GSK	GlaxoSmithKline
h	Hour
HAC	Heavy atom count

hba	Hydrogen bond acceptor
hbd	Hydrogen bond donor
HIV	Human immunodeficiency virus
HOBT	Hydroxybenzotriazole
HPLC	High performance liquid chromatography
HRMS	High resolution mass spectrometry
HTS	High throughput screening
hu	Human
H2L	Hit to lead
iCl_i	*In vitro* intrinsic clearance
IC_{50}	Concentration of drug that gives 50% of inhibition *in vitro*
IPA	Isopropyl alcohol
iv	Intravenous
IRS	Indoor residual spraying
LAH	Lithium aluminium hydride
LBF	Liver blood flow
LCMS	Liquid chromatography mass spectrometry
LSHTM	London School of Hygiene and Tropical Medicine
LE	Ligand efficiency
LLE	Lipophilic ligand efficiency
LLIN	Long-lasting insecticidal nets
LMIV	Laboratory of Malaria Immunology and Vaccinology
LMVR	Laboratory of Malaria and Vector Research
logP	Logarithm of the partition coefficient of a compound between *n*-octanol and water $\log(c_{octanol}/c_{water})$
LO	Lead optimisation
M	Molarity
mCPBA	*meta*-Chloroperbenzoic acid
MDR	Multidrug resistance
MedChem	Medicinal chemistry
MeOH	Methanol
m	Mouse
malERA	Malaria eradication
min	Minute

MMV	Medicines for Malaria Venture
mp	Melting point
MRM	Multiple reaction monitoring
MsCl	Mesyl chloride
MTD	Maximum tolerated dose
MVI	Malaria Vaccine Initiative
Mw	Molecular weight
NBS	*N*-Bromosuccinimide
NCS	*N*-Chlorosuccinimide
NIAID	National Institute of Allergy and Infectious Diseases
NIH	National Institute of Health
NIS	*N*-Iodosuccinimide
NITD	National Institute for Tropical Diseases
NMP	*N*-Methyl-2-pyrrolidone
NMR	Nuclear magnetic resonance spectroscopy
Papp	Apparent permeability
PBPK	Physiological based pharmacokinetic
Pf	*Plasmodium falciparum*
Pfcarl	*P. falciparum cyclic amine resistance locus*
Pf DHODH	*Plasmodium falciparum Dihydroorotate Dehydrogenase*
PFI	Property forecast index
PGP	P-Glycoprotein
PHS	Public Health Institute
PK	Pharmacokinetics
pK_a	Acid dissociation constant
po	From latin *per os*: by mouth
PPB	Plasma protein binding
PRR	Parasite reduction rate
PSA	Polar surface area
PTS	Platform Technology & Science
Py	Pyridine
quant.	Quantitative
r	Rat
rb	Rotatable bonds

RBC	Red blood cells
r.t.	Room temperature
RM	Reaction mixture
Ro5	Rule of five
RVW	Rabbit ventricular wedge
R & D	Research and development
SAR	Structure-activity relationship
SCF	Supercritical fluid
SCID	Severe combined immunodefiency disorder
SCX	Strong cation exchange
sec	Second
SEC	Single exposure chemoprotection
SERCaP	Single encounter radical cure and prophylaxis
SGF	Simulated gastric fluid
SM	Starting material
^{t}BuOMe	*tert*-Butylmethyl ether
TCAMS	Tres Cantos Antimalarial Set
TFA	Trifluoroacetic acid
THF	Tetrahydrofuran
TLC	Thin layer chromatography
T_3P	*n*-Propane phosphonic acid anhydride
TPH	Tropical and Public Health Institute
TPP	Target product profile
TrB	Transmission blocking
t_R	Time retention
$t_{1/2}$	Half-life
UK	United Kingdom
USA	United States of America
UV	Ultraviolet
Vss	Volume of distribution
WHO	World Health Organization
WRAIR	Walter Reed Army Institute of Research
Xantphos	4,5-Bis(diphenylphosphino)-9,9-dimethylxanthene
%F	Oral bioavailability; fraction of drug available after oral dosing

°C	Degrees Celsius or centigrade
ΔG	Gibbs free energy
2-MeTHF	2-Methyltetrahydrofuran

2. INTRODUCTION

3.1. MALARIA OVERVIEW

3.1.1. Malaria facts and data

Malaria is a major global disease caused by parasites of the genus *Plasmodium*, which are transmitted to people during the blood meal of a female anopheles mosquito. Five species of the parasite are able to infect humans: *Plasmodium malariae, Plasmodium ovale, Plasmodium knowlesi, Plasmodium vivax, and Plasmodium falciparum*, the latter two being the most deadly.[1]

This infectious disease occurs worldwide (**Figure 1**), showing a major prevalence in Sub-Saharan Africa. In 2016, 216 million cases of malaria were reported; of these over 445,000 were lethal, mainly in children and pregnant women.

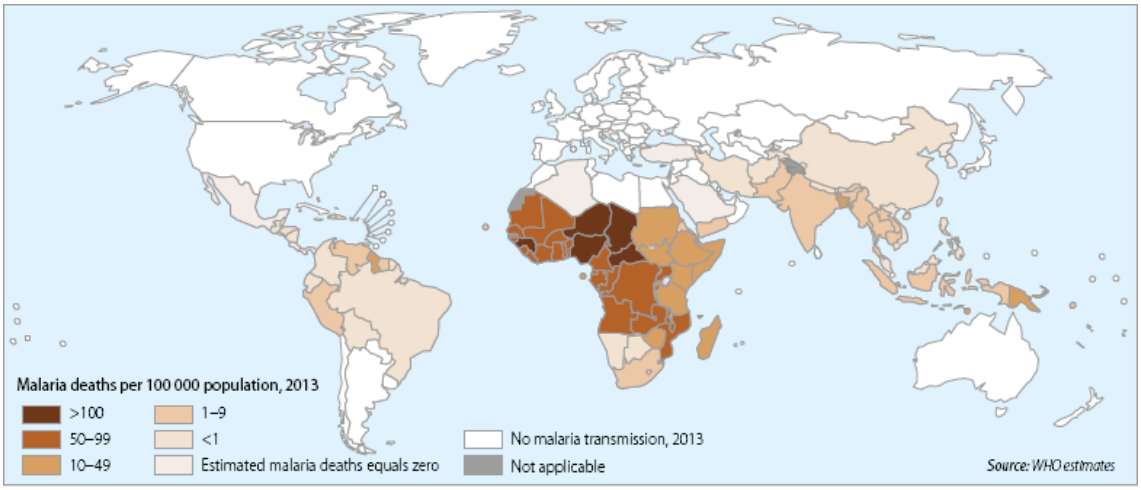


Figure 1. Trends in reported malaria incidence.[2]

The *plasmodium* life cycle starts (**Figure 2**) during the bite of a female anopheles mosquito which, while taking blood from the human, injects sporozoites into the bloodstream of the victim. In some minutes the sporozoites will arrive at the hepatocytes, where they will find the requisite machinery to develop into schizonts that will reproduce asexually into merozoites. Once mature, merozoites will be released into the bloodstream where they invade the red blood cells and replicate rapidly. It is at this stage that the symptoms of malaria become evident. These include high fever and shivers. Also in pregnant women and children under five there is a high probability of

death. What is still unknown is the mechanism whereby some of these merozoites will differentiate into male and female gametocytes that will wait in the bloodstream to be taken by another mosquito during its blood meal. Once in a mosquito they will form diploid zygotes and then ookinetes. These latter forms will travel to the midgut of a mosquito to develop into oocyst which, by meiotic division, will form the sporozoites which will migrate to the salivary glands of a mosquito, waiting for the next bite to go into a human and, in this way, close the cycle. In the case of *Plasmodium vivax* and *ovale*, a proportion of dormant hypnozoites will remain in the liver (for months or years) and can therefore start a new infection without the need of a bite.[3]

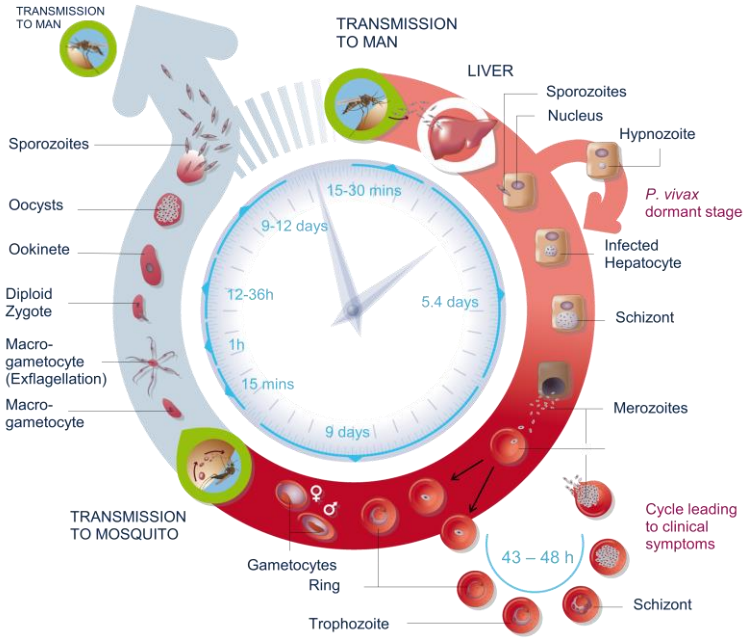


Figure 2. Life cycle of Plasmodium.[3]

As in other parasitic diseases, different programmes aimed at controlling the vector, like long-lasting insecticidal nets (LLINs) and indoor residual spraying (IRS), have been pursued. In fact, both strategies have been progressively adopted across an increasing number of malaria-endemic countries, which has led to a significant reduction in the number of disease cases. Nevertheless, resistance to the insecticides employed (mainly pyrethroids) has been reported and, consequently, chemotherapy is the cornerstone in malaria control. However, resistance to all the marketed antimalarials has been reported (**Figure 3**). Consequently, there is an urgency to find new molecules able to cure malaria.[4] Although malaria as a disease has been known for centuries, not many chemotypes have been developed. So, new strategies to find new drugs as soon as possible are needed.

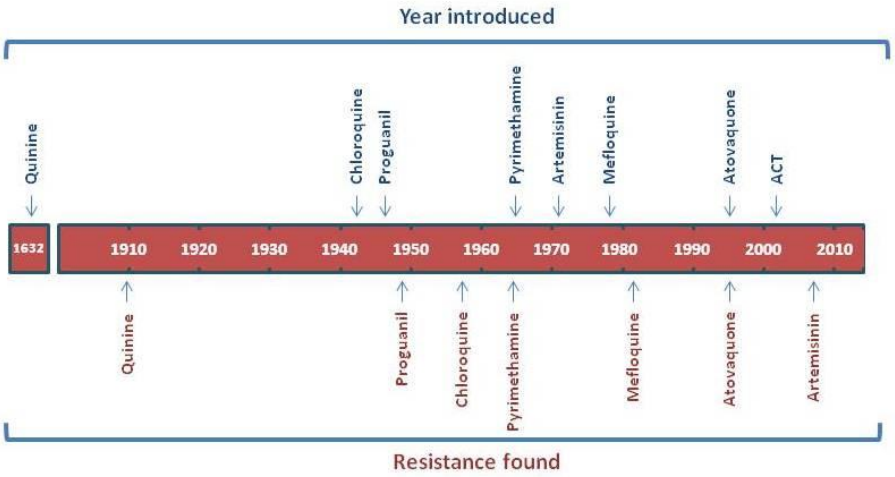
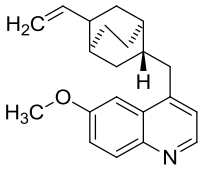


Figure 3. Year of introduction of new antimalarial therapies vs. the emergence of clinical resistance.[5]

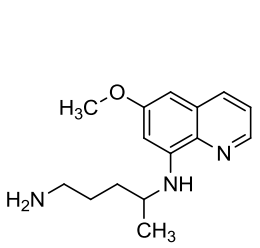
3.1.2. Malaria chemotypes

3.1.2.1. Quinine

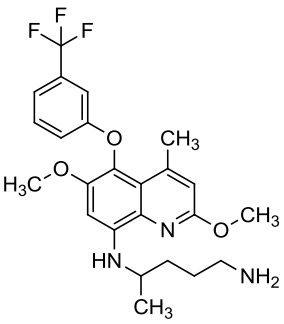


3.1

Quinine (**3.1**) is a natural alkaloid which presents antipyretic, antimalarial, analgesic, and anti-inflammatory properties.[6] Historically, it was the first antimalarial to be used and it was the reference therapeutic agent until the 1940s, when other drugs such as chloroquine (**3.4**; see **Section 3.1.2.2**), with fewer unpleasant side effects, replaced it. However, the quinoline scaffold at the heart of quinine has inspired the development of other antimalarials such as primaquine (**3.2**),[7] and tafenoquine (**3.3**).[7]



3.2



3.3

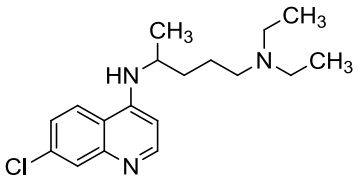
The antimalarial mode of action of quinine is not yet fully resolved, but the most widely accepted hypothesis is based on the closest related quinoline drug: chloroquine (**3.4**).[8]

This model involves the disruption of the established haemoglobin digestion process that occurs during the intra-erythrocytic growth of the parasite. Quinine (**3.1**) shows rapid schizonticidal action against *intra*-erythrocytic malaria parasites. It is also gametocytocidal for *P. vivax* and *P. malariae*, but not for *P. falciparum*.

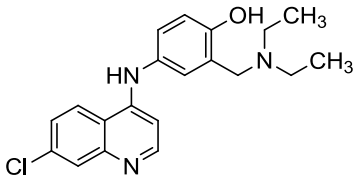
Quinine has a low therapeutic index and presents serious adverse effects such as vertigo, nausea and vomiting, abdominal pain, diarrhea, marked auditory loss, visual symptoms, including blindness, tinnitus, and headache.[6]

Primaquine (**3.2**) and tafenoquine (**3.3**) are clinical antimalarials delivering a cure for *P.vivax*.[9] Primaquine is nowadays the only drug approved to prevent the relapse of the dormant hypnozoites. However, it is a 14-day treatment that is given to patients who are effectively asymptomatic, but it has known gastro-intestinal side effects and, more importantly, presents a risk of haemolytic anaemia to certain populations. Consequently, the development of new molecules is a priority. The communities' immediate hope in this regard is the 8-aminoquinoline, tafenoquine, which benefits from being a single dose. Tafenoquine is undergoing Phase III studies with GSK and MMV, with the aim of evaluating its efficacy and safety. The phase III programme includes two randomised, double-blind treatment studies to investigate tafenoquine in adult patients with *P. vivax* malaria. The "DETECTIVE" study (TAF112582) aims to evaluate the efficacy, safety, and tolerability of tafenoquine as a radical cure for *P. vivax* malaria, co-administered with chloroquine, as blood stage (when the malaria symptoms become more evident) anti-malarial treatment.[3] The "GATHER" study (TAF116564) aims to assess the incidence of haemolysis, and safety and efficacy of tafenoquine compared to primaquine.[10]

3.1.2.2. Chloroquine and amodiaquine



3.4



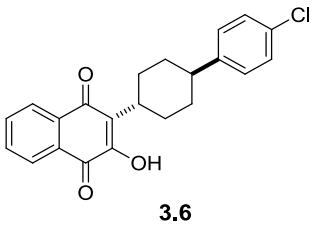
3.5

Chloroquine (**3.4**) is a 4-aminoquinoline discovered in 1934 by Bayer laboratories, although it was during World War II when its therapeutic value was observed.[11] This agent is, without any doubt, the most important antimalarial in history due to its high efficacy and low cost of production. Its excellent efficacy can be explained by its capacity to accumulate in the digestive acid vacuoles as a diprotonated molecule.[6] A number of studies have led to the conclusion that chloroquine accumulation can be explained on the basis of an ion-trapping mechanism: chloroquine is a diprotic weak base (pK_{a1}= 8.1, pK_{a2}= 10.2).[12,13] In its unprotonated form, it traverses the membranes of the parasitized erythrocyte and moves down the pH gradient to accumulate in the acidic food vacuole (pH= 5-5.2). Once protonated, the drug becomes membrane impermeable and is trapped in the acidic compartment of the parasite. According to this "weak base" model, the level of chloroquine accumulation depends only on the difference in pH between the external medium and the food vacuole.

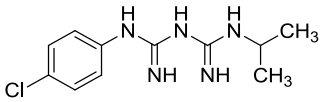
Chloroquine also acts by interaction with DNA, by inhibition of parasite feeding, and by polymerising the heme group of the parasite.[8] All these mechanisms of action make chloroquine a very potent and widely used drug, despite the serious side effects which it presents, especially related to ocular toxicity, as well as in pregnant women.

Amodiaquine (**3.5**) is an analogue of chloroquine that has shown efficacy against chloroquine (**3.4**) resistant strains. However, clinical use has been limited due its association with hepatotoxicity and agranulocytosis.[11]

3.1.2.3. Atovaquone



3.6



3.7

Atovaquone (**3.6**) is marketed in combination with proguanil (**3.7**) as Malarone®[14,15,16] for the treatment and prevention of malaria. Currently it is the preferred prophylaxis for travellers. This combination is very effective but the mechanism of such interaction is still unknown.

Atovaquone acts as an inhibitor of cytochrome bc_1 complex, which is a key mitochondrial enzyme that catalyses transfer of electrons maintaining the membrane potential of mitochondria.[17] The catalytic core of the cytochrome is composed of three subunits; cytochrome *b* (43 kDa), cytochrome c_1 (27 kDa) and the Rieske iron–sulfur protein ([$2Fe_2S$] ISP, 21 kDa), with these subunits participating directly in the electron-transfer pathway. The function of the remaining subunits is not well understood, but they are likely to contribute to complex stability and the assembly process.[18]

Inhibition of the cytochrome bc_1 complex (such as by the anti-malarial compound, atovaquone) leads to prevention of the pyrimidine biosynthetic pathway. This is an essential process in *Plasmodium* given their inability to salvage pyrimidines from the host or present an alternative biosynthetic pathway. Consequently, inhibition of bc_1 leads to death of the parasite.[17,18] A crystal structure for atovaquone-inhibited bc_1 is currently not available, but EPR spectroscopy of the Rieske [$2Fe_2S$] protein (**Figure 4**), site-directed mutagenesis of model organism cytochrome *b*, and gene sequencing of atovaquone-resistant *Plasmodium* species have demonstrated that atovaquone is a competitive inhibitor of bc_1.[17] **Figure 4** shows the interaction between the atovaquone and cytochrome bc_1. bc_1 is shown as a ribbon structure in orange (with the *ef* and cd_1 helical components of the Q_o site indicated). The Rieske protein is in green. Hydrogen bonding ligands *His*-181 (Rieske) and *Glu*-272 (cyt *b*) are shown in wireframe, with the bridging water molecule represented as a sphere.[17]

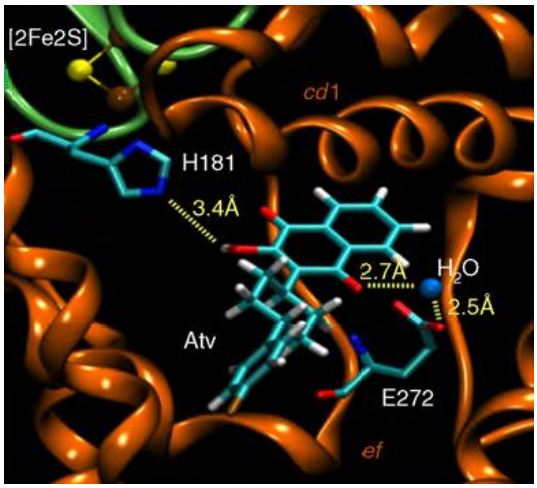
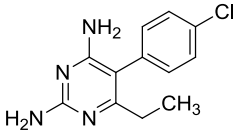


Figure 4. Inhibition of P.falciparum bc1 by atovaquone.[16]

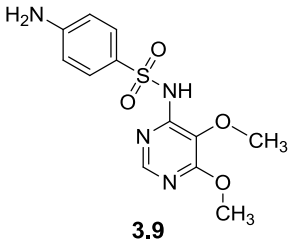
Currently, atovaquone is the only drug in clinical use targeting the *Plasmodium falciparum* bc_1 complex. The rapid emergence of resistance to atovaquone monotherapy resulted in its use in combination with proguanil, which acts as an inhibitor of the folate pathway.[19]

Some of the most widely used antimalarial drugs belong to the folate antagonist class, and a wide number are related to nucleic acid metabolism; the inhibition of enzymes of the folate pathway results in a decrease in pyrimidine synthesis, reducing DNA formation.

3.1.2.4. Pyrimethamine



3.8



3.9

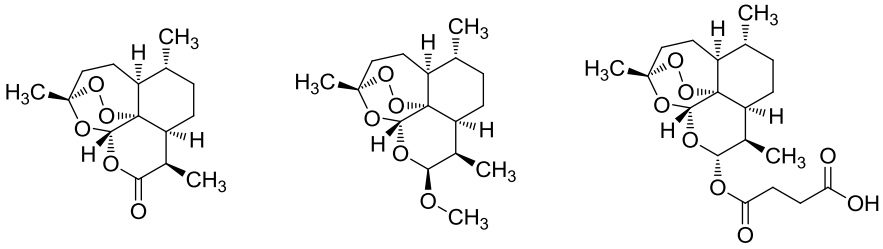
Pyrimethamine (**3.8**) is the most important representative of the well-known antimalarial chemical class of diamine-pyrimidines that are antifolates (*cf*. proguanil). Pyrimethamine interferes with tetrahydrofolic acid synthesis from folic acid by inhibiting the enzyme dihydrofolate reductase (DHFR).[19,20]

Pyrimethamine is marketed in combination with the sulfonamide antibiotic sulfadoxine (**3.9**), as the antimalarial drug Fansidar®[21] (often used as an alternative antimalarial

treatment). Pyrimethamine and sulfadoxine differently act on folic acid synthesis. Combination of them synergistically improve their antimalarial efficacy. [21,22]

Due to the side effects that are produced, this combination is just recommended in severe malaria cases or as a means of prevention in areas where other drugs may not work.

3.1.2.5. Artemisinin



3.10 **3.11** **3.12**

Artemisinin (**3.10**) and its derivatives are a group of drugs that possess the most rapid mode of action of all the current drugs against *Plasmodium falciparum*.[11] Although this agent has been used in China for centuries, it has not been used widely until the end of the last century. Structurally, the endoperoxide represents a key feature.

The mechanism of action for artemisinin is still unknown, however, several lines of evidence indicate that artemisinins exert their antimalarial action by radical formation that depends on their endoperoxide bridge. Two possible modes of action are described:[23]

- Reductive scission (cleavage) (**Figure 5**): Early work by Posner[24,25,26] and Jefford[27,28] proposed that these oxygen centred radicals subsequently rearrange to form carbon centred radicals, although the nature of the proposed radical and the mechanistic pathways giving rise to their formation were different in each case. Low valent transition ions (ferrous heme or non heme exogenous Fe^{2+}) were found to bind to artemisinin and after subsequent electron transfer induce reductive scission of the peroxide bridge to produce oxygen-centred radicals, which rearrange to give carbon-centred radicals. Due to the unsymmetrical nature of the endoperoxide bridge, iron was found to interact with the peroxide in different ways to produce either a primary carbon-centred radical or a secondary carbon-centred radical. Both primary and secondary radicals,[29,30] have

been efficiently spintrapped by electroparamagnetic resonance spin trapping techniques after being activated by iron.[31]

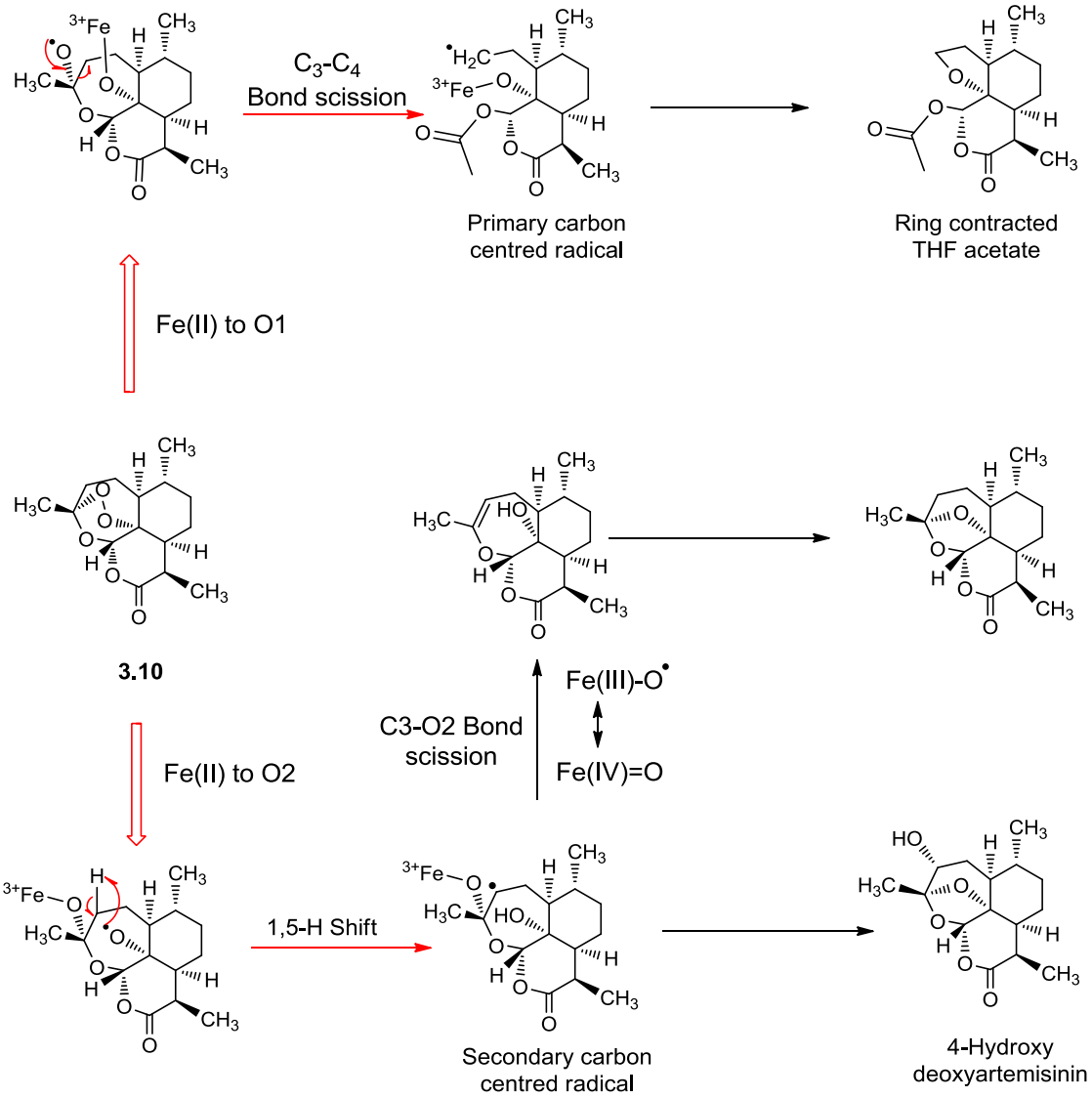


Figure 5. Bioactivation of artemisinin: reductive scission model.

- The alternative model suggests that ring opening is driven by protonation of the peroxide or by complexation of Fe^{2+} (**Figure 6**). Haynes and co-workers have proposed that iron acts as a Lewis acid to facilitate ionic, rather than radical bioactivation of the artemisinins (which, it is proposed, are too short-lived to have any intermolecular interaction).[32] In addition, it has also been suggested that non-peroxidic oxygen plays a role in facilitating ring opening of the peroxide to generate the open hydroperoxide.[33–35] The oxygen atom provides stabilization of the positive charge and, according to transition state theory, lowers the energy required for ring opening. Heterolytic cleavage of the

endoperoxide bridge and subsequent capture of water leads to the formation of an unsaturated hydroperoxide, capable of irreversibly modifying protein residues by direct oxidation. Subsequent Fenton degradation[36] of the hydroperoxide produces a hydroxyl radical, a species that can subsequently oxidize target amino acid residues. To support this theory artemisinin has been shown to mediate *N*-oxidation of tertiary alkylamine derivatives *via* the intermediacy of such a ring-opened peroxide form of artemisinin. This alternative mechanism may have the potential to produce a whole host of reactive oxygen species that may have implications for the antimalarial activity of these compounds.

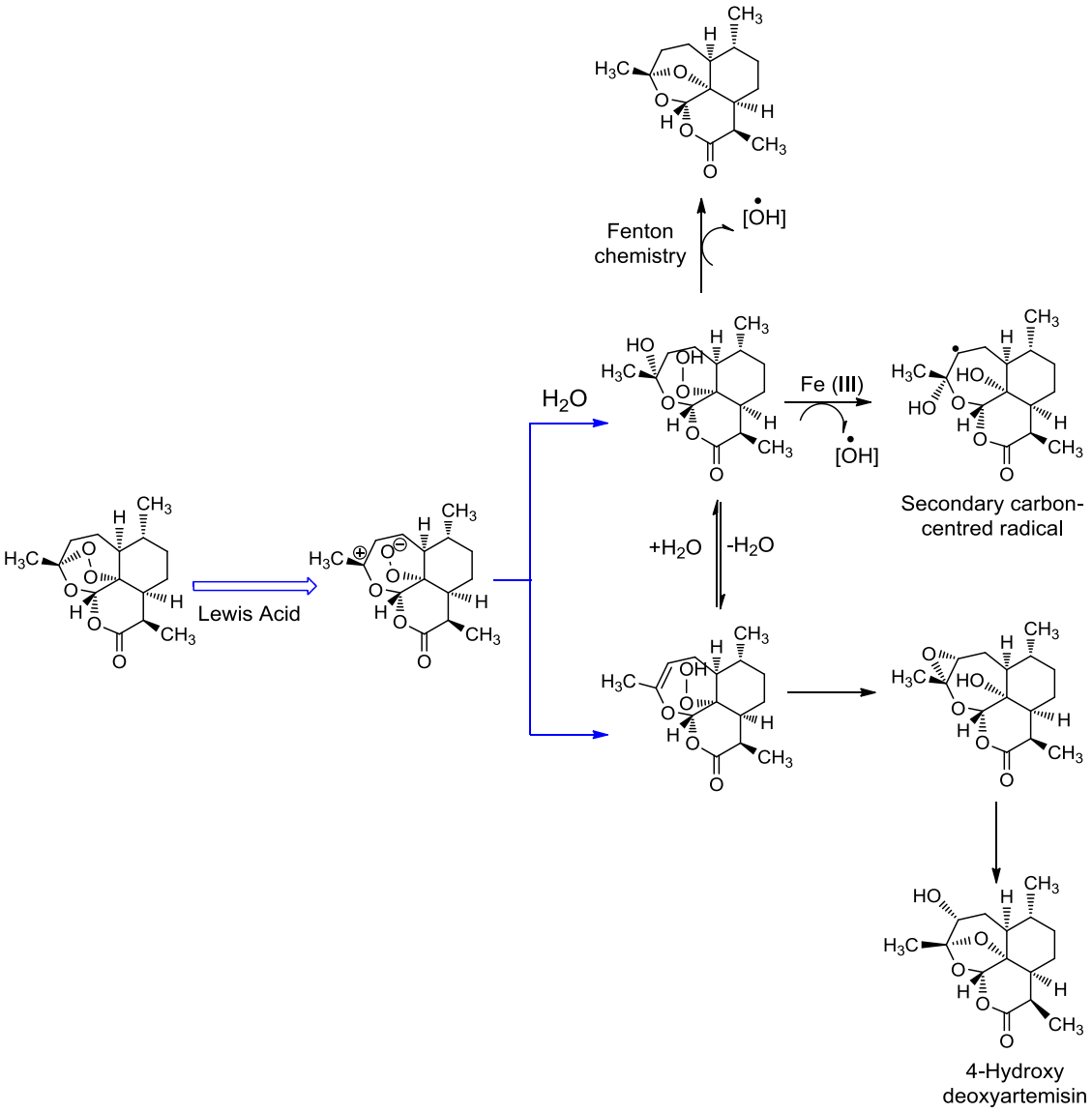
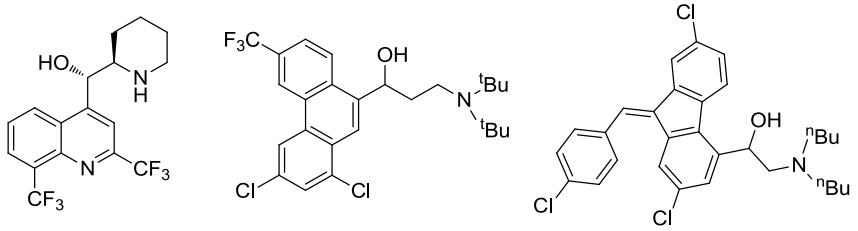


Figure 6. Bioactivation of artemisinin: open peroxide model.

Currently the ACTs (artemisinin combination therapies), which include artemisinin or any of its derivatives (*e.g.* artemeter (**3.11**) or artesunate (**3.12**)), are the preferred treatment for acute infection of *P. falciparum*.[37]

3.1.2.6. Mefloquine, Halofantrine, and Lumefantrine



3.13 **3.14** **3.15**

Mefloquine[38] (**3.13**) was developed with urgency when resistance to chloroquine was observed first in Asia (Thailand in 1957) and South-America (Colombian-Venezuelan border in 1959), and almost two decades later in Africa (Kenya and Tanzania in 1978). Associated with this, in 1960, the Experimental Therapeutics Division of the Walter Reed Army Institute of Research (WRAIR) ran an extensive screening campaign with the intention of finding a replacement for chloroquine. Two related compounds presenting a phenyl-amino-alcohol scaffold were selected: compound 142,490 (Mefloquine (**3.13**)-Lariam®) and 171,699 (Halofantrine (**3.14**)-Halfan®). Due to the special critical historic conditions, these compounds were marketed without phase III trials and some years later, in the early 1980's, randomized controlled trials were carried out in healthy populations, confirming these aminoalcohols as a potential source of psychological illnesses. Toxicity associated with mefloquine (**3.13**) includes adverse effects in the central nervous system and gastrointestinal tract. Further cardiotoxicity was shown for halofantrine (**3.14**). Despite these latter issues and although this chemical class presents many unknowns, in the last 40 years such species have been attractive to the antimalarial community. Indeed, a third representative of this chemical series, lumefantrine (**3.15**), is currently the preferred partner to combine with artemeter (**3.11**) for first-line acute therapies. This combination, marketed as Coartem®, constitutes 75% of the current ACTs (artemisinin combination therapies) used in the clinic and provides a rapid relief of symptoms due to the fast killing mode of action of the artemisinin derivative and prevents recrudescence due to the long half-life (4 days) exhibited by lumefantrine (**3.15**).

These drugs,[39] as well as the quinoline class, artemisinin, and other artemisinin peroxides, exert their mechanism of action by concentration in the food vacuole and are thought to interact with haemoglobin. The quinolines are thought to disrupt or prevent effective formation of haemozoin by binding to haem through π–π stacking of their planar aromatic structures, resulting in haem-mediated toxicity to the parasite. This may occur by inducing lipid peroxidation, although there are other possible explanations.

3.1.3. Global malaria research

The huge problem malaria represents worldwide has made many industries, universities, national institutes of health, and federal agencies engage in significant effort and invest economic resources in attempts to eliminate this terrible pandemia. Data from www.clinicaltrials.gov,[40] indicates that at present 975 clinical trials have occurred or are on-going around the world, most of them localized in Africa (**Figure 7**) due to the large number of cases that occur in this location every year (**Figure 1**, **page 14**).

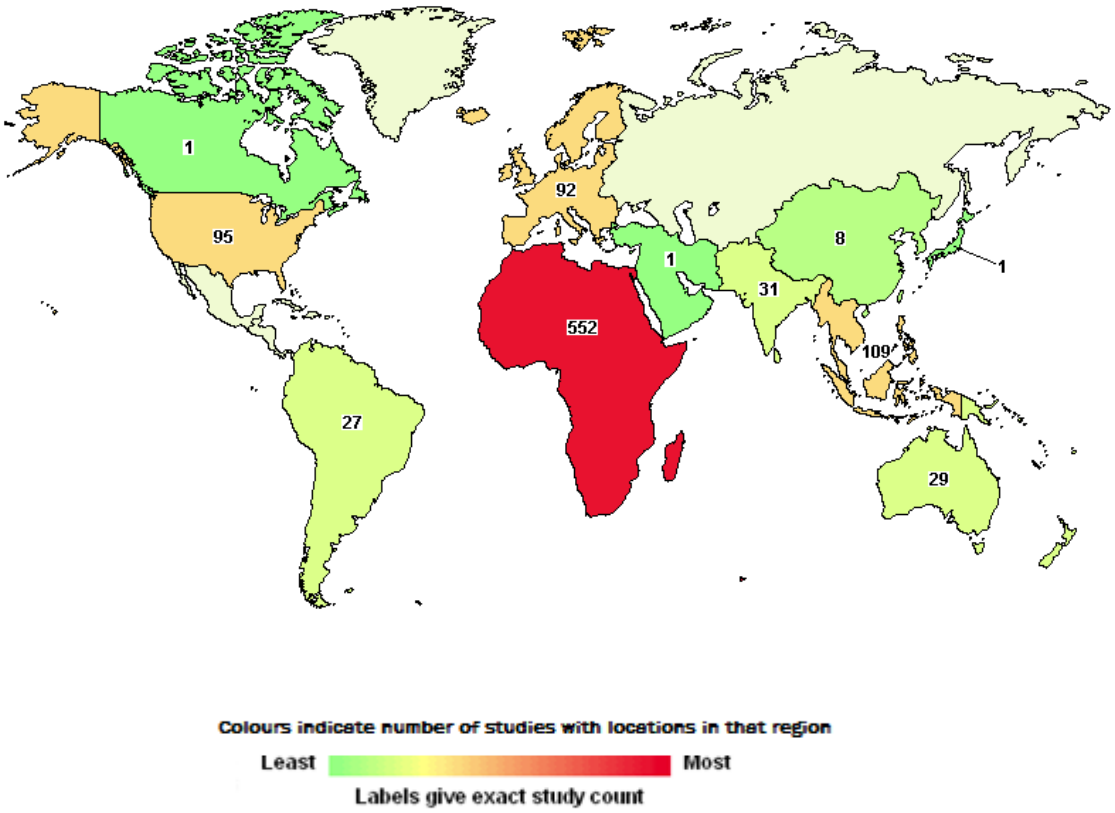


Figure 7. Clinical trials of Malaria worldwide.[40]

These trials are sponsored by many different sources but around 75% of them are supported by only 13 institutions. The National Institute of Allergy and Infectious

Diseases (NIAID), the London School of Hygiene and Tropical Medicine (LSHTM), and the University of Oxford are the centres that are contributing most appreciably (**Figure 8**).

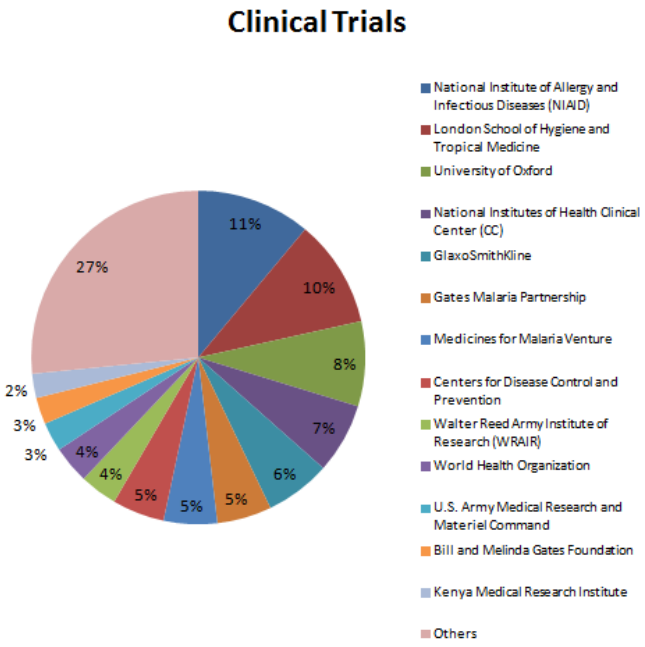


Figure 8. Clinical trials on Malaria.

NIAID:[41] National Institute of Allergy and Infectious Diseases is a division of the National Institute of Health (NIH). NIAID is the most important federal agency in the USA that supports malaria research and development with two important goals: to reduce the mortality and morbidity, and the eradication of malaria. In order to achieve its objectives, NIAID focuses on understanding the biology of malaria parasites, and their interactions with mosquitoes as vectors and humans as hosts. Meanwhile, this institute is also developing tools to prevent, treat, and control the sickness. NIAID is involved in malaria projects worldwide, and they also work to enhance research infrastructure in malaria-endemic countries. In 2010, NIAID established the International Centres of Excellence in Malaria Research, a global network of independent research centers in malaria-endemic settings.

In fiscal year 2007 (FY07), NIAID spent $88.9 M on malaria research, including support for 175 projects in the following categories:

- 98 projects in basic biology and pathogenesis: $36.2 million (including 27 projects in vector biology: $10.7 million)
- 36 projects in drug discovery and development: $16.3 million

- 38 projects in vaccine discovery and development: $35.3 million
- 3 projects in diagnostics: $1.2 million

NIAID counts in its portfolio a laboratory dedicated to research about malaria's vector and drug resistance (LMVR). There is also a laboratory focused on malaria immunology and vaccinology (LMIV) with a focus on research, development, and production of prototype malaria vaccines, which carries out early-phase clinical trials of promising vaccine candidates. LMIV is developing three groups of experimental vaccines, each of which targets a different stage of the malaria life cycle (asexual blood-stage, transmission blocking, and pre-erythrocytic stage).

LSHTM:[42] The Malaria Centre at the London School of Hygiene and Tropical Medicine houses at present over 300 researchers. It was established in 1998 to facilitate interdisciplinary research at the School, as well as support and promote networking within malaria endemic countries. In the centre, the research is performed from a genomic perspective, and focuses on two areas: 1) discovery of parasite mechanisms that can be targeted for future development of drugs and vaccines, and 2) parasite susceptibility to existing drugs and immune responses in endemic populations.

Three different prevention methods are studied in the centre: (i) protection against the mosquito through vector control, (ii) protection against the parasite through chemoprevention, and (iii) vaccination.

In addition to the scientific and policy work conducted under the remit of the Centre, members also provide teaching and training on malaria both in London and other centres in the UK, and many countries overseas. One of the main functions of the Centre is to organise seminars, conferences, and retreats that are open to participants outside of the school. Although the Centre is based in London, many members work overseas. *Via* association with LSHTM, members are conducting malaria research in over 40 countries.

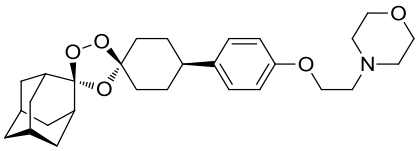
UNIVERSITY OF OXFORD- TROPICAL MEDICINE AND GLOBAL HEALTH[43]

The University of Oxford has a Department of Clinical Medicine (Nuffield Department) that includes research groups permanently based in Africa and Asia, as well as a growing number of groups in Oxford. The research in these groups ranges from clinical

studies to behavioural sciences and it is mainly supported by the Wellcome Trust. Three programmes are based in Kenya, Thailand, and Vietnam, respectively, and there are also a significant number of sister groups in Laos, Tanzania, Indonesia, and Nepal, as well as additional collaborators around the world.

Currently most of the research centres dedicated to malaria research are supported by the **MMV** (Medicines for Malaria Venture) portfolio that presently has 8 compounds in Phase I/II or in Preclinical Development.[44] A selection of these is described below:

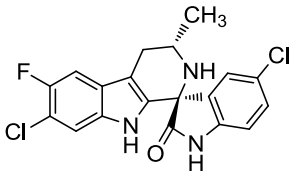
- OZ439 (**3.16**)



OZ439 (**3.16**) is a second generation endoperoxide, that has been designed to have superior pharmacokinetics to the artemisinins. OZ439 has demonstrated clinical efficacy as a single agent (in phase IIa studies, the so-called proof-of-concept), it has being tested in combination safety studies, and combination efficacy clinical trials are currently on going.[40]

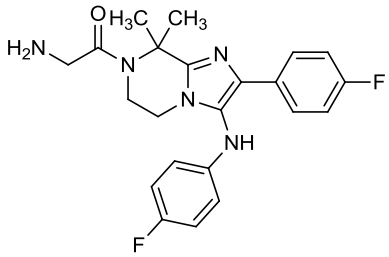
OZ439 (**3.16**) was delivered by a project team led by Jonathan Vennerstrom at Nebraska University, in collaboration with the Swiss Tropical and Public Health Institute (Swiss TPH), Monash University, and MMV.

- NITD609 (**3.17**)



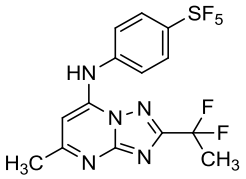
A second compound in Phase II trials is a novel, synthetic spiroindolone (**3.17**),[45,46,47] developed by the Novartis Institute for Tropical Diseases (NITD) in Singapore in a collaboration that included the Swiss TPH Institute, the Dutch Biomedical Primate Research Center (DBPR), the Genomics Institute of the Novartis Research Foundation (GNF), and MMV. It was the first compound to be developed using whole-cell screening of a library of natural product and natural product-like compounds at Novartis against the parasite and started human proof-of-concept studies in 2012, just under 5 years after the initial screen began. It has now passed proof-of-concept, and has been shown to clear parasites in patients infected with either *P. vivax* or *P. falciparum*. The compound inhibits the P-type sodium transporter ATPase 4 (PfATP4), and this results in an increase in the concentration of sodium ions in the parasite, which is toxic to the cell.

- KAF156 (**3.18**)



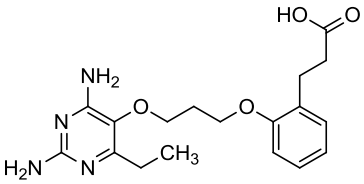
A second initiative from the Novartis consortium, led this time from the Genomics Institute of the Novartis Research Foundation (GNF) in San Diego, delivered KAF156 (**3.18**) as a new clinical compound, again in collaboration with the MMV.[44,48] The compound, an imidazolopiperazine, has high potency against blood and liver stages *in vitro* and *in vivo*, and has been shown to kill gametocytes, demonstrating potential in transmission blocking. The compound series acts *via* a novel mechanism, involving a previously unknown gene, now called *P. falciparum* Cyclic Amine Resistance Locus (Pfcarl). The compound is well tolerated in preclinical safety studies, and has now safely completed phase I studies.

- DSM265 (**3.19**)



The *P. falciparum* enzyme dihydroorotate dehydrogenase (*Pf*DHODH) is known to be essential for the survival of the parasite. A team led by Meg Phillips at the University of Texas Southwestern, in collaboration with the University of Washington, Monash University, and the MMV, identified a potent and selective triazolopyrimidine-based inhibitor *via* a high-throughput enzyme screen.[48,49] The three-dimensional structure of the enzyme-inhibitor complex was resolved and the subsequent lead optimization programme led to the identification of the preclinical candidate; this was an interesting molecule, but with non-optimal pharmacokinetics, on repeat dosing, and insufficient potency. First of all, progress was made to improve pharmacokinetics, and a second breakthrough came using X-ray crystal structure studies. These changes, that were made in collaboration with GSK, delivered DSM265 (**3.19**), which is now in Phase IIa clinical trials for the treatment of *P. falciparum* or *P. vivax.*

- P218 (**3.20**)



Dihydrofolate reductase (DHFR) inhibitors such as pyrimethamine (**3.8**) have been widely used for the treatment of malaria, although their clinical efficacy has been compromised by resistance. P218 (**3.20**) is a next generation inhibitor of DHFR that has been delivered by the Thai BIOTEC group in collaboration with Monash University, the London School of Hygiene and Tropical Medicine, and MMV.[50,51] This agent has now largely completed preclinical development. Impressively, the team designed P218 (**3.20**) using structure-based design methods to have a high affinity to both the parent and mutated enzymes, and to also kill both wild type and clinically-relevant resistant strains.

Vaccine against malaria[52]

The life cycle of the parasite is complex, it changes several times as it goes from mosquito to human blood and then in and out of the liver. At each stage, the parasite can exhibit different surface proteins - the common target used to develop a vaccine against an infection. So, while one bout of measles allows our immune system to develop immunity for life, it usually takes several bouts of malaria to provide even partial immunity. The malaria vaccines team[53] at GSK has been investigating how they could develop an immune response to *P. falciparum* – the malaria parasite most common in sub-Saharan Africa. The breakthrough for the team came in the mid-1990s with the candidate vaccine RTS,S, also known as Mosquirix. This vaccine can 'train' the immune system to recognise and defend against the malaria parasite as soon as it enters the body.

Finally, on July 2015, another important milestone was achieved after more than 30 years of R&D, and Africa's biggest-ever vaccine trial: the European Medicines Agency (EMA) gave a positive scientific opinion on the vaccine for the prevention of malaria in children aged 6 weeks to 17 months in sub-Saharan Africa. This was a key milestone as it marked the first step in the regulatory process toward making the vaccine available to children who need it most. As well as being the world's first malaria vaccine, it is also the first to target a human parasite.

This important milestone was achieved thanks to an innovative public-private partnership with the PATH Malaria Vaccine Initiative (MVI),[54] supported by grants from the Bill & Melinda Gates Foundation.

The world's first malaria vaccine will be rolled out in pilot projects in sub-Saharan Africa, WHO confirmed on 17th November 2016. Funding is now secured for the initial phase of the programme and vaccinations are due to begin in 2018.[55]

Once the vaccine is finally available, it will be provided at cost price plus 5 per cent, which will be reinvested into further research to fight malaria and other tropical diseases.

3.1.4. Physicochemical properties:
An important challenge in drug discovery

Through the last twenty-five years, the drug discovery process has been importantly affected by different factors: from the poor human pharmacokinetics in the 1990's to the low efficacy, toxicology outcomes, or commercial decisions (normally based on possible safety outcomes) that led to a direct affect on the drug attrition by 2000 (**Fig. 9**). Luckily, almost all of these parameters can be modified by designing and synthesising molecules with better physicochemical properties.[56]

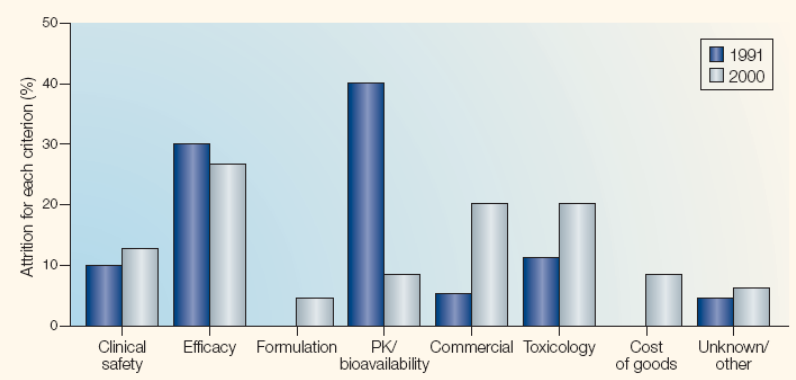


Figure 9. Causes of drug candidate attrition in 1991 and 2000.[56]

In 1997, Lipinski *et al.*[57] published a more than relevant, seminal paper containing that what is now known worldwide within the medicinal chemistry community: as Lipinski's 'Rule of Five' (Ro5), which identified physicochemical properties associated with good oral absorption, and has subsequently been developed towards the prediction of a wide range of properties with an essential role in drug discovery. Recently, and due to the high attrition in the pharmaceutical industry mainly caused by toxicological issues, this has now led to the identification of those properties which correlate with a reduced risk of toxicity.

Since Lipinski's 'Rule of Five', an appreciable number of guidelines have been developed and a selection of these is described below. These guidelines are based on *in silico* measurements to profile the compounds prior to synthesis. This includes properties such as solubility, lipophilicity, and polar surface area (PSA). The *in silico* profiling of compounds during the medicinal chemistry design process is critical to the identification of high-quality drug molecules. It can also reduce time and costs by eliminating the synthesis and biological profiling of compounds which do not present

drug-like properties. The range of the different physicochemical guidelines shows some aspects across all of the common approaches: low lipophilicity, reduced size, and reduced flatness.

- **Lipinski's Ro5**, developed within the Pfizer research laboratories, is based on the premise that good oral activity is a result of not only high intrinsic potency, but good oral bioavailability.[57] Oral bioavailability is primarily governed by the solubility and permeability of compounds, and by following the Ro5 the probability of identifying a compound with good solubility and permeability is increased. In Lipinski's Ro5, lipophilicity is defined as the octanol/water partition coefficient (logP). #hbd and #hba denote the number of hydrogen bond donors and acceptors, respectively.

  Lipinski's Ro5 for the prediction of good oral bioavailability:

$Mw \leq 500$
$logP \leq 5$
$\#hbd \leq 5, \#hba \leq 10$

- A similar study by **Veber *et al.***, within our laboratories, also identified physicochemical properties which predicted good oral bioavailability.[58] It was proposed that molecular weight had been identified by Lipinski as a result of its close correlation with polar surface area. This led to an updated set of criteria to predict good oral bioavailability, focused instead on polar surface area (PSA) and the number of rotatable bonds (#rb).

  Criteria described by Veber et al.[58] *to predict good oral bioavailability*:

$\#rb \leq 10$
$PSA \leq 140$ Å^2

- It has since been shown that physicochemical properties can be used to predict not only oral bioavailability, but a wide range of other drug properties. This is evidenced by the detailed analysis performed by **Ghose *et al***. within the Amgen research laboratories.[59] In this study, the physicochemical properties of known drug molecules were calculated, and a set of rules for drug-likeness developed, with the aim of designing better chemical libraries. As with the Ro5,

lipophilicity is amongst the descriptors, here as a calculated logP (clogP) value.

Criteria identified for drug-likeness, as described by Ghose et al.:[59]

$1.3 \leq \text{clogP} \leq 4.1$
$70 \leq \text{cmr} \leq 110$
$230 \leq \text{Mw} \leq 390$
$30 \leq \#\text{atoms} \leq 55$

clogP denotes the calculated logP, cmr the calculated molar refractivity, Mw the molecular weight, and #atoms the number of atoms.

- Ghose's rules for drug-likeness were supported by the work of Gleeson, again within our laboratories, which also identified molecular weight and clogP as the key predictors of drug-likeness.[60] In this case, physicochemical properties were correlated with absorption, distribution, metabolism, excretion, and toxicity (ADMET) parameters, such as solubility, plasma protein binding and *in vivo* clearance. This work resulted in the simple and easily applied guidelines shown below.

  '4/400' rule for good ADMET properties identified by Gleeson:[60]

$\text{clogP} \leq 4$
$\text{Mw} \leq 400$

- In 2008-2009, research within the Pfizer research laboratories to predict both good oral properties and toxicity was identified as the gold standard of good drug discovery. This work is well represented by two publications, the first showing a rule for increasing the likelihood of a good pharmacokinetic profile,[61] and the second showing a rule for lowering the probability of toxicity of compounds.[62] To optimise the key pharmacokinetic parameters of clearance and oral absorption, the **'Golden Triangle'** was developed, giving an optimum combination of molecular weight and lipophilicity, here denoted by the estimated octanol/buffer distribution coefficient $\text{elogD}_{\text{pH7.4}}$ (**Figure 10**).[61] The estimated $\text{logD}_{\text{pH7.4}}$ (elogD) is plotted on the x-axis, and the molecular weight (Mw) on the y-axis. Compounds with properties which are predicted to lead to a good pharmacokinetic profile lie inside the depicted 'golden triangle' area.

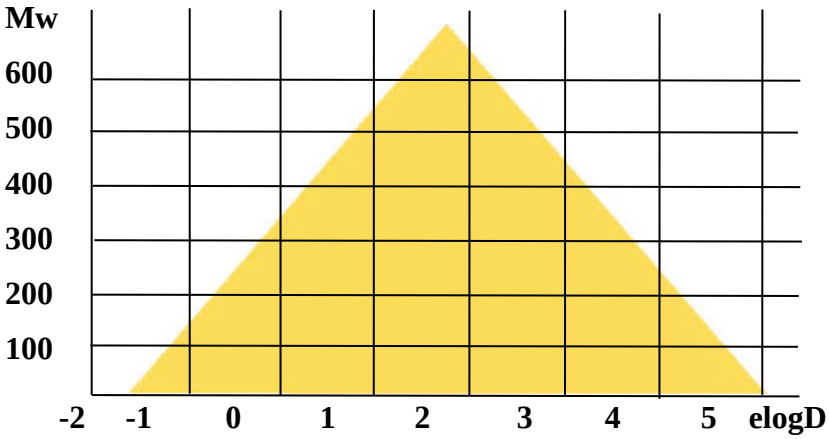


Figure 10. The 'Golden Triangle' rule for good pharmacokinetic profile.

This work was supplemented by a second set of guidelines which identified physicochemical parameters associated with a reduced likelihood of *in vivo* toxicology, commonly named the *'3/75' rule*.[62]

clogP ≤ 3
PSA ≥ 75 Å^2

This study showed that compounds with a polar surface area less than 75 Å^2 and a calculated lipophilicity (clogP) greater than 3.0 were over six times more likely to be toxic than compounds with a polar surface area greater than 75 and a clogP less than 3.0. An increased lipophilicity may lead to increased toxicity for a number of reasons, including the compound having increased pharmacological promiscuity and non-specific accumulation effects.[63]

The overall theme of these various rules is focused on the control of both lipophilicity (clogP), as well as molecular weight wherever possible during the optimisation process. This last factor was identified as being particularly critical when developing compounds which were designed to be either *in vivo* tool molecules or pre-clinical candidates. It is, however, important to note that these various specific rules were, therefore, being used as guidelines for improved compound design rather than as specific cut-off values. The risks of adhering strictly to one set of rules has also been documented,[64] and using these

various parameters as guidelines to improve the probability of success rather than blanket rules is in line with Lipinski's original intentions.[65]

The importance of lipophilicity is supported by the fact that the parabolic relationship of lipophilicity with oral bioavailability (**Figure 11**) can be rationalised scientifically as well as predicted by computational modelling.[66] Low intestinal absorption for compounds which are very hydrophilic, combined with an increase in first-pass metabolism as lipophilicity increases, drive the parabolic relationship. Although the permeability of biological membranes, and therefore oral absorption of drug molecules, is governed by both passive diffusion and carrier-mediated mechanisms, passive diffusion is probably the primary route of intestinal permeation in most drugs.[67] Intestinal absorption is therefore higher for more lipophilic compounds. The positive effect from lipophilicity of increased absorption is overtaken by an increase in first pass metabolism for more lipophilic compounds due to their greater affinity for enzymes such as cytochrome P450's.[68]

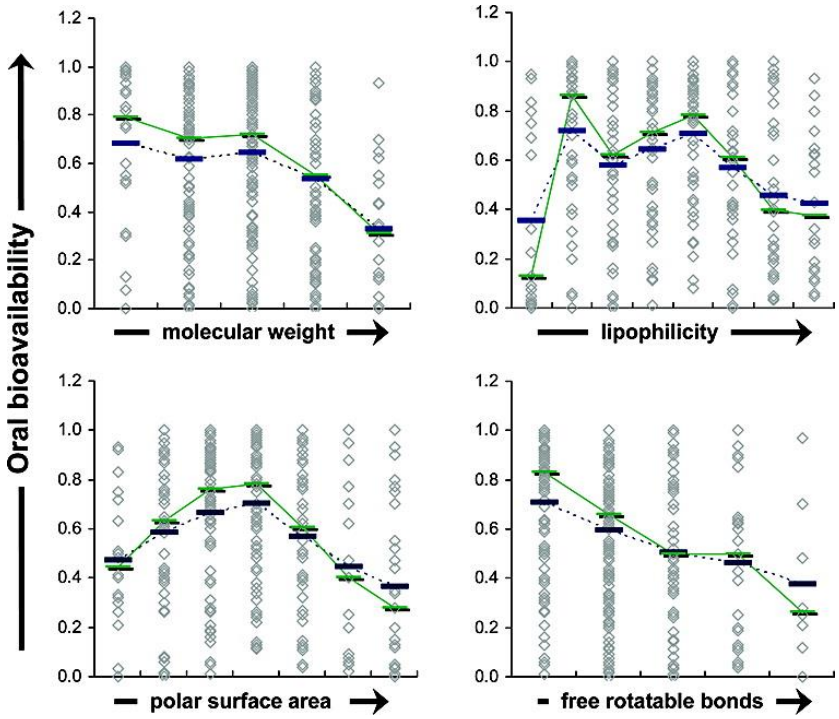


Figure 11.[65] The relationship between oral bioavailability and a range of physicochemical characteristics. The molecular weight range is 200–500; the lipophilicity range is a clogP of -2–5; the polar surface area range is 25–150 Å^2; the free rotatable bonds range is 0-12.

To aid decision making in medicinal chemistry, various descriptors have been proposed which simultaneously monitor potency and physicochemical properties. Commonly used descriptors include ligand efficiency (LE) and lipophilic ligand efficiency (LLE).[68,69]

- **Ligand efficiency** (LE) is a measure of potency with relation to heavy atom count.

$$LE = -\Delta G/HAC \sim (1.36 \times pIC_{50})/HAC$$

- **Lipophilic ligand efficiency** (LLE) is a measure of potency with relation to lipophilicity.

$$LLE = pIC_{50} - clogP$$

ΔG is the change in Gibbs Free Energy, pIC_{50} is the negative logarithm of the half maximal inhibitory concentration, HAC is the heavy atom count, and clogP is the calculated logP.

A typical compound with a pIC_{50} of 7, molecular weight of 350 and clogP of 3 will have an LE of 0.36 and an LLE of 4. The optimum values of LE and LLE depend on the stage of the programme, but during the drug discovery process the aim is to keep both these values as high as possible.

During the last five years, there have been further developments in using physicochemical parameters to reduce attrition during pre-clinical and clinical development. A significant contribution to this area comes from within our laboratories, with the **property forecast index** (**PFI**). This is the sum of a chromatographically measured distribution coefficient mChromlog$D_{pH7.4}$ and the number of aromatic rings.[70] The number of aromatic rings has previously been described as being a factor in compound-related attrition.[71] However, the key development associated with the use of PFI is the incorporation of a chromatographically measured value of lipophilicity rather than an *in silico* prediction. This was shown to significantly increase the correlation of lipophilicity with a number of key factors, including solubility and intrinsic clearance, in comparison with previous approaches.[70] PFI is the reference parameter used

throughout this programme of work (see **Section 3.1.6.** for further information about PFI range in the candidate selection process).

3.1.5. Requirements for oral administration of drugs

When designing a compound where the ultimate aim is the production of a drug treatment for patients, the behaviour of the compound in a whole animal system is critical for its ultimate efficacy. In medicinal chemistry, it is therefore important to control the pharmacokinetic (PK) profile of compounds during the design process. Pharmacokinetics is the study of the absorption, distribution, metabolism, and excretion (ADME) of drug molecules.[72]

To achieve *in vivo* activity from an orally administered drug, the compound must reach the site of action at sufficient concentration, and thus must be orally bioavailable. The main requirements for this are solubility in gastrointestinal fluids, permeability across the gut wall, and low first pass metabolism by the liver.

The requirements for oral bioavailability can be measured separately during drug discovery to aid compound design. **Solubility** may be measured in many relevant systems, the most informative for oral dosing being fasted state simulated intestinal fluid (**FaSSIF**).[73] This provides a measure of solubility relevant to the likely site of oral absorption. FaSSIF solubility is not performed for all the compounds, but all of them are tested in an assay as such as **CLND** (Chemoluminiscent nitrogen detection), which is a HTS assay that measures the solubility of the compounds in DMSO.[74] Nowadays, **CLND** has been replaced in our laboratories by **CAD** (Charged Aerosol Detector), which is also a HTS assay which measures the solubility using an HPLC with a charged aerosol detector.

An estimate of the **permeability** of compounds can be gained using *in vitro* systems, such as artificial membrane permeability assays, Caco-2 cells, and PGP substrate assays.[75] In combination, these can be used to assess the intestinal permeability of molecules. For oral dosing, moderating the clearance of compounds by the liver in the form of first pass metabolism is critical for gaining **good oral exposure**. This can initially be estimated using *in vitro* systems such as **microsomal**,[76] **hepatocyte**,[77] and

S9 fraction[78] **stability assays**. Translation to an *in vivo* animal model allows the rate of **clearance** of the molecule from the bloodstream to be measured, as well as the **half-life** of the compound. The *in vivo* clearance is considered appropiate, moderate, or inappropiate depending on the **liver blood flow** (LBF) of the different species (see **Table 1** & **2**).

A full assessment of a compound's suitability for oral dosing, which combines the parameters described above, can be made by performing an orally dosed *in vivo* PK study. **Oral bioavailability %F** can then be determined, and is a dose-normalised ratio of oral to intravenous **AUC's (areas under the curve)**. The AUC is a measure of the quantity of the drug in the body, and is the total area under the blood/plasma concentration–time curve.[79]

$$\%F = \frac{AUC_{po}}{AUC_{iv}} \cdot \frac{dose_{iv}}{dose_{po}}$$

The AUC measured after oral dosing is impacted by both oral absorption of the compound across the gut wall, and clearance of the compound from the blood. Measuring the bioavailability, by comparing the AUC after oral and intravenous dosing, indicates the fraction of the orally administered dose which enters the systemic circulation and is available to perform its predicted biological effect.

As previously described (**Section 3.1.4**), the PK properties of a compound, such as its oral absorption, may be improved by altering the physicochemical properties of the molecule. If these are not successful, alternative strategies may also be employed, such as the design of prodrugs of the active molecule. **Prodrugs** are pharmacologically inactive, or less active, chemical derivatives of the drug which are transformed within the body in order to release the active drug molecule.[80] The prodrug strategy has inspired the science of targeted drug discovery, where the chemical modification of molecules alters their pharmacodynamic, as well as pharmacokinetic, properties. This is achieved by modifications such as **antibody-drug conjugates**[81] or **esterase sensitive motifs**,[82] which result in molecules being targeted to specific tissues or cell types. In these cases, the aim is to deliver a higher concentration of active compound to tissues of

interest than to the rest of the body. In contrast to the prodrug strategy, the delivered drug molecules may retain a significant level of potency at the biological target.

Within **Sections 3.1.4** and **3.1.5**, parameters for the selection of new starting points for directing the research pathways as part of the drug discovery process have been described. The candidate quality panel (see **Section 3.1.6.**) collects the most representative in order to communicate how the candidate selection process is facilitated by these parameters.[83]

3.1.6. Compound quality panel

The Quality Team within our laboratories[83] has defined a desirable range of values for some key parameters used to assess the quality of the lead compounds and for drug candidates. **Tables 1** & **2** summarises collects the physicochemical, toxicity-related, and biological parameters that are used throughout this work, alongside colour-code criteria, as an easy-way to identify, and follow the progress within the medicinal chemistry process in the identification of a candidate from a Lead Compound.

Most of the parameters are detailed in **Sections 3.1.4** and **3.1.5** with the exception of IC_{50}, hERG and HepG2. Regarding the toxicity, the safety margin must be around 15-20 times the IC_{50} value, this means that Hep G2 (cytotoxicity) or hERG (cardiotoxicity) values have to be 15-20 times over the IC_{50} value to allow consideration that the compound is clean regarding these two types of toxicity.

In the case of the *in vitro* activity (pIC_{50}), the values are determined for individual projects.[84] In this project, an IC_{50}<100 nM is considered favourable, moderate activity is when the IC_{50} is found to be between 100 nM and 1 μM, and activity is unfavourable over 1 μM.

Parameter	Green	Yellow	Red
PFI	≤ 6	6 < PFI < 9	≥ 9
ChromLogD	≤ 4	4 < ChromLogD < 6	≥ 6
Sol. Index (µg/mL)	CLND N/A FaSSIF ≥ 100	CLND >5 FaSSIF 5 < sol < 100	CLND ≤ 5 FaSSIF ≤ 5
Mw (g/mol)	≤ 420	420 < Mw < 700	≥ 700
IC_{50} (µM)	to be determined by team		
Hep G2	pIC_{50} ≤ 4	4< pIC_{50} <4.5	pIC_{50} ≥ 4.5
hERG	pIC_{50} <4.3	N/A, risk score is either red or green	pIC_{50} ≥ 4.3
in vitro Cl (mL/min.g)	≤ 3	3 < Cli < 10	≥ 10
Permeability (nm/sec)	≥ 100	10 < Papp < 100	≤ 10
Protein binding	≤ 90%	90% < %bound < 99%	≥ 99%
In vivo Cl (rat or other def. species, %LBF, Qh) (mL/min.Kg)	≤ 30% Qh	30%Qh < Cl < 70%Qh	≥ 70%Qh
Oral Bioavailability (PO %F)	≥ 70	30 < %F < 70	≤ 30

Table 1. Candidate quality panel.

Liver Blood Flow values (Qh)	mL/min.Kg
Mouse	126
Rat	77.6
Dog	56.1

Table 2. Liver blood flow values.

3.2. PROGRAMME AIMS AND OBJECTIVES

Currently, the development of an effective antimalarial drug is an objective with appreciable challenges. Any emerging entity is required to satisfy a range of factors relating to: safety, efficacy, a new mode of action, and activity against all of the resistant strains isolated in the clinic, whilst, ideally, delivering an overall cost per treatment of less than $ 1. Moreover, with the eradication of the disease in mind, a drug which can act against hepatic and/or sexual forms, as well as mosquito stages, is also being sought. Any of these characteristics would add extra value to the new drug.

In attempts to identify suitable approaches, whole-cell screening was found to be the best choice within our laboratories for the discovery of new antimalarials with a novel mode of action. Our previous experience in the antimicrobial field has shown that it is often difficult to identify potent enzymatic inhibitors that retain whole cell activity.[85] Consequently, in 2009 GlaxoSmithKline (GSK) carried out a HTS of its collection of >2 M compounds. As a result of this, in 2010 the Tres Cantos Antimalarial Set (**TCAMS**)[86,87] was published. This portfolio contains more than 13,000 compounds presenting a growth inhibition over 80% at 2 μM against the 3D7 strain (reference strain used in laboratories to measure the activity against *P. falciparum*). This set (publicly available: http://www.ebi.ac.uk/chemblntd)[88] is a useful source to find new antimalarials, although the identification of high quality hits remains a persistent challenge.

Further additions to the GSK collection from 2010 delivered what is now known within our laboratories as the GSK Top-Up collection (not published). This new set comprises around 10,000 compounds that were selected by their physical chemistry properties, and which might offer new chemical diversity when compared to the historical GSK collection used to build the TCAMS. Moreover, in alignment with the current approaches within drug discovery laboratories, aimed at reducing attrition in clinical phases by selecting appropriate leads at early stages, there is a drive to find molecules in a chemical space in which the physicochemical properties (*e.g.* low lipophilicity, high aqueous solubility) allows leads to be developed with high probability of success in preclinical phases.[70]

Within our laboratories the phenotypic approach is combined with *in vitro* and/or *in vivo* screening platforms. This means that compounds that have shown whole cell activity are also tested in experiments like the PRR assay that determines the mode of action, the murine mouse model to test the efficacy, and a male/female gamete assay, which can determine the transmission blocking potential. Using these tools the compounds are classified in two different categories:

The first TPP, a Single Encounter Radical Cure and Prophylaxis (SERCaP) as proposed by the malERA (malaria eradication) Consultative Group on Drugs in 2011,[89] remains a priority. Such a single-dose treatment would be effective against resistant strains of malaria, cure clinical malaria, stop transmission and prevent relapses. It would also simplify case management and improve compliance.

The second TPP is for chemoprotection drugs for non-infected people entering an area of high-malaria endemicity. The goal is to develop a Single Exposure Chemoprotection (SEC). Compounds used for this indication would need to have distinct mechanisms of action compared to those used for treatment.

3.2.1. Aminoindoles

When the GSK collection was tested against *Pf.*, one compound stood out due to its activity at the asexual stage (*Pf* IC_{50}= 0.07 μM) but also because it was the first compound active against the sexual forms of the parasite (gametocytes GC= 2.6 μM and gametes 0.23/0.83 μM) that was not related to the ATP_4 pathway[90,91] (Ratio 3D7 *vs*. *Pf*ATP4 clones = 1) (**Table 3**).

This compound was GSK2420221A[91] (**3.21**), an aminoindole with the 1-position functionalised with a *p*-chlorobenzyl group and a sulfone in the 3-position.

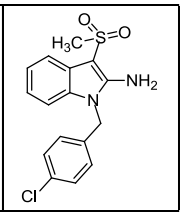
	
	GSK2420221A HIT
	3.21
***Pf* IC_{50} (µM)**	**0.07**
GC *Pf* IC_{50} (µM)	**2.6**
***Pf* male/female IC_{50} (µM)**	**0.23/0.83**
Ratio 3D7 vs. W2, V1/S, 2B12 & *Pf* ATP_4 clones	**1**
Hep G2 XC_{50} (µM)	**>100**
hERG XC_{50} (µM)	**>50.1**
CLND/FaSSIF (µg/mL)	**13/3**
ChromlogD/PFI	**5.3/8.3**
Cl (mL/min/Kg) (Mouse)	**32.4**
%F (Mouse)	**2.2**
ED_{90} (mg/Kg)	**>100**
AUC $_{ED90}$ (µg.h/mL/day)	**0.92**

Table 3. GSK2420221A Profile.

Compound **3.21** presents a poor physicochemical and ADMET profile: low solubility in CLND and FaSSIF, high PFI (ChromLogD + nAr), and very poor oral bioavailability (%F) (**Table 3**). Regarding the toxicity data (cytotoxicity and cardiotoxicity), these are good but, unfortunately, they are not reliable due to the low solubility of the molecule (when the solubility is under 30 µg/mL the toxicity data must not be taken into account). The compound presents a good to moderate *in vivo* clearance in mouse (32.4 mL/min/Kg), however, when this compound was tested in the *in vivo* efficacy study it did not reduce the parasitemia at a dose of 100 mg/Kg (**Table 3**; ED_{90}>100 mg/Kg).

A Hit to Lead programme started with three main issues having to be overcome:

- Is there a SAR associated with a series of compounds or is this just a single effective compound?
- Is it possible to improve the solubility of this class of compounds?
- Does the aniline moiety cause a problem within this compound class? If so, can it be removed or modified?

In attempts to answer those questions, a range of molecules with different substitutions in the 2-position of the indole were synthesized. All these derivatives showed *in vitro* activity (IC_{50} < 400 nM) so it could be concluded that the compound GSK2420221A (**3.21**) was not a singleton hit. The solubility problem was addresed by changing the amino group in position 2 to CH_2NH_2 (GSK3277997A) or by introducing a long chain primary amine (GSK3338579A). These compounds and other examples are represented in **Figure 12**.[84]

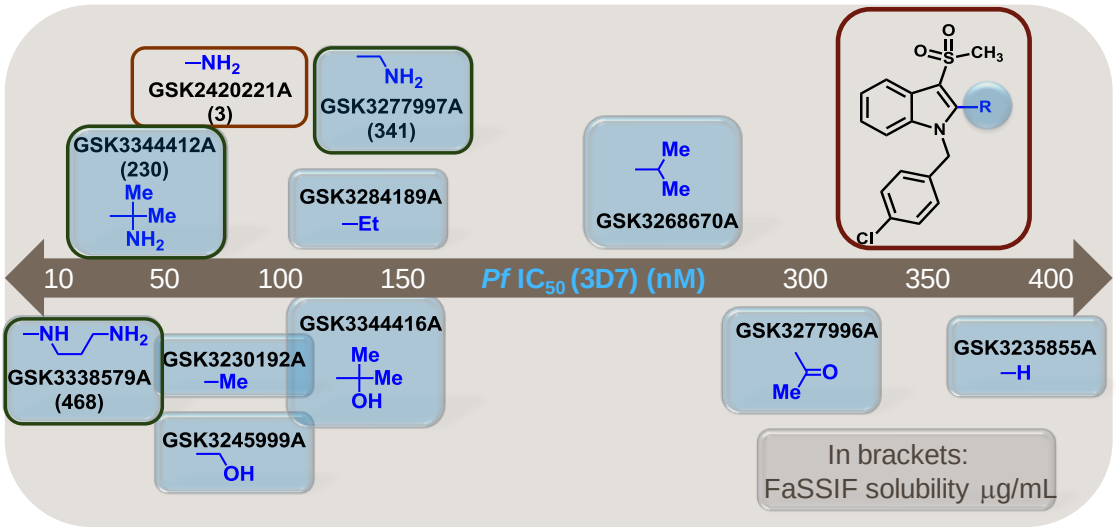
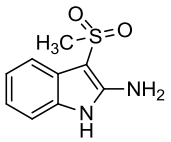


Figure 12. Some derivatives around GSK2400221A.

Also, at the same time, compound **3.21** and the minimum predicted aromatic amine metabolite (3-(methylsulfonyl)-1*H*-indol-2-amine, **3.22**)[92] were assayed in the AMES test[93,94] and were found to be non-mutagenic. Moreover, those compounds did not lose their transmission blocking character.



3.22

Accordingly and based on these additional outputs, a new preferred compound was identified: GSK3359992A (**3.23**).

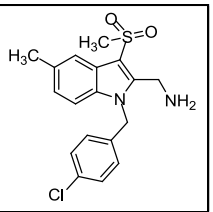
	
	GSK3359992A LEAD
	3.23
***Pf* IC_{50} (µM)**	**0.01**
GC *Pf* IC_{50} (µM)	**0.99**
***Pf* male/female IC_{50} (µM)**	**<0.13/0.07**
Ratio 3D7 vs. W2, V1/S, 2B12 & *Pf* ATP4	**1**
Hep G2 XC_{50} (µM)	**50.1**
hERG XC_{50} (µM)	**31.6**
CLND/ FaSSIF (µg/mL)	**108/ 52**
ChromlogD/PFI	**5/8**
Cl (mL/min/Kg) (Mouse)	**36.4**
%F (Mouse)	**6**
ED_{90} (mg/Kg)	**9.7**
AUC $_{ED90}$ (µg.h/mL/day)	**0.3**

Table 4. GSK3359992A Profile

As shown in **Table 4**, this compound presents an improved solubility (108/52 µg/mL) and *in vitro* activity in sexual and asexual forms (0.01 and < 0.13/0.07 µM). However, some liabilities regarding cytotoxicity (HepG2= 50.1 µM) and cardiotoxicity (hERG= 31.6 µM) emerged, and low oral bioavailability (F= 6%), persisted.

When this compound, which exhibits an *in vitro* activity in the same range as **3.21**, was tested in the *in vivo* efficacy test in *P. falciparum* murine model[95] it reduced completely the parasitemia after 4 days of treatment to a previously infected mouse with *plasmodium falciparum* (ED_{90}= 9.7 mg/kg and AUC=0.3 µg.h/mL/day). In **Fig. 13**, the curve on the top represents the percentage of parasitemia in the mouse that is zero before starting the infection and from the third day, when the treatment starts (100 mg/Kg of compound **3.23** per day for four consecutive days), the parasitemia starts to decrease until it is under the limit of detection at day seven. The curve on the bottom represents the average daily exposure in whole blood of the compound. Consequently the compound **3.23** was declared a Lead in June, 2014.

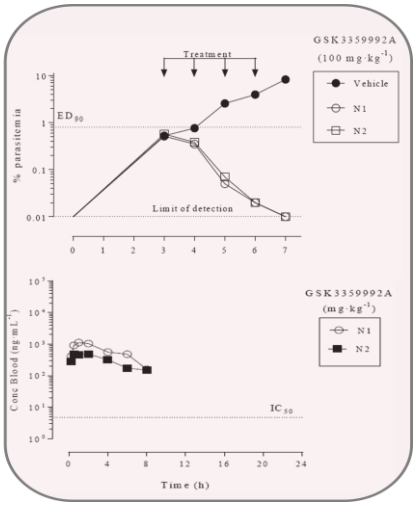


Figure 13. Parasitemia reduction *vs.* Time.

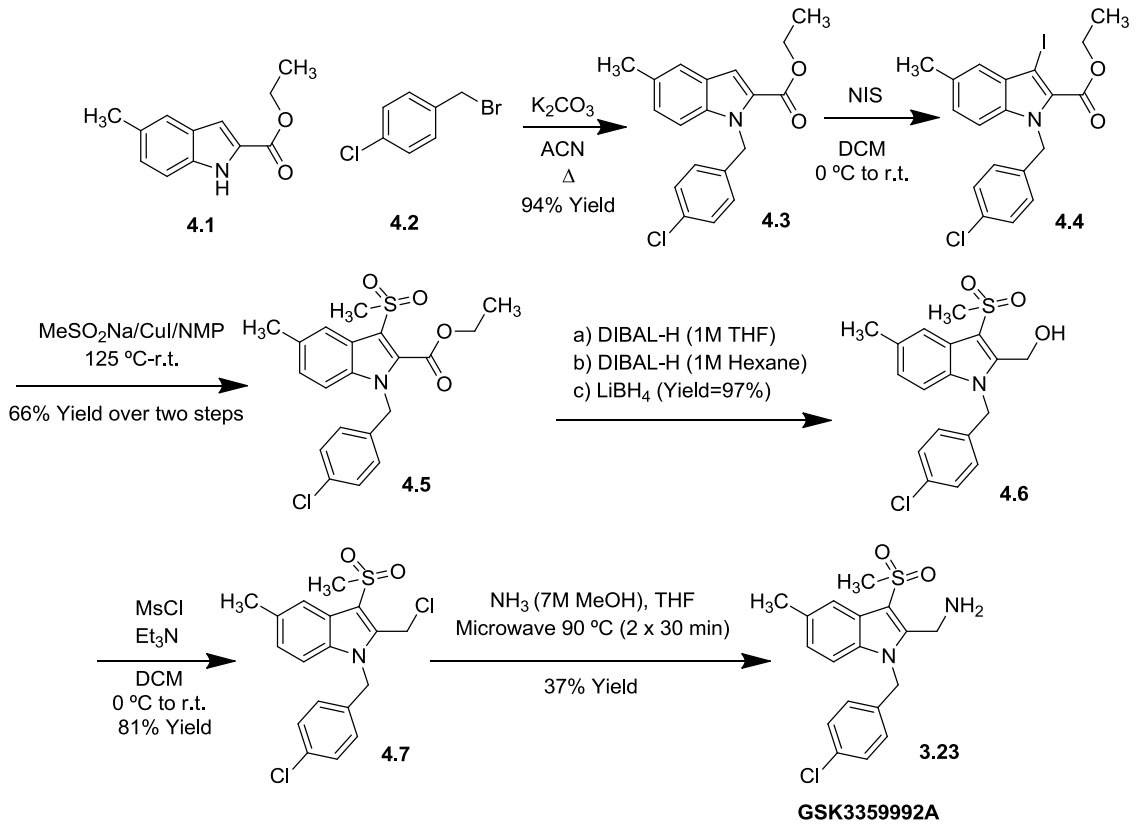
Before the Lead Optimization programme started, the full profile of the Lead (**3.23**) needed to be determined in terms of safety and pharmacokinetics. The most relevant parameters are represented in **Table 1** in order to justify the strategy to be followed in the Lead Optimization.

The first action that was taken within our laboratories was to resynthesize compound **3.23** in 100-200 mg scale. The synthesis of **3.23** was carried out following the same procedure as developed previously within our laboratories[84] (see **Section 4.1**).

4. LEAD OPTIMISATION PROGRAM

4.1. SYNTHESIS OF THE LEAD COMPOUND: GSK3359992A (3.23)

The synthesis of the key molecule **3.23** starts with the *N*-alkylation of the indole **4.1** using *p*-chlorobenzylbromide (**4.2**) as reagent under acetonitrile reflux with potassium carbonate as base. With a very good yield of **4.3** obtained, the next step consists of the iodination on position 3 with *N*-iodosuccinimide in DCM at room temperature to obtain **4.4**. Iodine was substituted by a sulfone group using an Ullmann type reaction.[96] Consequently, the reduction of the carboxylic ester **4.5** was carried out using solid $LiBH_4$ yielding the alcohol **4.6** almost quantitatively; two other reducing agents were applied and these experiments are described in the experimental section. Attempts at introducing a mesyl group afforded the chloride **4.7** instead of the expected mesylate. However, this caused no direct preparative issues since this chloride unit could also be used as a leaving group in the last step of the synthesis, in a nucleophilic substitution using NH_3 to afford **3.23**. The yields in this synthesis are moderate to good, but in the last step the limited solubility of the compound **3.23** was the most likely cause of the low yield (**Scheme 1**).



Scheme 1. Synthesis of GSK3359992A (3.23)

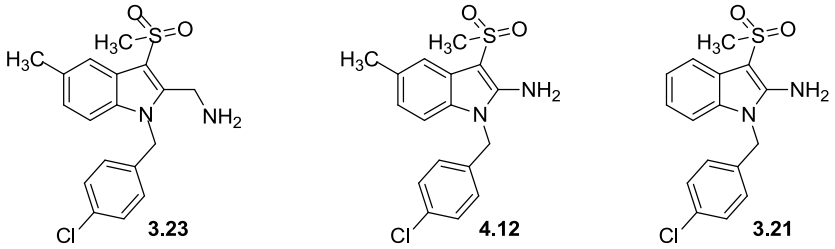
4.2. LEAD OPTIMIZATION PROGRAMME 1st ROUND

Taking into account the physicochemical (low solubility) and pharmacokinetic (poor oral exposure) liabilities of the Lead compound, three different strategies that involved the introduction of polar moieties, the disruption of the planarity of the core to improve the solubility, or the reduction on the number of the aromatic rings to reduce the lipophilicity were designed:

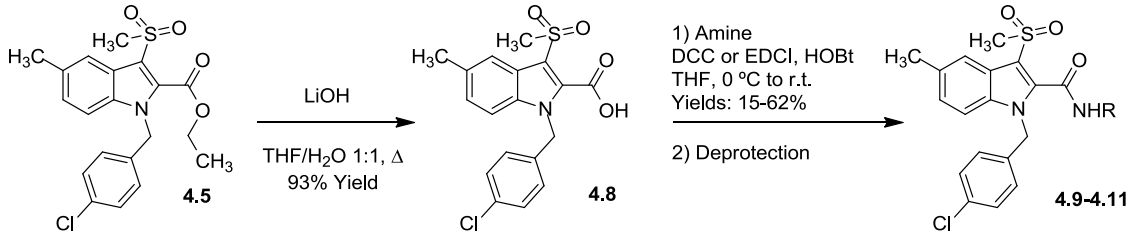
- Amides (type I & II) & Amines (type I & II)
- Breaking the planarity (pyrrole and tetrahydroindole approaches)
- Replacing one or two aromatic rings

4.2.1. Amides

Using the Lead compound (**3.23**, **page 43**) and the 5-methyl derivative (**4.12**) of the Hit (**3.21**, **page 44**) as tool compounds, different polar moieties were introduced in the 2-position in order to avoid the solubility issue presented in **3.23**.



Amides type I: These amides could be compared with the Lead (**3.23**) regarding the substitution in position 2, and, in fact, the synthetic route to obtain them is the same until the intermediate **4.5**, that in the case of the amides is hydrolysed to obtain the carboxylic acid **4.8**. This was then reacted with the corresponding amine using DCC or EDCI with HOBt as coupling conditions in THF at room temperature (**Scheme 2**). The amines used were protected with benzyl or *tert*-butoxycarbonyl groups and the last step of the synthesis was the deprotection to obtain the free amine.



Scheme 2. Synthesis of amides type I

Three compounds were synthesized with this substructure (**Table 5**). All of them show a good *in vitro* activity and better solubility than the Lead (**3.23**). Unfortunately, the compounds **4.9** and **4.10** showed cytotoxicity and cardiotoxicity liabilities. This is probably due to the recognised trend between the presence of a basic amine and cytotoxity; the compound **4.11**, which does not have this moiety, has no issues regarding cytotoxicity.

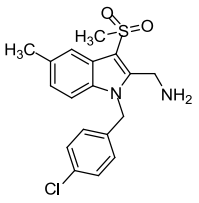
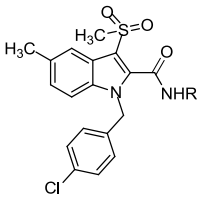
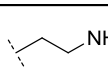
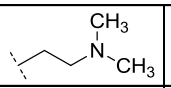
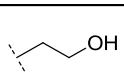
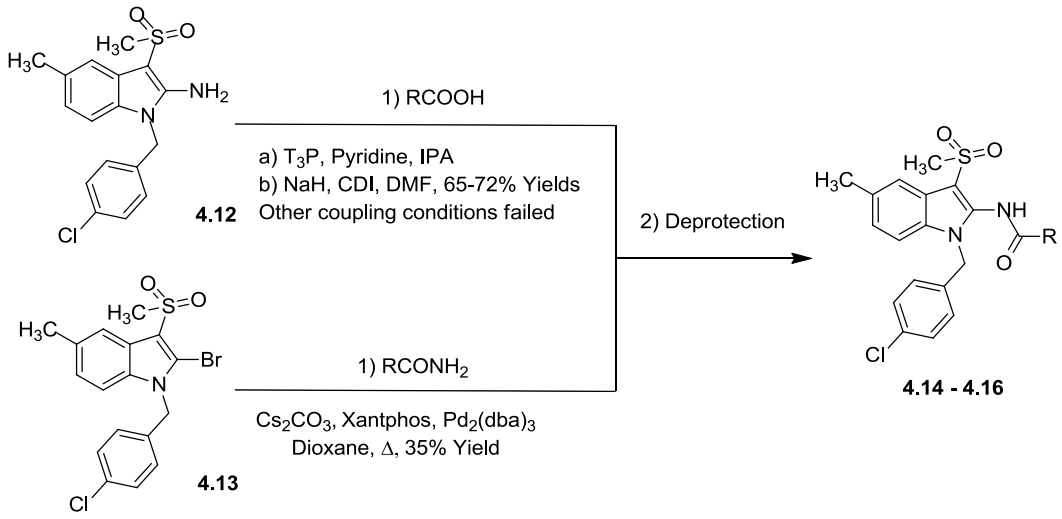
				
	LEAD			
	GSK3359992A	**GSK3390160A**	**GSK3389588A**	**GSK3393599A**
	3.23	**4.9**	**4.10**	**4.11**
Pf IC_{50} (µM)	**0.01**	**0.03**	**0.04**	**0.06**
Hep G2 XC_{50} (µM)	**50.1**	**15.8**	**31.6**	**>100**
hERG XC_{50} (µM)	**31.6**	**39.8**	**3.9**	**>50.1**
CLND/ FaSSIF (µg/mL)	**108/52**	**130/ND**	**185/ND**	**164/131**
ChromlogD/PFI	**5/8**	**3.2/6.2**	**4.4/7.4**	**4.5/7.5**

Table 5. Amides type I.

Amides type II: these amides that have the 5-methyl derivative (**4.12**) of the hit (**3.21**) as a scaffold were prepared using two different synthetic routes (**Scheme 3**):

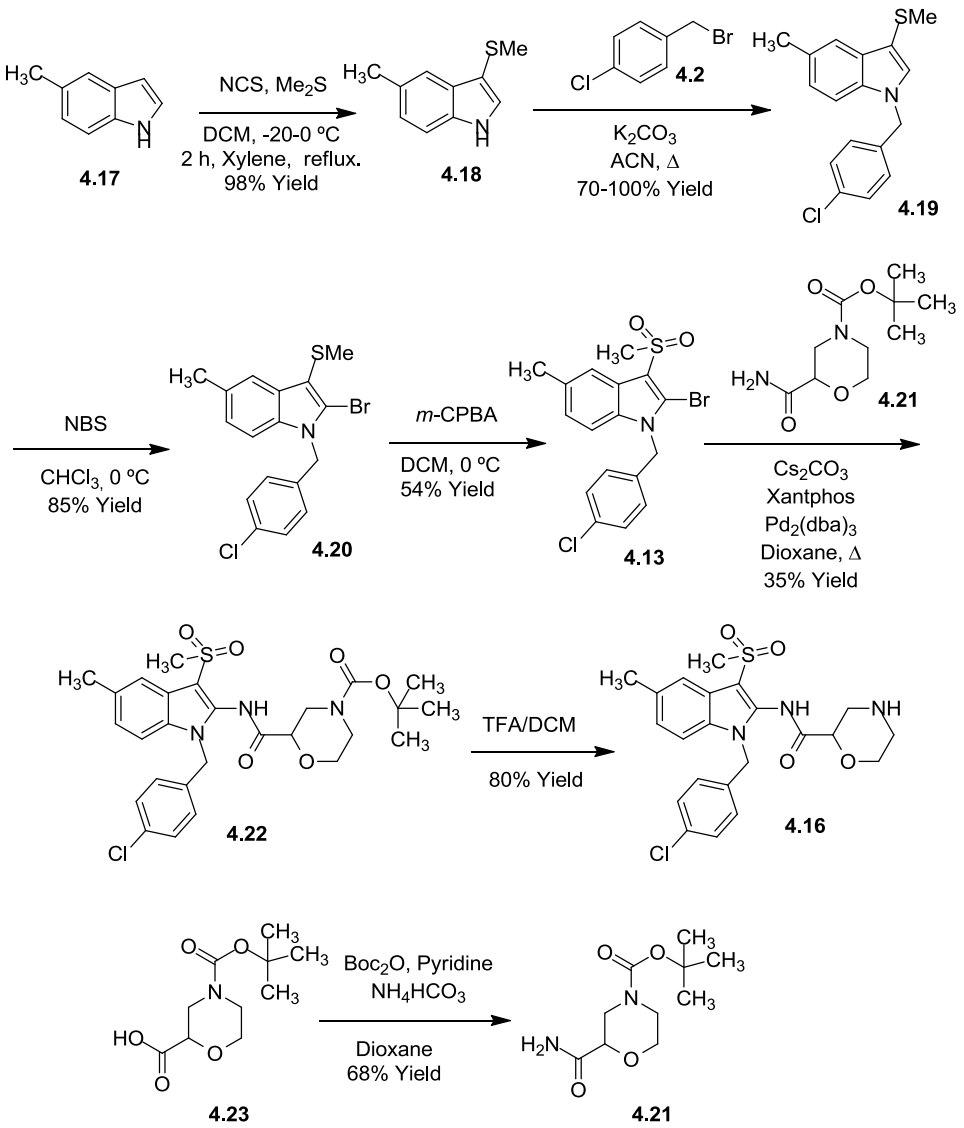
- Using the aminoindole **4.12**[97,98] as starting material and a carboxylic acid gave the corresponding amide (and a by-product). The synthesis of **4.14** and **4.15** by this method was performed elsewhere in our laboratories.[97]

- Buchwald-type reaction[99] between the bromine derivative **4.13** and an amide, followed by a Boc-deprotection, gave the desired compound **4.16**. The advantage of this route is that no by-product was formed as in the previous case.



Scheme 3. Synthesis of amides type II

The synthesis of key intermediate **4.13** and compound **4.16** (**Scheme 4**) started with the introduction of a thioether group on the 3-position of the indole **4.17**,[100] followed by the alkylation of the nitrogen using the benzyl bromide derivative (**4.2**) and potassium carbonate as base in acetonitrile at reflux. The third step is the bromination of **4.19** in position 2 using NBS in chloroform, followed by the oxidation of the thioether group with *m*-chloroperbenzoic acid to give sulfone (**4.13**). This intermediate reacted with amide **4.21** using a Buchwald-type reaction to obtain the amide **4.22** in a moderate yield. Removal of the Boc unit in DCM using TFA as acid gave the compound **4.16** as a TFA salt that was then converted to the free base using an SCX column.



Scheme 4. Synthesis of compound 4.16

Three amides (**Table 6**) with this scaffold were synthesized and all of them (**4.14-4.16**) showed good *in vitro* activity and solubility values, but they were also cytotoxic, as noted with the type I amides that also possess a basic amine unit.

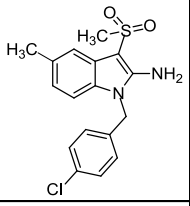
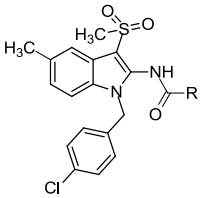
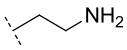
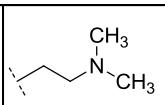
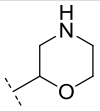
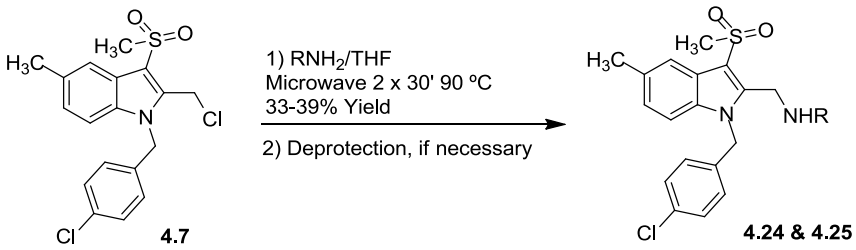
				
				
	GSK3276866A	**GSK3447367A**	**GSK3446876A**	**GSK3448747A**
	4.12	**4.14**	**4.15**	**4.16**
Pf IC_{50} (µM)	**0.02**	**0.01**	**0.01**	**0.02**
Hep G2 XC_{50} (µM)	**ND**	**15.8**	**15.8**	**39.8**
CLND/ FaSSIF (µg/mL)	**23/10**	**130/264**	**205/669**	**170/ND**
ChromlogD/PFI	**5.9/8.9**	**3.5/6.5**	**4.7/7.7**	**4.6/7.6**

Table 6. Amides type II.

4.2.2. Amines

As in the case of the amides, the purpose of this approach is the introduction of polar moieties to improve the physicochemical properties. These amines utilise the same scaffolds employed with the amides and very similar substituents.

Amines type I: these amines are designed based on the structure of the Lead compound **3.23** bearing a longer-chain primary amine in order to improve the physicochemical properties of the Lead in relation to the solubility or the PFI while keeping the *in vitro* activity.



Scheme 5. Synthesis of amines type I

Both compounds (**4.24** & **4.25**, in **Table 7**) were synthesised by a nucleophilic substitution (**Scheme 5**) with the compound **4.7**, as a common starting material, and the corresponding amine in THF using microwave irradiation. The yields were low due to purification being performed by preparative HPLC.

As in the case of the previous amines and amides, the compounds synthesised (**4.24** & **4.25**) presented good *in vitro* activity and lower PFI, but the same liability regarding the cytotoxicity, again, probably due to the basic amine that most of these molecules possess.

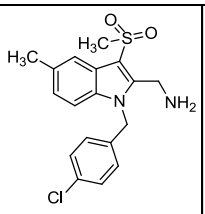
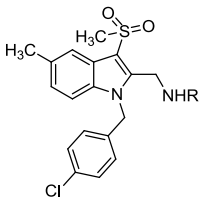
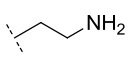
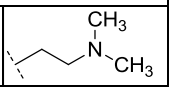
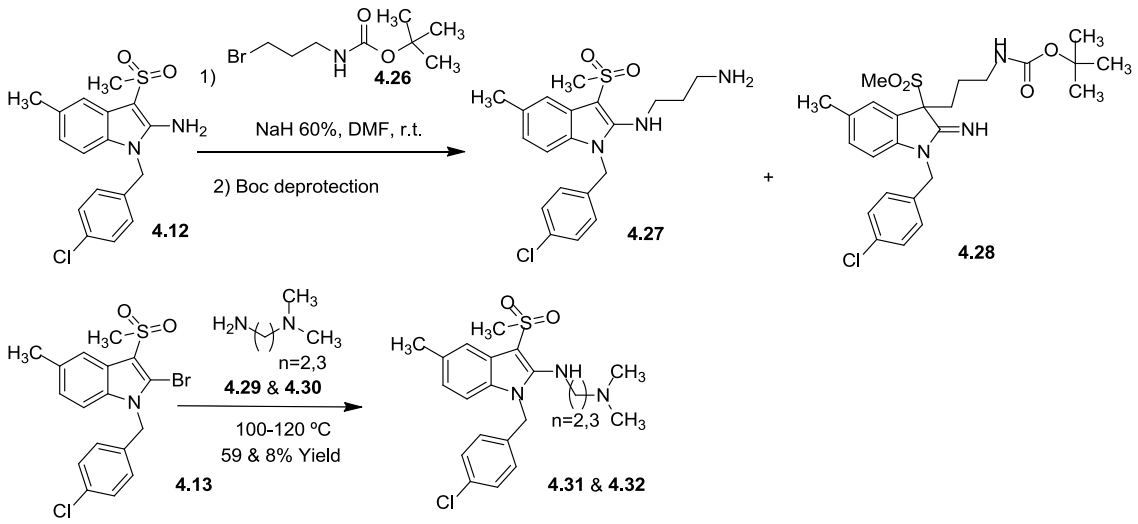
			
	LEAD		
	GSK3359992A	**GSK3390159A**	**GSK3438198A**
	3.23	**4.24**	**4.25**
Pf IC_{50} (µM)	**0.01**	**0.03**	**0.01**
Hep G2 XC_{50} (µM)	**50.1**	**15.8**	**25.1**
hERG XC_{50} (µM)	**31.6**	**25.1**	**ND**
CLND/ FaSSIF (µg/mL)	**108/52**	**50/ND**	**96/ND**
ChromlogD/PFI	**5/8**	**3.5/6.5**	**4.4/7.4**

Table 7. Amines type I.

Amines type II: This class of amines, derived from the 5-methyl derivative (**4.12**) of the hit compound **3.21**, were synthesised using two different routes (**Scheme 6**).



Scheme 6. Synthesis of amines type II.

Compound **4.27** was synthesised elsewhere in our laboratory[101] by a nucleophilic substitution using the aminoindole **4.12** as starting material. The problem with this route is the formation of the by-product **4.28**. As part of this programme, an alternative synthetic strategy was employed using the bromine derivative **4.13** in an aromatic nucleophilic substitution under neat conditions, to provide the desired compounds (**4.31** & **4.32**) in moderate yields but with no by-products.

As with the amides, the amines were designed and synthesised to improve the physicochemical properties and the results were similar: very active compounds were obtained with better solubility than the 5-methyl derivative of the hit (**4.12**)[98](**Table 8**). Unfortunately, the same cytotoxicity issue related to the presence of a basic amine was observed for compounds **4.27**, **4.32** & **4.31** (**Table 8**).

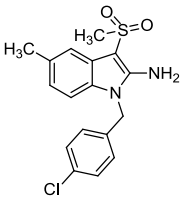
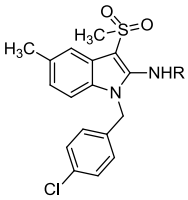
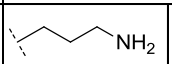
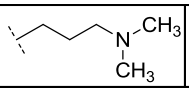
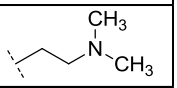
				
				
	GSK3276866A	**GSK3438453A**	**GSK3494130A**	**GSK3454773A**
	4.12	**4.27**	**4.32**	**4.31**
Pf IC_{50} (µM)	**0.02**	**0.009**	**0.01**	**0.001**
Hep G2 XC_{50} (µM)	**ND**	**15.8**	**15.8**	**15.8**
CLND/ FaSSIF (µg/mL)	**23/10**	**121/96**	**121/ND**	**76/ND**
ChromlogD/PFI	**5.8/8.8**	**3.9/6.9**	**4.9/7.9**	**6.4/9.4**

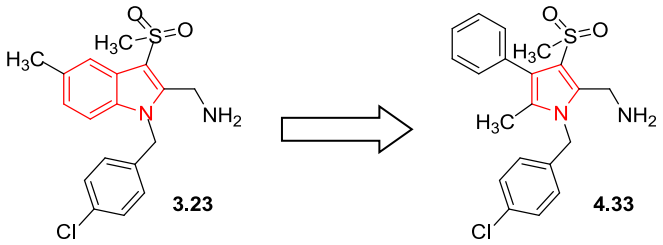
Table 8. Amines type II.

Taking into account the data coming from the compounds derived from the amines and the amides, it is possible to predict a trend between the presence of a basic amine and a cytotoxicity alert.

4.2.3. Breaking the planarity

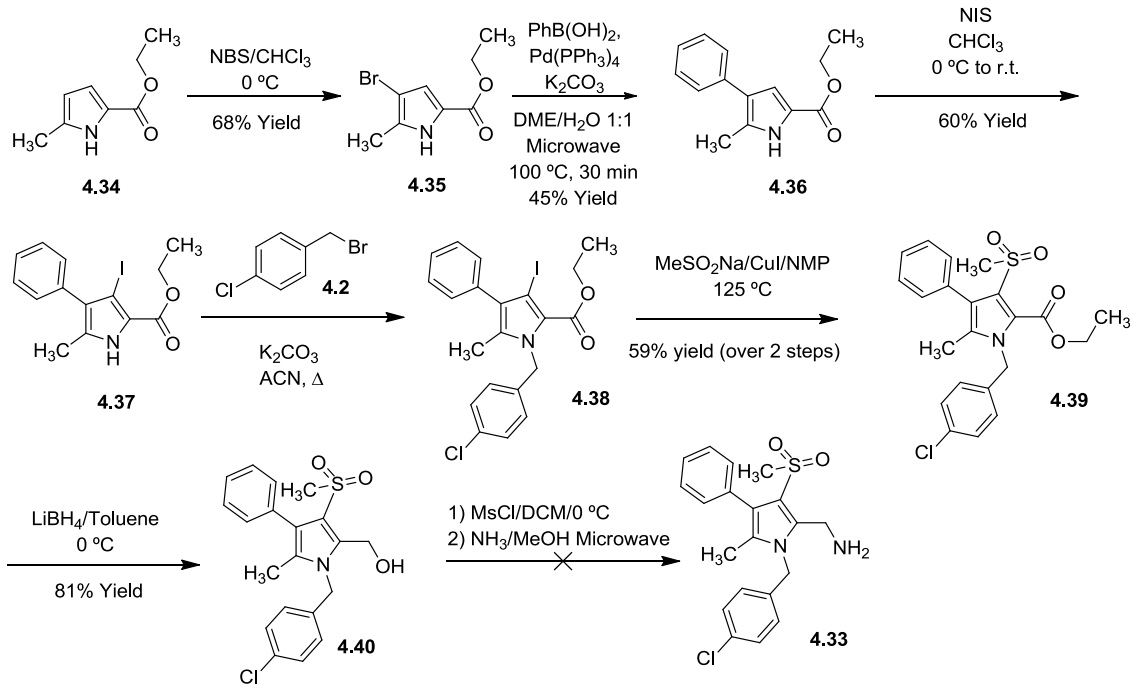
The second strategy consists in breaking the planarity of the indole core in order to gain flexibility and, therefore, improve the solubility of the Lead compound (**3.23**). In order to carry out this general strategy, two approaches were taken as described below.

4.2.3.1. Amend the indole core by replacing it with a monocyclic heteroaromatic ring (Scheme 7)



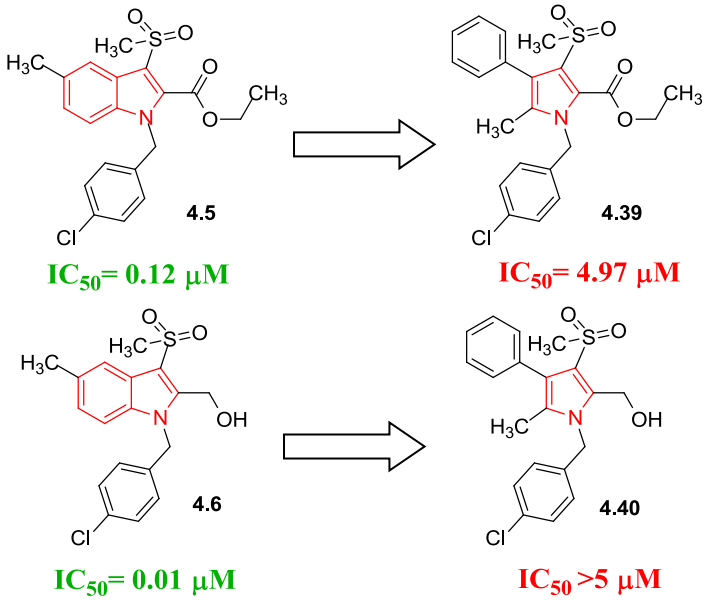
Scheme 7. Replacement of an indole by a pyrrole ring.

The first step in the synthetic route towards **4.33** was the selective bromination[102] of ethyl 5-methyl-1*H*-pyrrole-2-carboxylate (**4.34**) in position 4, followed by Suzuki[103] reaction with phenyl boronic acid using microwave irradiation to provide intermediate **4.36** in a moderate yield. Pyrrole **4.36** reacted with NIS to introduce an iodide in position 3. The next reaction was the *N*-alkylation of **4.37** with 1-(bromomethyl)-4-chlorobenzene (**4.2**). This compound (**4.38**) was then reacted with methanesulfinic acid, sodium salt to substitute the iodide group by the sulfone. A $LiBH_4$ mediated reduction in toluene at 0 ºC gave the alcohol **4.40** in 81% yield.



Scheme 8. Synthetic route to obtain 4.33.

As shown in **Scheme 8**, the last two steps of the proposed synthesis failed when attempted on two occasions. Consequently, other synthetic options were considered. However, the two discouraging biological results detailed below led us to terminate this strategy. Compounds **4.39** and **4.40** were tested and, as shown in **Scheme 9**, the IC_{50}s were around or higher than 5 μM. Comparing these data with the corresponding outputs from the equivalent indole core (**4.5** & **4.6**), it can be seen that the activity has been markedly reduced. Consequently, this general strategy for enhancing solubilility was abandoned.



Scheme 9. Comparison between indole and pyrrole derivatives.

4.2.3.2. Saturation of the homoaryl portion of the indole ring

An approach to amend the homoaryl portion of the indole nucleus was considered in order to keep a bicyclic core while increasing the flexibility and sp^3 character of the central framework in order to probe whether the solubility improves, whilst attempting to decrease the lipophilicity of the molecule. The first compound that was synthesized[104] **4.41** (**Table 9**) was the direct analogue to the Lead compound (**3.23**). Compound **4.41** showed a very high solubility in CLND and FaSSIF, a clean *in vitro* cardiotoxicity profile (hERG> 50 µM), moderate cytotoxicity on HepG2 cells (HepG2> 50 µM), and better oral bioavailability (%F= 22.3). However, the *in vivo* clearance increased from an acceptable value (36.4 mL/min/Kg; LBF_{mouse}= 29%) to a less favourable clearance of 47.2 mL/min/Kg. Based on all of these data, a new set of compounds containing the tetrahydroindole core were designed to try to improve the metabolic stability whilst keeping the antimalarial activity and the good physical chemistry properties and the clean safety profile (see **Section 4.3.2**).

This approach also incorporates the strategy to reduce the number of aromatic rings by one (3rd approach) and, therefore, to reduce the PFI (Chromlog D + nAr rings).

The product **4.41** was prepared elsewhere within our laboratories;[104] consequently it is not described in the experimental section.

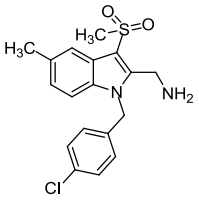
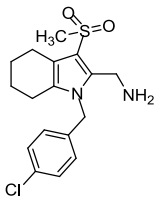
		
	GSK3359992A LEAD	**GSK3398235A**
	3.23	**4.41**
***Pf* IC_{50} (μM)**	**0.01**	**0.03**
Hep G2 XC_{50} (μM)	**50.1**	**50.1**
hERG XC_{50} (μM)	**31.6**	**>50.1**
CLND/ FaSSIF (μg/mL)	**108/ 52**	**141/ 746**
ChromlogD/PFI	**5/8**	**4.4/6.4**
Cl (mL/min/Kg) (Mouse)	**36.4**	**47.2**
%F (Mouse)	**6**	**22.3**

Table 9. Comparison between the Lead (3.23) and the tetrahydroderivative analog (4.41).

4.2.4. Replacing one or two aromatic rings

As noted in the previous section, one of the strategic options within this programme was the replacement of the indole core and this gave us the compound **4.41** (**Table 9**).[104] The other possibility was to replace the benzyl group in position 1 for a non-aromatic moiety. Some analogues were designed and synthesised in this strategy, and one of such compound **4.42**[104] gave a promising result : IC_{50}= 0.99 μM (**4.42**) (**Table 10**). The introduction of a methyl group in position 5 increased the activity by one order of magnitude (**4.43**,[101] IC_{50}= 0.14 μM). When the prolyl chain with a primary amine moiety was introduced in position 2 the potency of the compound reached 0.01 μM (**4.44**)[105] and, most importantly, the PK of this compound were outstanding showing a clearance of 7.1 mL/min/Kg and a half life time of 10 h; the oral bioavailability (F= 24.5%) was also better than that observed in the hit compound **3.21** but, unfortunately, not good enough.

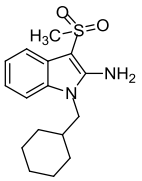
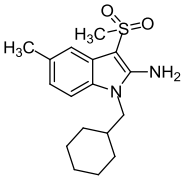
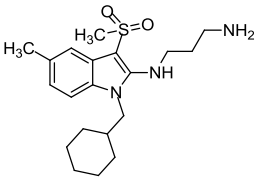
			
	GSK3277843A	**GSK3387392A**	**GSK3408466A**
	4.42	**4.43**	**4.44**
***Pf* IC_{50} (µM)**	**0.99**	**0.14**	**0.01**
Hep G2 XC_{50} (µM)	**ND**	**31.6**	**12.6**
CLND/ FaSSIF (µg/mL)	**50/16**	**31/113**	**90/ND**
ChromlogD/PFI	**6/8**	**6.6/8.6**	**4/6**
Cl (mL/min/Kg)/ t1/2 (h) (Mouse)	**ND**	**86.7/0.6**	**7.1/10**
%F (Mouse)	**ND**	**7**	**24.5**

Table 10. Best results replacing the *p*-chlorophenyl by a cyclohexyl moiety.

Following these results, literature outputs[106] supported the idea of the introduction of fluorine atoms to improve the oral bioavailability. Due to this, two fluorines were introduced in the 4-position of the cyclohexyl moiety (**Table 11**). This change increased the oral bioavailability to 60% (**Table 11**, compound **4.45**)[104] but, unfortunately, the potency was not improved (**Table 11**, compound **4.45**, IC_{50}= 1.36 µM). Accordingly, the same strategy as used before was applied: introduction of a methyl group in position 5 of the indole afforded the compound **4.46**[105,107] that shows an *in vitro* activity of 0.33 µM; when the aliphatic chain is introduced in position 2 the activity increased to 0.01 µM (compound **4.47**)[105,107] in a molecule with a PFI < 6. Compounds **4.44** and **4.47** demonstrated that it is possible to remove one aromatic ring, decrease the PFI, and keep good *in vitro* activity. In contrast and, unfortunately, the introduction of a basic amine increased the cytotoxicity risk. Whilst this was greater than 100 µM in the case of the compounds **4.45** and **4.46**, molecule **4.47** showed a level of 12.6 µM.

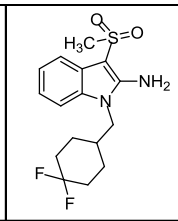
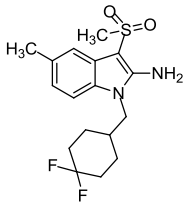
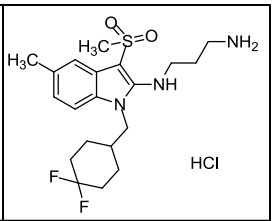
			
	GSK3378052A	**GSK3438655A**	**GSK3447358A**
	4.45	**4.46**	**4.47**
***Pf* IC_{50} (µM)**	**1.36**	**0.33**	**0.01**
Hep G2 XC_{50} (µM)	**>100**	**>100**	**12.6**
CLND/ FaSSIF (µg/mL)	**105/31**	**78/ND**	**131/627**
ChromlogD/PFI	**5/7**	**5.6/7.6**	**3.6/5.6**
Cl (mL/min/Kg)/ $t_{1/2}$ (h) (Mouse)	**59.2**	**ND**	**27.1/6**
%F (Mouse)	**60**	**ND**	**57.4**

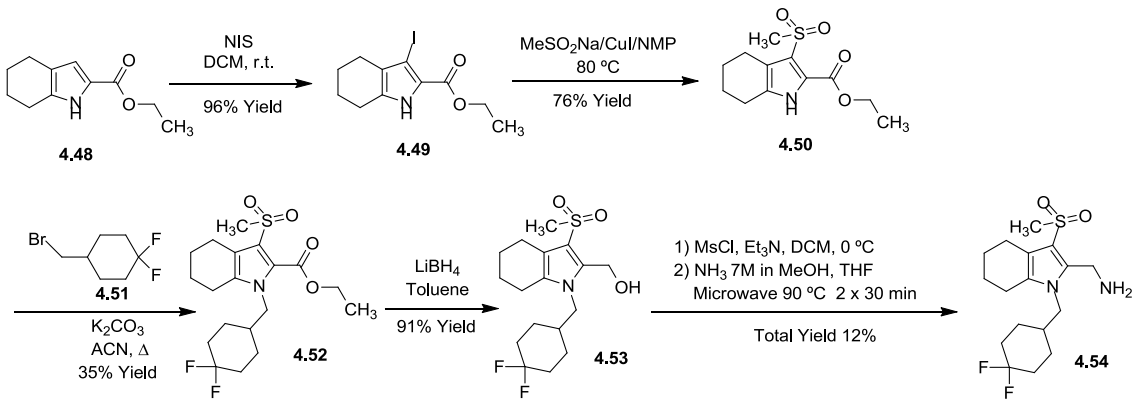
Table 11. Best results introducing two fluorines in the cyclohexyl ring.

Compounds **4.42-4.47** were prepared elsewhere in our laboratory,[107] and are not described in the experimental work.

Removing two aromatic rings: based on the outcomes described above and, in particular, the encouraging profiles of compounds **4.41** and **4.45-4.47**,[104,105,107] the hybrid compound (**4.54**) was designed (**Scheme 10**) and synthesised.

In order to approach the desired compound (**4.54**), a similar procedure was used as had been applied for the synthesis of compound **3.23** using the commercially available 4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid ethyl ester (**4.48**) as starting material and 4-(bromomethyl)-1,1-difluorocyclohexane (**4.51**) (synthesised in 10 g scale within our laboratory)[107] as the electrophilic reagent to be introduced in the 1-position of the molecule. The synthesis started with the introduction of an iodine in the 3-position of the indole **4.48** using *N*-iodosuccinimide as the iodination reagent to give the intermediate **4.49** in a very high yield. An Ullmann-type reaction to introduce the sulfone unit afforded **4.50**. Subsequently, *N*-alkylation using the bromide derivative **4.51** gave the intermediate **4.52** in a moderate yield despite the reaction appearing to be complete by HPLC (basic pH). Further reduction of the ester in the 2-position yielded the alcohol **4.53**. Reaction with mesyl chloride in the presence of triethylamine gave a mixture of two products that were assigned as the mesylate and the chlorine derivative (see compound **4.144** in the experimental section, page 167), the reaction was worked-

up and the crude material was used without any further purification as starting material in the nucleophilic substitution using NH_3 to afford **4.54**.



Scheme 10. Synthesis of compound 4.54.

As shown in **Table 12**, this compound (**4.54**) presents a moderate *in vitro* activity but appreciably low PFI, it is not cytotoxic, and shows a slight improvement in solubility. This scaffold that provides progression into a better chemical space will be taken into account when a more beneficial substituent in the 2-position is established in order to build up a more developed compound.

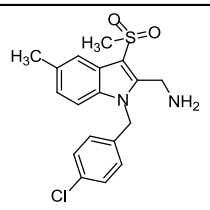
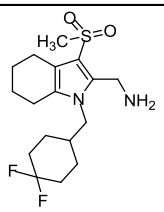
		
	GSK3359992A LEAD	**GSK3480472A**
	3.23	**4.54**
***Pf* IC_{50} (µM)**	**0.01**	**0.27**
Hep G2 XC_{50} (µM)	**50.1**	**>100**
CLND/ FaSSIF (µg/mL)	**108/ 52**	**155/ND**
ChromlogD/PFI	**5/8**	**3.7/4.7**

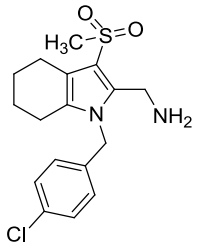
Table 12. Profile of compound 4.54 *vs.* Lead compound 3.23

4.2.5. Best results and conclusions from the first round of the LO programme

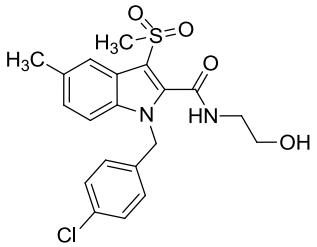
In this first round of this Lead Optimisation programme, a series of new molecules was obtained as part of three strategies, with some promising results having been obtained. In this regard, more soluble molecules were identified, by introducing an amine or an

amide. Moreover, a group of other compounds with improved properties has also been identified. For example, in compound **4.41** the replacement of the indole by a tetrahydroindole core maintained the *in vitro* activity and presented a clean safety profile, while the solubility was also improved. Regarding oral bioavailability, replacement of the *p*-chlorophenyl group in position 1 by a 4,4'-difluorocyclohexyl group has afforded compounds such as **4.47** with an oral exposure (bioavailability) of approaching 60%. Furthermore, a compound (**4.44**) with an outstanding PK profile that presents a very low clearance and a half-life of 10 h in mouse has been identified. We have also identified compounds as **4.11** containing an amide moiety that displayed good *in vitro* activity and did not show cytotoxicity issues.

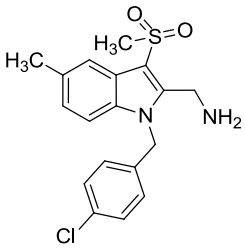
In the **Scheme 11**, the current set of key compounds are shown along with the parameters that have been improved when compared with the Lead compound (**3.23**).



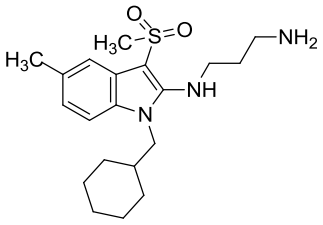
GSK3398235A (4.41)
IC_{50}= 0.03 µM
FaSSIF= 746 µg/mL
PFI= 6.4



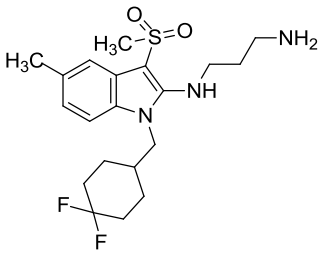
GSK3393599A (4.11)
IC_{50}= 0.06 µM
Hep G2 >100 µM



GSK3359992A (3.23)
IC_{50}= 0.01 µM
Hep G2 = 50.1 µM
CLND/FaSSIF= 108/52 µg/mL
PFI= 8
% F= 6
Cl= 36.4 mL/min/ Kg



GSK3408466A (4.44)
IC_{50}= 0.01 µM
Cl // $t_{1/2}$ = 7.1 mL/min/Kg // 10 h



GSK3447358A (4.47)
IC_{50}= 0.01 µM
F= 57%

Scheme 11. Current front runners and initial Lead GSK3359992A (3.23).

The combination of compound **4.41** that contains a tetrahydroindole core with the 4,4'-difluorocyclohexyl group in position 1, afforded an interesting compound (**4.54**) in terms of developability (PFI= 4.7 and CLND solubility of 155 μg/mL) and cytotoxicity (HepG2> 100 μM). This scaffold could be used in a future with a more developed substitution in position 2 in attempts to improve the *in vitro* activity (IC_{50}= 0.27μM) of **4.54**.

Regarding the synthesis of these compounds, some steps have been optimised, such as the reduction of the ester **4.5**; these new conditions have then been applied in the related synthetic routes. Moreover, some new possible synthetic approaches have been postulated, such as the synthesis of the bromine derivative **4.13** that is used as starting material for the Buchwald reaction that yields amides, and for the aromatic nucleophilic substitution to obtain amines of type II.

As this point, it is possible to conclude that the lack of solubility and low oral bioavailability affecting lead compound **3.23** has been circumvented, and that additional knowledge has been gathered relating to what drives the toxicity in this family of compounds.

From this point, a new series of compounds, taking into account the knowledge gained through the initial portions of this research endeavour, have informed the initiation of the 2nd round in the Lead Optimization programme.

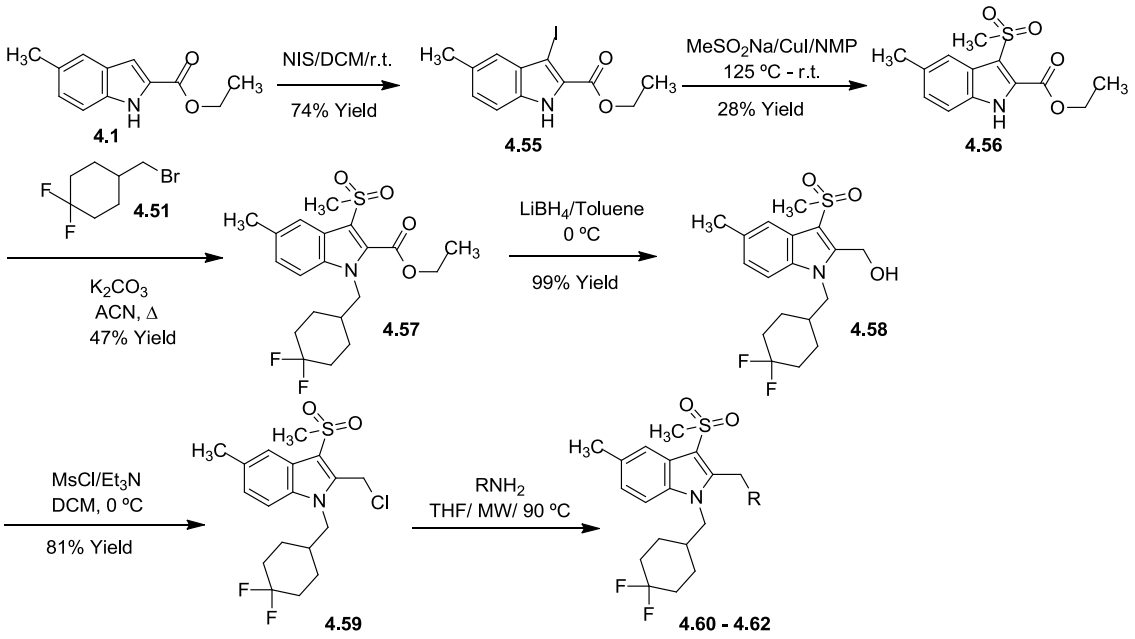
4.3. LEAD OPTIMIZATION PROGRAMME 2nd ROUND

4.3.1. Avoiding toxicity of a series of compounds with a good oral bioavailability

In order to solve the cytotoxicity problem present within compound **4.47** (F= 57%, HepG2= 12.6μM) , some compounds without basic amines were designed.[84] The most interesting substitutions from the amines and amides approaches were tested, as well as the introduction of new moieties in attempts to amend the cytotoxicity issue found in compound **4.47**.

In this piece of work, the amines of type I approach will be applied to this scaffold. The moieties to be introduced were selected due to their low basicity, low molecular weight, and the presence of polar goups such as hydroxyl or carboxylic acid units.

The synthesis of the starting material **4.59** for this approach is described in **Scheme 12**. This route started with the iodination of ethyl 5-methyl-1*H*-indole-2-carboxylate **4.1** in the presence of NIS to yield the intermediate **4.55**. The sulfone was then introduced with an Ullmann-type reaction in a low yield due to the prevalent reductive elimination of the iodine that gave **4.1**; this by-product was detected by mass spectroscometry but it was not isolated. The alkylation with intermediate **4.51** gave **4.57** in moderate yield, despite HPLC analysis (basic pH) showing that the conversion was complete. The reduction of the ester yielded alcohol **4.58** almost quantitatively using lithium borohydrate as the reducing agent in toluene at 0 ºC. Mesylation reaction in attempts to form the mesyl derivative afforded, again, chlorine **4.59**, identified by ^{1}H NMR and LCMS. This intermediate was then used as starting material to synthesise three of the derivatives by nucleophilic substitution of the chloride unit with the appropiate amine (**Scheme 12** & **Table 13**). Derivatives **4.60**-**4.62** were obtained in low yields (**Table 13**) despite the reaction conversion being complete. The low yield is probably due to the purification process as these compounds do not show a high UV absorbance.



Scheme 12. Synthesis of difluorocyclohexylderivatives 4.60-4.62.

Compounds **4.67-4.69** and the corresponding reaction yields are depicted in **Table 13**.

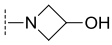
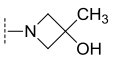
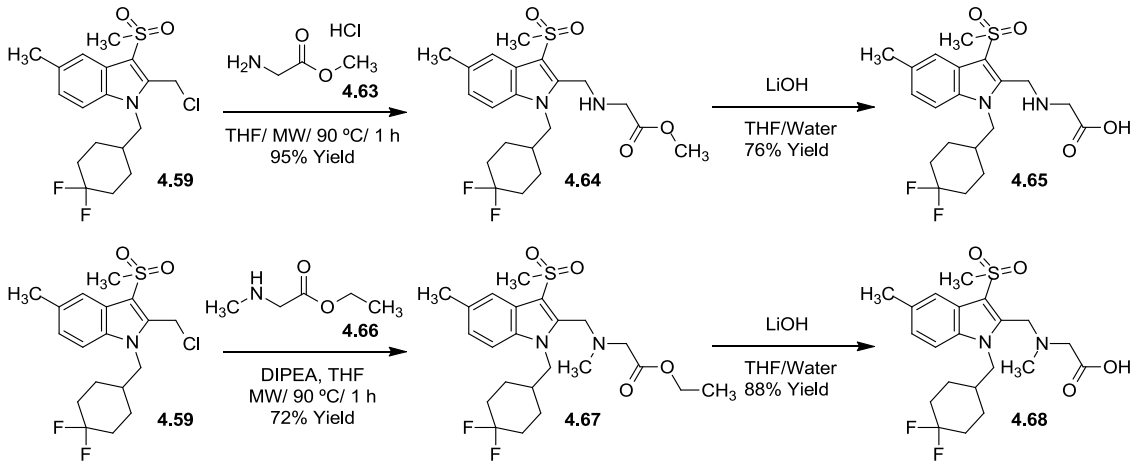
R	Product	Yield (%)
	**4.60**	14
	**4.61**	21
	**4.62**	25

Table 13. Compounds 4.60-4.62.

Two more compounds within this series were designed but the synthesis required further transformations. These compounds contained a carboxylic acid and were synthesised by nucleophilic substitution of the chlorine **4.59** with two different amines (**4.63** & **4.66**) bearing a terminal ester and gave the compounds **4.64** & **4.67**. The hydrolysis of these esters in basic conditions yielded the final acids (**4.65** & **4.68**) in good yield.



Scheme 13. Synthesis of compounds 4.65 & 4.68.

These products (**4.60**-**4.62**, **4.65** & **4.68**; **Table 14**) possessed less basic amines in attempts to obtain active and non-toxic compounds, with the aim of keeping the improved oral bioavailability that the compounds **4.45** and **4.47** showed (60 and 57 %, respectively). Unfortunately, none of the amines synthesised showed activities against *Pf*. under 100 nM. The compounds **4.65** & **4.68**, bearing a carboxylic acid group, lost completely the *in vitro* activity. This effect is probably due to the very low permeability that these molecules exhibited (8.6 and 42 nm/sec, respectively; see **Table 14**). In contrast, and more positively, the hepatotoxicity data reveals that using these amines in other scaffolds with better *in vitro* activity, may lead to avoidance of the cytotoxicity liabilities presented in the compounds with more basic amines.

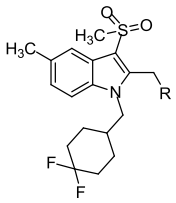
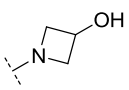
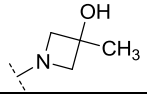
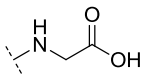
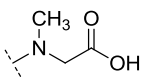
					
					
	GSK3487842A	**GSK3487843A**	**GSK3487252A**	**GSK3488128A**	**GSK3488190A**
	4.60	**4.61**	**4.62**	**4.65**	**4.68**
***Pf* IC_{50} (µM)**	**0.62**	**0.87**	**0.16**	**>5**	**>5**
Hep G2 XC_{50} (µM)	**>100**	**>100**	**>100**	**>100**	**ND**
CLND/ FaSSIF (µg/mL)	**115/ND**	**128/ND**	**144/ND**	**149/ND**	**158/ND**
ChromlogD/PFI	**4.7/6.7**	**5.1/7.1**	**5.8/7.8**	**2.9/4.9**	**3.2/5.2**
$pK_{a\ (theoretical)}$	**7.7**	**4.2**	**4.4**	**8.1**	**6.4**
Papp (nm/sec)	**310**	**260**	**270**	**8.6**	**42**
Cli (m,r,h) mL/min.g	**ND**	**3/4/5.6**	**7.6/7.6/4.4**	**<0.5/<0.5/<0.5**	**<0.5/<0.5/<0.5**

Table 14. Analogs with difluorocyclohexyl designed to avoid cytotoxicity.

4.3.2. Improving the PK profile of compound 4.41, while retaining the solubility and avoiding toxicity

Based on the promising profile of compound **4.41**, regarding its antimalarial activity and its physical chemistry properties, the objective of this approach is to enhance the metabolic stability of this series of compounds and, therefore, to increase the oral bioavailability (%F) and extend the half-life ($t_{1/2}$) that should enable lower and less frequent dosing. A better metabolic stability would also serve to diminish the number and significance of active metabolites, reducing the need for further studies on drug metabolites in animals and humans.[108,109]

There are different strategies in order to enhance the metabolic stability:

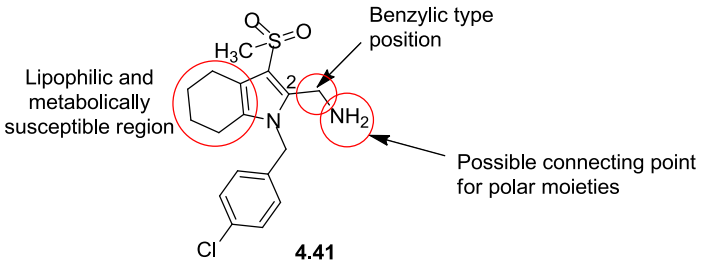
- **Reducing lipophilicity**:[108]

- By the introduction of isosteric atoms or functional groups into the molecule that increase polarity. The incorporation of a heteroatom or transformations to produce a more polar group (e.g. conversion of a ketone into the corresponding carboxamide) could lead to an increased polarity.

- By reducing the logP and logD. This is because the binding site of metabolizing enzymes is generally lipophilic in nature and, therefore, these enzymes more readily accept lipophilic molecules.

- **Blocking metabolically labile groups**:[108,109] a much more elegant strategy towards improving metabolic stability is to remove or to block the vulnerable site of metabolism. For example, sites that have been identified as being potentially labile towards oxidation (*e.g.* benzylic or allylic positions) can be blocked through the introduction of a halogen atom onto the carbon of the site, or by replacing the benzylic CH_2 with an isostere such as an oxygen atom.

Taking into account the possible strategies to improve the metabolic stability, the compound **4.41** was studied in order to design new derivatives that allow these stated main objectives to be reached without losing the antimalarial activity, keeping the physicochemical profile, and avoiding the toxicity issues.



4.41

The first strategy to improve the metabolic stability of the compound **4.41** focuses on the introduction of polar moieties in position 2 of the tetrahydroindole ring in a similar approach to that taken with the Lead compound (**3.23**). Two types of compounds were synthesised: amides and amines.

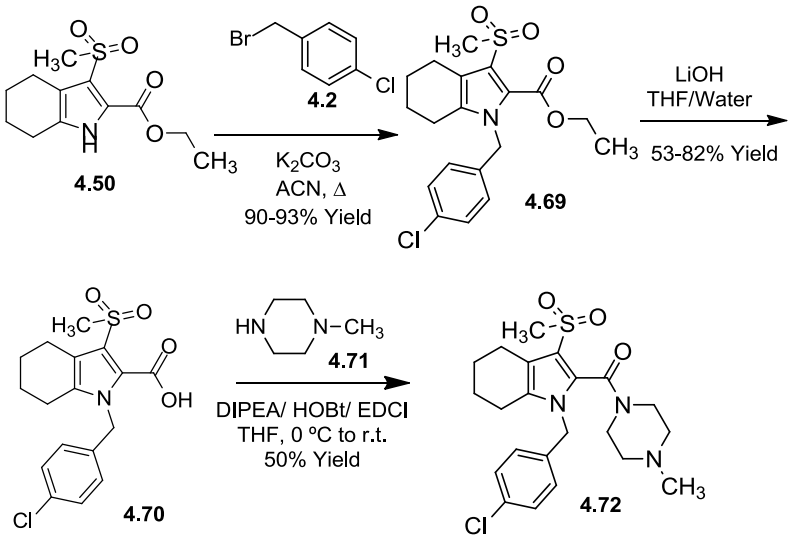
4.3.2.1 Amides

The amines to be introduced to the carboxylic acid **4.70** were chosen based on the predicted basicity and lipophilicity of the final products in order to avoid cytotoxicity and increase solubility, while keeping the metabolic stability, since it has been previously observed that there seems to be a relationship between basicity and cytotoxicity in the compounds included in **Sections 4.2.1** and **4.2.2**.

These amides were synthesised by the coupling between the acid **4.70** and the corresponding amine using HOBt/EDCI and DIPEA as base in THF with moderate to good yields being achieved. The synthesis of the key intermediate **4.70** started with alkylation of the intermediate **4.50** in very high yields, followed by hydrolysis of the

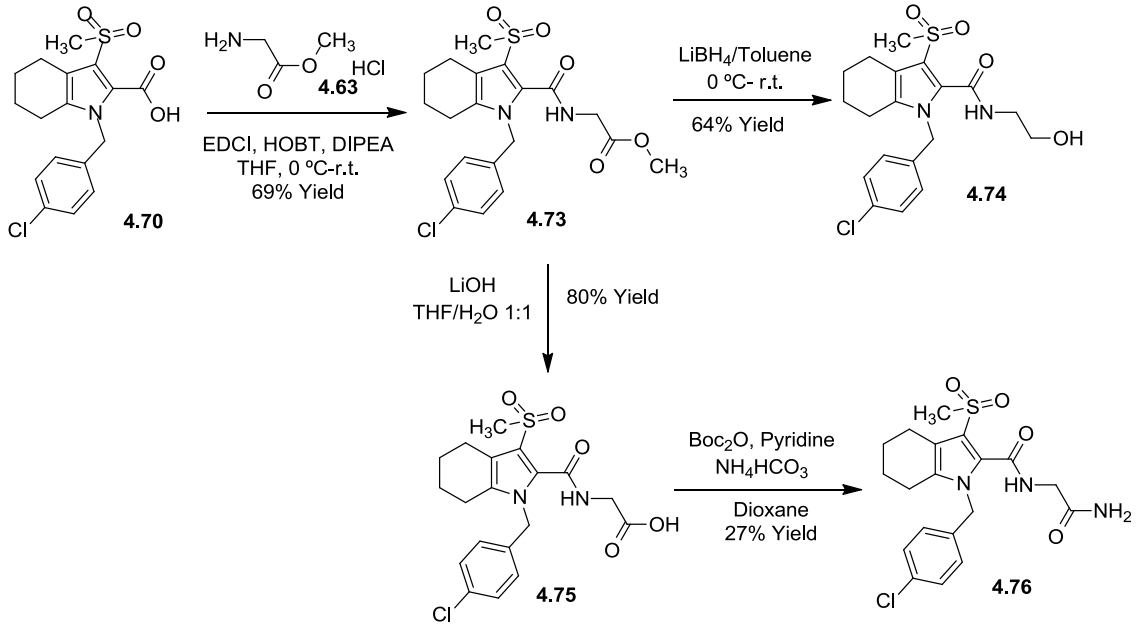
ester **4.69** under basic conditions to yield the carboxylic acid **4.70** in good yields (**Scheme 14**). Amide **4.72** was synthesised by direct coupling between the acid **4.70** and the corresponding amine (**Scheme 14**); for the rest, some transformations were required after the coupling, which are described in **Schemes 15-17**.

Synthesis of amide **4.72** is depicted in **Scheme 14**: acid **4.70** reacted with the amine **4.71** to give the amide **4.72** in a moderate yield using HOBT and EDCI in dry THF as coupling conditions.



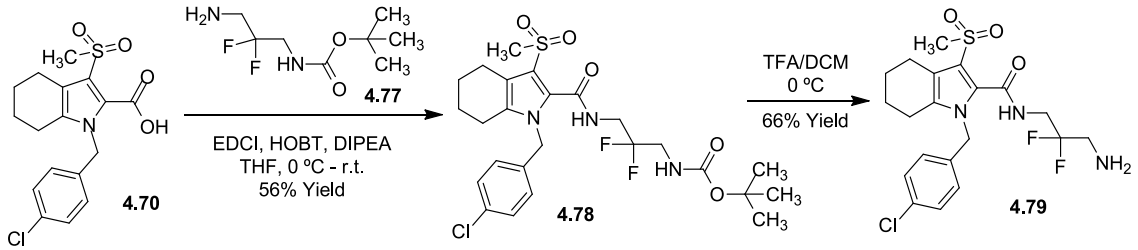
Scheme 14. Synthesis of key intermediate 4.70 and amide 4.72.

Synthesis of amides **4.74** & **4.76** is depicted in **Scheme 15**: acid **4.70** was reacted with the amine **4.63** to give the ester intermediate **4.73** that either was reduced by $LiBH_4$ to give the alcohol **4.74** or hydrolysed to give the carboxylic acid **4.75**, that was then reacted with Boc-anhydride and ammonium bicarbonate to give the amide **4.76** in a low yield due to HPLC purification.



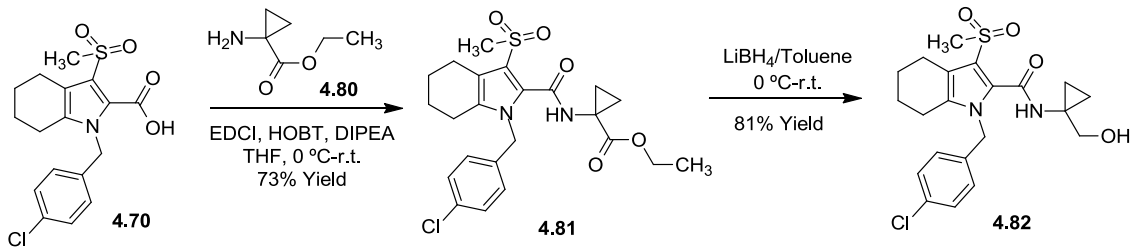
Scheme 15. Synthesis of amides 4.74 & 4.76.

Amide **4.79** was obtained by the Boc-deprotection with TFA of the intermediate compound **4.78**, which was formed by the coupling between carboxylic acid **4.70** and the amine **4.77** (see **Scheme 16**).



Scheme 16. Synthesis of amide 4.79.

For the synthesis of amide **4.82**, the intermediate **4.70** was reacted with amine **4.80** to give the ester intermediate **4.81**, that was reduced with lithium borohydride to afford the alcohol **4.82** in 81% yield (see **Scheme 17**).



Scheme 17. Synthesis of amide 4.82.

The compounds **4.74**, **4.76**, and **4.82** (**Table 15**) do not present any basic amine groups and most of those units possess a polar group in attempts to improve the physicochemical properties of the molecule. The presence of fluorine atoms decreases the basicity of the amine (calculated pK_a = 6.8) by their electron-withdrawing character, as in the case of compound **4.79**. Regarding compound **4.72**, this contains a *N*-methyl cyclic amine with a low basic character (calculated pK_a= 5.3); it is presumed that the pK_a values of the compounds **4.72** and **4.79** are low enough to avoid cytotoxicity issues.

The five amides (**4.72**, **4.74**, **4.76**, **4.79** & **4.82**, **Table 15**) synthesised within this approach, showed good to moderate *in vitro* activity, a clean safety profile regarding hepatotoxicity and cardiotoxicity (Hep G2 and hERG), and good solubility. Unfortunately, these compounds were unstable in microsomes (high intrinsic clearance for the three species). This instability may be due either to the presence of an amide that is metabolised by esterases present in blood (stability in plasma data are not available); or to the intrinsic instability of the tetrahydroindole core.

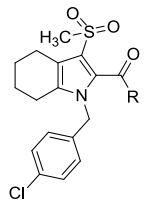
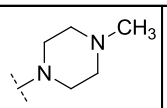
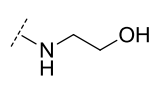
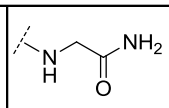
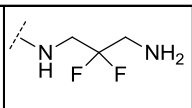
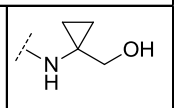
					
					
	GSK3512997A	**GSK3494202A**	**GSK3494131A**	**GSK3494133A**	**GSK3510594A**
	4.72	**4.74**	**4.76**	**4.79**	**4.82**
***Pf* IC_{50} (µM)**	**0.34**	**0.11**	**0.27**	**0.07**	**0.04**
Hep G2 XC_{50} (µM)	**>100**	**>100**	**>100**	**>100**	**>100**
hERG XC_{50} (µM)	**ND**	**>50.1**	**>50.1**	**>50.1**	**>50.1**
CLND/ FaSSIF (µg/mL)	**154/ND**	**157/211**	**183/ND**	**184/333**	**187/50**
ChromlogD/PFI	**5.1/7.1**	**4.4/6.4**	**4.2/6.2**	**4.9/6.9**	**4.8/6.8**
$pK_{a\ (theoretical)}$	**5.29**			**6.76**	
Cli (m/r/h) mL/min.g	**31.1/44.6/25.6**	**10.5/5.5/6.7**	**ND**	**13.5/6.0/1.8**	**17.4/6.9/10.2**

Table 15. Amides 4.72, 4.74, 4.76, 4.79 & 4.82.

The data in **Table 16** shows the effect on the *in vivo* clearance of the replacement of the tetrahydroindole core (**4.41**) by 5-F-indole (**4.83**)[105] or 6-F-indole (**4.84**)[110,111] with the latter two homoaryl units reducing this property considerably. Taking this into account,

other tetrahydro derivatives will be designed in order to obtain more stable compounds (see **Sections 4.3.2.3-4.3.2.5**).

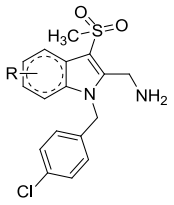
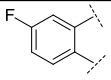
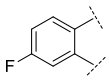
			
			
	GSK3398235A (HCl)	**GSK3400129B (HCl)**	**GSK3396694A**
	4.41	**4.83**	**4.84**
***Pf* IC_{50} (µM)**	**0.03**	**0.04**	**0.09**
Cli (m/r/h) mL/min.g	**1.8/2.2/1.1**	**ND/1.3/ND (Dog <0.4)**	**2.1/1/<0.6**
Cl (m/r) mL/min.Kg	**47.2/53.4**	**12.3/11.2**	**23/18.4**

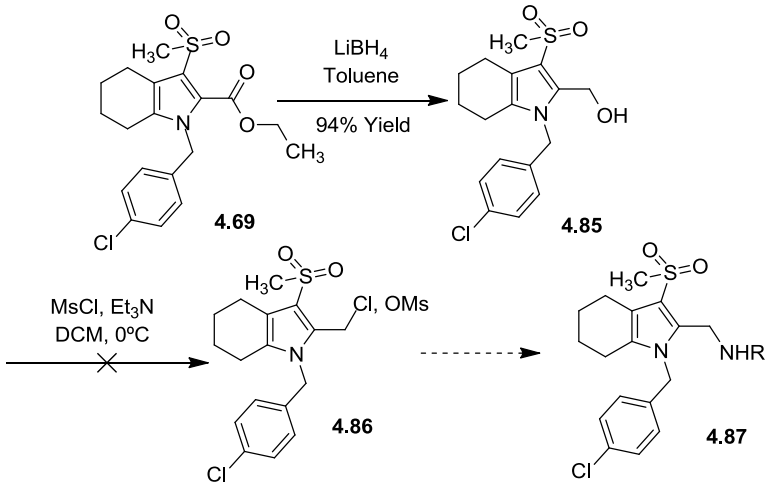
Table 16. Comparison between a tetrahydroderivative and two more stable scaffolds.

4.3.2.2. Amines

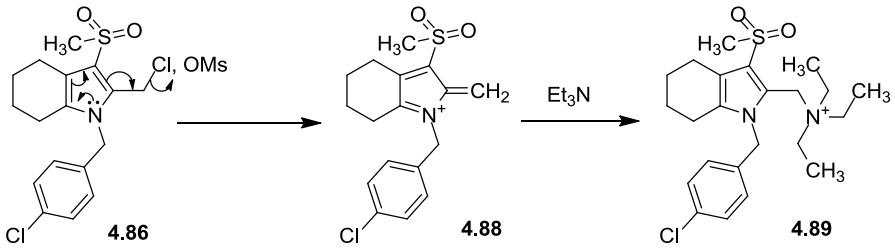
Two amines, already used in the amides approach, were chosen in order to introduce a polar moiety in the 2-position of the tetrahydroindole ring. This structural modification aimed to reduce the lipophilicity and, therefore, increase the metabolic stability whilst keeping the good physicochemical properties of the compound **4.41**, but avoiding the presence of basic amines, which have been related previously with cytotoxicity issues. The compounds' synthesis was approached from two different routes, as described below.

Route A (**Scheme 18**): Started with the reduction of the ester moiety present in the intermediate **4.69** to efficiently yield alcohol **4.85**. When this intermediate was submitted to reaction with mesyl chloride in DCM in the presence of triethylamine, LCMS showed a mixture of products that did not correspond either to the mesylate or the chloride (**4.86**). Both species, mesylate and chlorine, would yield expected product **4.87**. A possible explanation for this failure, is that the chloride or mesylate, that are both good leaving groups, are removed from the core as it is described in **Scheme 19** forming a very reactive iminium species (**4.88**) that could give many secondary products from further reaction; one example is shown in **Scheme 19** by reaction with triethylamine to give **4.89**, which was observed in the reaction mixture. This reaction

with mesyl chloride was performed twice with the same outcome and, accordingly, this route was abandoned.



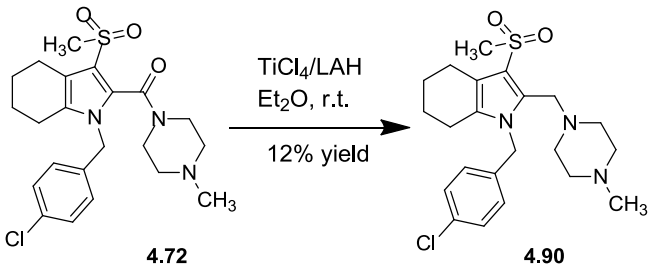
Scheme 18. Route A to synthesise the amines.



Scheme 19. Mechanism of leaving group cleavage and possible by-product formed in the reaction.

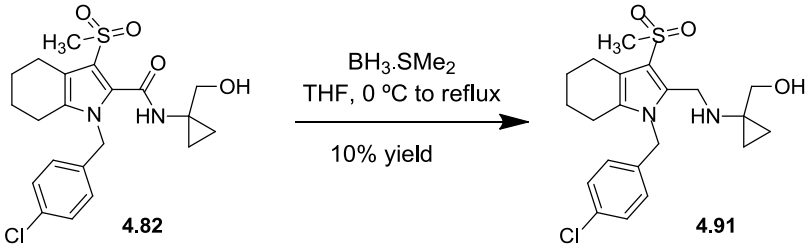
The mechanism shown in **Scheme 19** could be applied to explain the failed reaction in the pyrrole approach (see **Section 4.2.3.1**), as well as the very low yield in the last step of the synthetic route to obtain the compound **4.54** (bearing a tetrahydroindole core), as detailed in **Section 4.2.4**.

Route B: consisted of the reduction of the carbonyl moiety of the amides **4.72** & **4.82**. Amine **4.90** was obtained by reduction of the carbonyl group of the amide **4.72** that was dissolved in anhydrous Et_2O using LAH as reducing agent in the presence of $TiCl_4$, that enhances the electrophilic character of the carbonyl portion of the amide (**Scheme 20**).



Scheme 20. Synthesis of amine 4.90.

As the conditions used to reduce the amide **4.72** provided the amine **4.90** in a poor yield, a different reducting agent was used with the amide **4.82**. A previously cooled at 0 ºC solution of amide **4.82** in dry THF, was treated with borane-dimethylsulfide complex in THF to give amine **4.91** also in a very low yield (**Scheme 21**).



Scheme 21. Synthesis of amine 4.91.

The two amines (**Table 17**) prepared as part of this endeavour, showed very good *in vitro* activity. However, the less than optimal *in vitro* stability of all the tetrahydroindole derivatives described in this section, confirmed the need for a new scaffold design that can address this instability.

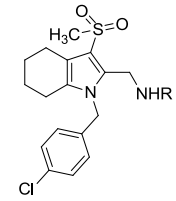
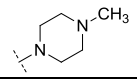
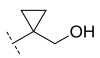
		
		
	GSK3519977A	**GSK3513311A**
	4.90	**4.91**
***Pf* IC_{50} (µM)**	**0.04**	**0.005**
Hep G2 XC_{50} (µM)	**31.6**	**>100**
CLND/ FaSSIF (µg/mL)	**165/ND**	**98/ND**
ChromlogD/PFI	**5.4/7.4**	**6/8**
pKa (theoretical)	**6.5**	**7.06**
Cli (m/r/h) mL/min.g	**9.8/10.4/4.5**	**43.7/36.2/26.4**

Table 17. Amine derivatives (4.90 & 4.91).

As the introduction of polar moieties in the 2-position of the tetrahydroindole core did not afford any appreciably promising outcome, a more challenging strategy (from a synthetic point of view) was envisaged: the introduction of polar moieties in the tetrahydroindole ring.

4.3.2.3. Reducing lipophilicity by introducing polar moieties in the tetrahydroindole core.

As previously mentioned, the reduction of the lipophilicity is one of the possible strategies to improve the metabolic stability. In this approach, it is proposed that the polarity is introduced in the tetrahydroindole ring aiming to stabilise the saturated part of the core (see **Figure 14**).

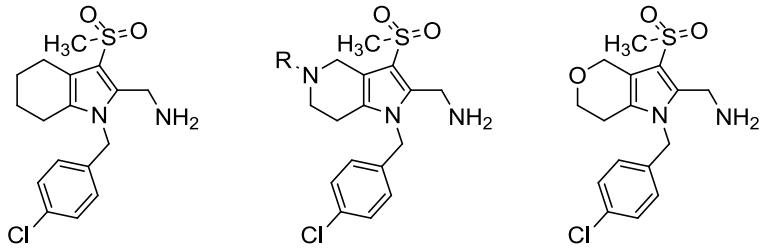


Figure 14. Reduction of the lipophilicity by introducing polar moieties in the tetrahydroindole ring.

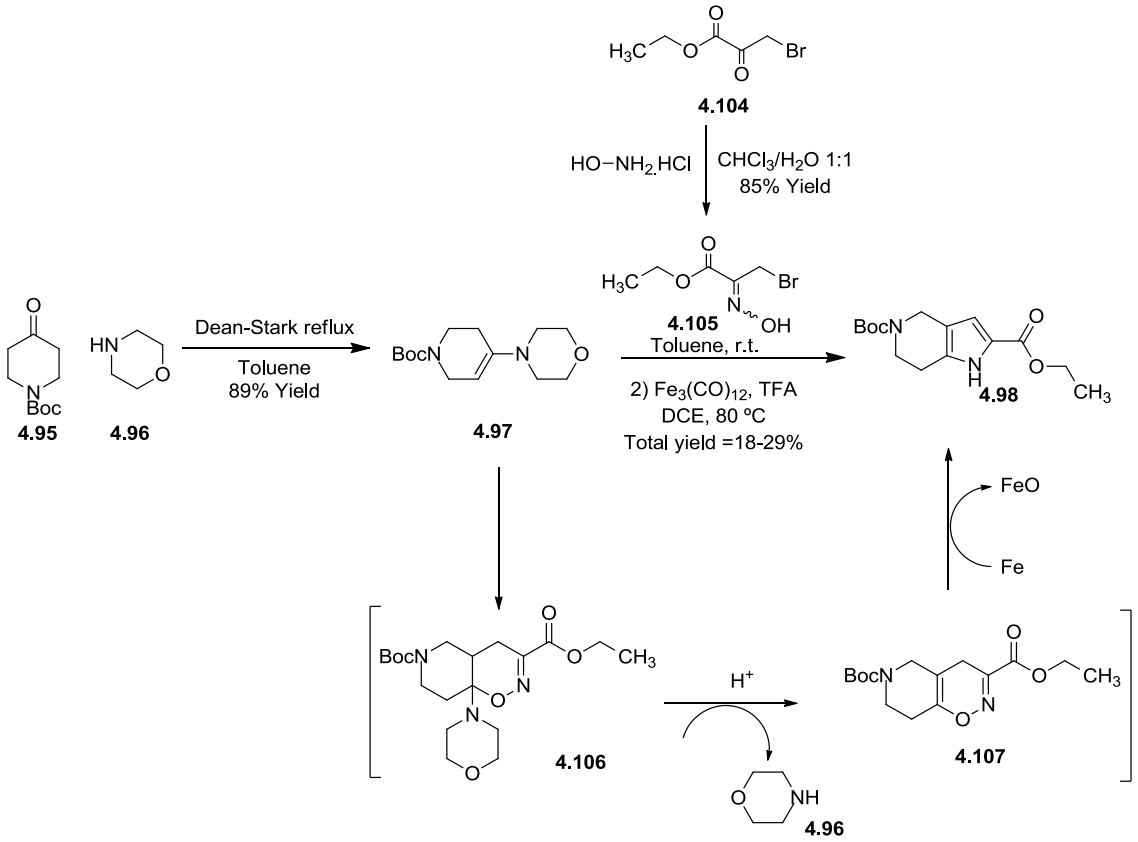
In the case of the introduction of a nitrogen, two compounds were designed: the NH in position 5 (compound **4.92**) and the corresponding methylated derivative **4.93**. The calculated ChromlogD for both compounds showed an important reduction of 3.4 and 2.5 units, respectively, comparing with our reference compound **4.41**. The compound **4.94**, that contains an oxygen on the 5-position of the tetrahydroindole ring also showed an important decrease on the theoretical chromlogD.

- ***Synthesis of compounds 4.92 & 4.93 (Scheme 22 & 23).***

The synthesis started with the reaction between *tert*-butyl-4-oxopiperidine-1-carboxylate and morpholine under reflux in a round bottomed-flask equipped with a Dean-Stark apparatus and a condenser to give the reactive enamine **4.97** along with the remaining starting materials in a good yield. The next step consisted of reaction between **4.97** and **4.105** that were stirred at room temperature for 3 h in toluene to form the intermediate **4.106** (see **Scheme 22** for **4.106** and a plausible reaction pathway). The reaction mixture was quenched with water and the phases were separated in a funnel. TFA and $Fe_3(CO)_{12}$[112] were added and the mixture was refluxed for 3 h in DCE to give

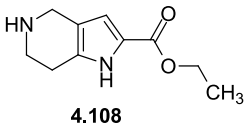
a dark solution that was filtered, and the mother liquors were evaporated under vacuum to give a crude product that was purified by flash chromatography to provide **4.98** in low yield.

The mechanism to obtain the compound **4.98** from the intermediate **4.106** is not confirmed, but a plausible pathway is depicted in **Scheme 22**. As shown, protonation of the compound **4.106** and elimination of morpholine to form the double bond (**4.107**) is followed by the reductive elimination of oxygen catalysed by the iron complex $Fe_3(CO)_{12}$ to give the desired compound **4.98**. FeO is removed from the reaction mixture by filtration.



Scheme 22. Synthesis of intermediate 4.98.

The presence of trifluoroacetic acid in the reaction mixture could have caused the deprotection of the Boc group. However, the product **4.108** was not detected nor isolated.



Intermediate **4.105** was obtained as 1:0.15 mixture of *E*/*Z* isomers by the reaction between the pyruvate **4.104** and hydroxylamine hydrochloride, in a very good yield. The computational modelling[113] (see **Figure 15**) of the *E*- and *Z*-isomers of **4.105**, using the computational program MOE (Molecular Operational Environment), showed that the *E*-isomer was the most stable (33.6 Kcal/mol) from both isomers. This is probably related to the hydrogen bond between the hydrogen of the hydroxy group and the oxygen of the ester moiety that occurs in the *E*-isomer, but does not happen in the *Z*-isomer. The proportion between both forms was calculated based on ^{1}H NMR peak integration.

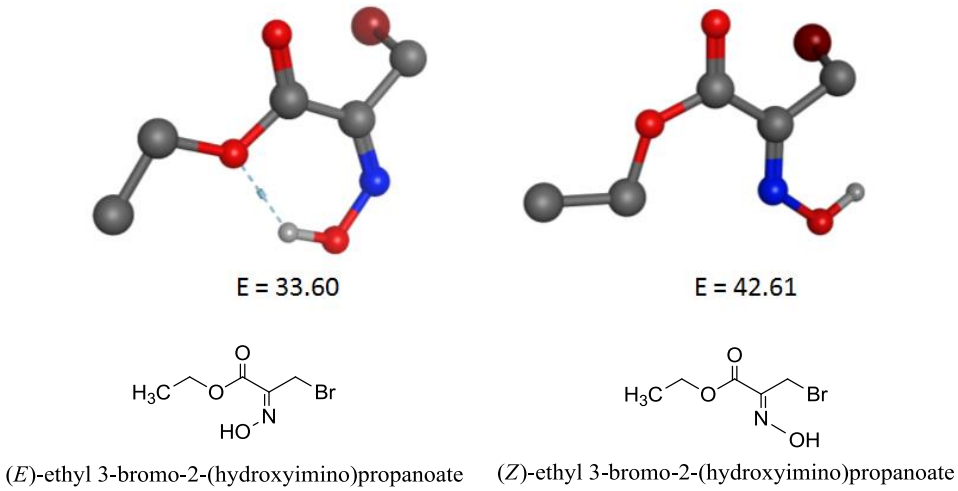
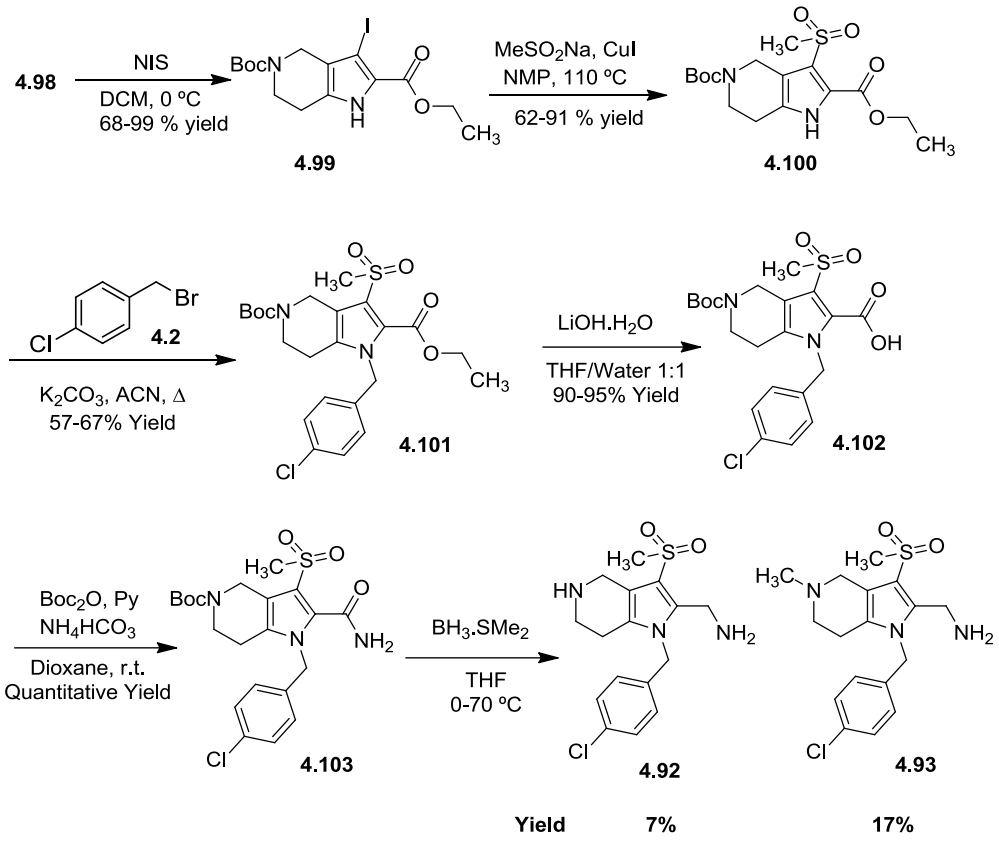


Figure 15. MOE and ChemDraw representations of *E*- & *Z*-isomers of 4.105.

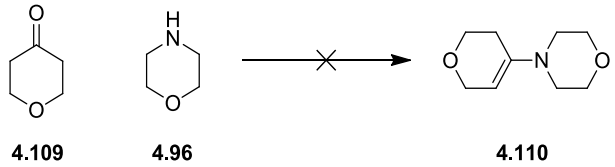
In turn, **4.98** was reacted with NIS to give the intermediate **4.99** in very good yield along with the reactant **4.95** in a proportion of 9:1 by ^{1}H NMR. This mixture was subsequently submitted to an Ullmann-type reaction to introduce the methylsulfone moiety in moderate to very good yield. The remaining **4.95** was eliminated in the purification. Alkylation with **4.2** gave ester **4.101** that was hydrolysed with lithium hydroxide to give the carboxylic acid **4.102**. The acid was reacted with Boc_2O in the presence of pyridine to form a reactive mixed anhydride, that reacted further with hydrogen ammonium carbonate to give the amide **4.103**. The amide moiety was reduced in the presence of the borane dimethylsulfide complex in THF under reflux to give the amines **4.92** & **4.93** (**Scheme 23**).



Scheme 23. Synthesis of compounds 4.92 & 4.93.

Synthetic efforts to obtain the compound 4.94.

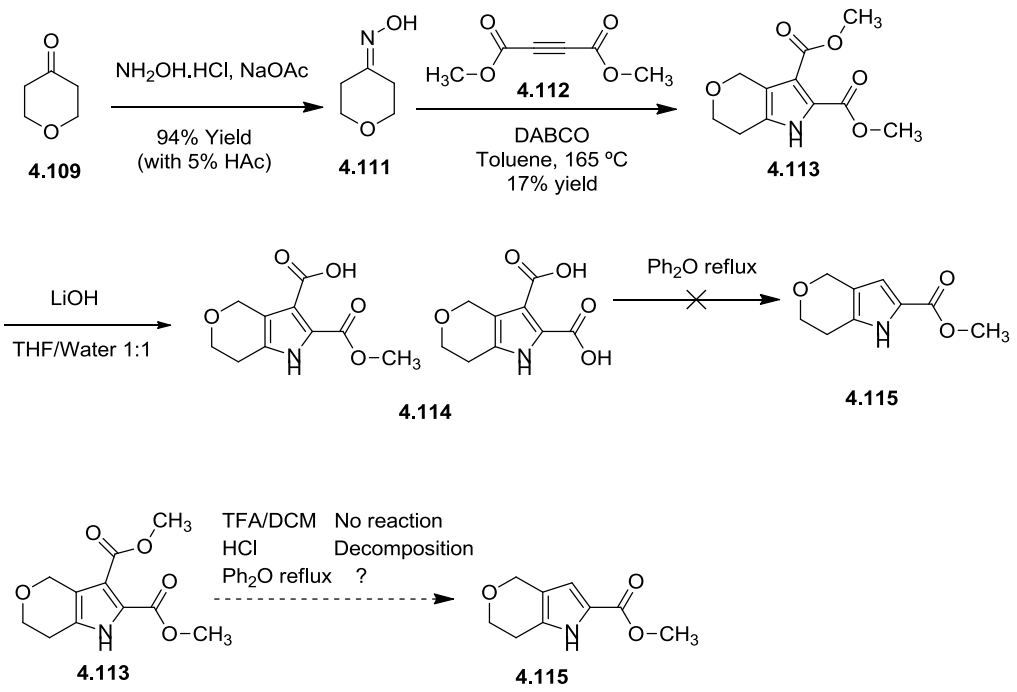
The same synthetic route employed to obtain the key intermediate **4.98** was applied to obtain the equivalent intermediate with an oxygen in the 5-position of the bicyclic structure. Two unsuccessful attempts to obtain the enamine **4.110** (**Scheme 24**) by reacting the appropiate ketone and morpholine were carried out at refluxing conditions (using a Dean-Stark apparatus and a condenser). The reaction yielded a complex mixture of products but the starting material was pure by ^{1}H NMR so a further synthetic approach was required.



Scheme 24. Failed reaction to obtain the desired reactive enamine 4.110.

A deeper literature search of reaction conditions to obtain these compounds revealed the Trofimov reaction. In particular, authors have used the nucleophilic catalyst DABCO in

the reaction between an oxime[114,115] and an alkyne to give a 1,2-dicarboxylic compound. These conditions were adapted to obtain the intermediate **4.113** (see **Scheme 25**).



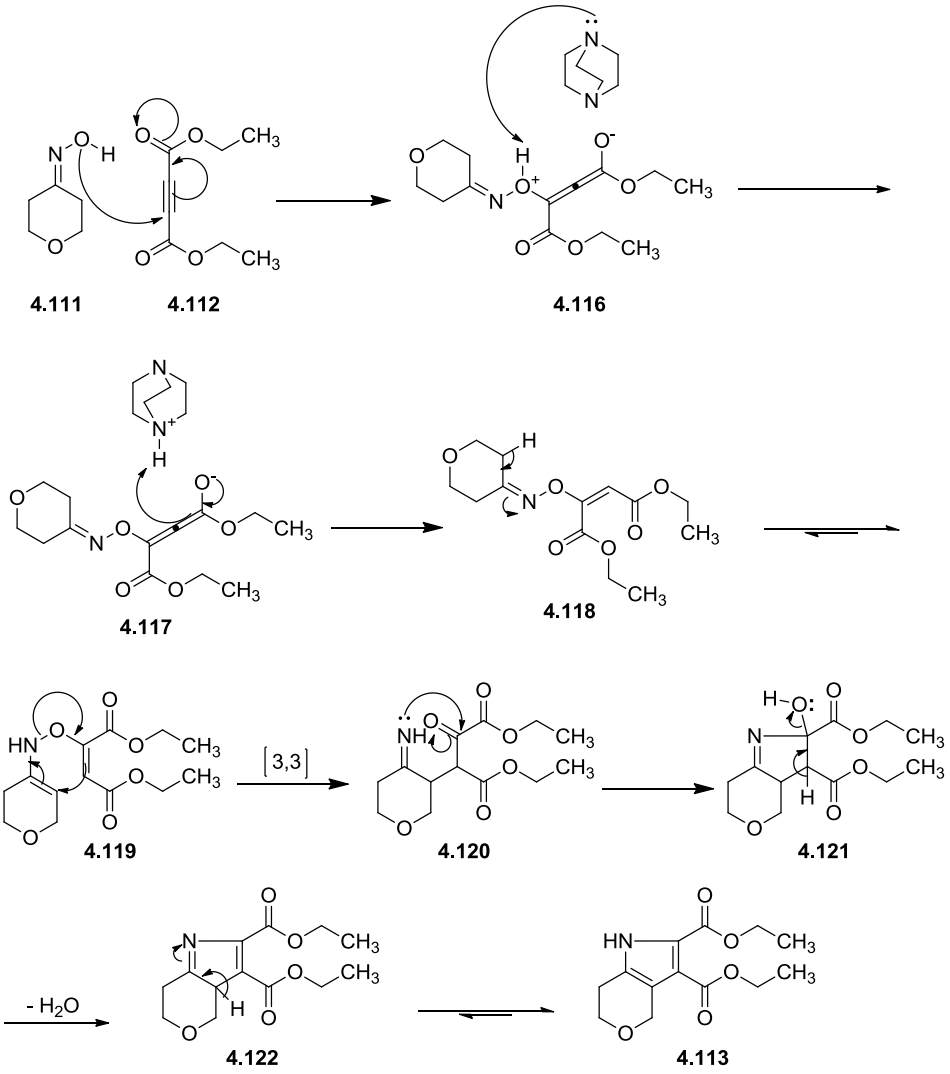
Scheme 25. Failed synthetic route to obtain 4.115.

The synthesis started with the formation of the oxime[116] **4.111**, that subsequently reacted with dimethyl but-2-ynedioate (**4.112**) *via* a Trofimov-type reaction[114,115] (see mechanism in **Scheme 26**) to give the diester **4.113**. The decarboxylation of the compound **4.113** to give the desired compound **4.115** did not proceed in neither occured in acidic conditions (TFA or HCl) or under thermal conditions in diphenyl ether at reflux (250 ºC). The reaction gave a complex mixture of products in all cases. Diester intermediate **4.113** was hydrolysed in the presence of LiOH to give a mixture of two products that was tentatively identified as **4.114**. This crude product was refluxed in diphenyl ether to give a complex mixture.

Trofimov reaction mechanism (Scheme 26)

The synthesis starts with the nucleophilic attack of the oxygen of the oxime moiety in the compound **4.111** to the triple bond of the alkyne **4.112** to give the allene-like intermediate **4.116**, that is subsequently deprotonated by DABCO to provide **4.117**. The enolate is protonated to give the ketone form **4.118**. The acidic proton in the morpholine ring is then taken by the base to form an endocyclic double bond (**4.119**). Further [3+3] sigmatropic rearrangement gives the imine **4.120**, that immediately reacts with the

ketone to form a 6,5-bicyclic compound (**4.121**). A double bond is formed by water elimination to give the imine intermediate **4.122** that tautomerises to the desired pyrrole **4.113**.



Scheme 26. Trofimov reaction mechanism.

Based on all of the above, two different synthetic routes were investigated in attempts to obtain the final target compound **4.94** without success. No further efforts were applied towards this compound due to other priorities within the chemistry of this project.

4.3.2.4. Reducing lipophilicity by reducing the size of the homoaryl part of the tetrahydroindole core.

A second approach to reduce the lipophilicity consisted of the reduction of the molecular weight. In this case, the homocyclic part of the tetrahydroindole ring was contracted to a five-membered ring reducing the calculated logD from 3.7 to 3.2 (**Figure 16**).

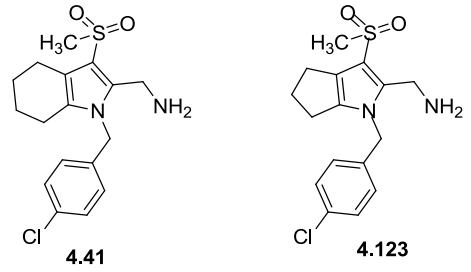
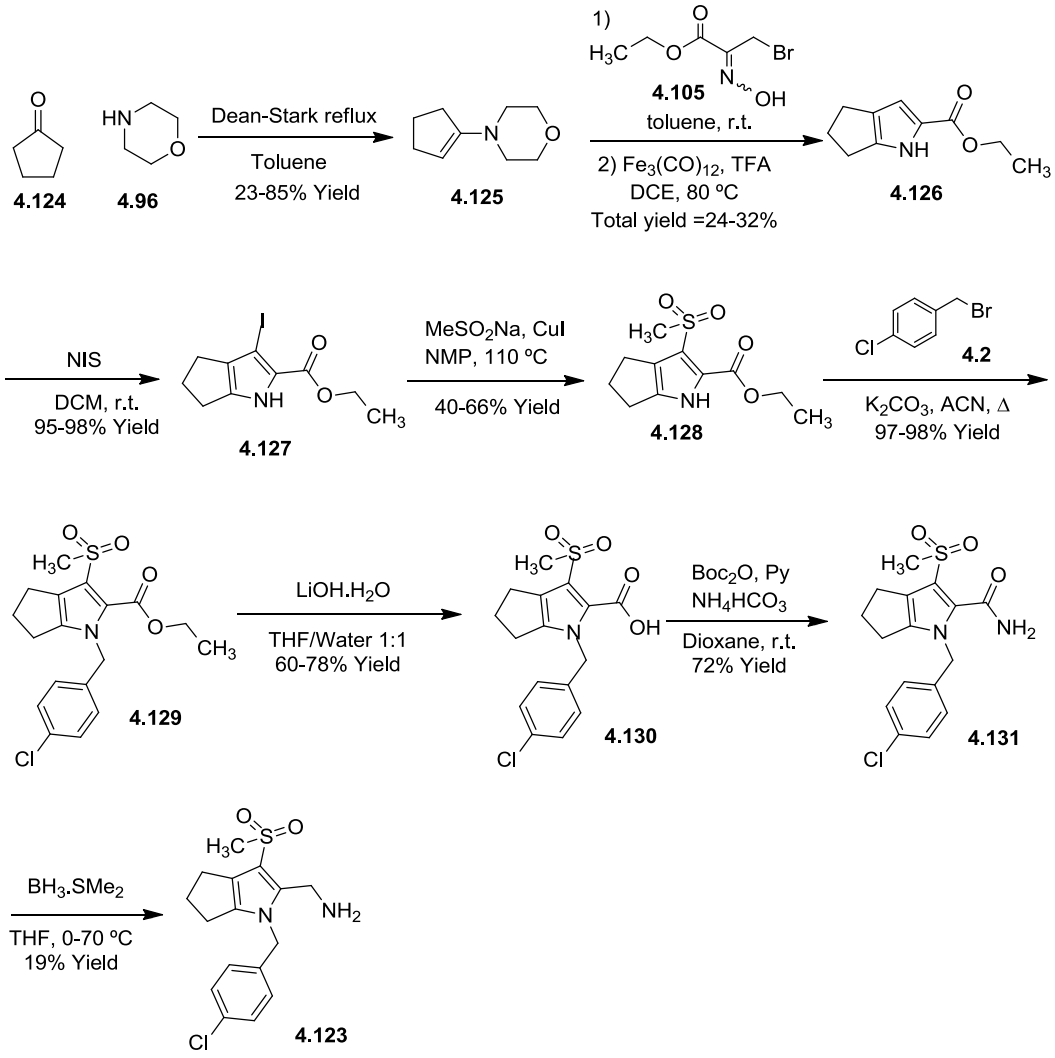


Figure 16. Reduction of the lipophilicity by contracting the size of the homocyclic part of the tetrahydroindole ring.

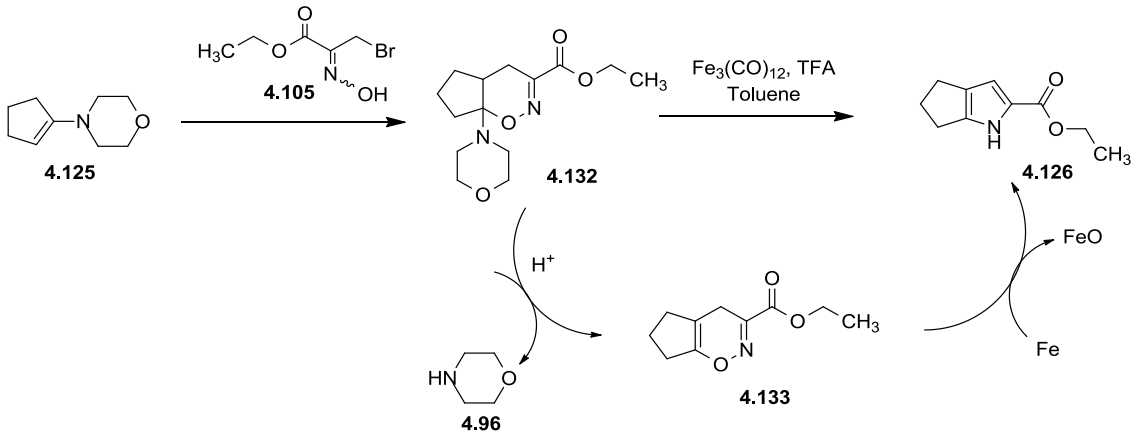
The synthesis towards **4.123** started with the reaction between cyclopentanone and morpholine under Dean-Stark to give the reactive enamine **4.125** in moderate to high yield. The next step consisted of the reaction between **4.125** and **4.105** that were stirred at room temperature for 3 h in toluene to form the intermediate **4.132** (see **Scheme 28** for a plausible reaction mechanism). The reaction mixture was quenched with water and the phases were separated in a funnel, the organic layer was washed with brine, dried over $MgSO_4$, filtered and evaporated under vacuum to dryness to afford a crude material that was dissolved in dichloroethane. TFA and $Fe_3(CO)_{12}$[112] were added to the solution and the mixture was refluxed for 3 h to give a dark solution that was filtered to remove the FeO. The mother liquors were evaporated under vacuum to give a crude product that was purified by flash chromatography to give **4.126** in low yield. The mechanism of the formation of the tetrahydroindole **4.126** from the intermediate **4.132** is equivocal at present. However, it is most likely that the reactions occur *via* a reductive deoxygenation of 4*H*-1,2-oxazine (**4.133**) that is produced by an acid-assisted elimination of morpholine from **4.132** (see **Scheme 28**).

4.126 reacted with NIS to give the intermediate **4.127** in almost quantitative yield. This intermediate was subsequently submitted to an Ullmann type reaction to introduce the

methylsulfone moiety in a moderate yield. Alkylation with **4.2** gave ester **4.129** that was hydrolysed with lithium hydroxide to give the carboxylic acid **4.130** that subsequently reacted with Boc_2O in the presence of pyridine to form a reactive mixed anhydride that reacted with hydrogen ammonium carbonate to give the amide **4.131**. The amide moiety was reduced in the presence of the borane dimethylsulfide complex in THF under reflux to give the amine **4.123** (**Scheme 27**).



Scheme 27. Synthesis of compound 4.123.



Scheme 28. Plausible mechanim for the formation of the intermediate 4.126.

4.3.2.5. Blocking metabolic labile positions

The last strategy to improve the metabolic stability of the tetrahydroindole ring is based on the blockage of metabolically labile positions. In order to stabilise the tetrahydroindole ring, one of the methylene groups of the tetrahydroindole ring was substituted by a CF_2 group (**Figure 17**, compound **4.134**).[84] The 5-position was selected because of the synthetic accessibility.

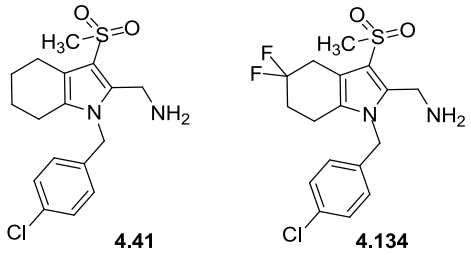


Figure 17. Introduction of fluorine atoms to block a metabolic labile position.

There are other physical and chemical factors, such as ionization, electronic character, configuration, and conformational factors, that influence the metabolic stability of the molecule, as well as biological factors, but these are not easily predictable.[109]

4.3.2.6. Biological results of the tetrahydroindole ring modification approaches.

As part of the strategy to improve the metabolic stability of the compound **4.41**, five compounds with different substitutions in the saturated part of the tetrahydroindole core were designed and four of these were synthesised. The biological results for these compounds are collected in **Table 18**, as well as that relating to the reference compound **4.41**.

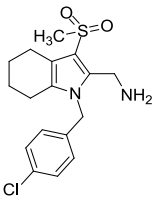
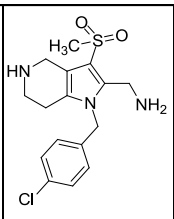
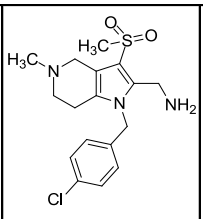
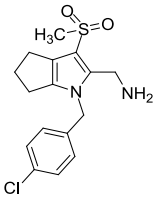
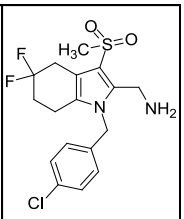
					
	GSK3398235A	**GSK3562205A**	**GSK3562204A**	**GSK3562959A**	**GSK3536838A**
	4.41	**4.92**	**4.93**	**4.123**	**4.134**
***Pf* IC_{50} (µM)**	**0.03**	**>5**	**0.29**	**0.15**	**0.22**
Hep G2 XC_{50} (µM)	**>50.1**	**>100**	**>100**	**63.1**	**>100**
CLND/ FaSSIF (µg/mL)	**141/746**	**149/ND**	**184/ND**	**137/ND**	
ChromlogD/PFI	**4.4/6.4**	**1.0/3.0**	**2.0/4.0**	**3.8/5.8**	
Papp (nm/sec)	**320**	**<10**	**82**	**470**	
Cli (m/r/h) mL/min.g	**1.8/1.3/<0.6**		**0.6/1/0.7**	**7.1/2.6/0.8**	**2.6/1.8/1.4**

Table 18. Tetrahydroindole ring modifications.

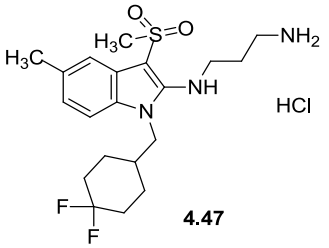
As it is shown in the **Table 18**, all the compounds showed a decreased lipophilicity (experimental values of ChromlogD) compared to **4.41**. For **4.92** the huge increase in polarity translated into a sharp decrease on the permeability, making **4.92** a completely inactive compound against *Pf*. Compounds **4.93**, **4.123**, and **4.134** showed a moderate *in vitro* activity that was insufficient to allow the progression of these compounds to PK studies, despite the *in vitro* clearance for compounds **4.93** and **4.134** showing values below 3 mL/min.g. In **Section 6**, details of the scaffolds of the compounds **4.93**, **4.123** and **4.134** being used as tool compounds will be communicated to introduce a more developed group in the 2-position of the ring and deliver more balanced compounds regarding antimalarial activity, pharmacokinetics, safety, and physico-chemical properties.

4.3.3. Highlights and conclusions

As described in section **4.2.5** (best results and conclusions from the first round of the LO programme) different changes in the Lead compound (**3.23**) were carried out in order to optimise the molecule and avoid the liabilities that it presented. Some of these changes have been applied in the second round of the lead optimisation to obtain hybrid compounds with improved properties (**Sections 4.3.1** & **4.3.2**).

In the case of compounds that maintain the core of the Lead compound (**3.23**), introduction of polar moieties resulted in a decreased lipophilicity and, consequently, an improvement on solubility (see sections **4.2.1** and **4.2.2**).

In this second round of the programme, when the amine approach was applied to compounds bearing the difluorocyclohexyl unit in the 1-position of the indole core, soluble and non-cytotoxic compounds (**4.60**-**4.62**, **4.65** & **4.68** collected in **Table 14**) were obtained. However, the *in vitro* whole cell activity was not good enough except in the case of the compound **4.62** that was moderate (**Table 14**, IC_{50} = 0.16 μM). Unfortunately, this compound was not stable in microsomes and, therefore, it was not progressed to pharmacokinetic studies. Other derivatives based on this series were synthesised elsewhere in our laboratories.[107] Most of these compounds showed an *in vitro* activity above 100 nM except when an aliphatic chain bearing a basic amine was introduced, as was observed with the compound **4.47**. This compound showed an *in vitro* activity of 10 nM, but a HepG2 value of 12.6 μM, which did not allow the progression of this compound due to an appreciable risk regarding cytotoxicity. This compound confirmed the trend observed between basicity and cytotoxicity. This series of compounds containing the difluorocyclohexyl moiety was put on hold until an optimal substitution for position 2 could be found.



4.47

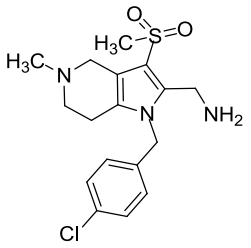
The different modifications proposed for the tetrahydroindole ring allowed the possibility of performing more challenging reactions, like the formation of the

intermediates **4.98** and **4.126**, which required the use of an iron complex to mediate the reductive scission of oxygen, or the Trofimov reaction that allowed the formation of pyrrole derivatives by reaction between an oxime and an alkyne.

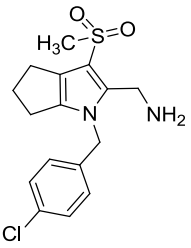
Four different strategies have now been carried out in attempts to improve the metabolic stability of the tetrahydroindole derivatives based on:

- the reduction of the lipophilicity by introducing polar groups in position 2 (amides and amines approaches);
- the reduction of the lipophilicity by introducing polar moieties in the tetrahydroindole core;
- the reduction of the lipophilicity by reducing the molecular weight in the tetrahydroindole core; and
- the blockage of metabolic positions in the tetrahydroindole core.

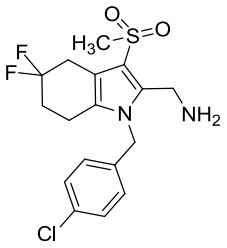
The modifications on the side chain afforded active but instable compounds with good physico-chemical properties and promising safety profiles. These moieties will be taken into consideration to be introduced in more stable scaffolds. Regarding the modifications in the aliphatic part of the ring, three new substructures (**4.93**, **4.123** & **4.134**) have been identified as possible scaffolds when an optimal substitution in the 2-position of the molecule is found.



4.93



4.123



4.134

A further analysis of the first round of the Lead Optimisation, allowed us to identify four compounds that shared the same scaffold and presented a similar substitution in the 2-position showing the 4 different connections studied in this work: amides type I & II, and amines type I & II. These compounds are shown in **Table 19**,[105,107] with all presenting a similar *in vitro* activity (0.01-0.06 μM). Regarding their metabolic stability, the compound **4.135** (Amide type II) showed the lowest intrinsic clearance in microsomes (8.4, 4.6 & 1.9 mL/min.g).

Compounds **4.135**, **4.136** & **4.137** were prepared elsewhere in our laboratories and they are not described in the experimental section.

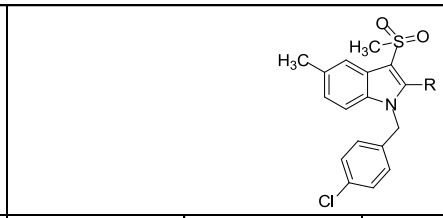
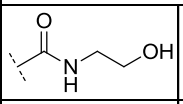
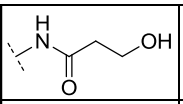
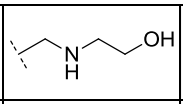
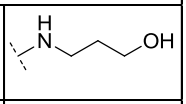
				
R				
	GSK3393599A	**GSK3515924A**	**GSK3510318A**	**GSK3490093A**
	4.11	**4.135**	**4.136**	**4.137**
Pf IC_{50} (μM)	**0.06**	**0.03**	**0.01**	**0.01**
Cli (m/r/h) mL/min.g	**78/14/35**	**8.4/4.6/1.9**	**19/14/6**	**69.5/35/9.3**

Table 19. Comparison of the 4 possible connective moieties.

Taking into account these results, the amides type II like **4.135** will now be explored further. For this purpose, a library of amides was computationally designed elsewhere in our laboratories,[117] in order to select the most promising commercially available amides to be introduced to the compounds targeted as a part of this programme. **Section 5** describes the selection of amides and the scaffold used for the library, as well as reaction conditions, results, and a discussion of the key outputs from this programme.

5. LIBRARY OF AMIDES

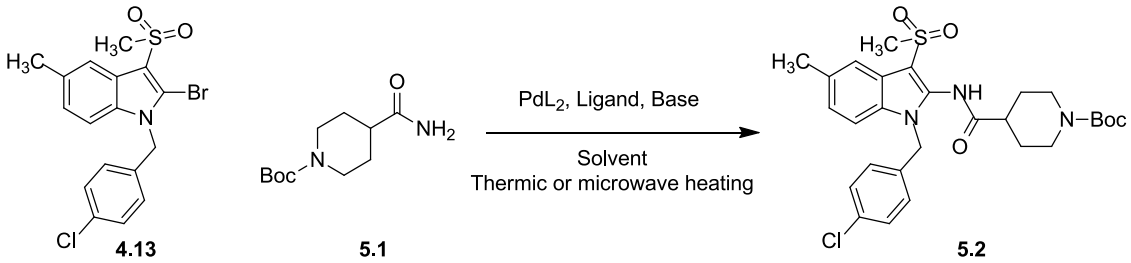
The targeted library as part of this programme aimed to cover an appreciable area of chemical space and a selection of the building blocks was performed *in silico* to avoid non drug-like functional groups. Additionally, some physico-chemical parameters, such as PFI and pK_a, were taken into account in order to obtain compounds with optimised properties regarding solubility, lipophilicity, and toxicity. Overall, once all data were available from the library, a selection of residues was introduced into more developed scaffolds.

Section **4.2.1** depicts the two approaches to synthesise this type of amide. At the time that the work on this library was started, yields for both routes were similar and a palladium-catalysed approach was chosen as the method for application here. Additionally, optimisation of the acid-amine coupling, was performed elsewhere in our laboratories,[111] which improved significantly the yields of these couplings. Accordingly, this route was also used to obtain a selection of compounds for this library.

As the yield of the Buchwald amidation shown in the **Scheme 3**, in **Page 51**, was low, a study to optimise the reaction conditions was performed.

5.1. INVESTIGATION OF THE PALLADIUM-CATALYSED AMIDATION CONDITIONS

Scheme 29 shows the reaction to be used in order to test different Buchwald-Hartwig conditions (**Table 20**) in terms of ligand, catalyst, base, temperature, and solvent.[99] The structures of the ligands used are shown in **Figure 18**.



Scheme 29. Reaction reference for Buchwald conditions study.

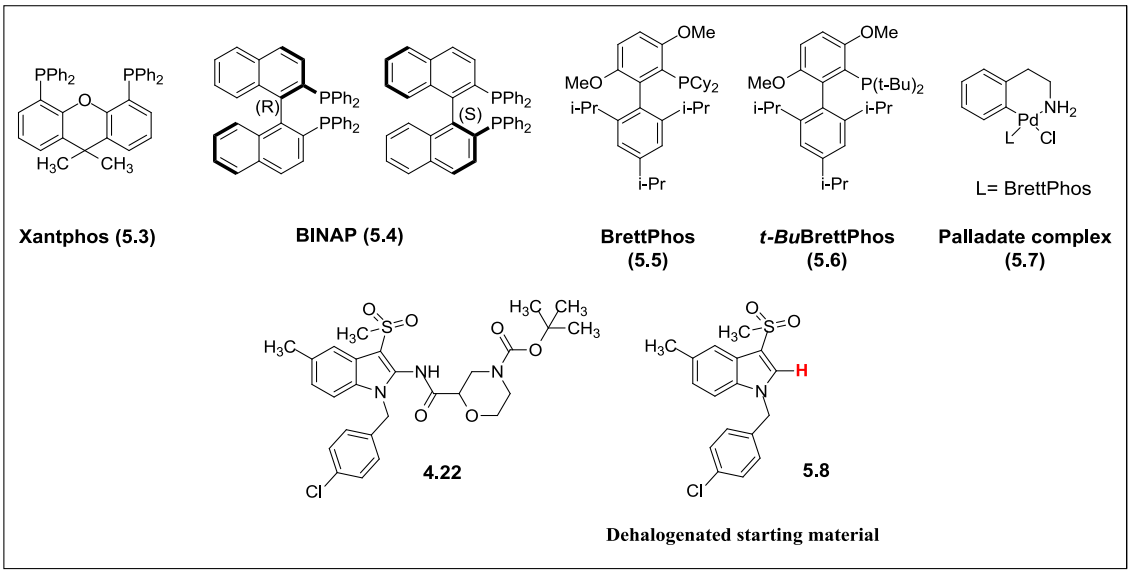


Figure 18. Ligands used in the study of the Buchwald conditions, intermediate 4.22 and dehalogenated starting material.

The thermal conditions used to synthesise compound **4.22** ($Pd_2(dba)_3$, Xantphos (**5.3**) and Cs_2CO_3 in dioxane) (**Entry 1**, **Table 20**) failed to give intermediate **5.2**, showing dehalogenation of starting material as the main product (**5.8**) as observed by HPLC (basic pH). Compound **5.8** was isolated, and characterised by ^{1}H NMR. The reaction was repeated under microwave conditions (130 ºC for 30 min) (**Entry 2**), and the expected product was detected along with a very small amount of **5.8**. In this case, the desired product was isolated in low yield (26%). Following the troubleshooting guide for Pd-catalyzed amination reactions,[99] the reaction temperature was decreased in order to avoid dehalogenation. The reaction was repeated at 90 ºC (**Entry 3**) but no reaction was observed, so the temperature was increased to 110 ºC (**Entry 3**) with a similar result by HPLC (basic pH) to the reaction at 130 ºC (**Entry 2**), however over longer reaction times (90 min *vs*. 30 min). Based on this outcome, decreasing the temperature did not deliver any advantage.

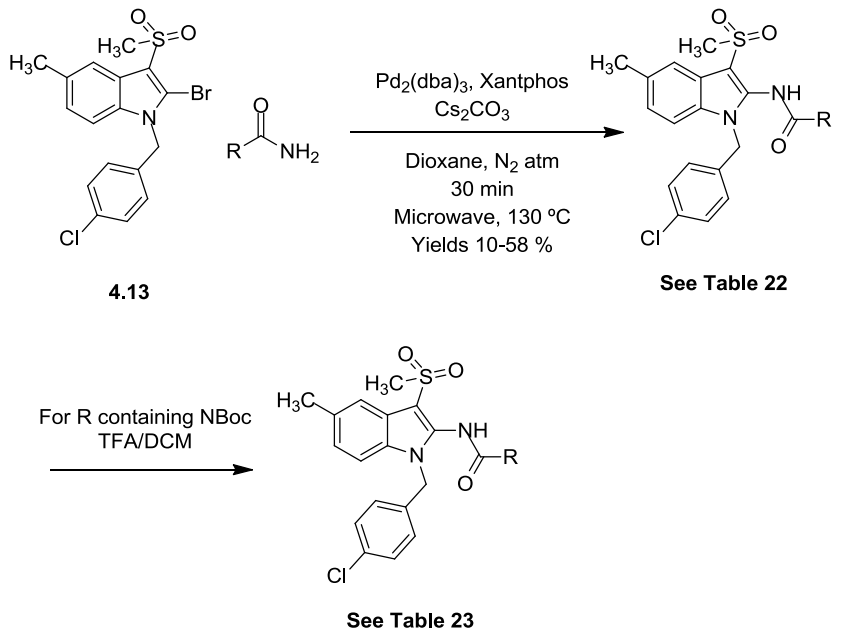
At this stage, the solvent was changed from 1,4-dioxane to tetrahydrofuran (**Entry 4**) with a higher dielectric constant (7.60 at 20 ºC *vs*. 2.2 at 25 ºC for dioxane) and the reaction was carried out twice, with yields of 7%, and 34%, respectively; in both cases the reaction was complete with a small amount of **5.8** observed. Based on this result, the ligand was changed to BINAP (**5.4**) commonly used in this type of cross-coupling reaction)[99,118] using THF as solvent at 130 ºC in the microwave and yielding the desired

product in a 25% yield but with a 1:1 proportion between desired product **5.2** and **5.8** (**Entry 5**). The same conditions were tried at lower temperature (100-115 ºC) and no changes in the HPLC (basic pH) were observed (**Entry 6**). Using the conditions described in the literature,[118] the reaction was repeated using toluene as solvent under thermal conditions (**Entry 7**) affording an unidentifiable product by LCMS. **Entries 8** and **9** show the use of the catalyst ^{t}*Bu*BrettPhos (**5.6**), specifically indicated for amidation reactions.[119] Unfortunately, no reaction was observed after various hours of heating. **Entry 10** shows the use of a palladate activated ligand[99] (**5.7**) to try to ensure the presence of the complex Pd-Ligand necessary to start the catalytic cycle. The chosen ligand was BrettPhos (**5.5**). This yielded an unidentifiable product by LCMS.

Entry	Pd Cat	Ligand	Base	Solvent	T (ºC) a= microwave b= thermal	Heating time	Crude HPLC Analysis	Yield
1	$Pd_2(dba)_3$	Xantphos	Cs_2CO_3	Dioxane	110 ºC (b)	20 h	A large proportion of dehalogenated SM (**5.8**)	
2	$Pd_2(dba)_3$	Xantphos	Cs_2CO_3	Dioxane	130 ºC (a)	30 min	Very small amount of dehalogenated SM (**5.8**) and no SM (**4.13**)	26%
3	$Pd_2(dba)_3$	Xantphos	Cs_2CO_3	Dioxane	90-110 ºC (a)	30 + 90 min	Similar to reaction at 130 ºC (**Entry 2**) but much longer reaction time and presence of a by-product was also detected	
4	$Pd_2(dba)_3$	Xantphos	Cs_2CO_3	THF	130 ºC (a)	30 min	Small amount of dehalogenated SM (**5.8**) and no SM (**4.13**)	7 & 34%
5	$Pd_2(dba)_3$	BINAP	Cs_2CO_3	THF	130 ºC (a)	30 min	Dehalogenated SM (**5.8**) and final product (**5.2**) (1:1 ratio)	25%
6	$Pd_2(dba)_3$	BINAP	Cs_2CO_3	THF	100-115 ºC (a)	1 h	No improvement decreasing the temperature compared to 130 ºC	
7	$Pd_2(dba)_3$	BINAP	Cs_2CO_3	Toluene	110 ºC (b)	16 h	Unknown product formed	
8	$Pd(OAc)_2$	^{t}BuBrettPhos	Cs_2CO_3	^{t}BuOH	100 ºC (b)	16 h	No reaction	
9	$Pd(OAc)_2$	^{t}BuBrettPhos	K_3PO_4	^{t}BuOH/ water	110 ºC (b)	3 h	No reaction	
10	Palladate complex	BrettPhos	LHMDS	THF	110 ºC (b)	1 h	Unknown product formed	

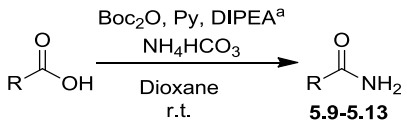
Table 20. Buchwald reaction conditions

Following these test reactions, the chosen conditions were those described in **Entry 2**, that used $Pd_2(dba)_3$ as Pd catalyst, Xantphos (**5.3**) as ligand, and caesium carbonate as base in dioxane under N_2 atmosphere and microwave irradiation at 130 ºC for 30 min (**Scheme 30**). The conditions described in **Entry 4** could have been used but the appearance of the HPLC chromatogram was much more complex.

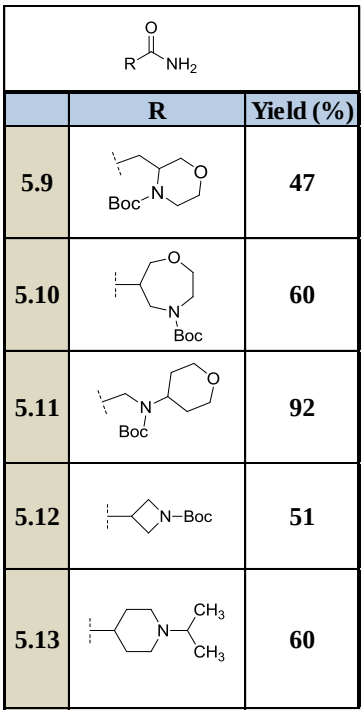


Scheme 30. Buchwald coupling and Boc-deprotection conditions.

Some of the amides used for this synthesis were not commercially available and they were synthesised (see **Scheme 31**) using the corresponding carboxylic acid with Boc anhydride in the presence of pyridine in dioxane as solvent. Ammonium hydrogen carbonate was added after 15 min of stirring and the reaction was left stirring at room temperature overnight to obtain 5 amides (**5.9-5.13**) in moderate to very good yields (**Table 21**).



Scheme 31. Conversion of carboxylic acid into amide.

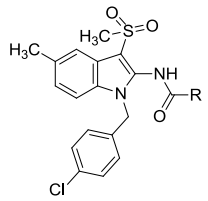


	R	Yield (%)
5.9		47
5.10		60
5.11		92
5.12		51
5.13		60

Table 21. Amides formed from the corresponding carboxylic acids.

[a]Used to obtain the compound 5.9.

The Buchwald coupling (**Scheme 30**) between the bromine derivative **4.13** and a selection of commercially available amides and amides **5.9-5.13** allowed the synthesis of 17 compounds (compounds **5.14-5.29** & **4.22** described in **Table 22**) with moderate to low yields (10-58%).



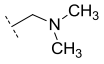
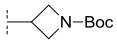
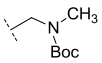
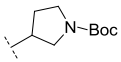
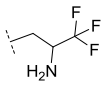
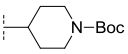
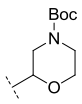
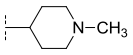
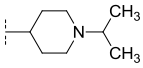
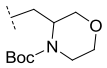
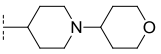
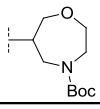
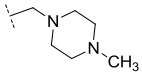
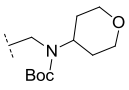
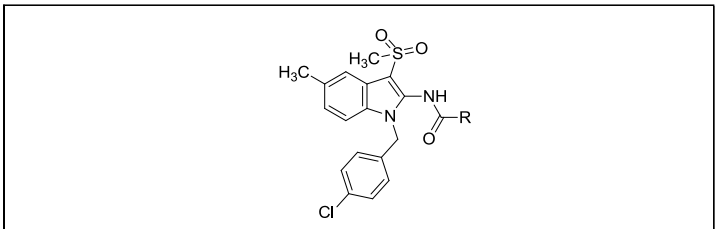
	R	Yield (%)		R	Yield (%)
5.14		**27**	**5.22**		**58**
5.15		**12**	**5.23**		**11**
5.16		**10**	**5.24**		**37**
5.17		**17**	**5.25**		**33**
4.22[a]		**35**	**5.26**[c]		**14**
5.18[b]		**14**	**5.27**		**22**
5.19		**ND**	**5.28**		**17**
5.20		**32**	**5.29**		**12**
5.21		**ND**			

Table 22. Buchwald coupling compounds.

[a]See Scheme 4, page 52, [b]The carboxylic amide was previously Boc-protected using conditions described in the experimental section (page 225), [c]Formic acid salt.

Some of these compounds were synthesised as *N*-Boc protected amines, so a further deprotection was needed (**Scheme 30**). The compounds were dissolved in DCM and TFA was added, and the reaction was stirred at room temperature until it reached completion. The 9 compounds are depicted in **Table 23** along with the corresponding reaction yields, most of them above 70% except in the case of the compound **5.34** (since the reaction was carried out with a non-pure starting material).



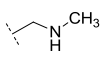
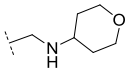
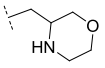
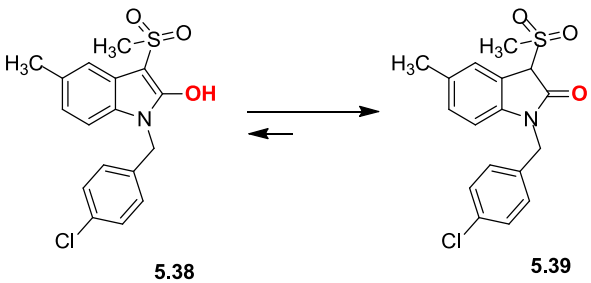
	R	Yield (%)		R	Yield (%)
5.30		**83**	**5.34**		**30**
4.16[a]		**80**	**5.35**		**87**
5.31		**89**	**5.36**		**71**
5.32		**79**	**5.37**		**97**
5.33		**76**			

Table 23. Boc-Deprotection yields.[a]See Scheme 4, Page 52.

The low yields observed in the Buchwald coupling step could be due either to the low basicity of the amides or to the formation, in most of the cases, of a major by-product: the hydroxy derivative of the starting material (**5.38**), formed by the presence of traces of water in the reaction mixture.[99] This by-product was isolated as its tautomeric form (**5.39**) that is a stable lactam (**Scheme 32**). This compound was isolated, characterised, and sent to biological test, showing a *Pf* IC_{50} = 2.5 μM.

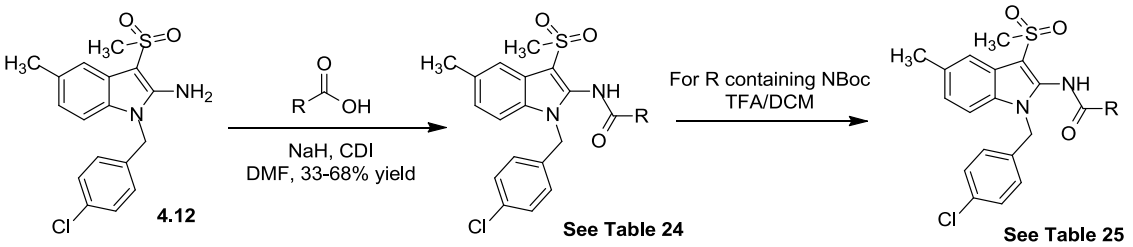


5.38 **5.39**

Scheme 32. Hydroxy-ketone tautomeric forms of the hydroxiderivative of the SM.

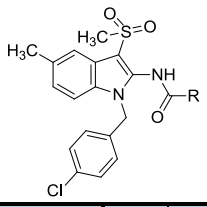
Compound **5.39** was formed in the reaction due to the presence of water,[99] that could be found in the solvent or in the reactants. In order to avoid the presence of water, the caesium carbonate was dried in a vacuum drying pistol (120 ºC, 10 mbar) for 2 days and a small reduction in the formation of **5.39** was observed by HPLC (basic pH). In other experiments, new bottles of dry dioxane and $Pd_2(dba)_3$ were used but no appreciable improvement was observed regarding the reduction of the formation of **5.39**.

During the time spent on the optimisation of the Buchwald conditions, and the synthesis of the first amides of the library, parallel efforts were performed elsewhere in our laboratory[111] to optimise the yield of the coupling between the amine **4.12** and the corresponding carboxylic acid (**Scheme 3**, **Page 51**). Since the Buchwald conditions were still low yielding, the remaining targets were synthesised using the developed conditions, that used CDI and NaH with DMF as solvent (**Scheme 33**).



Scheme 33. Coupling reaction conditions.

As shown in **Table 24**, the yields were generally increased over those obtained with Buchwald conditions. This increase in the yields, the fact that availability of carboxylic acids is greater, and that there is no need of an extra step to obtain the amides, led us to choose the acid-amine coupling as the synthetic route for further efforts in this area.



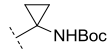
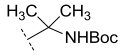
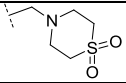
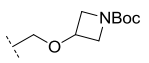
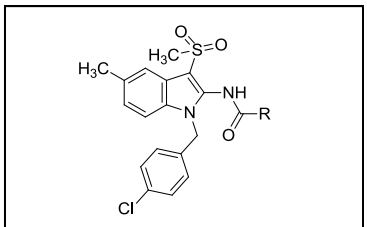
	R	Yield (%)		R	Yield (%)
5.40		**51**	**5.43**		**56**
5.41		**68**	**5.44**		**43**
5.42		**33**	**5.45**		**53**

Table 24. Coupling acid-amine yields.

As occured in the previous route, some of the compounds needed a further step to remove the Boc-protecting group. The conditions used are shown in **Scheme 33** and are the same as those used for the compounds described in **Table 23**. The results are described in **Table 25** and, as shown, the yields are considerably lower for two of the three compounds than the yields observed previously. In the case of the compound **5.46** the low yield is due to the low purity of the starting material **5.40**. For the compound **5.48** no ready explanation for the lower yield could be found.



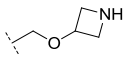
	R	Yield (%)
5.46		**35**
5.47		**98**
5.48		**38**

Table 25. Boc-Deprotection yields.

5.2. RESULTS AND ANALYSIS

A total of 23 new compounds were synthesised using Buchwald or acid-amine coupling reaction conditions, with low to moderate yields, and further Boc-deprotection when it was required. These compounds, and other analogues prepared by elsewhere else in our laboratories,[84] are collected in **Tables 26** and **27**.

This library of amides was designed using as model the scaffold of the compound **4.135** (**Tables 19**, **26** & **27** pages **89**, **101** & **102**) that showed better *in vitro* stability than the other three compounds depicted in **Table 19**, but still needed further modifications to enhance the microsomal stability and the physicochemical properties. Wider modifications were carried in the different positions of the molecule elsewhere in our laboratory,[84] whilst the library at the centre of this study with different substitutions (R) in the scaffold are depicted in the **Figure 19**.

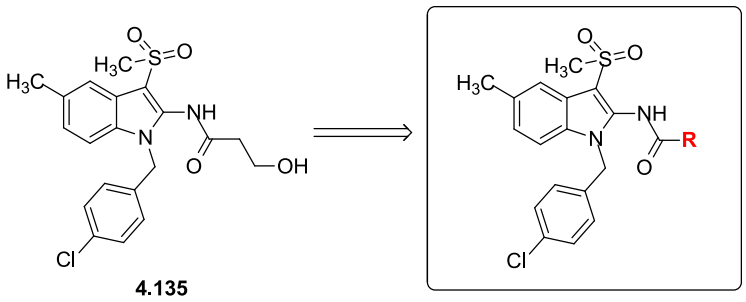


Figure 19. Compound 4.135 and library scaffold.

The reduction of one unit of the carbon chain of the compound **4.135** afforded the derivative **5.49**,[104] that showed a similar profile to **4.135** with a small improvement on the lipophilicity due to the lower molecular weight. When the hydroxy group was changed to an amino or dimethylamino group (**4.14** & **4.15**, respectively), the cytotoxicity was increased due to the presence of the basic amine. This effect could also be observed in **5.14** & **5.30**. In order to reduce the basicity of the terminal NH_2 of the compound **4.14**, a CF_3 was introduced in the carbon chain (**5.17**), producing a decrease on the pK_a from 8.7 to 4, that was translated to an increase on the HepG2 data from 15.8 to 61.7 μM. Unfortunately, the presence of the CF_3 group increased the PFI to 9.1 causing an important decrease on the solubility.

Blocking of the methylene position, considered as a metabolic labile point, by the introduction of a cyclopropyl (**5.16** & **5.46**) or a *gem*-dimethyl group (**5.47**), increased the lipophilicity causing a total loss of the solubility in the case of the compounds containing the cyclopropyl unit. Compound **5.47** was more soluble, allowing the identification of a potential issue regarding cytotoxicity (HepG2 = 42.7 μM).

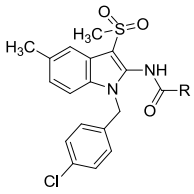
Product **4.16**, bearing a morpholine group, was separated by chiral chromatography into its corresponding enantiomers **5.50** & **5.51**. Either the racemic or the separate enantiomers showed a promising similar *in vitro* activity, solubility, moderate cytotoxicity, and *in vitro* stability. Other indole derivatives bearing this group in position 2 were synthesised elsewhere in our laboratory[97,110] showing promising results regarding the toxicity and the physicochemical profile but the PK studies showed high clearance for almost all the compounds tested.

Two more compounds with morpholine-type moieties were synthesised aiming to improve its metabolic stability whilst keeping the promising safety and physical

chemistry profiles but more unstable derivatives were obtained: **5.31** & **5.32**. Taking into account the cytotoxicity data of the compounds containing an oxygen atom, the compounds **5.33** and **5.34** were synthesised showing the best HepG2 values of the library of compounds (HepG2 = 79.4 & 74.1 μM). The solubility of these two compounds was moderate but the *in vitro* metabolic stability was insufficient to allow their progression to *in vivo* studies.

The cyclic amine series (**5.35**, **5.23**, **5.36**, **5.37**, **5.26**, **5.27**, **5.28** & **5.29**) represented active and more hydrophilic and soluble compounds. Almost all of them were showed *in vitro* activity below 100 nM and 4 of them were the most stable compounds of this amides collection. Unfortunately, the presence of a basic amine and the high pK_a values translated into an important cytotoxicity issue in this sub-series.

Four more compounds (**5.42**, **5.43**, **5.44** & **5.48**) were synthesised in this approach with no structural similarity with the rest or between them, and with the exception of **5.43** (see **Table 28**, **Page 103**) none of them showed any encouraging result.



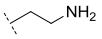
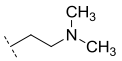
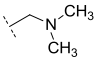
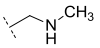
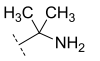
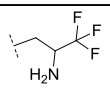
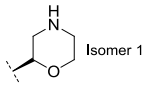
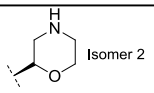
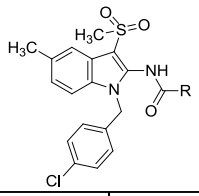
	R	*Pf* IC_{50} (µM)	Hep G2 XC_{50} (µM)	CLND/ FaSSIF (µg/mL)	ChromlogD PFI	pK_a (Theo)	Cli (m/r/h) mL/min.g
5.49		0.03	>100	25/ND	4.9/7.9		16.3/5.5/1.5
4.135		0.03	Cell Health:clean	26/24	4.5/7.5		8.4/4.6/1.9
4.14		0.01	15.8	141/264	3.4/6.4	8.72	
4.15		0.01	15.8	205/669	4.7/7.7	7.75	
5.14		0.01	44.7	23/ND	6/9	5.67	22.7/13.9/4.8
5.30		0.09	60.3	134/ND	4.7/7.7	8.22	9.8/3.8/3.4
5.16		0.02	>100	0	6.6/9.9		24.9/9.5/5.6
5.46		0.03	>100	2/4	5.7/8.7	7.71	8.3/8.4/2.2
5.47		0.04	42.7	170/	5.5/8.5	8.08	10.8/8.0/8.0
5.17		0.07	61.7	15/ND	6.1/9.1	4.02	2.4/4.4/1.7
4.16[a]		0.02	39.8	170/ND	4.6/7.6	7.53	3/2.5/1.6
5.50		0.05	46.8	152/ND	4.8/7.8	7.53	2/2.4/1.6
5.51		0.04	50.1	167/ND	4.8/7.8	7.53	2.9/2.1/1.3

Table 26. Library of amides results (part I). [a]See Scheme 4. Blue color indicates that the solubility for that compound is < 30 µg/mL, so toxicity and in vitro clearance data are not reliable.



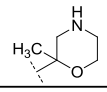
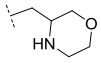
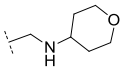
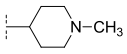
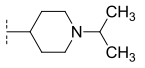
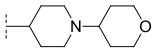
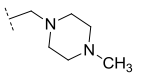
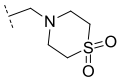
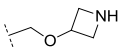
	R	*Pf* IC_{50} (µM)	Hep G2 XC_{50} (µM)	CLND/ FaSSIF (µg/mL)	ChromlogD PFI	pK_a (Theo)	Cli (m/r/h) mL/min.g
5.31		0.02	64.6	20/ND	5.3/8.3	7.69	9.9/6.7/6.1
5.32		0.05	58.9	188/ND	4.7/7.7	7.64	8.2/5/2.1
5.33		0.08	79.4	204/ND	4.7/7.7	8.78	8.7/5.1/2.3
5.34		0.17	74.1	68/ND	5.5/8.5	8.37	22.1/10.2/5.2
5.35		0.03	26.9	182/338	3.6/6.6	9.19	1.4/0.5/0.6
5.23		0.02	>100	29/ND	5.3/8.3	8.53	21.8/7.5/5.5
5.36		0.06	16.6	182/453	3.4/6.4	10.3	1/1.1/0.4
5.37		0.07	17.4	178/256	3.4/6.4	9.96	1/0.8/0.5
5.26[b]		0.05	13.8	240/ND	4.1/7.1	7.46	1.4/1.8/0.9
5.27		0.17	14.5	158/ND	4.3/7.3	8.01	2.4/7.3/2.6
5.28		0.65	20.0	115/92	4.5/7.5	8.26	4.5/1.8/2.7
5.29		0.05	31.6	166/245	4.7/7.7	6.2	92.6/12.5/18.6
5.42		1.43	>100	32/ND	5.2/8.2		35/18.9/6.9
5.43		0.09	50.1	84/664	3.8/6.8	7.34	1/1.8/0.9
5.44		0.11	>100	6/ND	5.6/8.6		4.9/3.9/3.8
5.48		0.08	20.0	200/ND	3.9/6.9	8.27	3.6/0.7/1.0

Table 27. Library of amides results (part II). [b]Formic acid salt. Blue color indicates that the solubility for that compound is < 30 µg/mL, so toxicity and in vitro clearance data are not reliable.

From all the compounds synthesised in this area of work, one of them stood out due to its promising profile. The compound **5.43** (**Tables 27 & 28**) bearing a quinuclidine unit, showed a good *in vitro* activity (90 nM), PFI under 7, a very high FaSSIF solubility (664 μg/mL), a moderate cytotoxicity, and a low *in vitro* clearance for the three species (mouse, rat, and human). This moiety will be introduced in a more developed scaffold when the optimal substitution of the indole ring is established.

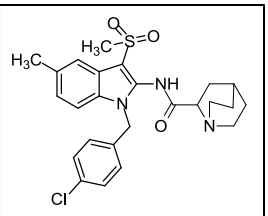
	
	GSK3533289A
	5.43
***Pf* IC_{50} (μM)**	**0.09**
Hep G2 XC_{50} (μM)	**50.1**
CLND/ FaSSIF (μg/mL)	**84/664**
ChromlogD/PFI	**3.8/6.8**
Cli (m,r,h) mL/min.g	**1/1.8/0.9**

Table 28. GSK3533289A (5.43).

A simultaneous study, elsewhere in our laboratories,[84] of the different substitutions on the homoaryl part of the indole ring allowed the identification of the 5-fluorine unit as the favoured substitution based on the full profile of the resultant compound **5.53** (**Table 29**), that combines the best substitutions found along the lead optimisation process. This compound was compared with the 5-hydrogen, 5-methyl, and 5-cyano analogues showing an improved profile regarding *in vitro* clearance in microsomes, a better solubility, and no major issues regarding cytotoxicity (HepG2 > 100 μM) (**Table 29**).

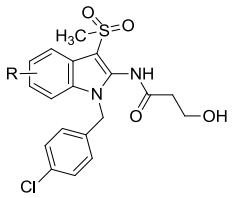
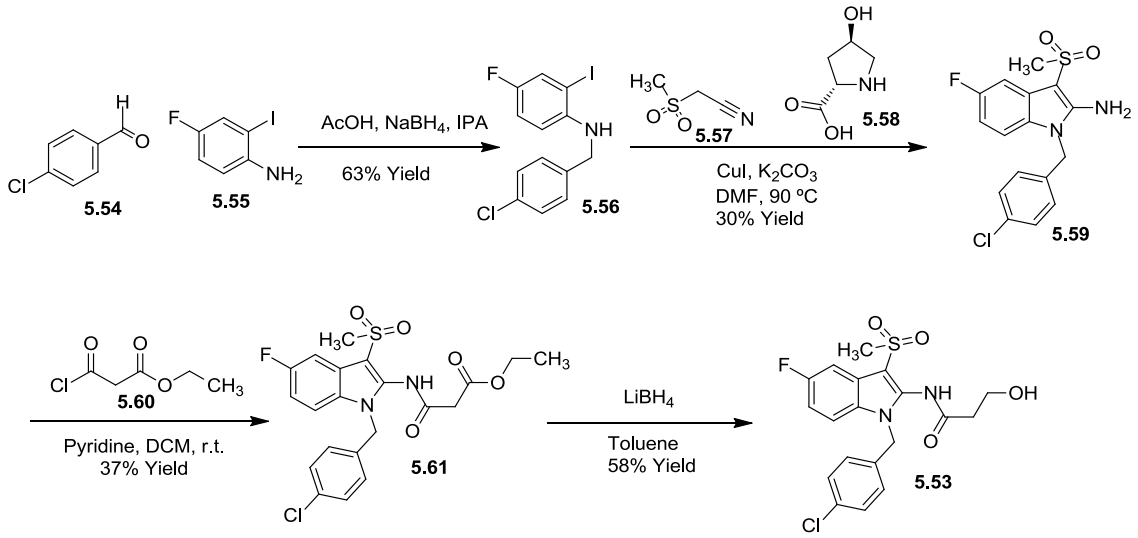
				
	5-Me	**5-H**	**5-CN**	**5-F**
	GSK3515924A	**GSK3384541A**	**GSK3510729A**	**GSK3531659A**
	4.135	**5.49**	**5.52**	**5.53**
***Pf* IC_{50} (μM)**	**0.03**	**0.03**	**0.06**	**0.03**
Hep G2 XC_{50} (μM)	**>100**	**>100**	**>100**	**>100**
FaSSIF (μg/mL)	**24**	**158**	**24**	**200**
Cli (m/r/h) mL/min.g	**8.4/ 4.6/ 1.9**	**14/ 1.6/ 0.9**	**0.5 /<0.4/<0.4**	**2.8 / 0.9/ 0.6**

Table 29. Comparison of the best substitutions on the homoaryl part of the indole ring.

The synthesis of compound **5.53** (**Scheme 34**) started with a reductive amination between *p*-chlorobenzaldehyde (**5.54**) and 4-fluoro-2-iodoaniline (**5.55**) using sodium borohydride as the reducting agent to give the intermediate **5.56** in an adequate yield. This intermediate reacted with 2-(methylsulfonyl) acetonitrile (**5.57**) using an *L*-Proline derivative as ligand (**5.58**) and CuI as catalysts to give the aminoindole **5.59** in a low yield. Acylation of **5.59** using ethyl 3-chloro-3-oxopropanoate (**5.60**) in the presence of pyridine gave **5.61**, which was reduced to the desired compound **5.53** with $LiBH_4$.



Scheme 34. Synthesis of GSK3531659A (5.53).

The promising profile of the compound **5.53** led the progression of this compound to an *in vivo* cardiotoxicity assay (Rabbit Ventricular Wedge (RVW)),[120] pharmacokinetic study in dog, *in vivo* efficacy in *P. Falciparum*,[121] and AMES test.[93,94] Having very promising data coming from these assays (**Table 30**), this compound was synthesised in a 50 g scale as a crystalline solid elsewhere in our laboratory.[110,111] When this crystalline compound was sent to solubility assay in different biorrelevant media (SGF, FaSSIF and FeSSIF), the compound showed a low solubility that did not allow its progression.

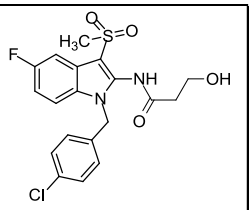
		
		GSK3531659A
		5.53
P. falciparum (in vitro)	***Pf* IC_{50} (µM)**	**0.03**
	DGFA IC_{50} male/female (µM)	**0.04/0.18**
SMFA (P.falciparum)	**Oocyst reduction IC_{50} (µM)**	**0.05**
In vivo efficacy	**ED_{90} (µg/Kg)**	**7.5**
	AUC ED_{90} (µg.h/mL.day)	**1.7**
PhysChem	**LE/LLE**	**0.36/4.8**
	SGF, FaSSIF, FeSSIF (µg/mL)	**10, 29.5, 106.5**
	PFI	**7.2**
***in vivo* ADME**	**Cl (mL/min.Kg) (m, r, d)**	**9/ 13.2/ 1.95**
	Vss(L/Kg) (m, r, d)	**1/ 9/ 1.52**
	$t_{1/2}$ (h) (m, r ,d)	**1.4/ 20.7β/ 12.2β**
	%F (m, r, d)	**34/ 53/ 29**
Cytotox	**HepG2 (µM)**	**>100**
	Cell Health (Mit, Nuc, Mem) (µM)	**>199/ 199/ 199**
Cardiotox	**hERG IC_{50} (µM)**	**>30 (45% @30 µM)**

Table 30. Full profile of compound 5.53

The low solubility of the crystalline form of compound **5.53** translated into a high predicted human dose (≈ 2 g). As result, a back-up approach was launched as part of this programme seeking to improve solubility, as well as, *in vitro* activity.

6. ATTEMPTS AT IMPROVING GSK3531659A (5.53)

As described in the previous section, the compound GSK3531659A (**5.53**) presented a very promising profile, but there was still room to improve its PFI and *in vitro* activity. For this reason, two strategies were followed:

- to replace the hydroxy aliphatic chain in position 2 by other groups to improve the *in vitro* activity; and
- to substitute the 5-fluoroindole ring by a tetrahydroderivative core (see **Section 4.3.2.3**) in order to diminish the number of the aromatic rings and, therefore, decrease the PFI and also to break the planarity of the molecule to improve solubility.

6.1. REPLACEMENT OF 3-HYDROXYPROPANAMIDE FROM GSK3531659A (5.53)

The first strategy consisted of the substitution of the 3-hydroxypropanamide group by other moieties. Considering the results obtained in **Section 5** and the study of the substitution on the 5-position of the indole, the first compound that was synthesised was a combination of compounds **5.53** and **5.43** to give the compound **6.1** (see **Table 31**).

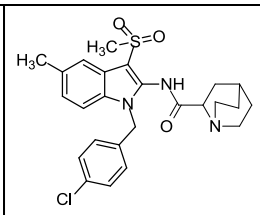
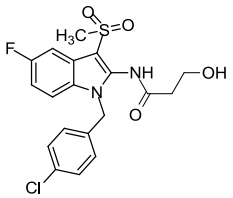
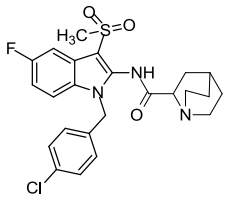
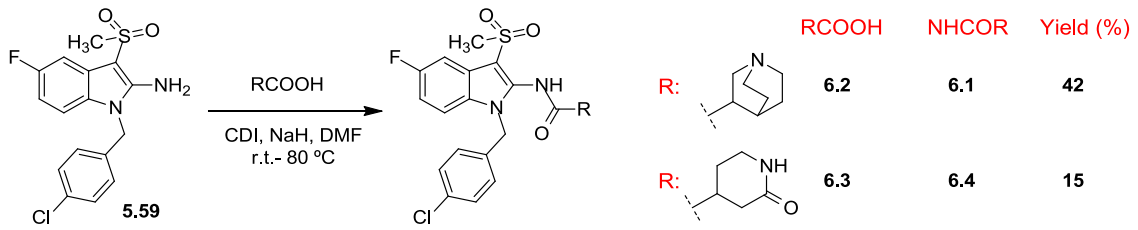
			
	GSK3533289A	**GSK3531659A**	**GSK3649413A**
	5.43	**5.53**	**6.1**
***Pf* IC_{50} (µM)**	**0.09**	**0.03**	**0.06**
Hep G2 XC_{50} (µM)	**50.1**	**>100**	**25.1**
CLND/ FaSSIF (µg/mL)	**84/664**	**178/200**	**150/503**
ChromlogD/PFI	**3.8/6.8**	**4.4/7.4**	**3.8/6.8**
pK_a	**7.3**		**7.3**
Cli (m,r,h) mL/min.g	**1/1.8/0.9**	**2.8/1/0.6**	**1.2/0.8/0.9**

Table 31. Comparison of compounds 5.43, 5.53 and 6.1.

In **Table 30** it is possible to appreciate that the compounds **5.43** and **6.1** that contained a quinuclidine moiety presented an improved solubility compared to that observed for the compound **5.53**, similar *in vitro* activity and *in vitro* stability, and also a lower PFI. Unfortunately, the presence of a basic amine led to a flag regarding cytotoxicity (Hep G2 of 50.1 and 25.1 µM for compounds **5.43** and **6.1**, respectively). In order to improve the cytotoxicity, 7 compounds with the scaffold of the compound **6.1** were designed and

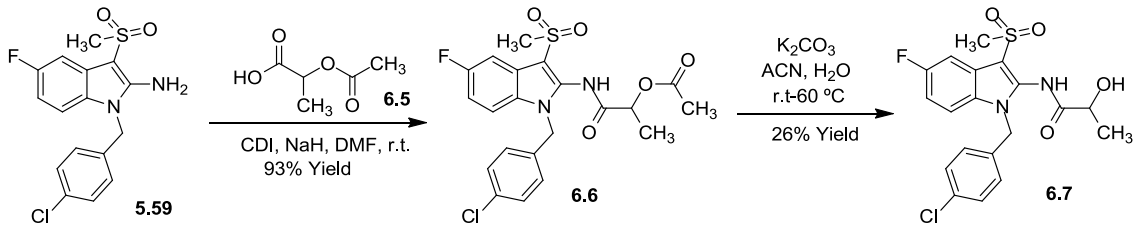
synthesised in this approach using the amine **5.59** as common starting material to be used in an acid-amine coupling using CDI as coupling agent, and sodium hydride as base. In some of the cases, further transformations were needed to obtain the desired products (see **Schemes 35-40**).

In **Scheme 35**, the synthesis of compounds **6.1** & **6.4** is also depicted, using the same conditions and the corresponding carboxylic acids **6.2** & **6.3** to afford to amides **6.1** & **6.5** in a low yield. This latter compound was designed to be an analogue to previously synthesised **5.37** but with an amide unit, which aimed to avoid the earlier observed cytotoxicity, as it did not present any basic amine.



Scheme 35. Synthesis of 6.1 & 6.5.

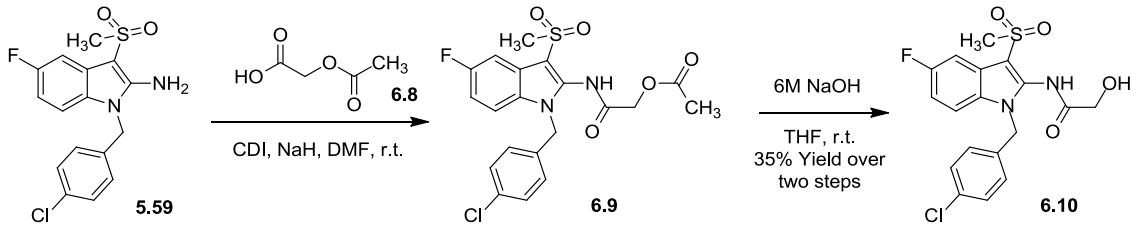
Using the idea of a carbon chain bearing a hydroxy group, as in compound **5.53**, two compounds were designed: the regioisomer **6.7** bearing a secondary alcohol instead of a primary unit, and the compound **6.10** with a shorter carbon chain. Both compounds were synthesised by basic hydrolysis of the corresponding acetate using a base. In the case of the compound **6.7**, the synthesis started with coupling between the aminoindole **5.59** and the carboxylic acid **6.5** to give the protected alcohol **6.6** in a high yield. Several additions of K_2CO_3 were used to remove the protecting group and complete the conversion that led to a low yield of **6.7** (**Scheme 36**).



Scheme 36. Synthesis of 6.7.

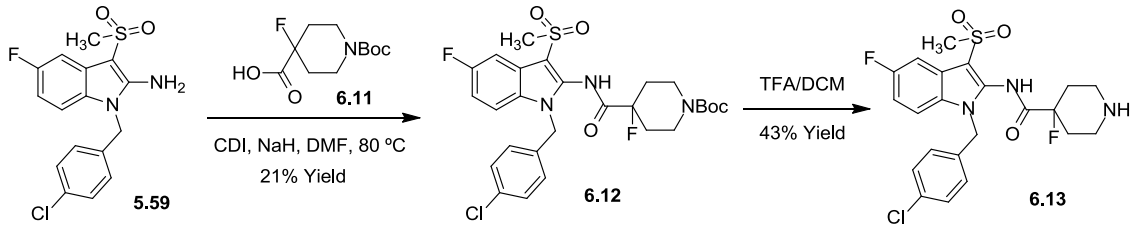
Scheme 37 depicts the synthesis of compound **6.10**, that started with the coupling between amine **5.59** and acid **6.8**, using the conditions previously described, to afford the amide **6.9** containing some starting material. The removal of the acetate was carried

out using a 6 M solution of sodium hydroxyde in THF to give the alcohol **6.10** in 3 h and no need of further additions of base.



Scheme 37. Synthesis of 6.10.

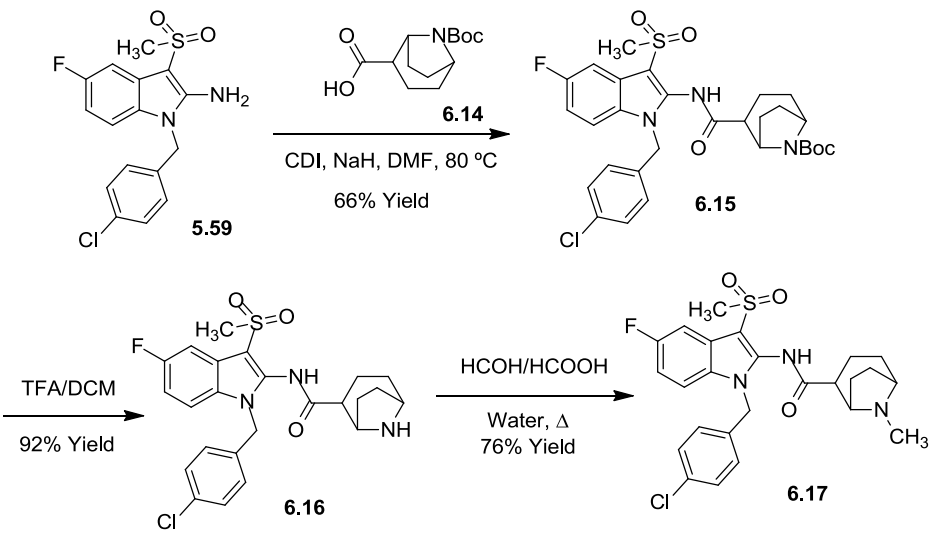
Taking into account the effect of the presence of fluorine atoms on the reduction of the basicity of the amines, the compound **6.13** was synthesised to combine the *in vitro* activity and stability of the piperidine compound **5.37** (**Section 5**) with an F unit to contribute to the reduction of the basicity, again aiming to avoid cytotoxicity issues. The compound was synthesised by Boc-deprotection of the compound **6.12** in the presence of TFA in DCM. Compound **6.12** was synthesised using CDI as coupling agent and NaH as base in a low yield, this was due to the reaction being incomplete even when it was heated at 80 ºC for 3 h (**Scheme 38**).



Scheme 38. Synthesis of 6.13.

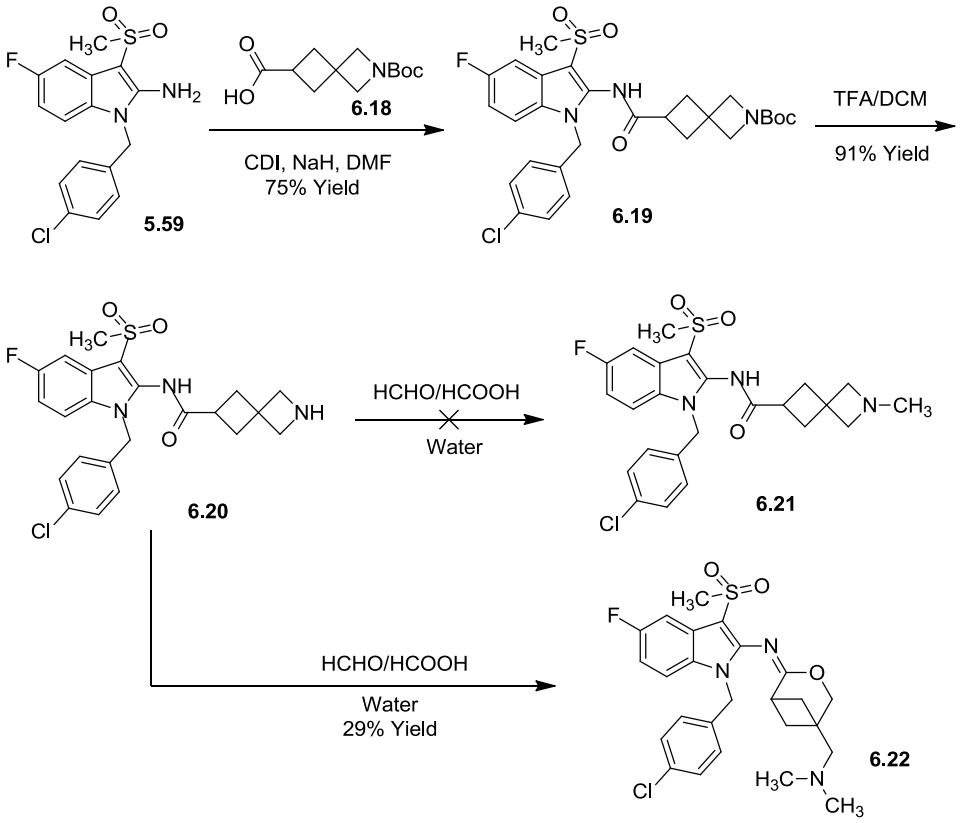
Based on the good levels of potency obtained in the library of amides (**Section 5**) when quinuclidine was introduced in the 5-methylindole derivative, a structural isomer was designed in attempts to establish the optimal substitution. The commercial availability of these type of compounds was the determining factor to choose carboxylic acid **6.14** to be used even when the calculated basicity of the amide **6.16** was quite high (pK_a= 10.4). In order to decrease the basicity, the amine was methylated (**6.17**), decreasing the pK_a to 8.1.

The synthesis (**Scheme 39**) started with the coupling between the amine **5.59** and the acid **6.14** with the previously used conditions in DMF at 80 ºC to give the Boc-protected amine **6.15**, that was subsequently treated with TFA in DCM to give the compound **6.16** in a high yield. Formaldehyde and formic acid were used to methylate the NH of the compound **6.16** to obtain **6.17** under water reflux.



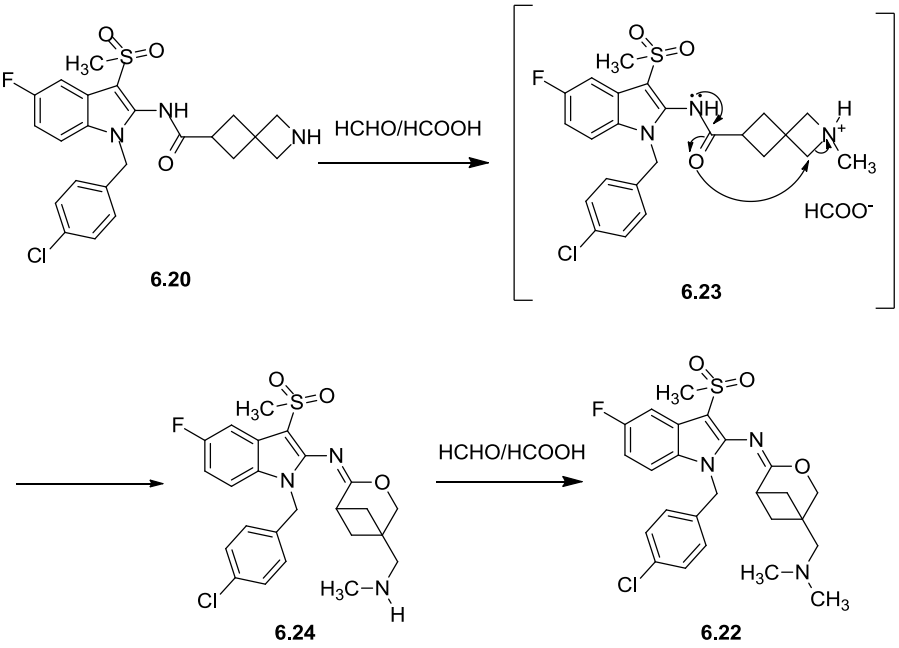
Scheme 39. Synthesis of 6.16 & 6.17.

In order to search for further chemical diversity, a spiroamine was selected to be introduced into this type of molecule. This azaspiro unit was introduced by coupling between the corresponding carboxylic acid **6.18** and the starting material **5.59** (**Scheme 40**) to give the Boc-protected amine **6.19**, that was then reacted with TFA to give the compound **6.20** in high yield. As the calculated pK_a of this compound was also quite high (9.9), methylation of the NH moiety of **6.20** was attempted to obtain **6.21**, using formaldehyde and formic acid. When the methylation reaction was carried out under the above-mentioned conditions, traces of the desired product (**6.21**) were observed by LCMS, along with a major unknown product. After the structural analysis by 1D and 2D NMR spectroscopy, the structure **6.22** was proposed for this product.



Scheme 40. Synthesis of 6.20 & 6.21.

A plausible mechanism for the formation of **6.22** is depicted in **Scheme 41**, and consists of the monomethylation of the NH of the spiro compound to give the proposed intermediated depicted in brackets as the quaternary salt (**6.23**), making the carbon in the α-position susceptible to be attacked by a nucleophile, i.e. the oxygen of the amide to form the compound **6.24**. Subsequently, the secondary amine **6.24** is methylated again with the system HCOH/HCOOH to give the dimethylated compound **6.22**.



Scheme 41. Plausible mechanism for the formation of 6.22.

6.1.1. Results and conclusions from the first strategy to improve 5.53

Eight new compounds were synthesised using the conditions initially described in **Section 5**. In the case of these compounds, stronger conditions were used regarding temperature, as most of the amine substrates did not react at room temperature. This may be an effect caused by the electronegative character of the fluorine present in the 5-position of the indole.

The profiles of these 8 amides and the reference compound **5.53** are depicted in **Table 32**.

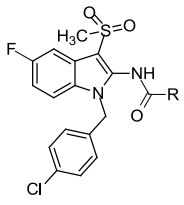
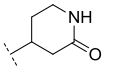
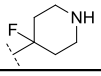
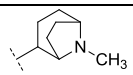
					
					
	GSK3531659A	**GSK3661742A**	**GSK3700573A**	**GSK3649416A**	
	5.53	**6.7**	**6.10**	**6.4**	
***Pf* IC_{50} (µM)**	**0.06**	**0.01**	**0.07**	**0.03**	
Hep G2 XC_{50} (µM)	**>100**	**63.1**	**>100**	**>100**	
hERG XC50 (µM)	**25.1/>30**	**15.8**		**>50.1**	
CLND/ FaSSIF (µg/mL)	**178/250**	**18/9**	**10/ND**	**14/10**	
ChromlogD/PFI	**4.4/7.4**	**5/8**	**4.5/7.5**	**4.1/7.1**	
pk_a					
Cli (m,r,h) mL/min.g	**2.8/1/0.6**	**3.9/2.2/0.8**	**10.8/1.7/0.5**	**1.1/1/1.2**	
					
	GSK3649412A	**GSK3649413A**	**GSK3649623A**	**GSK3649624A**	**GSK3682196A**
	6.13	**6.1**	**6.16**	**6.17**	**6.20**
***Pf* IC_{50} (µM)**	**0.08**	**0.06**	**0.02**	**0.03**	**0.08**
Hep G2 pXC_{50}/XC_{50}	**39.8**	**25.1**	**15.8**	**15.8**	**15.8**
hERG XC50 (mM)	**10**	**20**	**>50.1**	**50.1**	
CLND/ FaSSIF (µg/mL)	**66/ND**	**150/503**	**63/ND**	**119/124**	**223/ND**
ChromlogD/PFI	**3.7/6.7**	**3.8/6.8**	**3.6/6.6**	**3.5/6.5**	**3.8/6.8**
pk_a	**6.9**	**7.3**	**10.9**	**8.1**	**9.9**
Cli (m,r,h) mL/min.g	**1.3/1.1/0.7**	**1.2/0.8/0.9**	**1.1/1/0.7**	**1.2/10/0.7**	

Table 32. Library of amines using the scaffold of GSK3531659A (5.51).

All the compounds synthesised presented a similar *in vitro* activity to that observed with the compound **5.51**. The most promising compound from this group regarding activity was the structural isomer **6.7** but the solubility of this compound was 18 µg/mL, which was insufficient to rely on the rest of the data relating to toxicity or *in vitro* stability. The compound **6.10** showed a similar solubility, so they were both discarded.

Regarding the compound bearing the quinuclidine, the toxicity values made this compound not able to be progressed to further studies.

As it was observed in previous sections, the presence of a basic amine, meant a cytotoxicity issue as in the compounds **6.13**, **6.1**, **6.16**, **6.17** & **6.20**. In this series of compounds it is possible to confirm the tendency between pK_a and HepG2: decrease in the pK_a is translated into an increase on the HepG2 (less cytotoxic compounds).

6.2. REPLACEMENT OF THE 5-F-INDOLE CORE BY A TETRAHYDROINDOLE DERIVATIVE

Taking into account the *in vitro* stability observed for the compounds **4.41**, **4.93**, **4.123** & **4.134**, the corresponding derivatives with the alternative substitution in position 2 of the compound **5.51** were synthesised in order to reduce the lipophilicity and improve the physicochemical properties.

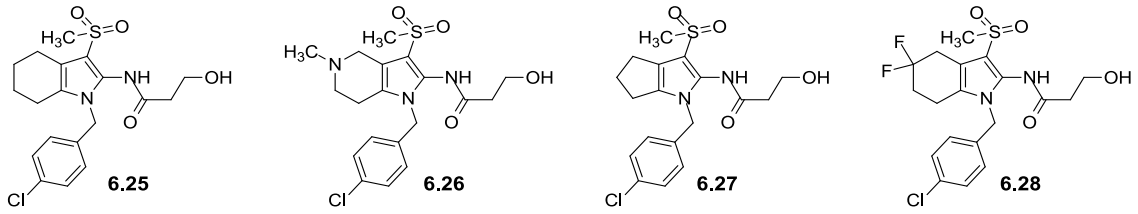
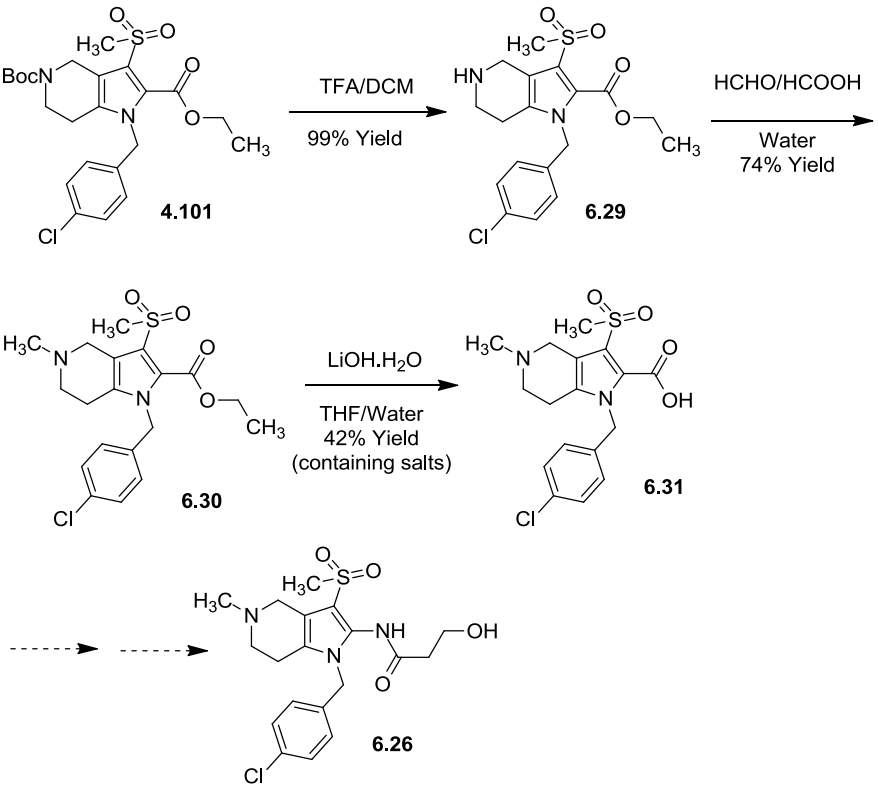


Figure 20. Target compounds for the replacement of the indole in 5.51 (6.25-6.28).

Compound **6.25** was synthesised elsewhere in our laboratories[84] and its synthesis is not described in this report.

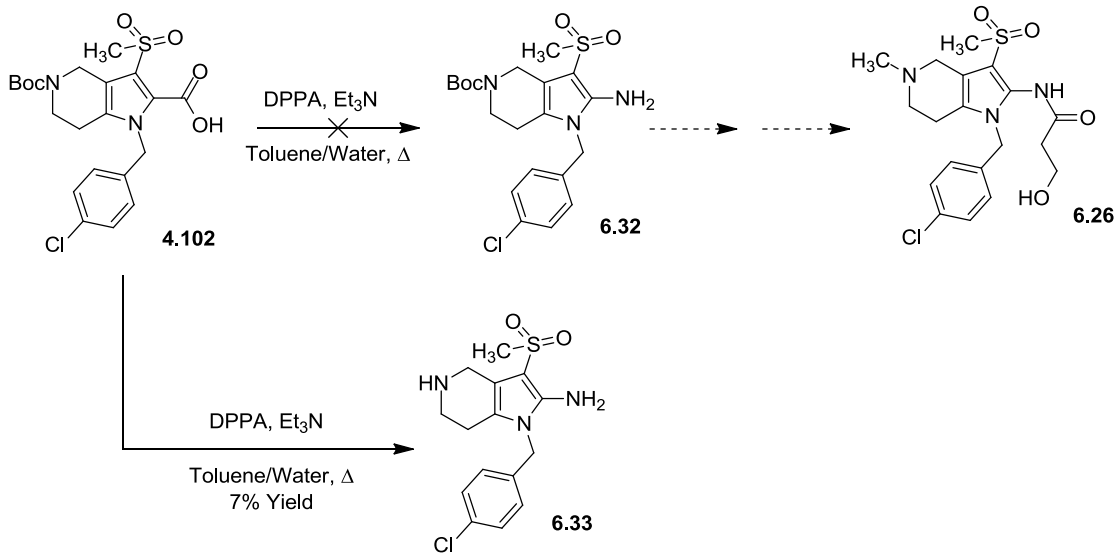
6.2.1. Synthesis of compound 6.26

In order to synthesise compound **6.26**, two different synthetic routes were approached:
In Route A (**Scheme 42**), the synthesis started with the deprotection of the *N*-Boc intermediate **4.101**, yielding the free amine **6.29** almost quantitatively. Compound **6.29** was subsequently alkylated with the formaldehyde/formic acid system to give **6.30** in a good yield. Then the ester moiety present in **6.30** was hydrolysed to the carboxylic acid **6.31** with the reaction perceived to have been completed to afford the desired product by LCMS. However, due to isolation problems, compound **6.31** was not obtained without the presence of inorganic salts. Several techniques were carried out in attempts to purify this desired material without any success and, therefore, this synthetic route was abandoned.



Scheme 42. Route A to synthesise compound 6.26.

Route B (**Scheme 43**) presents an alternative pathway starting with the Curtius rearrangement of the carboxylic acid **4.102** using water as co-solvent to obtain the amine **6.32**. This compound was not isolated but the deprotected amine **6.33** was isolated and sent for biological assay. This delivered a less positive result with an IC_{50} of 3.7 μM.

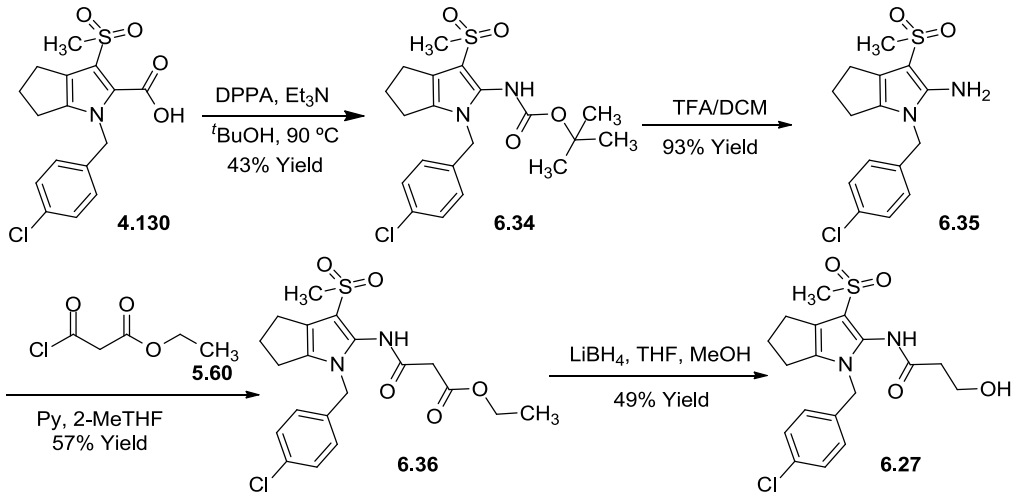


Scheme 43. Route B to synthesise compound 6.26.

Taking into account the synthetic difficulties encountered in attempting to obtain compound **6.26** and the preliminary biological results obtained for compound **6.27** (see **Sections 6.2.2** and **6.2.4**), the synthesis of **6.26** was abandoned.

6.2.2. Synthesis of compound 6.27

The synthesis of compound **6.27** (**Scheme 44**) started with the Curtius rearrangement of the carboxylic acid **4.130** using ^{t}BuOH as a solvent to obtain the Boc-protected amine **6.34**, that was then reacted with TFA in DCM to obtain the amine **6.35** in a high yield. Acylation with the corresponding acyl chloride **5.60** gave the intermediate **6.36**. The reduction of the ester moiety of the compound **6.36** afforded the desired alcohol **6.27** in a moderate yield.

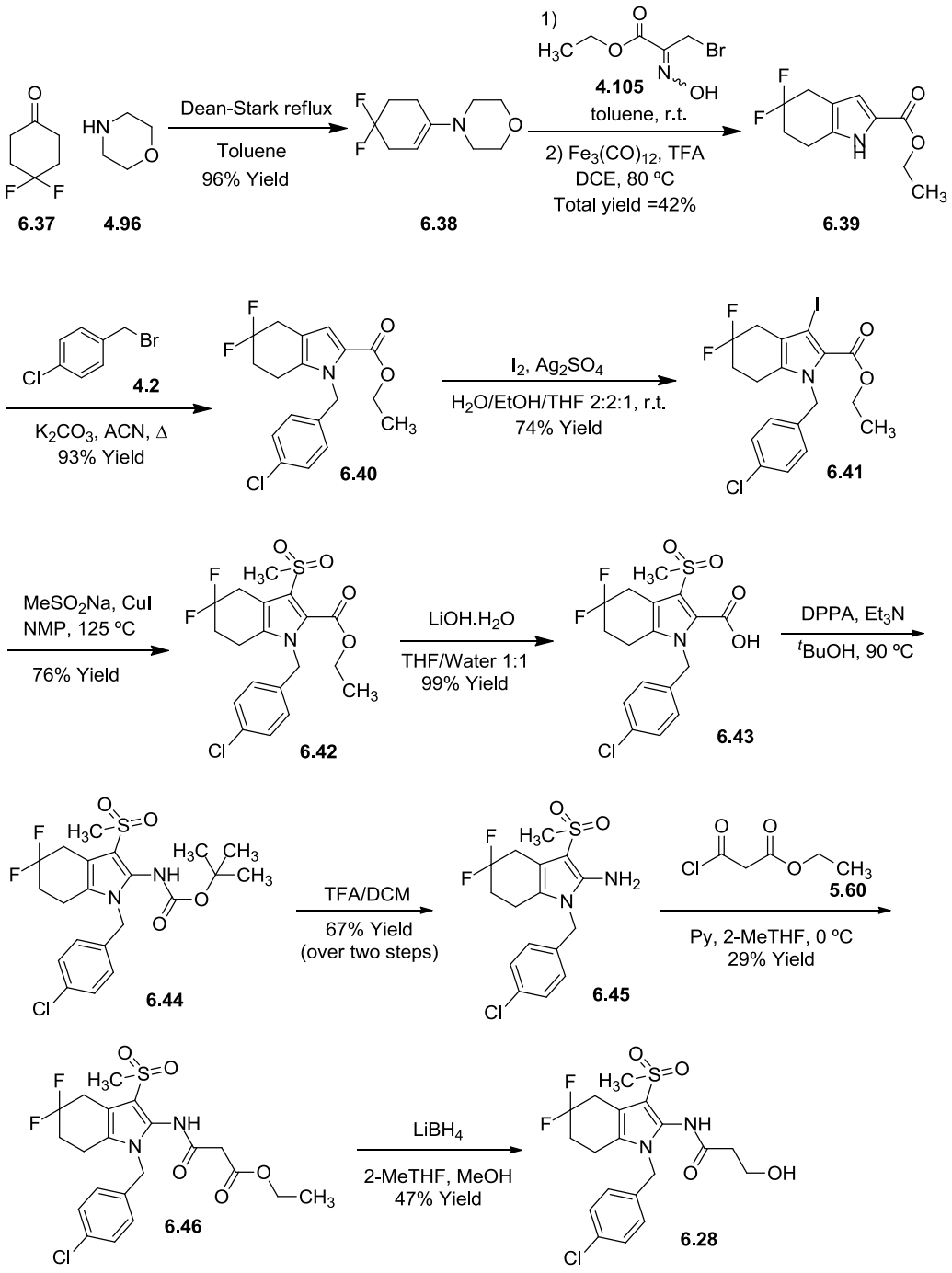


Scheme 44. Synthesis of compound 6.27.

6.2.3. Synthesis of compound 6.28

The synthesis of **6.28** (**Scheme 45**) started with the reaction between 4,4-difluorocyclohexanone and morpholine under reflux for 4 days under Dean-Stark conditions to give the reactive enamine **6.38** in a very good yield. The next step consisted of reaction between **6.38** and **4.105** that were stirred at room temperature for 2 h in toluene to form the corresponding oxazine intermediate (see **Scheme 22**, **page 78**). The reaction mixture was quenched with water and the phases were separated in a funnel. TFA and $Fe_3(CO)_{12}$[112] were added and the mixture was refluxed for 3 h in DCE to give a dark solution that was filtered, and the mother liquors were evaporated under

vacuum to give a crude product that was purified by Flash chromatography to provide **6.39**. Alkylation with **4.2** afforded **6.40** in a very good yield. This intermediate was then reacted with I_2 in the presence of Ag_2SO_4 to give the iodine intermediate **6.41**, that was submitted to an Ullmann-type reaction to introduce the sulfone moiety in position 3 in a high yield. Hydrolysis of the ester moiety afforded the carboxylic acid **6.43** that was subsequently reacted with DPPA and Et_3N in ^tBuOH to give the Boc-protected aminoindole **6.44** as Curtius rearrangement product. Deprotection of the amine in acid conditions gave **6.45** that was then reacted with the acid chloride **5.60** giving the ester intermediate **6.46**. The reduction of the ester in the presence of $LiBH_4$ afforded the desired alcohol **6.28** in a moderate yield.



Scheme 45. Synthesis of the compound 6.28.

6.2.4. Results of the tetrahydroderivative approach applied to improve the physicochemical properties of GSK3531659A (5.53)

As it was shown in **Tables 29 & 30**, compound GSK3531659A (**5.53**) showed a good *in vitro* antimalarial activity, and pharmacokinetic and safety profile, but the solubility of its crystalline form in FaSSIF media was insufficient to progress this compound. In order to improve the solubility, less planar scaffolds with one aromatic ring less were designed. Those compounds possessed the scaffolds of the tetrahydro derivative compounds **4.41**, **4.93**, **4.123** & **4.134**, and incorporated the 3-hydroxypropanamide unit present in compound **5.53**: compounds **6.25**, **6.26**, **6.27**, and **6.28**.

Synthesis of the compound **6.26** failed from two different approaches but it was possible to isolate the amino intermediate **6.33** that was sent to biological assays. The lack of activity of **6.33** (IC_{50}= 3.7 μM) and the synthetic difficulties led to no further attempts to synthesise **6.26**.

The compounds **6.25**[84] and **6.28** showed similar *in vitro* activity to the reference compound **5.53**. ChromlogD was also similar, but the removal of one aromatic ring caused a reduction in the PFI to 6.2 and 6, respectively (compound quality panel estimated PFI < 6 as a favourable value) moving to a better chemical space and also all the compounds were selective *versus* HepG2 (see **Table 33**). The compound **6.25** showed an *in vitro* stability that did not allow its progression to pharmacokinetic studies, but the modification of the metabolically susceptible 5-position with two fluorine atoms (compound **6.28**) ameliorated the *in vitro* stability. When this compound was tested in pharmacokinetic studies in mouse, the compound showed a good clearance (20.4 mL/min. Kg) and high oral bioavailability (74%). The half life time (0.6 h) of the compound was not enough for an antimalarial that would be used in a developing country, as the treatment would require to be administered once per day during three days. Having stated this, the discovery of this compound constitutes the appreciable development of a highly promising scaffold that will be of considerable future use with other substitutions in attempts to improve its half-life time, volume of distribution, and *in vitro* activity.

In order to increase the stability, a compound with a lower ChromlogD (removing also one metabolic labile position) was synthesised: **6.27**. This compound presented a ChromlogD of 3.7, and PFI of 5.7 (PFI < 6), and lower intrinsic clearance than **6.25**. Unfortunately, the solubility in CLND was too low (< 30 μg/mL) to rely on the toxicity and the stability data.

Compounds **6.27 & 6.28** required a ten-step synthetic route including a challenging step at the beginning of the route (see synthesis of carboxylic acid **4.130** on **page 84** and **Schemes 44** & **45** on pages **115** & **117**), so further efforts on improving the synthesis of these and other derivatives should be carried out.

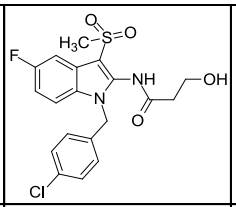
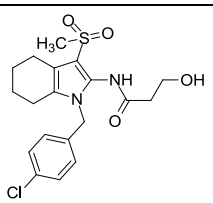
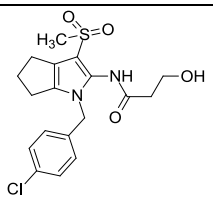
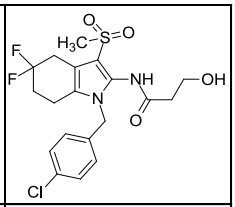
				
	GSK3531659A	**GSK3562852A**	**GSK3686839A**	**GSK3825153A**
	5.53	**6.25**	**6.27**	**6.28**
***Pf* IC_{50} (μM)**	**0.03**	**0.04**	**0.21**	**0.06**
Hep G2 XC_{50} (μM)	**> 100**	**> 100**	**>100**	**>100**
CLND/ FaSSIF (μg/mL)	**178/200**	**157/250**	**29/ND**	**207/142**
ChromlogD/PFI	**4.4/7.4**	**4.2/6.2**	**3.7/5.7**	**4/6**
Cli (m/r/h) mL/min.g	**2.8/1/0.6**	**13.1/2.2/2.4**	**6.6/ND/0.9**	**1.6/1.1/1.5**
Cl (m) (mL/min/Kg)				**20.4**
$t_{1/2}$ (m) (h)				**0.6**
%F (m)				**74**

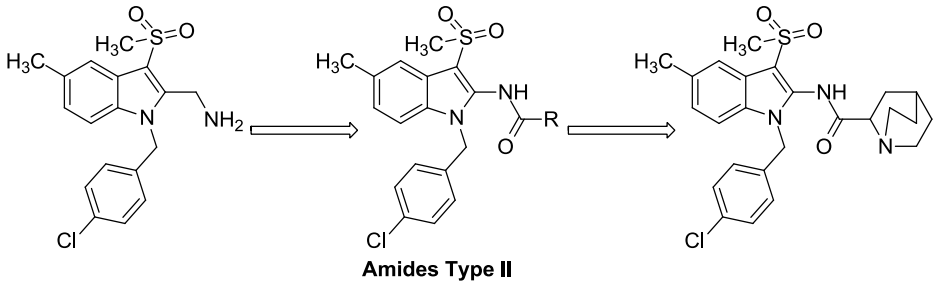
Table 33. Replacement of the 5-Fluoroindole from 5.53 for a tetrahydroindole core.

7. CONCLUSIONS

The studies within this programme targeting key aminoindoles have been focused on the improvement of the physical chemistry properties, and the safety profile of the Lead compound (**3.23**). With this objective, a substantial number of compounds have been designed and synthesised using different synthetic routes. Some of these syntheses were straightforward and afforded the compounds in good yields but in many cases, the synthesis needed more concentrated efforts.

At the outset of this project, a prominent objective was to find enhanced substitution of the Lead compound (**3.23**) in position 2. As part of this, Amides type II were identified providing optimal substitution in terms of *in vitro* activity and stability (see **Table 19**). In order to improve the physical chemical and safety profile of this type of compounds, a library of molecules bearing the same scaffold with different substitutions in position 2 was designed. Most of the compounds were obtained using a Buchwald-type reaction between the corresponding amide and a bromoindole derivative (**4.13**). Reaction conditions were also studied to improve the yields and avoid the appearance of by-products common in this type of reactions. Despite these efforts, the yields remained between 10-58% so another approach was chosen. The last compounds of this library were obtained by a more tractable synthetic route consisting of an acid-amine coupling with slightly better yields (33-68%) and more accesible starting materials. From this work, one compound bearing a quinuclidine (**5.43**) stood out due its balanced profile (see **Fig. 21**, **page 121**).

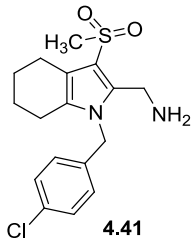
Compound **5.43** showed an *in vitro* activity in the same range as **3.23** and the same levels of cytotoxicity, however, this compound showed an appreciable improvement in FaSSIF solubility (from 52 to 664 μg/mL) and on *in vitro* stability. This quinuclidine moiety was further introduced in a more developed scaffold.

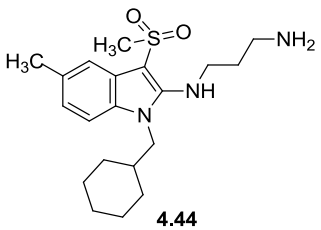


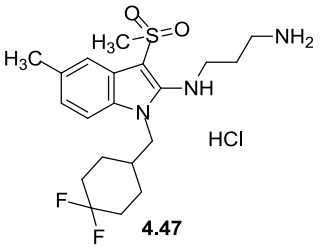
	GSK3359992A LEAD	GSK3533289A
	3.23	5.43
Pf IC_{50} (µM)	0.01	0.09
Hep G2 XC_{50} (µM)	50.1	50.1
FaSSIF (µg/mL)	52	664
ChromlogD/PFI	5/8	3.8/6.8
Cli (m,r,h) mL/min.g	16.7/13.7/4.3	1/1.8/0.9

Figure 21. Progression and data from Lead (3.23) to an enhanced compound 5.43.

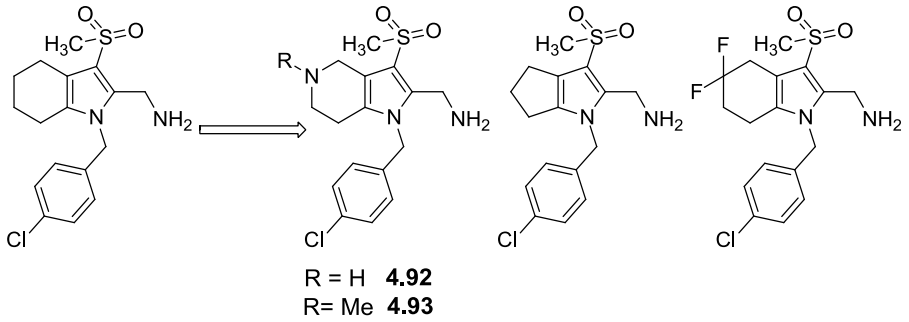
During these studies, different substitutions on other parts of the molecule were probed elsewhere in our laboratories.[84] From this effort, other molecules were identified with better physical chemistry properties such as the compound **4.41**, or better PK profile delivered by **4.44** and **4.47**, but with other liabilities.







Compound **4.41** presented a good solubility and PFI, but its PK properties needed to be improved. In order to ameliorate them, and supported by the literature, different molecules (**4.92**, **4.93**, **4.123**, & **4.134,** see **Fig. 22**) were designed and synthesised through a long synthetic route using a challenging step with an iron complex as reagent to obtain the key intermediates. The insufficient, or null in the case of **4.92**, *in vitro* activity of these molecules did not allow their progression but in some cases the *in vitro* stability was improved and the PFI was also decreased. Those scaffolds were used further with other substitutions.

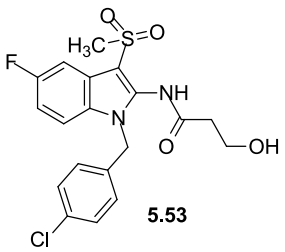


	4.41	4.92 / 4.93	4.123	4.134
Pf IC_{50} (µM)	0.03	>5/ 0.29	0.15	0.22
PFI	6.4	1 / 4	5.8	
Cli (m/r/h) mL/min.g	1.8/1.3/<0.6	ND 0.6/1/0.7	7.1/2.6/0.8	2.6/1.8/1.4

Figure 22. Modifications of the homoaliphatic part of 4.41 and relevant data.

The compounds previously described **4.44** & **4.47** showed an adequate PK profile, as well as a good *in vitro* activity, but the presence of a basic amine produced an important negative effect on the cytotoxicity, making these molecules not progressable to further *in vivo* efficacy studies. In order to avoid this issue, some molecules with the scaffold of **4.47** were designed and synthesised containing less basic amines. Those compounds (depicted in **Table 14**, **page 69**) were clean regarding cytotoxicity but the *in vitro* activity was not better than 150 nM making them not progressable. This approach was put on hold until a better substitution in the molecule was found.

As part of the overall programme aimed at accesing a more effective array of amides, a molecule (**5.53**) was declared a precandidate in June 2016. Unfortunately, the different crystalline forms of this compound were not sufficiently soluble to continue its progression and a back-up study, as part of this programme, was initiated in order to improve its profile.



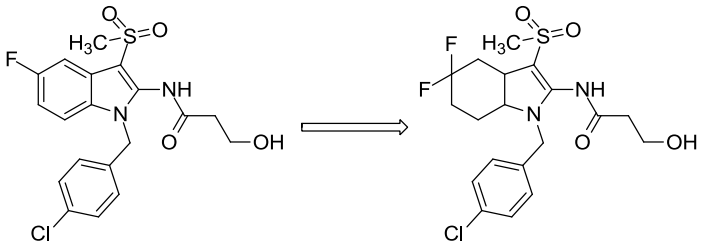
5.53

The studies to enhance the effectiveness over that found with compound **5.53** consisted of two different approaches:

- change the 3-hydroxypropanamide by other moieties; and
- change the indole scaffold by a tetrahydro derivative core to reduce the number of aromatic rings and reduce PFI in attempts to improve the solubility

From the first strategy, 8 new compounds with good *in vitro* activities were obtained. One of these species contained the quinuclidine from compound **5.43** but its cytotoxicity did not allow its progression. The rest of the compounds presented other issues that did not permit them to proceed to further studies. New molecules have now been designed to try to improve the profile of **5.53** maintaining the main scaffold (see **Section 8**).

As part of the second strategy, the replacement of the 5-fluoroindole core by a 5,5-difluoro-4,5,6,7-tetrahydroindole (as previously used in compound **4.134**) afforded an active compound (**6.28**), with a similar *in vitro* activity to **5.53**, adequate FaSSIF solubility, no cytotoxicity issues, and similar ChromlogD but lower PFI than **5.53** (from 7.4 to 6), due to the reduced number of aromatic rings from 3 to 2. Furthermore, the low *in vivo* clearance and high oral bioavailability made this a very interesting compound, and a molecule which constitutes a significant development emerging from this program of research. Additionally, **6.28** provides a highly promising scaffold which has the distinct potential to be improved upon, especially in terms of half-life time.



	5.53		6.28
Pf IC_{50} (µM)	0.03		0.06
Hep G2 XC_{50} (µM)	> 100		>100
CLND/ FaSSIF (µg/mL)	178/200		207/142
ChromlogD/PFI	4.4/7.4		4/6
Cli (m/r/h) mL/min.g	2.8/1/0.6		1.6/1.1/1.5
Cl (m) (mL/min/Kg)			20.4
$t_{1/2}$ (m) (h)			0.6
%F (m)			74

Figure 23. Comparison between the 5-fluoroindole derivative and the 5'5-difluorotetrahydroindole derivative.

8. FUTURE WORK

Future work on this project will continue focused on the improvement of the compounds **5.53** and **6.28**.

Based on the compound **6.28**, bearing a 5,5-difluorotetrahydroindole derivative, other analogues with substitutions already investigated as part of this project, could be synthesised to improve properties, such as the *in vitro* activity or increase the half-life of the molecule (**Figure 24**).

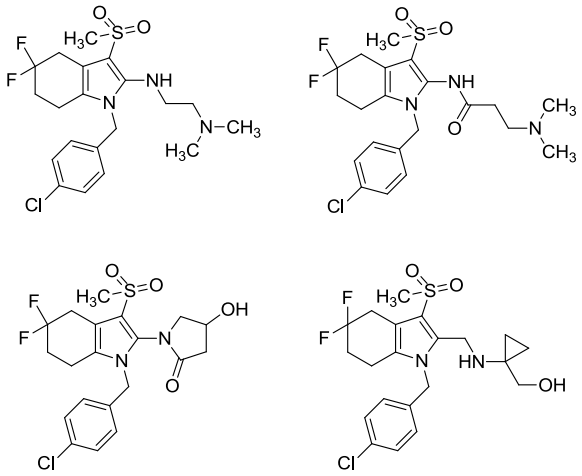


Figure 24. Target molecules derived from compound 6.28

One of the ideas is to replace the hydroxy group by other polar moieties; as we already know from this work, amines afforded compounds with important flags regarding cytotoxicity, so amino groups may be less well-tolerated. Other polar groups to be installed could be a carboxylic acid but, from previous experience with this type of compound, it is known that molecules bearing a carboxylic acid are inactive due to their low permeability (see **Section 4.3.1**, **Pages 66-69**, **Table 14**, compounds **4.65** & **4.68**). A good alternative to the carboxylic acid group is to use bioisosteres such as a tetrazole. Accordingly, some compounds could be synthesised bearing a tetrazole group (**Fig. 24**).

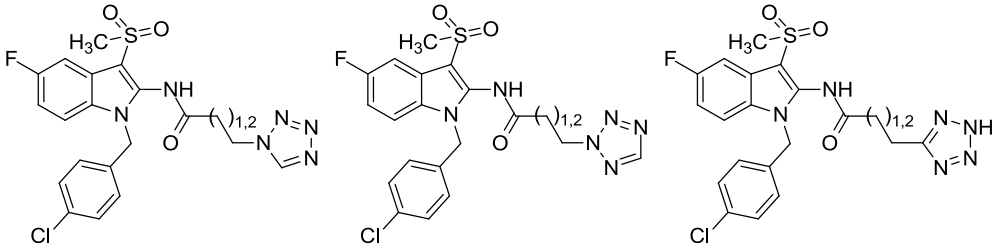


Figure 25. Target molecules bearing a tetrazole.

9. EXPERIMENTAL

9.1. GENERAL EXPERIMENTAL DETAILS

The names of the compounds prepared have been obtained using Chemdraw Ultra 12.0.

9.1.1.Purification by column chromatography

The Isolera Four is an automated multi-user flash chromatography system, available from Biotage, which utilises disposable, normal phase, silica gel cartridges. It provides quaternary solvent mixing to enable gradient methods to be run. Samples are queued using the multi-functional open access software, which manages solvents, flow-rates (10-200 mL/min), gradient profile, and collection conditions. The system is equipped with a variable wavelength UV-detector.

9.1.2. HPLC methodology

9.1.2.1. For analytic purpose: Method using ammonium bicarbonate modifier- " HPLC (basic pH)"

The HPLC analysis was conducted on an Agilent 1100 XBridge C_{18} column (4.6 mm x 50 mm, i.d. 3.5 µm packing diameter) at 35 ºC. The solvents employed were: A = ammonium hydrogen carbonate 0.1 M; B = acetonitrile. The gradient (A:B) employed was water $(NH_4)HCO_3$-ACN. 0-0.5 min 70:30; 0.5-4 min 0:100; 4-5 min 0:100. Flow: 1 mL/min. The UV detection wavelength was 254 nm and 210 nm.

9.1.2.2. For separative purpose: Method using ammonium bicarbonate modifier- " HPLC_sep (basic pH)"

The HPLC analysis was conducted on an Agilent 1200 or on an Agilent 1100, either on an X-Bridge C_{18} column (19 mm x 150 mm, i.d 5 µm packing diameter) or an X-Bridge C_{18} column (30 mm x 150 mm, i.d. 5 µm packing diameter) at 35 ºC. The solvents employed were: A = 0.1M ammonium bicarbonate in water; B = acetonitrile. The purification was run as a gradient (A:B) over either 20 min or 25 min, with a flow rate of 17 mL/min (19 mm x 150 mm, i.d. 5 µm packing diameter) or 35 mL/min (30 mm x 150 mm, i.d. 5 µm packing diameter). The UV detection wavelengths were 210 nm and 350 nm.

9.1.2.3. For separative purpose: Method using formic acid modifier- " HPLC_sep (acid pH)"

The HPLC analysis was conducted on an Agilent 1200 or on an Agilent 1100, either on an X-Bridge C_{18} column (19 mm x 150 mm, i.d 5 µm packing diameter) or an X-Bridge C_{18} column (30 mm x 150 mm, i.d. 5 µm packing diameter) at 35 ºC. The solvents employed were: A = 0.1M formic acid in water; B = 0.1M formic acid in acetonitrile. The purification was run as a gradient (A:B) over either 20 min or 25 min, with a flow rate of 17 mL/min (19 mm x 150 mm, i.d. 5 µm packing diameter) or 35 mL/min (30 mm x 150 mm, i.d. 5 µm packing diameter). The UV detection wavelengths were 210 nm and 350 nm.

9.1.3. LCMS methodology (LCMS Neutral)

The HPLC analysis was conducted on an Agilent HPLC. Method: Neutral with ammonium acetate pH 7. (4.5 min chromatogram). Initial conditions 70:30 ammonium acetate:ACN. The UV detection was an averaged signal from wavelength of 210 nm to 350 nm and mass spectra were recorded on a Waters ZMD mass spectrometer using alternate-scan positive and negative mode electrospray ionisation (ES +ve and ES -ve). Mass range 100-1200.

9.1.4. High resolution mass spectrometry

Positive ion mass spectra were acquired using a QSTAR Elite (AB Sciex Instruments) mass spectrometer, equipped with a turbospray source, over a mass range of 250 – 700, with a scan time of 1 sec. The elemental composition was calculated using Analyst QS 2.0 software. These experiments were performed by Jaime De-Mercado. Senior Scientist in the Pharmacology group at DDW-GSK Tres Cantos.

9.1.5. NMR spectroscopy

Unless otherwise specified, ^{1}H and ^{13}C NMR spectra were recorded in either $CDCl_3$ or DMSO-d_6 on a Bruker DPX 400 at 400 MHz and 100 MHz, respectively. The internal standard used was the residual protonated solvent at 7.27 ppm for $CDCl_3$ or 2.50 ppm for DMSO-d_6; and 77.1 ppm for $CDCl_3$ or 39.5 ppm for DMSO-d_6 in carbon spectra.

9.1.6. Microwave reactor

A Biotage Initiator microwave was used. The initial absorption was set as 'high' and 15 sec of pre–stirring was applied before heating commenced.

9.1.7. Infrared spectroscopy

IR spectra were recorded from solid samples using a Perkin Elmer Spectrum One FTIR spectrometer fitted with a Perkin Elmer Universal ATR (attenuated total reflectance) sampling accessory. Absorption frequencies are reported in cm^{-1}. These experiments were performed by Jesús Gómez, Scientist in the core chemistry group at DDW-GSK Tres Cantos.

9.1.8. Melting point

Melting points were measured using two different techniques depending on the mass availability:

- Differential scanning calorimetry (DSC) analysis (5 mg).

DSC is a thermoanalytical technique in which the difference in the amount of heat required to increase the temperature of a sample and reference is measured as a function of temperature. Both the sample and reference are maintained at nearly the same temperature throughout the experiment. Generally, the temperature program for a DSC analysis is designed such that the sample holder temperature increases linearly as a function of time. The reference sample should have a well-defined heat capacity over the range of temperatures to be scanned.[122]

These experiments were performed by Ana Álvarez, Associated Scientist in the core chemistry group at DDW-GSK Tres Cantos.

- Melting point microscope: Technoterm 7300 (Reichert-Jung Optische Werke) (≤ 1 mg)

9.1.9. Compound purity

The purity of compounds tested on *in vitro* and *in vivo* assays was greater than 95% using interpretation of a combination of LCMS (HPLC: Acquity UPLC BEH C18 1.7 µ 2.1 x 50 mm) at 35 ºC. Method: Acetate NH_4 25 mM + 10% ACN at pH 6.6/ACN. 0-0.2 min 100:0; 0.2-1.0 min 10:90; 1.0-1.8 min 10:90; 1.8-2.0 min 100:0. Flow: 0.8 mL/min. The UV detection wavelength was 254 nm and 210 nm)) and ^1H NMR data, unless stated otherwise. These analyses were performed by Ana Álvarez (Associated Scientist), María Teresa Quesada (Associated Scientist), and Jesús Gómez (Senior Scientist) in the core chemistry group at DDW-GSK Tres Cantos.

9.1.10. *In vitro* studies

9.1.10.2. Pf IC_{50} determination

Parasite growth inhibition assays and IC_{50} determination were carried out following standard methods using the ^{3}H-hypoxanthine incorporation assay.[123] Briefly, this assay relies on the parasite incorporation of labelled hypoxanthine that is proportional to *P. falciparum* growth. A culture of *Plasmodium falciparum* 3D7 parasitized red blood cells (RBC), (0.5% parasitemia, 2% haematocrit) in RPMI-1640, albumax 5%, and 5 μM hypoxanthine is exposed to drug serial dilutions. Plates are incubated for 24 h at 37 °C, with 5% CO_2, 5% O_2, and 95% N_2. After 24 h of incubation, ^{3}H-hypoxanthine is added and plates are incubated for an additional 24 h period. After this, parasites are harvested on a glass fiber filter using a TOMTEC Cell harvester 96. Filters are dried on melt-on scintillator sheets to determine the incorporation of ^{3}H-hypoxanthine. Radioactivity is measured using a microbeta counter. Data are normalised using the incorporation of the positive control (parasitised red blood cells without drug). IC_{50}s are determined using the Grafit 7 program. These assays were performed by the parasitology group at DDW-GSK Tres Cantos.

9.1.10.3. Gametocytes and male/female gamete inhibition

These assays were performed by the transmission blocking team at DDW-GSK Tres Cantos.

9.1.10.3.1. Gametocytocidal assay

This method is a sensitive ATP bioluminescence-based assay which determines the activity of antimalarial drugs by measuring the level of the intracellular ATP content of *Plasmodium falciparum* NF54 mature gametocytes after exposure to the drug. The erytrocytes are removed in a two-step procedure (Nycoprep cushion and magnetic column) and the purified gametocytes are exposed to the test compounds in RPMI 1640 supplemented with 10% AlbuMAX II. Plates are incubated in a 5% CO_2, 5% O_2, and 90% N_2 atmosphere, and 95% humidity at 37 ºC for 48 h. The ATP level is determined using the BacTiter-GloTM reagent (Promega) which generates a ‘‘glow-type’’ luminescent signal produced by the luciferase reaction, which consists of mono-oxygenation of luciferin catalysed by luciferase in the presence of Mg^{2+}, ATP, and molecular oxygen. The luminiscence is recorded in a 1420 Multilabel HTS counter Victor (PerkinElmer).

9.1.10.3.2. Inhibition of female gamete formation

This assay targets the inhibition of female gamete activation as an indicator of gametocyte functionality. *Plasmodium falciparum* NF54 mature gametocytes are incubated in RPMI 1640 supplemented with 10% human serum (Interstate Blood Bank) with the test compounds in a 5% CO_2, 5% O_2, and, 90% N_2 atmosphere, and 95% humidity at 37 ºC for 48 h. Then, the plates are transferred for activation to another incubator at 26 ºC for 24 h. The method is based on the specific expression of the Pfs25 protein at the surface of the female activated gamete detected by the use of fluorescent Cy3-Anti Pfs25 antibody (as primary parameter). The final output is the total number of activated female *Plasmodium falciparum* gametes per well and events are analysed by an algorithm (designed using the Acapella software or with Columbus blocks) according to their size, roundness, and the intensity of the fluorescence measured using an Opera High Content Screening System (PerkinElmer).

9.1.10.3.3. Inhibition of male gamete formation

This assay allows an estimation of exflagellation drug inhibition by addressing their ability to prevent male mature gametocytes to progress to microgametes. The method measures exflagellation of male microgametes by image analysis. *Plasmodium falciparum* NF54 mature gametocytes are incubated in RPMI-1640 supplemented with 10% human serum (Interstate Blood Bank) with the test compounds in conical TwistTop microcentrifuge vials. The microcultures are placed within a 37 °C gas-regulated incubator (5% O_2, 5% CO_2, and 90% N_2, and 95% humidity) for 48 h. After incubation, supernatant from each tube is removed and the cell pellet is resuspended in ookinete medium (RPMI medium with 25 mM HEPES, 50 mg/L hypoxanthine, 2 g/L sodium bicarbonate, 100 mM xanthurenic acid, and 20% human serum). The gametocyte suspension is then introduced into an individual chamber of a FastRead disposable hemocytometer. After 14 min incubation at room temperature (21 °C), the chamber is placed under a Direct Monitorized DM400B Microscope using a 10x magnification objective. Time-lapse movies of exflagellating cells in erythrocyte monolayers are captured and an algorithm which evaluates each frame and compares it to the preceding frame is written in the image manipulation software, Image J.

9.1.10.4. hERG inhibition determination

hERG (the human *Ether-à-go-go*-Related Gene) is a gene (*$KCNH_2$*) that codes for a protein known as $K_v11.1$, the alpha subunit of a potassium ion channel. This ion channel (sometimes simply denoted as 'hERG') is best known for its contribution to the electrical activity of the heart that coordinates the heart's beating (i.e., the hERG channel mediates the repolarizing I_{Kr} current in the cardiac action potential). When this channel's ability to conduct electrical current across the cell membrane is inhibited or compromised, either by application of drugs or by rare mutations in some families, it can result in a potentially fatal disorder called long QT syndrome, that is a rare inherited heart condition in which delayed repolarization of the heart following a heartbeat increases the risk of episodes of *torsades de pointes* (TdP, a form of irregular heartbeat that originates from the ventricles). These episodes may lead to palpitations, fainting, and sudden death due to ventricular fibrillation.

A number of clinically successful drugs in the market have had the tendency to inhibit hERG, and create a concomitant risk of sudden death, as a side-effect, which has made hERG inhibition an important antitarget that must be avoided during drug development. The hERG activity was measured using medium-throughput electrophysiology (IonWorks™ HT)[124,125,126] and automated Qpatch analysis.[127,128]

These assays were performed by Metul Patel and Brian Donovan at the PTS (Platform Technology Science) Department in GSK Stevenage.

9.1.10.5. Rabbit ventricular wedge assay[120]

Female rabbits were sedated with 6 mg/kg xylazine (*i.m.*), anticoagulated with 800 U/kg heparin (*i.v.)* and anaesthetised with ketamine (30–35 mg/kg, i.v.), or with pentobarbital (50 mg/kg, *i.v.*). The left circumflex or anterior descending branch of the coronary artery of the excised rabbit heart was cannulated and perfused in cardioplegic solution. A transmural left-ventricular wedge was dissected and placed in a tissue bath and arterially perfused with Tyrode's solution. After approximately 1 h of equilibration in the bath at a stimulation frequency of 1 Hz, the stimulation frequency was reduced to 0.5 Hz for 5 min of stabilisation where the baseline ECG was measured. The preparations were then returned to a stimulation frequency of 1 Hz and perfused with Tyrode's solution containing a test compound. For each test compound concentration,

the preparation was perfused for approximately 30 min at a frequency of 1 Hz followed by 5 min at a frequency of 0.5 Hz, where again the ECG was recorded.

These assays were performed by Sam Turner at the PTS (Platform Technology Science) Department in GSK Ware.

9.1.10.6. Cell cytotoxicity assays

The cytotoxicity is the quality of being toxic to cells. Regarding cytotoxicity assays, assessing cell membrane integrity is one of the most common ways to measure cell viability and cytotoxic effects since compounds that have cytotoxic effects often compromise cell membrane integrity.

These assays were performed by Vanesa Barrosa at the PTS (Platform Technology Science) Department in CIB at Tres Cantos, GSK. The methods to measure the cell cytotoxicity are described in the literature.[129]

9.1.10.7. CLND solubility determination

Solubility is the dose-limiting property for *in vitro* studies, and therefore is a critical physicochemical property to measure in drug discovery. Solubility data can be used to guide lead optimisation, troubleshoot erratic bioassay results, and identify potential downstream liabilities, such as insufficient solubility for bioassays or oral bioavailability. Typically, early *in vitro* studies are performed using library compounds prepared as dimethylsulfoxide (DMSO) stock solutions, resulting in *in vitro* test solutions containing DMSO at low concentration (<5% v/v). Since DMSO can affect the apparent solubility, it is desirable to obtain solubility data under conditions mimicking the *in vitro* study. The protocols used leading to the data presented in this document describe a general procedure for assessing kinetic aqueous solubility of early drug discovery compounds using a miniaturized shake flask method with chemiluminescent nitrogen detection (CLND).[74]

This assay was performed by the Physical Chemistry Group that is part of Analytical Chemistry located in Stevenage (UK) and Upper Providence (USA).

9.1.10.8. FaSSIF solubility determination

Determination of FaSSIF solubility was performed at pH 6.5 using 1-5 mg of substance, adding 1-5 mL of the appropiate solvent, and stopping the tube/vial. The sample was equilibrated at ambient temperature (~21–23 °C) or in a thermostatic water bath at 25 °C, unless a different temperature was desired or required, *e.g.* 37 °C. At each sample pull, the following was recorded: elapsed time, measured pH, and visual observations (*e.g.* colour changes, absence of solid). The sample was measured using HPLC. Typically, for this stage of development a generic chromatographic method was used.[130]

9.1.10.9. ChromlogD[131]

The Chromatographic Hydrophobicity Index (CHI)S8 values are measured using reversed phase HPLC column (50 x 2 mm 3 μM Gemini NX C18, Phenomenex, UK) with fast acetonitrile gradient at starting mobile phase of pHs 2, 7.4 and 10.5. CHI values are derived directly from the gradient retention times by using a calibration line obtained for standard compounds. The CHI value approximates to the volume % organic concentration when the compound elutes. CHI is linearly transformed into ChromlogDS9 by least-square fitting of experimental CHI values to calculated ClogP values for over 20K research compounds using the following formula: ChromlogD = 0.0857CHI-2.00. The average error of the assay is ±3 CHI unit or ±0.25 ChromlogD.

This assay was performed by the Physical Chemistry Group that is part of Analytical Chemistry located in Stevenage (UK) and Upper Providence (USA).

9.1.11. *In vivo* studies

“The human biological samples were sourced ethically and their research use was in accord with the terms of the informed consents”.

“All animal studies were ethically reviewed and carried out in accordance with Animals (Scientific Procedures) Act 1986 and the GSK Policy on the Care, Welfare and Treatment of Animals.”

9.1.11.1 Pharmacokinetic studies in mice

For pharmacokinetic studies in mice, animals were dosed either by intravenous (iv) or oral (po) route. For iv administration, the dosing volume was 10 mL/kg for a total dose of 1 mg/kg, and for po administration, the dosing volume was 10 mL/kg for a total dose of 10 mg/kg. The dosing solution for intravenous administration was prepared in 20% encapsine, 5% DMSO in saline solution (0.1 mg/mL), and for po administration a suspension in 1% methylcellulose (w:v) was formulated (C=1 mg/mL). Following iv dosing, blood samples were collected at 5, 15, and 30 minutes, 1, 2, 4, 6, 8, and 24 hours post-dose. Following oral dosing, blood samples were collected at 15, 30, and 45 minutes, 1, 2, 4, 6, 8, and 24 h post-dose. For whole blood samples analysis, 25 µL of fresh blood were mixed with 25 µL of saponine solution (0.1% in water) and immediately stored at -80 °C until analysis. Diluted blood samples were processed under standard liquid-liquid extraction procedures using acetonitrile and analysed by LC-MS/MS in positive ion mode with electrospray. Samples were assayed for the parent compound using a Sciex API 4000 Triple Quadrupole Mass Spectrometer (Sciex, Division of MDS Inc., Toronto, Canada), against a series of matrix matched calibration curve standards, using multiple reaction monitoring (MRM) at the specific transitions for the compound. Non-compartmental analysis was performed using Phoenix, version 6.3. (Phoenix™ WinNonlin® Copyright ©1998-2012, Certara L.P.), and the main pharmacokinetic parameters were estimated. Additional statistical analysis of the data was performed with GraphPad Prism® Version 5.01 (GraphPad Software Inc., San Diego CA).

The PK studies were performed by the Pharmacology Department at DDW-GSK Tres Cantos.

9.1.11.2. P. falciparum murine model

The efficacy of **3.21** & **3.23** against *P. falciparum Pf*3D7$^{0087/N9}$ was determined as previously described.[95] Briefly, a group of two female NSG (NOD-scid IL-2Rγ^{null}) mice engrafted with human erythrocytes (~ 50% human erythrocytes in peripheral blood) were infected by intravenous route with 2×10^7 parasitized erythrocytes on day 0. The compound was administered per oral route at 100 mg•Kg^{-1} in 1% methylcellulose once a day from day 3 to day 6 after infection. Parasitemia was assessed by FACS as previously described.[121] Fresh samples of peripheral blood from *P. falciparum*-infected

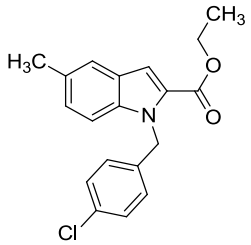
mice were stained with TER-119-Phycoerythrine (marker of murine erythrocytes) and SYTO-16 (nucleic acid dye) and then analysed by flow cytometry (FACSCalibur, BD). A qualitative analysis of the effect of treatment on *P. falciparum* Pf3D7$^{0087/N9}$ was assessed by microscopy and flow cytometry. Microscopy analysis was performed with Giemsa-stained blood smears. The levels in blood during a 23 h period after the first administration were measured in mice for the efficacy study.

These assays were performed by Therapeutic Efficacy Malaria group at DDW-GSK Tres Cantos.

9.2. LEAD OPTIMISATION

9.2.2. Synthesis of GSK3359992A (3.23)

Ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (4.3)



To a solution of ethyl 5-methyl-1*H*-indole-2-carboxylate (**4.1**) (5 g, 24.6 mmol) in dry acetonitrile (100 mL) was added potassium carbonate (6.8 g, 49.2 mmol), then 4-chlorobenzyl bromide (**4.2**) (6.1 g, 29.5 mmol) was added, and the resulting mixture was refluxed overnight. HPLC (basic pH) showed that reaction was complete. The solvent was evaporated under vacuum, and the resulting crude product was dissolved in water and extracted with EtOAc (x 3). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated affording an off-white solid that was analyzed by ^{1}H NMR showing little impurities. This was triturated with cyclohexane to give ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (**4.3**) (3.2 g, 40% yield) as a white solid.

The mother liquors were evaporated under vacuum to afford a solid that was triturated again with cyclohexane, this was filtered affording ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (**4.3**) (560 mg, 7% yield) as a white solid. The ^{1}H NMR spectrum was identical to the previous batch.

The second mother liquors were evaporated again affording 4.4 g of an off-white solid that was purified on a 100 g silica gel cartridge (Cy/EtOAc 0-50%). Appropriate fractions were evaporated together to give ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (**4.3**) (3.8 g, 47% yield) as a white solid. The ^{1}H NMR spectrum was identical to previous batches.

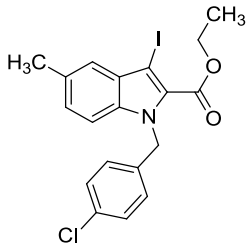
Total yield: 94%

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.49 (s, 1H), 7.46 (d, *J*=8.6 Hz, 1H), 7.32 (d, *J*=8.3 Hz, 2H), 7.28 (s, 1H), 7.15 (dd, *J*=8.6, 1.3 Hz, 1H), 7.01 (d, *J*=8.6 Hz, 2H), 5.81 (s, 2H), 4.27 (q, *J*=7.1 Hz, 2H), 2.38 (s, 3H), 1.28 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 162.5, 138.2, 138.1, 132.1, 130.4, 129.0, 128.6, 127.8, 127.5, 126.3, 122.2, 111.5, 110.8, 59.9, 47.4, 21.5, 14.1 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1 ;0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90 ; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.61 min, $[M+H]^+$ 328 (> 95% pure).

Ethyl 1-(4-chlorobenzyl)-3-iodo-5-methyl-1*H*-indole-2-carboxylate (4.4)

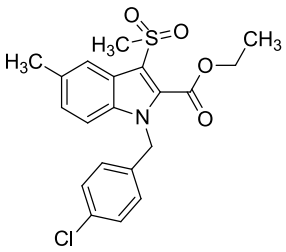


To a 0 ºC solution of NIS (1.67 g, 7.4 mmol) in dry DCM (40 mL), ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (**4.3**) (2.2 g, 6.71 mmol) was added. The reaction (pink suspension) was kept at 0 ºC for 2 h and then at room temperature overnight. After this time the solution colour had turned to dark pink (I_2 presence). HPLC (basic pH) did not show any starting material remaining so the reaction was considered finished. The reaction mixture was diluted with DCM and washed with water, sodium thiosulfate (solution turned green), brine, and dried over $MgSO_4$. The organic layer was filtered and evaporated under vacuum to obtain a crude product that was purified on a silica gel cartridge (80 g; Cy/EtOAc 0-15%) affording 2.7 g of a white product which corresponded to desired product **4.4** and starting material **4.3** (75:25). This material was used into the next reaction without further purification.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.51 (s, 1H)*, 7.49 (s, 0.3H), 7.46 (d, *J*=8.6 Hz, 0.3H), 7.33 (d, *J*=8.6 Hz, 2H)*, 7.32 (d, *J*=8.6 Hz, 0.6H), 7.28 (s, 0.3H), 7.27 (s, 1H)*, 7.24 (dd, *J*=8.6, 1.5 Hz, 1H)*, 7.15 (dd, *J*=8.6, 1.5 Hz, 0.3H), 7.01 (d, *J*=8.6 Hz, 0.6H), 7.00 (d, J=8.6 Hz, 2H)*, 5.81 (s, 0.6H), 5.78 (s, 2H)*, 4.32 (q, *J*=7.1 Hz, 2H)*, 4.27 (q, *J*=7.1 Hz, 0.6H), 2.43 (s, 3H)*, 2.38 (s, 0.9H), 1.3 (t, *J*=7.1 Hz, 3H)*, 1.28 ppm (t, *J*=7.1 Hz, 0.9H).

***Signals corresponding to final product **4.4**.

Ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (4.5)



A mixture of ethyl 1-(4-chlorobenzyl)-3-iodo-5-methyl-1*H*-indole-2-carboxylate (**4.4**) and ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (**4.3**) (75:25) (2.7 g, 6 mmol) was dissolved in NMP (3 mL). CuI (1.1 g, 6 mmol) and methanesulfinic acid, sodium salt (1:1) (1.5 g, 14.9 mmol) were added at room temperature. The reaction was heated at 125 ºC for 2 h, then left standing at room temperature for two days. TLC (Cy/EtOAc 0-20%) showed complete reaction. EtOAc was added and the resulting precipitate was filtered and the filtrate was washed with water, and brine, and dried over $MgSO_4$, filtered, and evaporated to afford a crude product that was purified by silica gel cartridge (10 g, 10-35% Cy/EtOAc) to give ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.5**) (1.59 g, 66% yield) as a yellowish sticky oil.

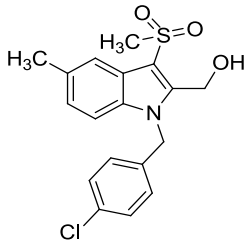
Ethyl 1-(4-chlorobenzyl)-5-methyl-1*H*-indole-2-carboxylate (**4.3**) (790 mg) was also recovered from the purification.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.83 (s, 1H), 7.58 (d, *J*=8.6 Hz, 1H), 7.34-7.45 (m, 2H), 7.24 (dd, *J*=8.7, 1.4 Hz, 1H), 7.13 (d, *J*=8.3 Hz, 2H), 5.60 (s, 2H), 4.34 (q, *J*=7.1 Hz, 2H), 3.32 (s, 3H), 2.43 (s, 3H), 1.26 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.0, 136.1, 134.4, 133.1, 133.0, 132.7, 129.1, 129.0, 127.6, 124.0, 120.4, 115.9, 112.3, 63.2, 48.0, 45.6, 21.6, 13.7 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1 ; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90 ; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.36 min, $[M+H]^+$ 406 (>95% pure).

(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (4.6)



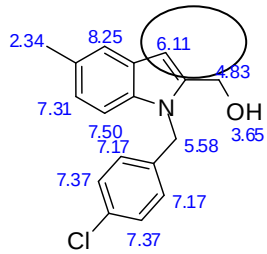
Three different conditions were carried out in attempts at synthesising compound **4.6**:

a) DIBAL-H in THF (1M solution).

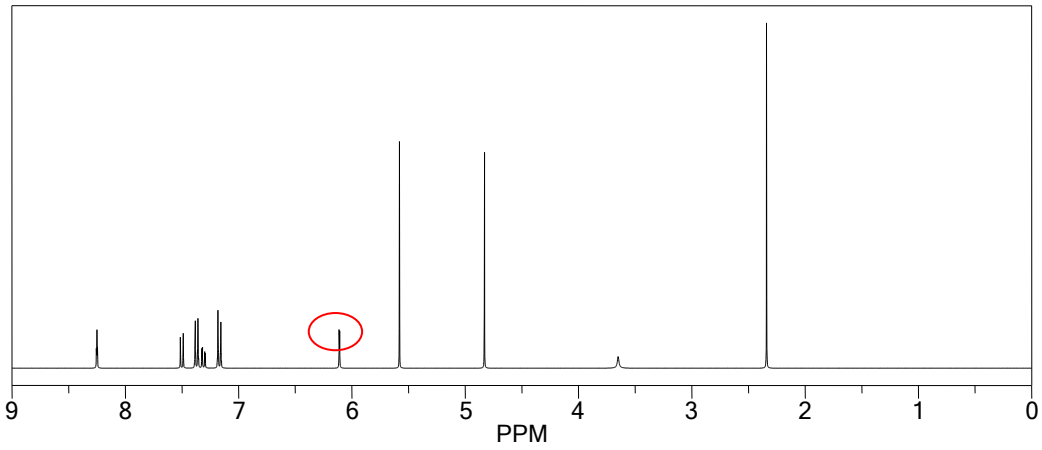
A solution of DIBAL-H in THF (1M, 16 mL, 16.00 mmol) was slowly added to a solution of ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.5**) (1.9 g, 4.7 mmol) in toluene (20 mL) at 0 ºC. The reaction mixture was allowed to stand under stirring at 0 ºC for 2 h and then at room temperature overnight. HPLC (basic pH) showed three peaks but none of them corresponded to starting material. The reaction mixture was cooled to 0 ºC and then a saturated solution of sodium potassium tartrate was added dropwise, EtOAc was added, and the phases were separated. The aqueous phase was washed with EtOAc (x 3) and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to afford 2.3 g of a crude product that was purified on a silica gel cartridge (Merck, 30 g), using Cy/EtOAc 0-35% as gradient. One product was isolated corresponding with the peak at 4.2 min in the HPLC (basic pH). The ^{1}H NMR spectrum matches reduction of the sulfone unit.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.31-7.35 (m, 2H), 7.30 (s, 1H), 7.17 (d, *J*=8.3 Hz, 1H), 7.03 (d, *J*=8.3 Hz, 2H), 6.88 (dd, *J*=8.5, 1.4 Hz, 1H), 6.35 (s, 1H), 5.76 (s, 2H), 5.43 (s, 2H), 5.29 (t, *J*=5.3 Hz, 1H), 4.56 (d, *J*=5.3 Hz, 2H), 2.34 ppm (s, 3H).

ChemNMR ^{1}H Estimation



Estimation quality is indicated by color: good, medium, rough



b) DIBAL-H in Hexane (1M solution).

A solution of DIBAL-H in hexane (1 M, 0.4 mL, 0.4 mmol) was slowly added to a solution of ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.5**) (50 mg, 0.1 mmol) in toluene (2 mL) at 0 ºC. The reaction was allowed to stand under stirring at 0 ºC for 1 h. HPLC (basic pH) showed one peak at 3.8 min identified as the desired product.

The reaction mixture was diluted with EtOAc and quenched with a saturated solution of sodium potassium tartrate, during which the organic phase turned to bright yellow. These conditions were deprioritized *vs.* the conditions c) because the solution colour did not change.

c) Solid $LiBH_4$

$LiBH_4$ (91 mg, 4.2 mmol) was added to a solution of ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.5**) (496 mg, 1.2 mmol) in toluene (20 mL) at 0 ºC. The reaction was allowed to stand under stirring at 0 ºC for 1 h. At this point HPLC (basic pH) analysis showed reaction completion. The reaction was quenched with a saturated solution of sodium potassium tartrate,

EtOAc was added, and the phases were separated. The aqueous phase was washed with EtOAc (x 3), and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated under vacuum to afford (1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (**4.6**) (430 mg, 97% yield) as a pale yellow solid.

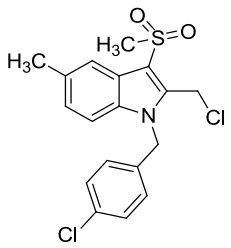
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.71 (s, 1H), 7.31-7.39 (m, 3H), 7.12 (d, *J*=8.3 Hz, 2H), 7.08 (dd, *J*=8.6, 1.3 Hz, 1H), 5.61 (s, 2H), 5.60 (br.s, 1H), 4.95 (d, *J*=3.8 Hz, 2H), 3.21 (s, 3H), 2.40 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 143.4, 136.7, 134.3, 132.4, 131.5, 129.0, 128.8, 125.5, 124.9, 119.7, 115.9, 111.5, 52.2, 46.7, 46.4, 21.5 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.24 min, $[M+H]^+$ 364 (>95% pure).

This reaction was repeated using 400 mg of ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.5**) to give 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (**4.6**) (380 mg, 100% yield) as a pale yellow solid. ^{1}H NMR showed that the product contained residual solvent. Both products were combined to be used as starting material in the next step of the synthesis.

1-(4-Chlorobenzyl)-2-(chloromethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (4.7)



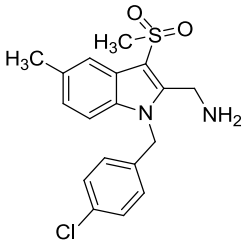
To (1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (**4.6**) (810 mg, 2.2 mmol) in dry DCM (5 mL) cooled at 0 ºC, methanesulfonyl chloride (0.2 mL, 2.67 mmol) and Et_3N (0.5 mL, 3.3 mmol) were added. The reaction was kept at this temperature for 1.5 h and then at room temperature for 60 h. The reaction mixture was diluted with DCM, washed with sat. aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered, and the solvent evaporated under vacuum affording 1-(4-chlorobenzyl)-2-(chloromethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.7**) (800 mg, 81% yield) as a pale yellow foam.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.72 (s, 1H), 7.34-7.41 (m, 3H), 7.15 (dd, *J*=8.6, 1.3 Hz, 1H), 7.10 (d, *J*=8.3 Hz, 2H), 5.65 (s, 2H), 5.32 (s, 2H), 3.24 (s, 3H), 2.42 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 138.7, 136.1, 134.7, 132.5, 132.3, 129.1, 128.8, 126.6, 124.6, 119.7, 112.6, 112.0, 46.4, 46.0, 34.2, 21.6 ppm.

LCMS No molecular ion was identified.

(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanamine (3.23)



To 1-(4-chlorobenzyl)-2-(chloromethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.7**) (800 mg, 1.81 mmol) in dry THF (10 mL), NH_3 in MeOH (7M, 9 mL, 1.81 mmol) was added and reaction was irradiated in the MW at 90 ºC for 30 min (2 cycles). Solvent was evaporated under vacuum affording 800 mg of a beige solid that was purified by semipreparative HPLC_sep (basic pH) to obtain two fractions:

- 80 mg of pure product (white solid) with traces of solvents; and
- 246 mg of (1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanamine as a white solid (**3.23**) (37% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.68 (s, 1H), 7.34-7.40 (m, 3H), 7.10 (d, *J*=8.6 Hz, 2H), 7.07 (dd, *J*=8.5, 1.4 Hz, 1H), 5.64 (s, 2H), 4.10 (s, 2H), 3.22 (s, 3H), 2.40 (s, 3H), 1.87 ppm (br. s., 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 146.5, 136.9, 134.4, 132.5, 131.5, 129.2, 128.7, 125.2, 125.1, 119.5, 111.4, 110.8, 46.4, 46.3, 35.1, 21.8 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.20 min, $[M+H]^+$ 363 (95% purity).

IR ν_{max} (neat) cm^{-1}: 3362.5, 3028.9, 1592.6, 1276.8, 1135.3.

MP 152 °C (Crystalline solid).

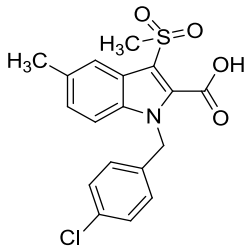
HRMS (ES) calcd for $C_{18}H_{20}{}^{35}ClN_2O_2S$, $(M + H)^+$ 363.0934, found 363.0919.

9.2.3. Lead optimisation programme (1st Round)

9.2.3.2. Amides & Amines

9.2.3.2.1. Amides

1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylic acid (4.8)

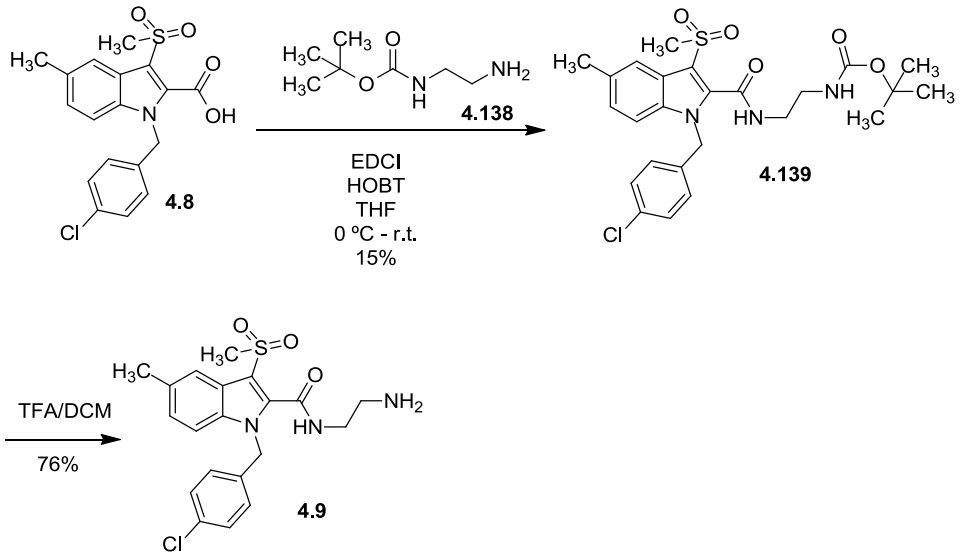


Ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.5**) (1.5 g, 3.7 mmol) was suspended in a mixture of THF (5 mL) and water (5 mL). $LiOH.H_2O$ (0.35 g, 14.8 mmol) was added, and the mixture was stirred under reflux for 2 h. HPLC (basic pH) showed reaction completion. The reaction mixture was acidified with 2 N HCl, and EtOAc was added, the phases were separated in a separating funnel, and the aqueous phase was isolated and washed with EtOAc (x 3). The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under vacuum affording 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylic acid (**4.8**) (1.3 g, 93% yield) as a white solid.

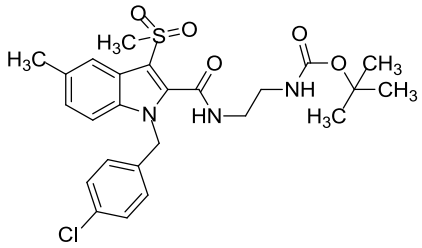
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.64-15.64 (m, 1H), 7.90 (s, 1H), 7.59 (d, *J*=8.6 Hz, 1H), 7.43-7.48 (m, 2H), 7.26 (dd, *J*=8.6, 1.3 Hz, 1H), 7.22 (d, *J*=8.6 Hz, 2H), 5.68 (s, 2H), 3.39 (s, 3H), 2.48 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 163.0, 136.3, 134.2, 132.7, 132.7, 132.8, 129.2, 129.2, 129.1 127.1, 124.4, 120.4, 112.3, 47.9, 45.6, 21.7 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.0 min, $[M+H]^+$ 378 (>95% pure).



Tert-Butyl(2-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamido)ethyl)carbamate (4.139)



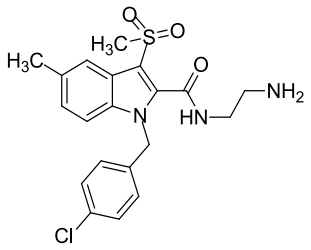
To a stirred solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylic acid (**4.8**) (200 mg, 0.53 mmol) dissolved in THF (6 mL) at 0 ºC was added EDCI (101 mg, 0.53 mmol) and HOBT (81 mg, 0.53 mmol). After 30 min *N*-Boc-ethylenediamine (**4.138**) (0.06 mL, 0.53 mmol) was added. The mixture was stirred overnight (18 h) at room temperature and LCMS Neutral showed reaction completion. Solvent was evaporated under vacuum and the residue was partitioned between EtOAc and water. The organic layer was washed two more times with water, brine, dried over Na_2SO_4, filtered, and evaporated to obtain 300 mg of a crude product that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-50%) to give impure product. This crude product was repurified by semipreparative HPLC_sep (basic pH). Appropriate fractions were evaporated to give *tert*-butyl (2-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamido)ethyl)carbamate (**4.139**) (42 mg, 15% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.00 (t, *J*=5.8 Hz, 1H), 7.66-7.69 (m, 1H), 7.45 (d, *J*=8.6 Hz, 1H), 7.37 (d, *J*=8.3 Hz, 2H), 7.29 (d, *J*=8.3 Hz, 2H), 7.15 (dd, *J*=8.6, 1.3 Hz, 1H), 6.72 (t, *J*=5.8 Hz, 1H), 5.37-5.42 (s, 2H), 3.26-3.32 (m, 2H), 3.24 (s, 3H), 3.06-3.14 (m, 2H), 2.41 (s, 3H), 1.36 ppm (s, 9H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.7, 155.9, 139.3, 135.9, 133.6, 132.8, 132.3, 129.7, 129.0, 126.3, 124.2, 119.7, 112.2, 111.5, 78.3, 55.5, 47.8, 45.8, 28.6, 26.7, 21.6 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.29 min, $[M+H]^+$ 520 (95% purity).

_N_-(2-Aminoethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indole-2-carboxamide (4.9)



To *tert*-butyl (2-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamido)ethyl)carbamate (**4.139**) (41 mg, 0.08 mmol) in DCM (3 mL), TFA (100 µL, 0.08 mmol) was added, and the reaction was stirred at room temperature for 15 min. HPLC (basic pH) showed reaction completion. Solvent and TFA were evaporated under vacuum affording 56 mg of a yellow oil that was loaded into a 1 g SCX column and eluted with 7 M ammonia in MeOH to give *N*-(2-aminoethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (**4.9**) (25 mg, 76% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.88-8.99 (m, 1H), 7.69 (s, 1H), 7.44 (d, *J*=8.6 Hz, 1H), 7.34-7.40 (m, 2H), 7.27 (d, *J*=8.3 Hz, 2H), 7.14 (dd, *J*=8.6, 1.3 Hz, 1H), 5.42 (s, 2H), 3.26-3.29 (m, 2H), 3.25 (s, 3H), 2.69 (t, *J*=6.2 Hz, 2H), 2.41 ppm (s, 3H) (NH_2 not observed in this experiment conditions).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.6, 139.5, 135.9, 133.7, 132.8, 132.3, 129.7, 129.0, 126.3, 124.2, 119.8, 112.1, 111.5, 47.7, 45.8, 43.0, 41.1, 21.6 ppm.

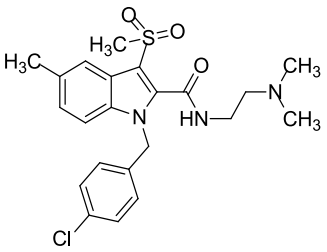
LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.05 min, $[M+H]^+$ 420 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2923.7, 1655.2, 1554.8, 1305.8, 1118.4.

MP Insufficient mass.

HRMS (ES) calcd for $C_{20}H_{23}{}^{35}ClN_3O_3S$, $(M + H)^+$ 420.1143, found 420.1146.

1-(4-Chlorobenzyl)-*N*-(2-(dimethylamino)ethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (4.10)



To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylic acid (**4.8**) (200 mg, 0.529 mmol) dissolved in THF (6 mL) at 0 ºC was added HOBT (81 mg, 0.529 mmol) and EDCI (101 mg, 0.529 mmol); 30 min later N^1,N^1-dimethylethane-1,2-diamine (0.046 mL, 0.529 mmol) was added. The reaction was stirred overnight at r.t. and LCMS Neutral showed reaction completion. Solvent was evaporated under vacuum, and residue was partitioned between EtOAc and water. Layers were separated, and the organic layer was washed two more times with water and then with brine, dried over Na_2SO_4, filtered, and evaporated to obtain 330 mg of a crude that was purified on a 12 g analogyx cartridge to give 1-(4-chlorobenzyl)-*N*-(2-(dimethylamino)ethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (**4.10**) (80 mg, 34% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.91 (t, *J*=5.6 Hz, 1H), 7.66-7.72 (m, 1H), 7.42 (d, *J*=8.1 Hz, 1H), 7.38 (d, *J*=8.3 Hz, 2H), 7.29 (d, *J*=9.3 Hz, 2H), 7.13 (dd, *J*=8.3, 1.3 Hz, 1H), 5.45-5.50 (s, 2H), 3.34-3.38 (m, 2H), 3.24 (s, 3H), 2.40 (s, 3H), 2.36 (t, *J*=6.8 Hz, 2H), 2.11 ppm (s, 6H).

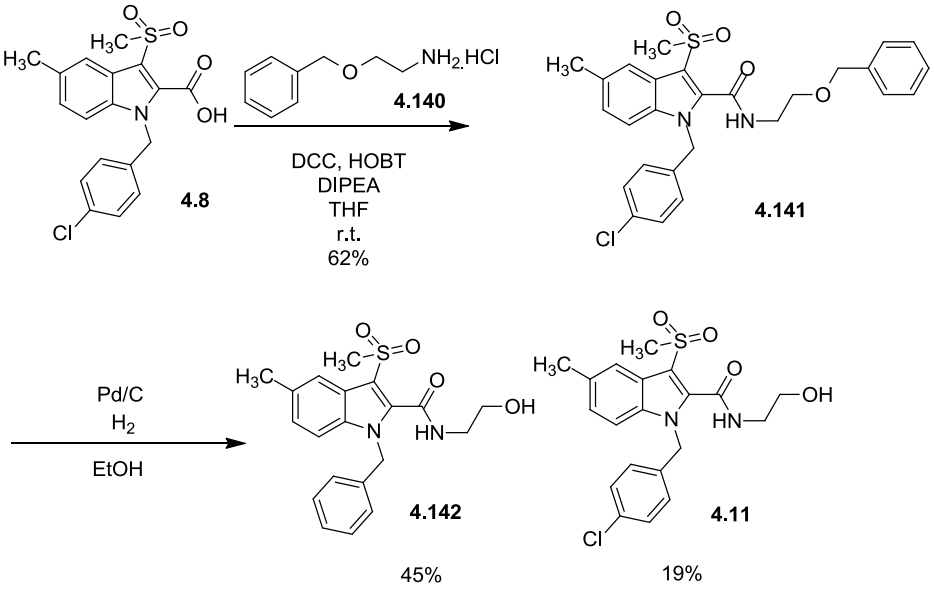
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.5, 139.6, 136.2, 133.7, 132.7, 132.3, 129.7, 129.0, 126.2, 124.4, 119.8, 112.1, 111.6, 58.1, 47.7, 45.9, 45.6, 37.8, 21.7 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.15 min, $[M+H]^+$ 448 (>95% purity).

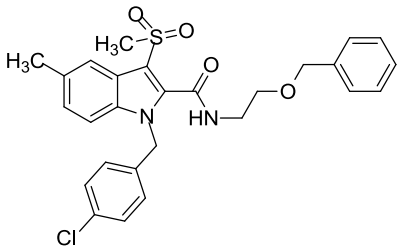
IR ν_{max} (neat) cm^{-1}: 3332.1, 2980.0, 2856.4, 2819.2, 2777.2, 1665.3, 1289.3, 1114.0.

MP 160.2 °C (Crystalline solid).

HRMS (ES) calcd for $C_{22}H_{27}{}^{35}ClN_3O_3S$, $(M + H)^+$ 448.1456, found 448.1470.



***N*-(2-(Benzyloxy)ethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide) (4.141)**



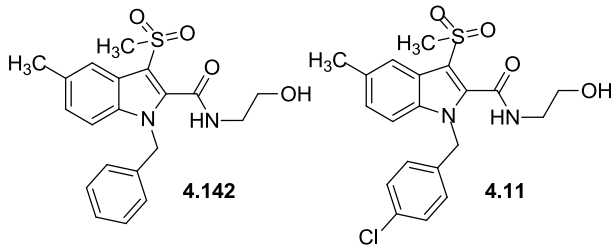
To a stirred solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylic acid (**4.8**) (150 mg, 0.4 mmol) dissolved in THF (2 mL) at 0 ºC was added DCC (82 mg, 0.4 mmol) and HOBT (60.8 mg, 0.4 mmol). After 30 min a solution of 2-(benzyloxy)-1-ethanamine, hydrochloride (**4.140**) (60 mg, 0.4 mmol) and DIPEA (0.07 mL, 0.4 mmol) in THF (2 mL) was added, and the reaction mixture was stirred at r.t. After 5 h HPLC (basic pH) showed the reaction had reached completion. Solvent was evaporated under vacuum and the residue was partitioned between EtOAc and water. The organic layer was washed two more times with water and then with brine, dried over Na_2SO_4, filtered, and evaporated to obtain 230 mg of a crude that was triturated with MeOH. The resulting solid was filtered to give *N*-(2-(benzyloxy)ethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (**4.141**) (120 mg, 62% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.12 (s, 1H), 7.68 (s, 1H), 7.40 (d, *J*=8.3 Hz, 1H), 7.18-7.37 (m, 9H), 7.09-7.17 (m, 1H), 5.38 (s, 2H), 4.45 (s, 2H), 3.53-3.60 (m, 2H), 3.45-3.53 (m, 2H), 3.22 (s, 3H), 2.40 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.7, 139.6, 138.8, 135.9, 133.6, 132.8, 132.3, 129.7, 129.0, 128.7, 128.1, 127.9, 126.2, 124.3, 119.8, 112.2, 111.7, 72.5, 68.6, 47.8, 45.8, 39.7, 21.8 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.35 min, $[M+H]^+$ 511 (95% purity).

***N*-(2-(Benzyloxy)ethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (4.142) & 1-(4-chlorobenzyl)-*N*-(2-hydroxyethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (4.11)**



N-(2-(Benzyloxy)ethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (**4.141**) (50 mg, 0.098 mmol) was suspended in EtOH (5 mL) and a N_2 stream was bubbled through it, then Pd(C) (10% wt, 10.41 mg, 9.78 µmol) was added and the reaction was hydrogenated with a balloon filled with hydrogen. After 2 h the reaction was checked by HPLC (basic pH) and showed two products. The reaction mixture was filtered through a PTFE filter joined to a syringe to remove Pd, the syringe and the PTFE filter were rinsed with more EtOH, and the colourless solution was evaporated under vacuum to give 40 mg of a crude product that was purified by semi-preparative HPLC_sep (basic pH).

Two products were isolated:

- *N*-(2-(Benzyloxy)ethyl)-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (**4.142**) (17 mg, 45% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.95-9.03 (m, 1H), 7.65-7.70 (m, 1H), 7.39-7.46 (m, 1H), 7.28 (d, *J*=2.5 Hz, 5H), 7.09-7.15 (m, 1H), 5.42 (s, 2H), 4.60-4.72 (m, 1H), 3.46-3.55 (m, 2H), 3.34-3.38 (m, 2H), 3.24 (s, 3H), 2.40 ppm (s, 3H).

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.10min, $[M+H]^+$ 387 (>95% purity).

- 1-(4-chlorobenzyl)-*N*-(2-hydroxyethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxamide (**4.11**) (8 mg, 19% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.99 (t, *J*=5.7 Hz, 1H), 7.68 (s, 1H), 7.43 (d, *J*=8.6 Hz, 1H), 7.33-7.40 (m, 2H), 7.25-7.31 (m, 2H), 7.14 (dd, *J*=8.6, 1.5 Hz, 1H), 5.42 (s, 2H), 4.67 (t, *J*=5.2 Hz, 1H), 3.51 (q, *J*=5.6 Hz, 2H), 3.35 (q, *J*=5.6 Hz, 2H), 3.24 (s, 3H), 2.41 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.7, 139.6, 136.0, 133.7, 132.9, 132.3, 129.8, 129.0, 126.2, 124.3, 124.2, 119.8, 112.2, 59.8, 47.8, 45.9, 42.5, 22.0 ppm.

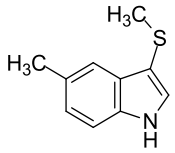
LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.16 min, $[M+H]^+$ 421 (95% purity).

IR ν_{max} (neat) cm^{-1}: 3519.6, 3277.9, 2923.7, 1647.4, 1304.63, 1117.8.

MP 164.8 °C (Crystalline solid).

HRMS (ES) calcd for $C_{20}H_{22}{}^{35}ClN_2O_4S$, $(M + H)^+$ 421.0983 found 421.0975.

5-Methyl-3-(methylthio)-1*H*-indole (4.18)[100]



To a solution of succinimide-dimethylsulfonium chloride, prepared by the addition of dimethylsulfide (1.2 mL, 16.8 mmol) to a solution of 1-chloropyrrolidine-2,5-dione (2.24 g, 16.8 mmol) in DCM (40 mL) at 0 ºC, was added 5-methyl-1*H*-indole (2 g, 15.2 mmol) in DCM (20 mL) at -20 ºC under N_2. After removal of the solvent, the oily residue was suspended in xylene (30 mL) and the mixture was heated to reflux for 2 h. After this, the reaction was concentrated under reduced pressure and the residue was purified by chromatographic column (40 g, cHex-EtOAc gradients from 100:0 to 90:10) to give 5-methyl-3-(methylthio)-1*H*-indole (**4.18**) (1.8 g, 67% yield) as a beige oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.17 (br. s., 1H), 7.42 (d, *J*=2.3 Hz, 1H), 7.37 (s, 1H), 7.29 (d, *J*=8.3 Hz, 1H), 6.97 (dd, *J*=8.2, 1.4 Hz, 1H), 2.41 (s, 3H), 2.29 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 135.2, 129.3, 129.0, 128.5, 123.8, 118.3, 112.2, 105.2, 21.7, 20.1 ppm.

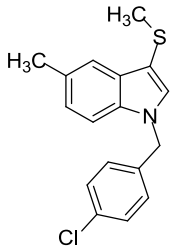
LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.26 min, $[M+H]^+$ 178 (> 95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain 5-methyl-3-(methylthio)-1*H*-indole (**4.18**) (7.3 g, 98% yield) as a beige oil:

- Dimethylsulfide (3.4 mL, 46.1 mmol).
- 1-Chloropyrrolidine-2,5-dione (6.16 g, 46.1 mmol) in DCM (100 mL) at 0 °C.
- Methyl-1*H*-indole (5.5 g, 41.9 mmol) in DCM (50.00 mL) at -20°C under N_2.

Xylene (50 mL).

1-(4-Chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (4.19)



To a solution of 5-methyl-3-(methylthio)-1*H*-indole (**4.18**) (1 g, 5.6 mmol) in dry ACN (30 mL) was added potassium carbonate (1.56 g, 11.3 mmol), then 1-(bromomethyl)-4-chlorobenzene (**4.2**) (1.39 g, 6.8 mmol) was added and reaction was refluxed overnight. Solvent was evaporated under vacuum, the crude mixture was dissolved in water, and extracted with EtOAc (x 3). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated affording a brownish oil that was purified three times by silica gel cartridge (0-10% Cy/EtOAc) to obtain 1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.19**) (1.2 g, 70% yield) as a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.61 (s, 1H), 7.32-7.40 (m, 4H), 7.18-7.24 (m, 2H), 6.98 (dd, *J*=8.5, 1.4 Hz, 1H), 5.37 (s, 2H), 2.39 (s, 3H), 2.31 ppm (s, 3H).

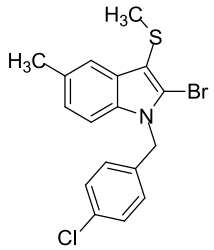
Purity by HPLC (basic pH) is 80%.

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain 1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.19**) (15 g, 100% yield) as a beige oil. The compound contained some impurities.

- 5-Methyl-3-(methylthio)-1*H*-indole (**4.18**) (8.5 g, 48 mmol) in dry ACN (200 mL)
- Potassium carbonate (13.2 g, 96 mmol)

- 1-(Bromomethyl)-4-chlorobenzene (**4.2**) (11.8 g, 57.5 mmol)

2-Bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (4.20)



NBS (465 mg, 2.6 mmol) was added to an ice-cooled solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.19**) (788 mg, 2.6 mmol) in chloroform (20 mL) and the mixture stirred for 15 min at 0 °C. HPLC (basic pH) showed that the reaction was complete after this time. The mixture was poured onto an ice-cooled 2 N NaOH solution (20 mL) and extracted with $CHCl_3$. The combined extracts were washed twice with water (20 mL) and dried (Na_2SO_4). Evaporation of the solvent gave 1.4 g of an orange oil that was purified by flash chromatography (Cy/EtOAc 0-10%) to give 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.20**) (840 mg, 85% yield) as a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.41-7.46 (m, 2H), 7.34-7.40 (m, 2H), 7.06 (d, *J*=8.3 Hz, 2H), 7.03 (dd, *J*=8.6, 1.3 Hz, 1H), 5.52 (s, 2H), 2.41 (s, 3H), 2.27-2.30 ppm (m, 3H).

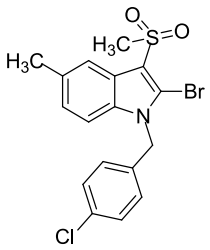
Purity by HPLC (basic pH) is 75%.

The product was used in the next step of the synthesis without any further purification.

This reaction was repeated on a bigger scale, using the same procedure and the amounts depicted below to obtain 1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.20**) (13.6 g, 72% yield). This product contained small impurities, but this was also used in the next step without any further purification.

- NBS (8.85 mg, 49.7 mmol).
- 1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.19**) (15 g, 49.7 mmol) in chloroform (200 mL).

2-Bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (4.13)



A solution of 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.20**) (0.4 g, 1.051 mmol) in anhydrous DCM (10 mL) under N_2 atmosphere was cooled to 0 ºC and *m*-CPBA (0.45 g, 2.6 mmol) was added and the resulting mixture was stirred for 1 h, and HPLC (basic pH) showed reaction completion after this time. The reaction was diluted with DCM (40 mL) and treated with 1 N NaOH (15 mL). The layers were separated, and the organic phase was washed with 10% $NaHCO_3$ (10 mL) and brine (10 mL). The organic phase was then dried over anhydrous $MgSO_4$, filtered, and concentrated under reduced pressure. The residue obtained was triturated with *t*-BuOMe, and the solid was filtered to give 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (236 mg, 54% yield) as an off-white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.77 (s, 1H), 7.56 (d, *J*=8.6 Hz, 1H), 7.40 (d, *J*=8.1 Hz, 2H), 7.08-7.19 (m, 3H), 5.62 (s, 2H), 3.22 (s, 3H), 2.42 ppm (s, 3H).

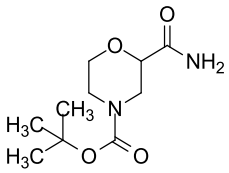
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 135.9, 134.7, 132.7, 132.2, 129.3, 128.9, 125.8, 125.2, 119.4, 119.2, 113.7, 111.6, 47.9, 45.2, 21.7 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.33 min, $[M+H]^+$ 412 (> 90% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (6.2 g, 40% yield) as an off-white solid.

- 2-Bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylthio)-1*H*-indole (**4.20**) (14.1 g, 37 mmol) in dry DCM (370 mL) under N_2 atmosphere was cooled to 0 ºC.
- *m*-CPBA (16 g, 93 mmol).

tert-Butyl 2-carbamoylmorpholine-4-carboxylate (4.21)

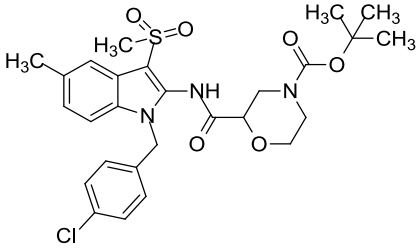


N-Boc-morpholine-2-carboxylic acid (**4.23**) (1 g, 4.3 mmol) was dissolved in 1,4-dioxane (25 mL) and pyridine (0.18 mL, 2.2 mmol) was added dropwise. Boc anhydride (1.04 g, 4.8 mmol) was added and the reaction was stirred for 15 min. After this time ammonium bicarbonate (0.342 g, 4.32 mmol) was added and the reaction was stirred overnight. EtOAc and water were added, the phases were separated, and the organic layer was washed with a 5% aqueous solution of H_2SO_4, dried over anhydrous Na_2SO_4, filtered, and evaporated under vacuum affording *tert*-butyl 2-carbamoylmorpholine-4-carboxylate (**4.21**) (681 mg, 68% yield) as an off-white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.37-7.52 (br.s, 1H), 7.32 (br. s., 1H), 3.99-4.14 (m, 1H), 3.88-3.99 (m, 1H), 3.84 (dd, *J*=10.5, 3.2 Hz, 1H), 3.71-3.81 (m, 1H), 3.53 (tq, *J*=11.6, 2.8 Hz, 1H), 2.70-3.07 (m, 2H), 1.48 ppm (s, 9H).

^{13}C NMR (DMSO-d_6, 101MHz): δ = 171.1, 154.3, 79.8, 75.1, 66.1, 65.1, 28.7, 27.3 ppm.

tert-Butyl 2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)morpholine-4-carboxylate (4.22)



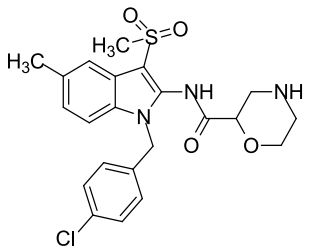
A round bottom flask was charged with $Pd_2(dba)_3$ (14.4 mg, 0.02 mmol), Xantphos (18.2 mg, 0.03 mmol), Cs_2CO_3 (154 mg, 0.47 mmol) and vacuumed and filled with N_2 several times. Then a solution of *tert*-butyl 2-carbamoylmorpholine-4-carboxylate (**4.21**) (72.5 mg, 0.3 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (130 mg, 0.3 mmol) in 1,4-dioxane (5 mL) was added, and the reaction was refluxed for 5 h. After this time, HPLC (basic pH) showed reaction completion. The mixture was filtered through a celite cartridge and the solvent was evaporated to give a crude product (400 mg) that was purified on a 12 g silica gel

cartridge (0-60% Cy/EtOAc) to afford *tert*-butyl 2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)morpholine-4-carboxylate (**4.22**) (63 mg, 35% yield) as an orange solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.32 (s, 1H), 7.64 (s, 1H), 7.31-7.39 (m, 3H), 7.18 (d, *J*=8.6 Hz, 2H), 7.09 (dd, *J*=8.6, 1.3 Hz, 1H), 5.32 (s, 2H), 4.07-4.15 (m, 1H), 3.93-4.04 (m, 2H), 3.67-3.77 (m, 1H), 3.53-3.63 (m, 1H), 3.15 (s, 3H), 2.87-3.09 (m, 2H), 2.40 (s, 3H), 1.42 ppm (s, 9H).

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.35 min, $[M+H]^+$ 562, (> 95% purity).

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)morpholine-2-carboxamide (4.16)



tert-Butyl 2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl) carbamoyl)morpholine-4-carboxylate (**4.22**) (55 mg, 0.1 mmol) was dissolved in DCM (2 mL) and TFA (300 µl, 0.1 mmol) was added. After stirring for 20 min HPLC (basic pH) showed reaction completion. DCM and TFA were evaporated under vacuum and the crude product was loaded on a SCX cartridge previously washed with MeOH; the product was eluted with a 7 M solution of ammonia in MeOH, and solvent was evaporated to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)morpholine-2-carboxamide (**4.16**) (36 mg, 80% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.61-10.75 (m, 1H), 7.64 (s, 1H), 7.36 (d, *J*=8.3 Hz, 2H), 7.33 (d, *J*=8.6 Hz, 1H), 7.18 (d, *J*=8.3 Hz, 2H), 7.06-7.11 (m, 1H), 5.31 (s, 2H), 4.05 (dd, *J*=9.6, 2.8 Hz, 1H), 3.91 (d, *J*=11.1 Hz, 1H), 3.58 (td, *J*=10.6, 3.0 Hz, 1H), 3.10-3.21 (m, 3H), 3.02 (dd, *J*=12.5, 2.4 Hz, 1H), 2.63-2.78 (m, 3H), 2.40 ppm (s, 3H). NH proton not visible in DMSO-d_6.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 171.3, 135.9, 135.8, 132.6, 131.7, 131.6, 129.4, 129.0, 125.3, 124.1, 119.3, 111.6, 107.8, 76.3, 67.3, 48.0, 45.7, 45.2, 44.1, 21.5 ppm.

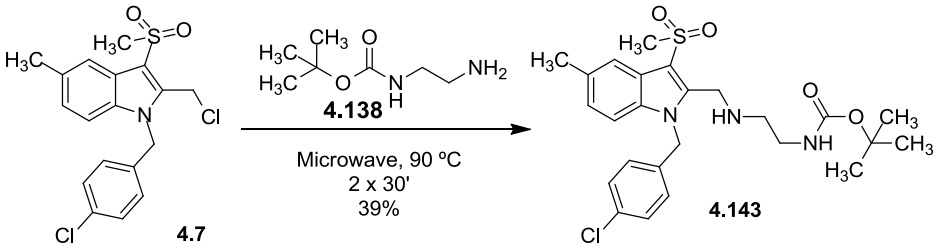
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.15 min, $[M+H]^+$ 462 (> 95% purity).

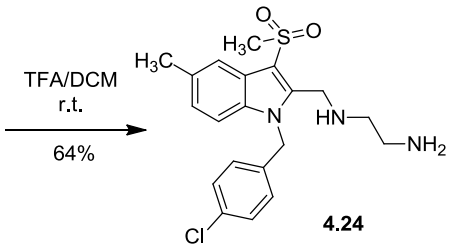
IR ν_{max} (neat) cm^{-1}: 2919.1, 1696.7, 1306.7, 1120.3, 1091.6.

MP 204.4 °C (Crystalline solid).

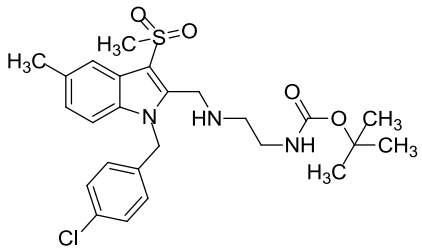
HRMS (ES) calcd for $C_{22}H_{25}{}^{35}ClN_3O_4S$, $(M + H)^+$ 462.1249 found 462.1247.

9.2.3.2.2. Amines





***Tert*-Butyl (2-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2 yl)methyl)amino)ethyl)carbamate (4.143)**



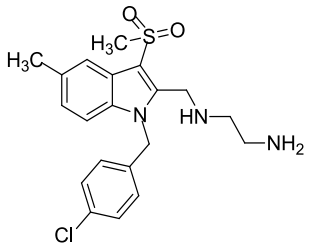
To 1-(4-chlorobenzyl)-2-(chloromethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.7**) (70 mg, 0.2 mmol) in dry THF (1 mL), *tert*-butyl (2-aminoethyl)carbamate (**4.138**) (733 µL, 6.4 mmol) was added and reaction was irradiated in the microwave at 90 ºC for 30 min (2 cycles). LCMS Neutral showed reaction completion after this time. Solvent was evaporated under vacuum affording a yellow oil that was purified by semi-preparative HPLC_sep (basic pH). Appropriate fractions were evaporated together to give *tert*-butyl-(2-(((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)ethyl)carbamate (**4.143**) (36 mg, 39% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.75 (s, 1H), 7.37-7.48 (m, 3H), 7.19 (d, *J*=8.3 Hz, 2H), 7.11-7.16 (m, 1H), 6.74-6.82 (m, 1H), 5.70 (s, 2H), 4.19 (br. s., 2H), 3.28 (s, 3H), 3.05 (d, *J*=5.8 Hz, 2H), 2.67 (br. s., 2H), 2.47 (s, 3H), 2.22-2.31 (m, 1H), 1.42 ppm (s, 9H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 156.3, 143.7, 136.7, 134.5, 132.5, 131.4, 129.1, 128.9, 125.3, 124.9, 119.5, 112.0, 111.5, 78.0, 49.3, 46.5, 46.3, 41.9, 28.7, 21.7 ppm (1 CH_2 is not visible under the analytical conditions).

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.35 min, $[M+H]^+$ 506 (>95% purity).

N^1-((1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)ethane-1,2-diamine (4.24)



To *tert*-butyl (2-(((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)ethyl)carbamate (**4.143**) (41 mg, 0.08 mmol) in DCM (4 mL), TFA (400 µL, 0.19 mmol) was added and reaction was stirred at room temperature over 3 h, then HPLC (basic pH) showed reaction completion. DCM and TFA were evaporated under vacuum to give N^1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl) methyl)ethane-1,2-diamine as the TFA salt. SCX column was used to obtain the compound as the free base, N^1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)ethane-1,2-diamine (**4.24**) (21 mg, 64% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.76 (s, 1H), 7.38-7.48 (m, 3H), 7.10-7.23 (m, 3H), 5.72 (s, 2H), 4.20 (s, 2H), 3.29 (s, 3H), 2.64 (br. s., 4H), 2.47 ppm (s, 3H).

The spectrum was obtained by adding some drops of TFA, and also in $CDCl_3$; however the NH units were not visible under any of these conditions.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 143.6, 136.9, 134.5, 132.4, 131.5, 129.2, 128.7, 125.4, 125.0, 119.5, 111.5, 52.2, 46.5, 46.4, 41.9, 41.6, 26.9, 21.7 ppm.

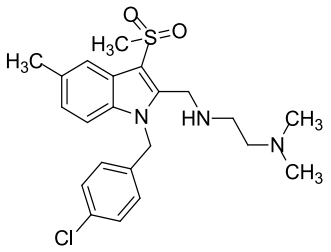
LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.08min, $[M+H]^+$ 406 (95% purity).

IR ν_{max} (neat) cm^{-1}: 3241.5, 2915.3, 1523.8, 1285.4, 1116.7.

MP 147°C (Crystalline solid).

HRMS (ES) calcd for $C_{20}H_{25}{}^{35}ClN_3O_2S$, $(M + H)^+$ 406.1351, found 406.1356.

N^1-((1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)-N^2,N^2-dimethylethane-1,2-diamine (4.25)



To 1-(4-chlorobenzyl)-2-(chloromethyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.7**) (70 mg, 0.183 mmol) in dry THF (1 mL), N^1,N^1-dimethylethane-1,2-diamine (700 µl, 6.4 mmol) was added and the reaction was irradiated in the microwave at 90 ºC for 30 min (2 cycles). LCMS Neutral showed reaction completion after this time. Solvent was evaporated under vacuum affording a colourless oil that was purified by semi-preparative HPLC_sep (basic pH). Appropriate fractions were evaporated together to give N^1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)-N^2,N^2-dimethylethane-1,2-diamine (**4.25**) (26 mg, 33% yield) as a yellowish solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.69 (s, 1H), 7.30-7.40 (m, 3H), 7.10 (d, *J*=8.3 Hz, 3H), 5.65 (s, 2H), 4.13 (s, 2H), 3.21 (s, 3H), 2.61 (t, *J*=5.3 Hz, 2H), 2.40 (s, 3H), 2.23 (t, *J*=7.1 Hz, 2H), 2.06 ppm (s, 7H).

^{13}C NMR (DMSO-d_6, 101MHz): δ = 143.7, 136.9, 134.5, 132.4, 131.5, 129.1, 128.8, 125.3, 125.0, 119.4, 112.0, 111.4, 59.1, 46.9, 46.5, 46.3, 45.7, 42.1, 21.6 ppm.

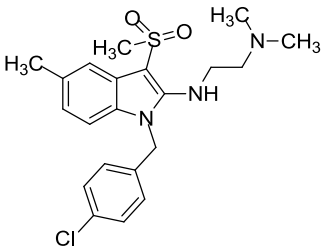
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.13min, $[M+H]^+$ 434 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3029.5, 2934.5, 2754.6, 1596.3, 1317.6, 1121.5.

MP Insufficient mass available.

HRMS (ES) calcd for $C_{22}H_{29}{}^{35}ClN_3O_2S$, $(M + H)^+$ 434.1664 found 434.1646.

N^1-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-N^2,N^2-dimethylethane-1,2-diamine (4.31)



In a microwave vial, were placed 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) and *N*,*N*-dimethylethylenediamine (**4.29**) (400 µL, 3.6 mmol) and heated under thermal conditions for 30 min at 120 ºC. LCMS Neutral showed that reaction had reached completion after this time. The amine was evaporated as much as possible in the rotavap and the crude product was purified by HPLC_sep (basic pH). Appropiate fractions were evaporated to give N^1-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-N^2,N^2-dimethylethane-1,2-diamine (**4.31**) (30 mg, 59% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.39-7.52 (m, 3H), 7.18 (dd, *J*=8.2, 6.4 Hz, 3H), 6.94 (dd, *J*=8.1, 1.0 Hz, 1H), 6.16 (t, *J*=5.7 Hz, 1H), 5.44 (s, 2H), 3.30 (q, *J*=5.8 Hz, 2H), 3.19 (s, 3H), 2.39-2.44 (m, 5H), 2.16 ppm (s, 6H).

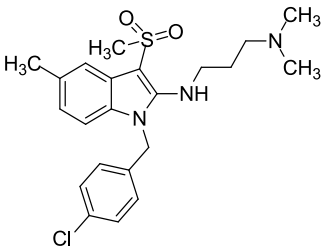
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 151.0, 136.5, 132.5, 132.3, 131.2, 129.2, 128.6, 125.4, 122.7, 117.5, 110.3, 94.8, 58.9, 46.6, 45.4, 45.3, 45.2, 18.3 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.31 min, $[M+H]^+$ 420 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3334.2, 2943.3, 2821.0, 2771.1, 1556.9, 1284.5, 1110.9.

MP Decomposes from 250 ºC (amorphous solid).

N^1-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1H-indol-2-yl)-N^3,N^3-dimethylpropane-1,3-diamine (4.32)



In a microwave vial, were placed 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (70 mg, 0.2 mmol) and *N*,*N*-dimethyl-1,3-propanediamine (**4.30**) (650 µl, 5.1 mmol) and heated under thermal conditions for 3 h at 100 ºC. LCMS Neutral showed that reaction had reached completion after this time. The amine was evaporated, as much as possible, in the rotavap and the crude product was purified by HPLC_sep (basic pH). Appropiate fractions were evaporated to give N^1-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-N^3,N^3-dimethylpropane-1,3-diamine (**4.32**) (5.8 mg, 8% yield) as a white solid.

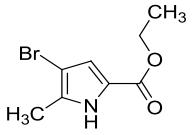
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.36-7.46 (m, 3H), 7.06-7.17 (m, 3H), 6.85-6.93 (m, 1H), 5.93-6.00 (t, *J*=6.3 Hz, 1H), 5.37 (s, 2H), 4.06-4.13 (q, *J*=5.1 Hz, 1H), 3.12-3.23 (m, 4H), 2.36 (s, 3H), 2.12-2.18 (t, *J*=6.6 Hz, 2H), 2.02 (s, 6H), 1.58 ppm (q, *J*=6.6 Hz, 1H). NH proton is not visible in the spectrum conditions.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.18 min, $[M+H]^+$ 434, (> 95% purity).

9.2.3.3. Breaking the planarity

9.2.3.3.1. Amend the indole core by replacing it with a monocylic heteroaromatic ring

Ethyl 4-bromo-5-methyl-1*H*-pyrrole-2-carboxylate (4.35)



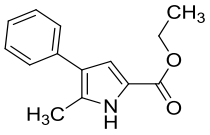
NBS (1.162 g, 6.53 mmol) was added to an ice-cooled solution of ethyl 5-methyl-1*H*-pyrrole-2-carboxylate (**4.34**) (1 g, 6.5 mmol) in chloroform (30 mL) and the mixture stirred for 30 min at 0 °C. HPLC (basic pH) showed reaction completion after this time. The mixture was poured onto an ice-cooled 2 N aq. NaOH solution (20 mL) and extracted with DCM. The combined extracts were washed twice with water (20 mL) and dried over Na_2SO_4. Evaporation of the solvent gave a beige solid (1.5 g) which was purified by flash chromatography (cyclohexane/EtOAc 0-50%) to give ethyl 4-bromo-5-methyl-1*H*-pyrrole-2-carboxylate (**4.35**) (1.04 g, 68% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.07 (br. s., 1H), 6.74 (s, 1H), 4.21 (q, *J*=7.1 Hz, 2H), 2.17 (s, 3H), 1.26 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.0, 132.8, 121.0, 116.8, 95.9, 60.2, 14.8, 11.8 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.23 min, $[M+2H]^+$ 232 (95% purity).

Ethyl 5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (4.36)



Ethyl 4-bromo-5-methyl-1*H*-pyrrole-2-carboxylate (**4.35**) (600 mg, 2.6 mmol), phenyl boronic acid (378 mg, 3.1 mmol) and K_2CO_3 (1.07 g, 7.8 mmol) were suspended in a mixture of DME (10 mL) and water (10 mL), and the mixture was degassed with an N_2 stream, and tetrakis(triphenylphosphine)palladium (0) (299 mg, 0.26 mmol) was added. The mixture was heated in the microwave at 100 ºC for 30 min. LCMS Neutral showed

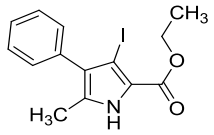
the reaction to be completed after this time. EtOAc and water were added and the phases were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to give a crude product (350 mg) that was purified on a 12 g silica gel cartridge using Cy/EtOAc 0-50% to give ethyl 5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.36**) (270 mg, 45% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.86 (br. s., 1H), 7.40-7.51 (m, 4H), 7.24-7.30 (m, 1H), 7.00 (d, *J*=2.5 Hz, 1H), 4.31 (q, *J*=7.1 Hz, 2H), 2.45 (s, 3H), 1.36 ppm (t, *J*=7.1 Hz, 3H)

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.1, 136.3, 132.1, 129.0, 127.5, 126.0, 122.7, 120.4, 115.6, 59.9, 15.0, 13.1 ppm

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.28 min, $[M+H]^+$ 230 (>90% purity).

Ethyl 3-iodo-5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (4.37)



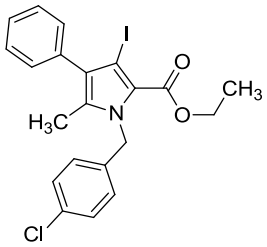
To a solution of ethyl 5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.36**) (230 mg, 1 mmol) in chloroform (10 mL) at 0 ºC was added NIS (226 mg, 1 mmol). The mixture was warmed to room temperature and stirred overnight (16 h). After this time HPLC (basic pH) showed some starting material remaining. More NIS (110 mg, 0.5 mmol) was added and the reaction mixture was stirred for an additional 3 h. After this time HPLC (basic pH) showed that the reaction had completed. $NaHSO_3$ was added, the phases were separated, the organic phase was washed with brine, dried over Na_2SO_4, filtered, and dried under vacuum to give 350 mg of a crude product that was purified on a 10 g Merck cartridge (0-50% Cy/EtOAc) to give ethyl 3-iodo-5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.37**) (215 mg, 60% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.07-12.20 (m, 1H), 7.37-7.45 (m, 2H), 7.23-7.35 (m, 3H), 4.27 (q, *J*=7.1 Hz, 2H), 2.20 (s, 3H), 1.32 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 159.6, 135.2, 132.1, 130.5, 128.2, 127.6, 126.9, 120.5, 74.0, 59.8, 14.6, 12.2 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.33 min, $[M+H]^+$ 356 (>90% purity).

Ethyl 1-(4-chlorobenzyl)-3-iodo-5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (4.38)



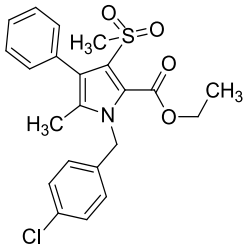
To a solution of ethyl 3-iodo-5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.37**) (210 mg, 0.6 mmol) in dry acetonitrile (15 mL) was added potassium carbonate (163 mg, 1.2 mmol), and 4-chlorobenzyl bromide (146 mg, 0.7 mmol). The reaction was refluxed for 1 h, and then stirred at room temperature for 60 h. LCMS Neutral showed only starting material. The reaction was then refluxed for 3 h, and LCMS Neutral showed some desired product but mainly starting material. The mixture was then refluxed 22 h, and after this time LCMS Neutral showed that the reaction had progressed but there was still SM remaining. More potassium carbonate (163 mg, 1.2 mmol) and 4-chlorobenzyl bromide (146 mg, 0.7 mmol) were added and the reaction was refluxed for 6 h. LCMS Neutral showed that the reaction had proceed to completion. Solvent was evaporated under vacuum, and the crude was dissolved in water, and extracted with EtOAc (x 3). The combined organic layers were dried over $MgSO_4$, filtered, and evaporated affording an orange oil that was purified on a 12 g silica gel cartridge using Cy/EtOAc 0-45% to give a pink solid (227 mg) corresponding to the desired product (**4.38**) along with an unknown product and a small amount of starting material.

This material was used as such in the next transformation.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.39-7.47 (m, 4H), 7.32-7.39 (m, 1H), 7.24-7.30 (m, 2H), 7.02 (d, *J*=8.3 Hz, 2H), 5.62 (s, 2H), 4.19 (q, *J*=7.1 Hz, 2H), 2.10 (s, 3H), 1.20-1.27 ppm (m, 3H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.60 min, $[M+H]^+$ 480 (>85% purity).

Ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4-phenyl-1*H*-pyrrole-2-carboxylate (4.39)



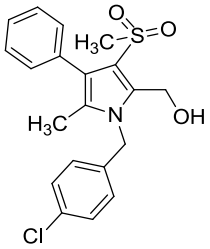
Ethyl 1-(4-chlorobenzyl)-3-iodo-5-methyl-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.38**) (220 mg crude, 0.5 mmol) was dissolved in NMP (5 mL), and copper (I) iodide (87 mg, 0.5 mmol) and sodium methanesulfinate (117 mg, 1.1 mmol) were added at room temperature. The reaction was heated at 125 ºC for 2 h, and then LCMS Neutral showed that the reaction was complete. EtOAc was added, the solid filtered off, and the filtrate washed with water, brine, and dried over $MgSO_4$, filtered, and evaporated to afford a crude that was purified by silica gel cartridge (Merck, 10 g, 10-35% Cy/EtOAc) to give ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.39**) (117 mg, 59% yield over two steps) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.46 (d, *J*=8.6 Hz, 2H), 7.26-7.41 (m, 5H), 7.13 (d, *J*=8.6 Hz, 2H), 5.37 (s, 2H), 4.23 (q, *J*=7.1 Hz, 2H), 3.15 (s, 3H), 1.97 (s, 3H), 1.21 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.3, 136.1, 133.4, 132.7, 131.6, 131.2, 129.3, 128.8, 128.3, 127.7, 126.3, 124.1, 122.9, 62.4, 48.6, 45.9, 14.0, 10.7 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.36 min, $[M+H]^+$ 432 (>95% purity).

(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4-phenyl-1*H*-pyrrol-2-yl)methanol (4.40)



$LiBH_4$ (29.8 mg, 1.370 mmol) was added to a 0 ºC solution of ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4-phenyl-1*H*-pyrrole-2-carboxylate (**4.39**) (110 mg, 0.3

mmol) in toluene (6 mL). The reaction was stirred at 0 ºC for 90 min. At this stage LCMS Neutral showed that the reaction had completed. A saturated solution of sodium potassium tartrate was added dropwise, EtOAc was added, and the phases were separated, the aqueous phase was washed with EtOAc (x 3), and the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent evaporated under vacuum to give (1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4-phenyl-1*H*-pyrrol-2-yl)methanol (**4.40**) (80 mg, 81% yield) as a white solid.

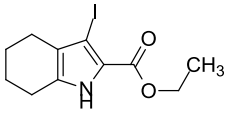
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.44 (d, *J*=8.6 Hz, 2H), 7.33-7.41 (m, 2H), 7.28 (s, 3H), 7.09 (s, 2H), 5.36 (s, 2H), 5.27 (t, *J*=5.6 Hz, 1H), 4.75-4.80 (d, *J*=5.3 Hz, 2H), 2.89 (s, 3H), 1.86 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 136.9, 135.4, 134.3, 132.5, 131.6, 129.3, 128.8, 128.5, 128.2, 127.4, 121.0, 119.0, 52.1, 47.1, 46.8, 10.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.26 min, $[M+H]^+$ 390 (>95% purity).

9.2.3.4. Replacing one or two aromatic rings

Ethyl 3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (4.49)



NIS (1.481 g, 6.58 mmol) was added to a solution of 4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid ethyl ester (**4.48**) (1.06 g, 5.5 mmol) in dry DCM (20 mL), and the reaction mixture was stirred at room temperature in the abscence of light for 1 h. After this time, HPLC (basic pH) showed reaction completion. A 20% aqueous solution of $Na_2S_2O_3.5H_2O$ and DCM were added to the reaction mixture and the phases were separated. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to afford ethyl 3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.49**) (1.66 g, 95% yield) as an orange solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.04 (br. s., 1H), 4.34 (q, *J*=7.2 Hz, 2H), 2.59 (t, *J*=5.8 Hz, 2H), 2.36 (t, *J*=5.8 Hz, 2H), 1.67-1.93 (m, 4H), 1.39 ppm (t, *J*=7.2 Hz, 3H).

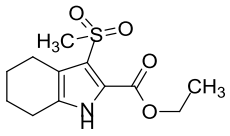
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 160.3, 133.6, 125.0, 120.4, 73.1, 60.3, 24.2, 23.2, 23.1, 22.9, 14.3 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.35 min, $[M+H]^+$ 320 (> 95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain ethyl 3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (8.1 g, 96% yield), as a beige solid.

- NIS (7.27 g, 32.3 mmol)
- 4,5,6,7-Tetrahydro-1*H*-indole-2-carboxylic acid ethyl ester (**4.48**) (5.1 g, 26.4 mmol) in dry DCM (80 mL)

Ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (4.50)



Ethyl 3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.49**) (660 mg, 2.1 mmol) was dissolved in NMP (3 mL), and CuI (394 mg, 2.1 mmol) and methanesulfinic acid, sodium salt (1:1) (528 mg, 5.2 mmol) were added at room temperature. The reaction was heated at 80 ºC for 1 h , HPLC (basic pH) showed SM remaining so it was refluxed for a further 1 h but no variation was observed. Methanesulfinic acid, sodium salt (1:1) (528 mg, 5.17 mmol) and CuI (394 mg, 2.1 mmol) were added and after 1 h at 80 ºC, HPLC (basic pH) did not show any starting material. EtOAc was added to the reaction mixture, the solid was filtered, and the filtrate was washed with water and brine, dried over $MgSO_4$, filtered, and evaporated to afford a crude (920 mg) that was purified using a silica gel cartridge (20 g, 10-35% cy/EtOAc) to give ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.50**) (420 mg, 75% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.27 (br. s., 1H), 4.39 (q, *J*=7.2 Hz, 2H), 3.23-3.40 (m, 3H), 2.84 (t, *J*=6.1 Hz, 2H), 2.61 (t, *J*=5.9 Hz, 2H), 1.68-1.89 (m, 4H), 1.34-1.46 ppm (t, *J*=7.2 Hz, 3H).

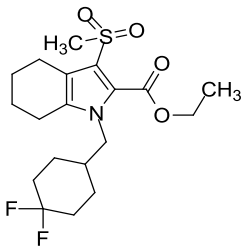
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 154.0, 127.2, 120.8, 118.1 x 2, 115.1, 56.8, 39.8, 18.1, 17.8, 17.3, 9.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.06 min, $[M+H]^+$ 272 (> 95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (5.2 g, 76% yield), as a white solid.

- Ethyl 3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.49**) (8.1 g, 25.4 mmol) was dissolved in NMP (100 mL).
- CuI (4.83 g, 25.4 mmol).
- Methanesulfinic acid, sodium salt (1:1) (6.48 g, 63.5 mmol).

Ethyl 1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (4.52)



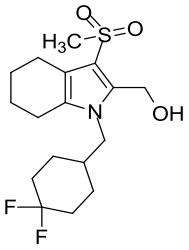
To a solution of ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.50**) (0.3 g, 1.106 mmol) in dry ACN (20 mL) were added potassium carbonate (0.31 g, 2.2 mmol) and 4-(bromomethyl)-1,1-difluorocyclohexane (**4.51**) (0.36 g, 1.7 mmol) and the reaction was refluxed for 18 h. HPLC (basic pH) showed starting material remaining. 4-(Bromomethyl)-1,1-difluorocyclohexane (0.2 g, 0.9 mmol) was added, and reaction was refluxed for 1 h. After this time, HPLC (basic pH) showed that the reaction had reached completion. Solvent was evaporated under vacuum, the crude material was dissolved in EtOAc, water was added, and phases were separated. The aqueous phase was extracted with EtOAc (x 3), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated affording an orange oil that was purified using a 10 g silica cartridge (cyclohexane/EtOAc 0-50%) affording ethyl 1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.52**) (155 mg, 35% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 101 MHz): δ = 4.40 (q, *J*=7.2 Hz, 2H), 4.02 (d, *J*=7.3 Hz, 2H), 3.24 (s, 3H), 2.81 (t, *J*=6.1 Hz, 2H), 2.53 (t, *J*=6.1 Hz, 2H), 2.04-2.21 (m, 2H), 1.58-1.89 (m, 9H), 1.42 (t, *J*=7.2 Hz, 3H), 1.27-1.39 ppm (m, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 160.4, 133.5, 125.5, 123.1, 122.9 (t, *J*=123.9 Hz), 120.9, 61.8, 49.9, 45.1, 37.6 (d, *J*=1.5 Hz), 33.1(t, *J*= 25.6 Hz), 33.0, 26.6 (d, *J*= 10.1 Hz), 22.8, 22.5, 22.4, 13.9 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.30 min, $[M+H]^+$ 404 (> 95% purity).

(1-((4,4-Difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanol (4.53)



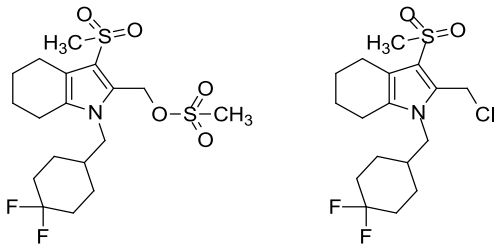
$LiBH_4$ (35.2 mg, 1.617 mmol) was added to a solution of ethyl 1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.52**) (145 mg, 0.36 mmol) in toluene (5 mL) that was previously cooled to 0 ºC, and the reaction mixture was allowed to reach room temperature overnight. HPLC (basic pH) showed reaction completion. A saturated solution of sodium potassium tartrate was added dropwise, EtOAc was added, and the phases were separated, the aqueous phase was washed three times more with EtOAc, the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and solvent was evaporated under vacuum to afford (1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanol (**4.53**) (120 mg, 91% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.77 (d, *J*=6.6 Hz, 2H), 3.82 (d, *J*=7.6 Hz, 2H), 3.15 (s, 3H), 3.06 (t, *J*=7.1 Hz, 1H), 2.77 (t, *J*=6.1 Hz, 2H), 2.57 (t, *J*=6.1 Hz, 2H), 2.15-2.26 (m, 2H), 1.67-1.98 (m, 9H), 1.35-1.48 ppm (m, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 134.7, 129.7, 123.1 (dd, *J*= 239.4, 242.5 Hz), 117.9, 117.5, 53.5, 48.6, 45.8, 37.8 (d, *J*=1.5 Hz), 33.1(t, *J*= 25.6 Hz), 26.6 (d, *J*= 10.0 Hz), 22.8, 22.7, 22.4, 21.7 ppm.

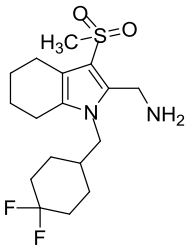
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.18 min, $[M+H]^+$ 362 (> 95% purity).

1-((4,4-Difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methyl methanesulfonate and 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole (4.144)



To a solution of (1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanol (**4.53**) (115 mg, 0.3 mmol) in dry DCM (5 mL), previously cooled to 0 ºC, mesyl chloride (0.03 mL, 0.4 mmol) and Et_3N (0.07 mL, 0.5 mmol) were added. The reaction mixture was stirred at 0 ºC for 2 h and HPLC (basic pH) showed two products. Et_3N (0.07 mL, 0.5 mmol) and MsCl (0.03 mL, 0.4 mmol) were added, and 1 h later HPLC (basic pH) showed that one peak had almost dissapeared. MsCl (0.03 mL, 0.4 mmol) and Et_3N (0.07 mL, 0.5 mmol) were added and 1 h later no changes were observed so the reaction was stopped. The reaction mixture was diluted with DCM, and washed with a saturated solution of $NaHCO_3$, brine, dried over $MgSO_4$, filtered, and the solvent was evaporated under vacuum affording 103 mg of a crude mixture (**4.144**) that was used in the next step of the synthesis.

(1-((4,4-Difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanamine (4.54)



To **4.144** (100 mg, 0.2 mmol) in dry THF (1 mL), was added ammonia in MeOH (1 mL, 7 mmol, 7 M) and the reaction was irradiated at 90 ºC in the microwave (2 x 30 min), after which time HPLC (basic pH) showed reaction completion. The solvent and the ammonia were evaporated under vacuum to give 90 mg of a crude mixture that was purified by HPLC_sep (basic pH). Appropriate fractions were evaporated to give (1-((4,4-difluorocyclohexyl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanamine (**4.54**) (10 mg, 12% yield) as a beige oil.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 4.02 (s, 2H), 3.77 (d, *J*=7.3 Hz, 2H), 3.05 (s, 3H), 2.69 (t, *J*=5.9 Hz, 2H), 2.49 (t, *J*=5.9 Hz, 2H), 2.07-2.22 (m, 2H), 1.59-1.93 (m, 11H), 1.30-1.41 ppm (m, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 129.2, 125.5, 123.1(dd, *J*= 239.4, 242.5 Hz), 120.7, 117.1, 48.4, 45.9, 37.8 (d, *J*=1.5 Hz), 35.4, 33.1(t, *J*= 25.6 Hz), 26.6 (d, *J*= 10 Hz), 23.0, 22.9, 22.5, 22.0 ppm.

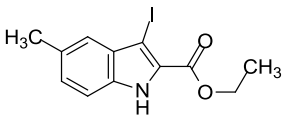
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1 ;0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90 ; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.07 min, $[M+H]^+$ 361 (> 95% purity).

HRMS (ES) calcd for $C_{17}H_{27}F_2N_2O_2S$, $(M + H)^+$ 361.1756 found 361.1756.

9.2.4. Lead optimisation programme (2nd Round).

9.2.4.2. Avoiding toxicity of a series of compounds with a good oral bioavailability.

Ethyl 3-iodo-5-methyl-1*H*-indole-2-carboxylate (4.55)



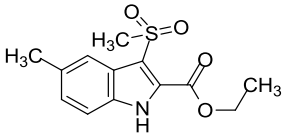
To a solution of ethyl 5-methyl-1*H*-indole-2-carboxylate (**4.1**) (5 g, 24.6 mmol) in dry DCM (100 mL) was added NIS (5.54 g, 24.6 mmol) and the reaction was stirred at room temperature overnight. At this stage HPLC (basic pH) showed that the reaction had finished. A solution of $Na_2S_2O_3$ (20%) was added to the reaction mixture, phases were separated, and the organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 8 g of a yellowish solid. This solid was triturated with ^{t}BuOMe, and filtered to give ethyl 3-iodo-5-methyl-1*H*-indole-2-carboxylate (**4.55**) (6 g, 74% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.20 (br. s., 1H), 7.43 (d, *J*=8.3 Hz, 1H), 7.21-7.28 (m, 2H), 4.43 (q, *J*=7.1 Hz, 2H), 2.48 (s, 3H), 1.45 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.8, 135.8, 131.2, 130.6, 128.3, 127.2, 122.0, 113.2, 65.6, 61.2, 21.6, 14.7 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.39 min, $[M+H]^+$ 328 (> 95% purity).

Ethyl 5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (4.56)



Ethyl 3-iodo-5-methyl-1*H*-indole-2-carboxylate (**4.55**) (6 g, 18.2 mmol) was dissolved in NMP (40 ml), and CuI (3.47 g, 18.2 mmol) and methanesulfinic acid, sodium salt (4.65 g, 45.6 mmol) were added at room temperature. The reaction mixture was heated at 125 ºC for 2 h, and then left to stand at room temperature overnight. After this time HPLC (basic pH) showed that the reaction had reached completion. EtOAc was added,

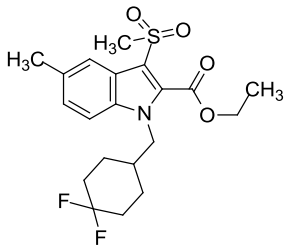
the solid was filtered, and the filtrate was washed with water, brine, and dried over $MgSO_4$, filtered, and evaporated to afford a crude material that was purified on a silica gel cartridge (2 Merck cartridges, 100 g, 10-50% Cy/EtOAc) to give ethyl 5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.56**) (1.46 g, 28% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.91 (br. s., 1H), 7.95 (s, 1H), 7.47 (d, *J*=8.6 Hz, 1H), 7.23 (dd, *J*=8.5, 1.4 Hz, 1H), 4.42 (q, *J*=7.1 Hz, 2H), 3.38 (s, 3H), 2.41 (s, 3H), 1.38 ppm (t, *J*=7.2 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 159.9, 133.5, 132.3, 128.6, 127.8, 125.6, 120.8, 117.5, 113.3, 62.4, 45.2, 21.3, 14.7 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.20 min, $[M+H]^+$ 282 (> 95% purity).

Ethyl 1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (4.57)



To a solution of ethyl 5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.56**) (1.46 g, 5.2 mmol) in dry ACN (50 mL), was added potassium carbonate (1.43 g, 10.38 mmol), and 4-(bromomethyl)-1,1-difluorocyclohexane (**4.51**) (1.44 g, 6.8 mmol). The reaction mixture was refluxed for 17 h, after this time HPLC (basic pH) showed starting material remaining. 4-(Bromomethyl)-1,1-difluorocyclohexane (**4.51**) (0.78 g, 3.6 mmol) was added and the reaction was refluxed for 6 h, after which time HPLC (basic pH) showed that the reaction was complete. EtOAc and a saturated aqueous solution of $NaHCO_3$ were added, phases were separated, and the organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated to give 2.7 g of a dark brown solid that was purified on a 70 g silica gel cartridge using Cy/EtOAc 0-40% to give ethyl 1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.57**) (990 mg, 47% yield) as a yellow solid.

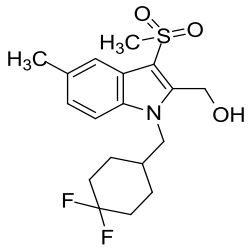
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.80 (s, 1H), 7.72 (d, *J*=8.6 Hz, 1H), 7.28 (dd, *J*=8.7, 1.4 Hz, 1H), 4.43 (q, *J*=7.2 Hz, 2H), 4.28 (d, *J*=7.3 Hz, 2H), 3.30 (s, 3H), 2.44 (s,

3H), 1.90-2.03 (m, 3H), 1.62-1.82 (m, 2H), 1.53 (d, *J*=13.1 Hz, 2H), 1.37 (t, *J*=7.1 Hz, 3H), 1.30 ppm (d, *J*=12.9 Hz, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.2, 134.7, 133.1, 132.7, 127.3, 124.5 (t, *J*=241.5 Hz), 123.9, 120.1, 115.4, 112.7, 63.2, 49.7, 45.7, 36.5, 32.7 (t, *J*=22.7 Hz), 26.6 (d, *J*=9.5 Hz), 21.7, 14.1 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.36 min, $[M+H]^+$ 414 (> 95% purity).

(1-((4,4-Difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (4.58)



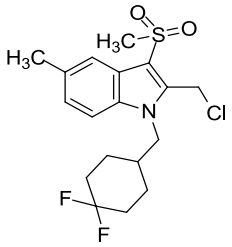
$LiBH_4$ (0.16 g, 7.4 mmol) was added to a 0 ºC solution of ethyl 1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.57**) (1 g, 2.4 mmol) in toluene (30 mL). The reaction was allowed to stand under stirring at 0 ºC for 4 h, after which time HPLC (basic pH) showed that the reaction had reached completion. A saturated aqueous solution of sodium potassium tartrate was added dropwise, EtOAc was added and the phases were separated, the aqueous phase was extracted with EtOAc (x 3), the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated under vacuum to afford 1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (**4.58**) (0.8 g, 99% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.70 (s, 1H), 7.59 (d, *J*=8.6 Hz, 1H), 7.14 (dd, *J*=8.5, 1.4 Hz, 1H), 5.48 (t, *J*=5.6 Hz, 1H), 4.97 (d, *J*=5.6 Hz, 2H), 4.27 (d, *J*=7.6 Hz, 2H), 3.18 (s, 3H), 2.42 (s, 3H), 1.92-2.14 (m, 3H), 1.59-1.83 (m, 2H), 1.55 (d, *J*=12.9 Hz, 2H), 1.36 ppm (d, *J*=14.4 Hz, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 143.7, 134.7, 131.3, 127.1, 125.2, 124.7, 119.4, 112.1, 111.1, 52.1, 48.4, 46.7, 36.3, 32.9 (dd, *J*=22.7, 24.9 Hz), 26.8 (d, *J*=9.5 Hz), 21.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.25 min, $[M+H]^+$ 372 (> 95% purity).

2-(Chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (4.59)



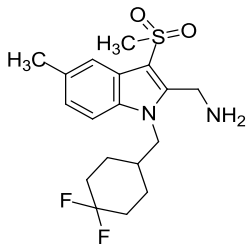
To (1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanol (**4.58**) (878 mg, 2.4 mmol) in dry DCM (10 mL) previously cooled to 0 ºC, Et_3N (0.5 mL, 3.6 mmol) and mesyl chloride (0.22 mL, 2.8 mmol) were added. The reaction mixture was stirred at room temperature for 2 h. HPLC (basic pH) showed the reaction was completed. The reaction mixture was diluted with DCM, and washed with sat. aq. $NaHCO_3$, brine, dried over $MgSO_4$, filtered, and the solvent was evaporated under vacuum affording a crude product (980 mg) that was purified on a 20 g silica gel cartridge (Cy/EtOAc 0-45%) to give 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.59**) (750 mg, 81% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.69 (s, 1H), 7.66 (d, *J*=8.6 Hz, 1H), 7.21 (dd, *J*=8.6, 1.5 Hz, 1H), 5.31 (s, 2H), 4.26 (d, *J*=7.6 Hz, 2H), 3.20 (s, 3H), 2.43 (s, 3H), 1.99 (br. s., 3H), 1.65 (br. s., 2H), 1.49-1.60 (m, 2H), 1.42 ppm (d, *J*=15.4 Hz, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 138.6, 135.0, 132.1, 126.3, 124.6, 124.5 (t, *J*=241.5 Hz), 119.5, 112.3, 112.1, 48.2 (d, *J*=1.5 Hz), 45.9, 36.5, 34.4, 33.0 (t, *J*=24.1 Hz), 26.5 (d, *J*= 10.2 Hz), 21.5 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.35 min, $[M+H]^+$ 390 (> 95% purity).

(1-((4,4-Difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanamine (4.60)

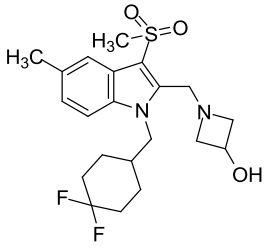


In a microwave vial was dissolved 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.59**) (60 mg, 0.2 mmol) in dry THF (0.5 mL), and a 7 M solution of ammonia in MeOH (0.5 mL, 3.5 mmol) was added and the reaction mixture was irradiated for 1 h in the microwave at 90 ºC. HPLC (basic pH) showed reaction had completed after this time, with the additional formation of a by-product. Solvent and ammonia were evaporated under vacuum to give a crude material that was purified by semi-preparative HPLC_sep (basic pH). Appropiate fractions were evaporated together to give (1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methanamine (**4.60**) (8 mg, 14% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.65 (s, 1H), 7.57 (d, *J*=8.3 Hz, 1H), 7.11 (dd, *J*=8.3, 1.3 Hz, 1H), 4.25 (d, *J*=7.6 Hz, 2H), 4.12 (s, 2H), 3.17-3.21 (s, 3H), 2.41 (s, 3H), 1.98 (br. s., 3H), 1.61-1.83 (m, 2H), 1.51-1.61 (m, 4H), 1.31-1.46 ppm (m, 2H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.15 min, $[M+H]^+$ 371 (> 95% purity).

1-((1-((4,4-Difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)azetidin-3-ol (4.61)



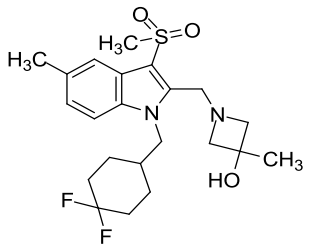
To 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.59**) (60 mg, 0.2 mmol) in dry THF (1 mL), azetidin-3-ol hydrochloride (84 mg, 0.8 mmol) was added and reaction was irradiated in the microwave at 90 ºC for 1 h.

After this time, HPLC (basic pH) showed starting material remaining. The reaction was irradiated at 90 ºC for one more hour and HPLC (basic pH) showed that the reaction was almost completed. The solvent was evaporated under vacuum and the crude mixture was purified by HPLC_sep (basic pH). Appropiate fractions were evaporated to give 1-((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)azetidin-3-ol (**4.61**) (14 mg, 21% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.65 (s, 1H), 7.56 (d, *J*=8.1 Hz, 1H), 7.13 (d, *J*=7.8 Hz, 1H), 5.27-5.39 (m, 1H), 4.28 (d, *J*=7.3 Hz, 2H), 4.09 (br. s., 3H), 3.46 (br. s., 2H), 3.18 (br. s., 3H), 3.00 (br. s., 2H), 2.42 (s, 3H), 1.98 (br. s., 3H), 1.66 (br. s., 2H), 1.53 (d, *J*=12.4 Hz, 2H), 1.29-1.46 ppm (m, 2H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.18 min, $[M+H]^+$ 427 (> 95% purity).

1-((1-((4,4-Difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)-3-methylazetidin-3-ol (4.62)

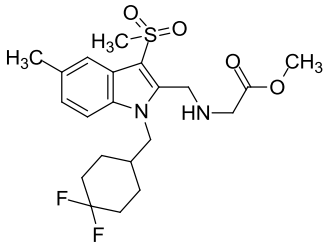


To 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.59**) (70 mg, 0.2 mmol) in dry THF (1 mL), 3-hydroxy-3-methylazetidine hydrochloride (111 mg, 0.9 mmol) was added and the reaction mixture was irradiated in the microwave at 90 ºC for 1 h. HPLC (basic pH) showed that the reaction had reached completion. The solvent was evaporated under vacuum to give a crude that was purified in a 10 g silica gel cartridge (Cy/EtOAc 10-65%) to give 1-((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)-3-methylazetidin-3-ol (**4.62**) (20 mg, 25% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.65 (s, 1H), 7.56 (d, *J*=8.6 Hz, 1H), 7.13 (dd, *J*=8.5, 1.1 Hz, 1H), 5.22 (s, 1H), 4.27 (d, *J*=7.3 Hz, 2H), 4.12 (s, 2H), 3.17 (s, 3H), 3.10-3.16 (m, 4H), 2.42 (s, 3H), 2.05-2.18 (m, 1H), 1.92-2.04 (m, 2H), 1.59-1.80 (m, 2H), 1.51 (br. s., 2H), 1.40 (s, 2H), 1.32 ppm (s, 3H).

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.28 min, $[M+H]^+$ 441 (> 95% purity).

Methyl 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)acetate (4.64)

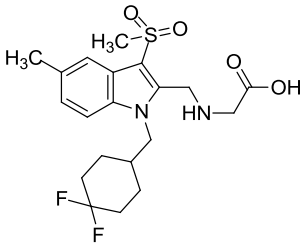


To 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.59**) (100 mg, 0.3 mmol) in dry THF (3 mL), methyl 2-aminoacetate hydrochloride (**4.63**) (193 mg, 1.5 mmol) was added and the reaction was irradiated in the microwave at 90 ºC for 1 h. HPLC (basic pH) showed that the reaction had reached completion. EtOAc and a saturated solution of NH_4Cl were added and the phases were separated, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated to give methyl 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)acetate (**4.64**) (140 mg, 95% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.66 (s, 1H), 7.56 (d, *J*=8.6 Hz, 1H), 7.09-7.15 (m, 1H), 4.32 (d, *J*=7.3 Hz, 2H), 4.18 (s, 2H), 3.61 (s, 3H), 3.43 (s, 2H), 3.18 (s, 3H), 2.55-2.70 (m, 1H), 2.41 (s, 3H), 1.99 (s, 3H), 1.51-1.79 (m, 4H), 1.29-1.48 ppm (m, 2H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.25 min, $[M+H]^+$ 443 (> 90% purity).

2-(((1-((4,4-Difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)acetic acid (4.65)



Methyl 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)acetate (**4.64**) (125 mg, 0.3 mmol) was suspended in a mixture of THF) (2 mL) and water (2 mL). LiOH (27 mg, 1.1 mmol) was added and the mixture was stirred under reflux. After 1 h, the LCMS Neutral showed that the reaction had reached completion. The reaction mixture was acidified with a 2 N aqueous solution of HCl and EtOAc was added, the phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give a white solid that corresponded with the desired product also containing a small amount of impurities. The product was triturated with ^{t}BuOMe and filtered to give 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)amino)acetic acid (**4.65**) (92 mg, 76% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.07-11.19 (br. s, 1H), 7.62-7.75 (m, 2H), 7.23 (d, *J*=8.3 Hz, 1H), 4.66 (br. s., 2H), 4.36 (d, *J*=7.3 Hz, 2H), 3.92 (br. s., 2H), 3.32 (s, 3H), 3.08 (s, 1H), 2.41-2.47 (m, 3H), 1.98 (br. s., 3H), 1.50-1.82 (m, 4H), 1.31-1.48 ppm (m, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 134.8, 131.8, 127.0, 126.2, 124.4, 119.3, 113.4, 112.4, 48.8, 48.1, 45.8, 36.7, 32.9 (dd, *J*=23.4, 25.6 Hz), 27.4, 26.5 (d, *J*=10.2 Hz), 21.7 ppm (carboxylic carbon and CF_2 not visible under the analytical conditions).

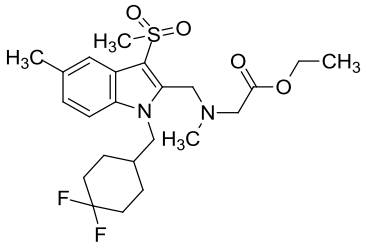
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$ 1.0 min, $[M+H]^+$ 429 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2983.54, 1737.85, 1289.14, 1596.38, 1113.83, 952.97.

MP Amorfous solid that decomposes from 250 ºC.

HRMS (ES) calcd for $C_{20}H_{27}F_2N_2O_4S$, $(M + H)^+$429.1654 found 429.1640.

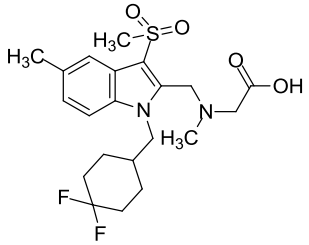
Ethyl 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)(methyl)amino)acetate (4.67)



To 2-(chloromethyl)-1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.59**) (120 mg, 0.308 mmol) in dry THF (4 mL), ethyl 2-(methylamino)acetate hydrochloride (**4.66**) (284 mg, 1.8 mmol) and DIPEA (0.3 mL, 1.8 mmol) were added and the reaction mixture was irradiated in the microwave at 90 ºC for 1 h. LCMS Neutral showed that the reaction had reached completion. EtOAc and a saturated solution of NH_4Cl were added and the phases were separated, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated to give 105 mg of ethyl 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)(methyl)amino)acetate (**4.67**) (105 mg, 72% yield) with 20% (by ^{1}H NMR) of ethyl 2-(methylamino)acetate remaining. The next step of the synthesis was carried out without any further purification.

^{1}H NMR (DMSO-d_6, 400MHz): δ = 7.68 (s, 1H), 7.58 (d, *J*=8.6 Hz, 1H), 7.14 (dd, *J*=8.6, 1.3 Hz, 1H), 4.36 (q, *J*=7.3 Hz, 2H), 4.15 (s, 2H), 4.05-4.13 (m, 2H), 3.36 (s, 2H), 3.17 (s, 3H), 2.42 (s, 3H), 2.21-2.32 (m, 4H), 2.03-2.17 (m, 1H), 1.99 (s, 3H), 1.60-1.82 (m, 2H), 1.31-1.47 (m, 2H), 1.19 ppm (m, 3H).

2-(((1-((4,4-Difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)(methyl)amino)acetic acid (4.68)



Ethyl 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)(methyl)amino)acetate (**4.67**) (125 mg, 0.3 mmol) was suspended in a mixture of THF (2 mL) and water (2 mL). LiOH (25.4 mg, 1.1 mmol) was added and the mixture was stirred under reflux for 2 h. After this time, the LCMS Neutral showed

that the reaction was finished. The reaction mixture was acidified with a 2 N aqueous solution of HCl and EtOAc was added, phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 2-(((1-((4,4-difluorocyclohexyl)methyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)methyl)(methyl)amino)acetic acid (**4.68**) (103 mg, 88% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.33-12.82 (br. s., 1H), 7.53-7.78 (m, 2H), 7.19 (d, *J*=8.3 Hz, 1H), 4.43 (d, *J*=7.3 Hz, 4H), 3.4 (br. s., 2H), 3.24 (s, 3H), 2.44 (s, 3H), 1.93-2.07 (m, 3H), 1.59-1.83 (m, 2H), 1.46-1.60 (m, 2H), 1.30-1.44 ppm (m, 5H).

The **^{13}C NMR** was collected but the resolution of the spectrum was not good enough to identify all of the signals.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$ 1.03 min, $[M+H]^+$ 443 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2926.60, 2582.44, 1729.84, 1112.26, 952.97.

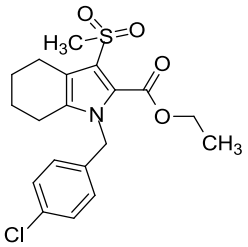
MP Amorphous solid that decomposes from 300 ºC.

HRMS (ES) calcd for $C_{21}H_{29}F_2N_2O_4S$, $(M + H)^+$ 443.1811 found 443.1798.

9.2.4.3. Improving the PK profile of compound 4.48 while retaining the solubility and avoiding toxicity.

9.2.4.3.1. Amides

Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (4.69)



To a solution of ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.50**) (1.7 g, 6.27 mmol) in dry ACN (30 mL) was added potassium carbonate (1.732 g, 12.53 mmol). After 15 min stirring at room temperature, 1-(bromomethyl)-4-chlorobenzene (**4.2**) (1.54 g, 7.5 mmol) was added and reaction was refluxed overnight. Solvent was evaporated under vacuum and the crude mixture was dissolved in water, extracted with EtOAc (x 3), and then the combined organic layers were dried over $MgSO_4$, filtered, and evaporated affording a whitish solid that was purified to obtain ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.69**) (2.3 g, 93% yield)

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.38-7.48 (m, 2H), 7.04 (d, *J*=8.6 Hz, 2H), 5.32 (s, 2H), 4.20 (q, *J*=7.1 Hz, 2H), 3.23 (s, 3H), 2.67 (t, *J*=5.6 Hz, 2H), 2.42 (t, *J*=5.7 Hz, 2H), 1.59-1.75 (m, 4H), 1.20 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.5, 136.5, 133.7, 132.4, 129.2, 128.6, 124.5, 123.8, 119.9, 62.0, 47.9, 45.3, 22.8, 22.4, 22.2, 21.9, 13.9 ppm.

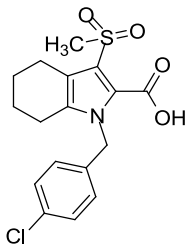
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R= 1.36 min, $[M+H]^+$ 396 (> 95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.69**) (6.6 g, 90% yield), as a pale yellow solid.

- Ethyl 3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.50**) (5 g, 18.4 mmol) in dry ACN (100 mL)

- Potassium carbonate (5.1 g, 36.9 mmol).
- 1-(Bromomethyl)-4-chlorobenzene (**4.2**) (4.5 g, 22.1 mmol)

1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (4.70)



Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.69**) (723 mg, 1.8 mmol) was suspended in a mixture of THF (5 mL) and water (5 ml). LiOH (175 mg, 7.3 mmol) was added and the mixture was stirred under reflux. After 6 h, LCMS Neutral showed that the reaction was not complete. More LiOH (175 mg, 7.3 mmol) and water (3 mL) were added and the reaction was stirred at room temperature overnight. After that time the reaction had progressed but was not complete. The reaction mixture was refluxed for 6 h and LCMS Neutral showed that the reaction had reached completion. The reaction mixture was acidified with a 2 N aqueous solution of HCl and EtOAc was added, the phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**4.70**) (550 mg, 82% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.42-13.90 (m, 1H), 7.38-7.44 (d, *J*=8.3 Hz, 2H), 7.04 (d, *J*=8.3 Hz, 2H), 5.37 (s, 2H), 3.23-3.26 (m, 3H), 2.67 (t, *J*=5.4 Hz, 2H), 2.40 (t, *J*=5.6 Hz, 2H), 1.58-1.73 ppm (m, 4H).

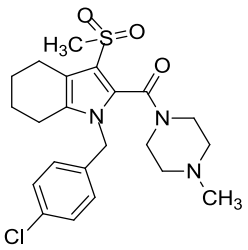
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.8, 136.6, 132.9, 132.3, 129.2, 128.5, 125.1, 123.9, 119.6, 47.7, 45.2, 22.8, 22.4, 22.1, 21.8 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.01 min, $[M+H]^+$ 368 (> 95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**4.70**) (1.48 g, 53% yield) as a beige solid.

- Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**4.69**) (3 g, 7.6 mmol) was suspended in a mixture of THF (25 mL) and water (25 ml).
- LiOH (730 mg, 30.3 mmol).

(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)(4-methylpiperazin-1-yl)methanone (4.72)



To a 0 ºC solution of 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**4.70**) (200 mg, 0.5 mmol) in dry THF (3 mL), HOBT (125 mg, 0.8 mmol), EDCI (156 mg, 0.8 mmol), and DIPEA (0.15 mL, 0.8 mmol) were added and reaction was stirred at room temperature for 30 min, then 1-methylpiperazine (**4.71**) (0.09 mL, 0.8 mmol) was added and the reaction mixture was stirred at room temperature for 5 h. After this time, HPLC (basic pH) showed that the reaction had reached completion. The solvent was evaporated under vacuum to give a crude product that was redissolved in EtOAc, a saturated solution of $NaHCO_3$ was added, and the phases were separated, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 180 mg of a crude material that was redissolved in MeOH and evaporated again to give (1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)(4-methylpiperazin-1-yl)methanone (**4.72**) (122 mg, 50% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.34 (d, *J*=8.3 Hz, 2H), 7.04 (d, *J*=8.3 Hz, 2H), 4.82-4.98 (m, 2H), 3.50-3.62 (m, 1H), 3.22 (br. s., 1H), 2.93 (s, 3H), 2.80 (br. s., 1H), 2.51 (br. s., 2H), 2.44-2.48 (m, 1H), 2.37-2.40 (m, 1H), 2.22-2.32 (m, 2H), 2.15 (br. s., 1H), 1.99 (s, 3H), 1.92 (br. s., 1H), 1.64 (br. s., 4H), 1.38 ppm (br. s., 1H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.8, 136.1, 132.7, 130.8, 129.4, 129.2, 129.1, 118.1, 116.5, 53.8, 46.8, 45.9, 45.2, 41.6, 22.7, 22.5, 21.7, 21.5 ppm.

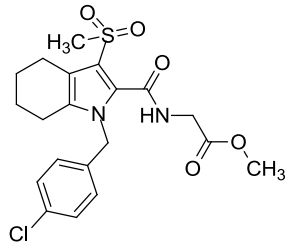
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=1.18 min, $[M+H]^+$ 450 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2927.1, 2802.8, 1624.5, 1292.6.

MP Crystalline solid that melts at 172 ºC.

HRMS (ES) calcd for $C_{22}H_{29}{}^{35}ClN_3O_3S$, $(M + H)^+$ 450.1613 found 450.1614.

Methyl 2-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetate (4.73)



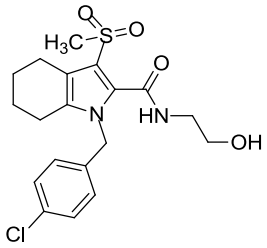
To a previously cooled at 0 ºC and stirred solution of 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**4.70**) (200 mg, 0.5 mmol) in dry THF (6 mL) was added HOBT (83 mg, 0.5 mmol) and EDCI (104 mg, 0.5 mmol); 30 min later glycine methyl ester hydrochloride (**4.63**) (68.3 mg, 0.5 mmol) and DIPEA (0.1 mL, 0.5 mmol) were added. The reaction mixture was allowed to reach r.t. and stirred for 15 h. HPLC (basic pH) at this time showed that the reaction had reached completion. The solvent was evaporated under vacuum to give 210 mg of a crude material that was purified on a 12 g silica gel cartridge (0- 45% Cy/EtOAc) to give methyl 2-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetate (**4.73**) (165 mg, 69% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.01-9.18 (m, 1H), 7.41 (d, *J*=8.6 Hz, 2H), 7.20 (d, *J*=8.3 Hz, 2H), 5.20 (s, 2H), 4.00 (d, *J*=6.1 Hz, 2H), 3.58 (s, 3H), 3.14 (s, 3H), 2.54-2.66 (m, 2H), 2.25-2.38 (m, 2H), 1.51-1.78 ppm (m, 4H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 170.4, 161.9, 136.4, 132.6, 130.5, 130.3, 129.5, 128.9, 118.7, 117.4, 52.3, 47.6, 45.6, 41.6, 22.7, 22.4, 21.8 (2C) ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.25 min, $[M+H]^+$ 439 (> 95% purity).

1-(4-Chlorobenzyl)-*N*-(2-hydroxyethyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (4.74)



$LiBH_4$ (8.5 mg, 0.4 mmol) was added to a previously cooled at 0 ºC solution of methyl 2-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetate (**4.73**) (50 mg, 0.1 mmol) in toluene (2 mL). The reaction mixture was allowed to stand at room temperature for 18 h. After this time HPLC (basic pH) mainly showed starting material. $LiBH_4$ (17 mg, 0.8 mmol) was added to the cooled (0 ºC) reaction, then it was allowed to reach room temperature and stir during 1 day, after which time the reaction was stopped. A saturated solution of Rochelle's salt and EtOAc were added to the mixture, phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 1-(4-chlorobenzyl)-*N*-(2-hydroxyethyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (**4.74**) (30 mg, 64% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.55 (t, *J*=5.7 Hz, 1H), 7.38 (d, *J*=8.6 Hz, 2H), 7.18 (d, *J*=8.1 Hz, 2H), 5.09 (s, 2H), 4.58 (t, *J*=5.8 Hz, 1H), 3.44 (q, *J*=6.3 Hz, 2H), 3.24 (q, *J*=6.1 Hz, 2H), 3.14 (s, 3H), 2.59 (t, *J*=6.1 Hz, 2H), 2.29-2.35 (m, 2H), 1.58-1.70 ppm (m, 4H).

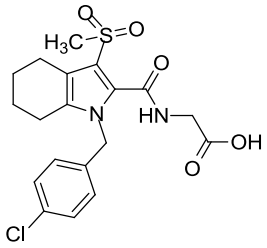
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.2, 136.4, 132.6, 131.3, 130.1, 129.5, 129.0, 118.4, 117.0, 59.8, 47.5, 45.6, 42.4, 22.8, 22.5, 21.8, 21.7 ppm

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.25 min, $[M+H]^+$ 411 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3476.0, 3317.6, 2932.8, 1655.3, 1285.4, 1103.9, 837.4.

HRMS (ES) calcd for $C_{19}H_{24}{}^{35}ClN_2O_4S$, $(M + H)^+$ 411.1140 found 411.1130

2-(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetic acid (4.75)



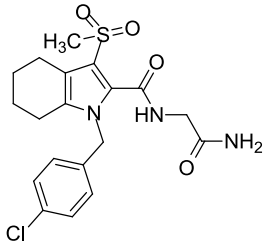
Methyl 2-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetate (**4.73**) (70 mg, 0.2 mmol) was suspended in a mixture of THF and water (2.5 mL)/(2.5 mL). LiOH (15 mg, 0.6 mmol) was added and the mixture was stirred at room temperature. After 1 h, HPLC (basic pH) showed that the reaction had finished. The reaction mixture was acidified with 2 N HCl and EtOAc was added, the phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 2-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetic acid (**4.75**) (54 mg, 80% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.44-12.84 (m, 1H), 9.01 (t, *J*=5.9 Hz, 1H), 7.39 (d, *J*=8.3 Hz, 2H), 7.21 (d, *J*=8.3 Hz, 2H), 5.24 (s, 2H), 3.89 (d, *J*=6.1 Hz, 2H), 3.14 (s, 3H), 2.54-2.63 (m, 2H), 2.25-2.36 (m, 2H), 1.63 ppm (br. s., 4H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 162.0, 136.0, 132.6, 130.6, 129.8, 129.2, 129.0, 123.4, 118.9, 117.2, 47.4, 45.4, 40.6, 22.6, 22.3, 21.6, 21.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$0.99 min, $[M+H]^+$ 425 (> 95% purity).

***N*-(2-Amino-2-oxoethyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (4.76)**



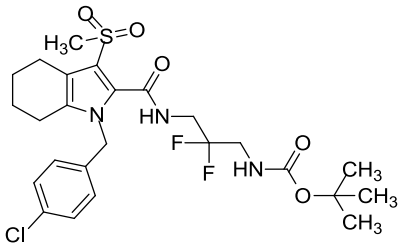
2-(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)acetic acid (**4.75**) (37.4 mg, 0.1 mmol) was dissolved in 1,4-dioxane (2

mL) and pyridine (5 µL, 0.04 mmol) was added. Boc anhydride (21.1 mg, 0.1 mmol) was added and the reaction was stirred for 15 min. After that time, ammonium bicarbonate (7 mg, 0.1 mmol) was added and the reaction was stirred for 4 h. HPLC (basic pH) at that time showed that the reaction had reached completion. EtOAc and water were added, the phases were separated, and the organic layer was washed with a solution of H_2SO_4 (5%), dried over anh. Na_2SO_4, filtered, and evaporated under vacuum affording 23 mg of desired product with a little of an impurity. The product was stirred in a small amount of ^{t}BuOMe and filtered to give *N*-(2-amino-2-oxoethyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (**4.76**) (10 mg, 27% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.89 (t, *J*=5.9 Hz, 1H), 7.39 (d, *J*=8.3 Hz, 2H), 7.25 (br. s., 1H), 7.18 (d, *J*=8.3 Hz, 2H), 7.14 (br. s., 1H), 5.17 (s, 2H), 3.73 (d, *J*=6.1 Hz, 2H), 3.17 (s, 3H), 2.59 (br. s., 2H), 2.33 (br. s., 2H), 1.64 ppm (br. s., 4H)

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.12 min, $[M+H]^+$ 424 (> 95% purity).

tert-Butyl (3-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)-2,2-difluoropropyl)carbamate (4.78)

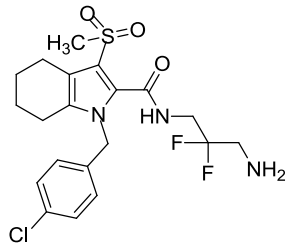


To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**4.70**) (55 mg, 0.15 mmol) dissolved in THF (6 mL), was added HOBT (23 mg, 0.15 mmol) and EDCI (29 mg, 0.15 mmol); 30 min later *tert*-butyl (3-amino-2,2-difluoropropyl)carbamate (**4.77**) (31.4 mg, 0.15 mmol) and DIPEA (0.03 mL, 0.15 mmol) were added. The reaction was allowed to reach r.t. and stirred for 2 h. After this time, HPLC (basic pH) showed that the reaction had reached completion. Solvent was evaporated under vacuum to give 90 mg of a crude material that was purified on a 4 g silica gel cartridge (0- 45% Cy/EtOAc) to give *tert*-butyl (3-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)-2,2-difluoropropyl)carbamate (**4.78**) (47 mg, 56% yield).

^{1}H NMR (DMSO-d_6, 400MHz): δ = 8.94 (t, *J*=5.8 Hz, 1H), 7.38 (d, *J*=8.3 Hz, 2H), 7.16 (d, *J*=8.3 Hz, 2H), 5.09 (s, 2H), 3.65 (tq, *J*=14.9, 6.3 Hz, 2H), 3.42 (tq, *J*=14.1, 6.3 Hz, 2H), 3.14 (s, 3H), 2.60 (t, *J*=5.1 Hz, 2H), 2.32 (t, *J*=5.6 Hz, 2H), 1.57-1.71 (m, 4H), 1.38 ppm (s, 9H) one of the NH units is not visible in the spectrum conditions.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.35 min, $[M+H]^+$ 560 (> 95% purity).

_N_-(3-Amino-2,2-difluoropropyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1_H_-indole-2-carboxamide (4.79)



To a stirred solution of *tert*-butyl (3-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)-2,2-difluoropropyl)carbamate (**4.78**) (44 mg, 0.08 mmol) dissolved in DCM (3 mL) at 0 ºC was added TFA (0.5 mL, 0.08 mmol). After 1 hour, HPLC (basic pH) showed the reaction had reached completion. The solvent was evaporated under vacuum to give 40 mg of a yellowish oil that was loaded onto a SCX cartridge and eluted with 7 M NH_3 in MeOH to give *N*-(3-amino-2,2-difluoropropyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (**4.79**) (24 mg, 66% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.99 (t, *J*=6.1 Hz, 1H), 7.40 (d, *J*=8.3 Hz, 2H), 7.16 (d, *J*=8.6 Hz, 2H), 5.07 (s, 2H), 3.70 (td, *J*=14.0, 5.8 Hz, 2H), 3.13 (s, 3H), 2.88 (t, *J*=14.5 Hz, 2H), 2.59 (br. s., 2H), 2.32 (br. s., 2H), 1.65 ppm (br. s., 4H). The NH_2 unit is not visible in the spectrum conditions.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 162.0, 136.1, 132.5, 130.4, 130.3, 129.3, 128.9, 125.3, 122.9 (t, *J*=242.9 Hz), 118.6, 117.1, 47.5, 45.4, 43.8 (t, *J*=27.8 Hz), 41.2 (t, *J*=29.3 Hz), 22.6, 22.4, 21.6 ppm.

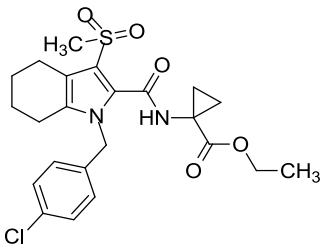
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.18 min, $[M+H]^+$ 460 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3301.2, 2933.6, 2854.2, 1289.9, 1108.1.

MP Crystaline solid that melts at 142 ºC and decomposes from 250 ºC.

HRMS (ES) calcd for $C_{20}H_{25}{}^{35}ClF_2N_3O_3S$, $(M + H)^+$ 460.1268 found 460.1278.

Ethyl 1-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)cyclopropanecarboxylate (4.81)



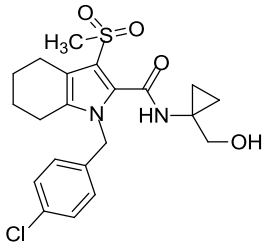
To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**4.70**) (200 mg, 0.5 mmol) in dry THF (3 mL), HOBT (83 mg, 0.5 mmol), EDCI (104 mg, 0.544 mmol), and DIPEA (0.2 mL, 1.1 mmol) were added and reaction was stirred at room temperature for 30 min, then ethyl 1-aminocyclopropanecarboxylate hydrochloride (**4.80**) (90 mg, 0.5 mmol) was added and the reaction mixture was stirred at room temperature overnight (15 h). After this time, HPLC (basic pH) showed that the reaction had reached completion. The reaction mixture was diluted with EtOAc and quenched with water, the phases were separated in a funnel, and the organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to give 250 mg of a pale yellow oil that was purified on a 12 g silica gel cartridge (0-50% Cy/EtOAc) to give ethyl 1-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)cyclopropanecarboxylate (**4.81**) (190 mg, 73% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.18 (s, 1H), 7.34 (d, *J*=8.6 Hz, 2H), 7.18 (d, *J*=8.3 Hz, 2H), 5.10 (s, 2H), 3.85 (q, *J*=7.1 Hz, 2H), 3.01 (s, 3H), 2.47 (br. s., 2H), 2.14-2.23 (m, 2H), 1.53 (br. s., 4H), 1.27-1.34 (m, 2H), 1.02-1.09 (m, 2H), 0.85 ppm (t, *J*=7.2 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 172.2, 162.3, 136.2, 132.5, 131.0, 130.1, 129.5, 128.9, 118.3, 116.6, 61.2, 47.5, 45.6, 33.2, 33.2, 22.6, 22.4, 21.6, 21.5, 17.1, 13.5 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.29 min, $[M+H]^+$ 479 (> 95% purity).

1-(4-Chlorobenzyl)-*N*-(1-(hydroxymethyl)cyclopropyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (4.82)



$LiBH_4$ (26.3 mg, 1.2 mmol) was added to a previously cooled at 0 ºC solution of ethyl 1-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamido)cyclopropanecarboxylate (**4.81**) (170 mg, 0.4 mmol) in toluene (2 mL). The reaction was stirred at room temperature for 18 h, after which time HPLC (basic pH) showed some starting material remaining. The reaction mixture was cooled to 0 ºC and more $LiBH_4$ (26.3 mg, 1.2 mmol) was added; the reaction was then allowed to reach room temperature. After 4 h HPLC (basic pH) showed that the reaction had reached completion. A saturated solution of Rochelle's salt and EtOAc were added to the mixture, the phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 1-(4-chlorobenzyl)-*N*-(1-(hydroxymethyl)cyclopropyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (**4.82**) (125 mg, 81% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.64 (s, 1H), 7.28-7.35 (d, *J*=8.3 Hz, 2H), 7.12 (d, *J*=8.3 Hz, 2H), 4.98 (s, 2H), 4.43 (t, *J*=5.9 Hz, 1H), 3.39 (d, *J*=5.8 Hz, 2H), 3.02 (s, 3H), 2.44-2.51 (m, 2H), 2.18-2.32 (m, 2H), 1.47-1.64 (m, 4H), 0.49-0.62 ppm (m, 4H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 162.1, 136.4, 132.6, 131.4, 130.0, 129.6, 128.8, 118.4, 116.8, 63.9, 47.5, 45.5, 35.1, 22.8, 22.5, 21.7, 21.7, 10.5 ppm.

LCMS (Acetate NH_4 25 mM + 10%ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.18 min, $[M+H]^+$ 437 (> 95% purity).

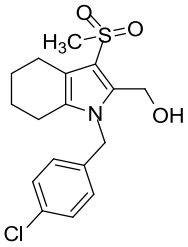
IR ν_{max} (neat) cm^{-1}: 3283.3, 2933.4, 2851.6, 1220.3, 1053.8.

MP Polimorfous solid that contains two crystaline forms that melt at 182 ºC and 331 ºC.

HRMS (ES) calcd for $C_{21}H_{26}{}^{35}ClN_2O_4S$, $(M + H)^+$ 437.1296 found 437.1294.

9.2.4.3.2. Amines

(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanol (4.85)

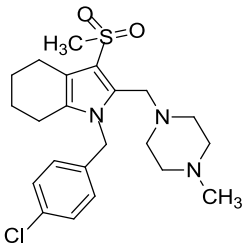


$LiBH_4$ (0.274 g, 12.56 mmol) was added to a solution of ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole-2-carboxylate (**4.69**) (1.5 g, 3.7 mmol) in toluene (20 mL) that was previously cooled to 0 ºC. The reaction was allowed to stand under stirring at room temperature overnight. After this time LCMS Neutral showed that the reaction had reached completion. The reaction mixture was cooled to 0 ºC and then a saturated solution of sodium potassium tartrate was added dropwise, EtOAc was added, and the phases were separated, the aqueous phase was washed with EtOAc (x 3), the combined organic layers were washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated under vacuum to afford (1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methanol (**4.85**) (1.35 g, 94% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.37-7.44 (m, 2H), 7.04 (d, *J*=8.3 Hz, 2H), 5.21 (s, 2H), 5.17 (t, *J*=5.4 Hz, 1H), 4.67 (d, *J*=5.3 Hz, 2H), 3.07 (s, 3H), 2.56-2.63 (m, 2H), 2.24-2.35 (m, 2H), 1.64 ppm (d, *J*=5.3 Hz, 4H).

^{13}C NMR (DMSO-d_6, 101MHz): δ = 137.1, 134.6, 132.1, 129.7, 129.1, 128.6, 117.8, 116.8, 51.7, 46.6, 46.3, 23.0, 22.6, 22.0, 21.6 ppm.

1-(4-Chlorobenzyl)-2-((4-methylpiperazin-1-yl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole (4.90)



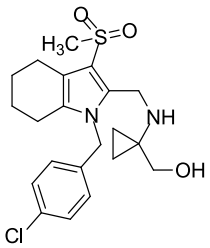
(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)(4-methylpiperazin-1-yl)methanone (**4.72**) (170 mg, 0.4 mmol) was suspended in diethyl

ether (12 mL) and $TiCl_4$ (0.4 mL, 3.6 mmol) and, subsequently, LAH (43 mg, 1.1 mmol) were added. The reaction was stirred at room temperature for 1 h and LCMS Neutral after this time showed appreciable SM remaining. The reaction was kept in the freezer overnight and the next morning it was allowed to reach room temperature and more $TiCl_4$ (0.4 mL, 3.6 mmol) and LAH (43 mg, 1.1 mmol) were added. The reaction became much thicker and more ether was added (4 mL). Reaction monitoring by HPLC (basic pH) indicated that more $TiCl_4$ (0.4 mL, 3.6 mmol) and LAH (43 mg, 1.1 mmol) were needed. After 5 h HPLC (basic pH) showed that the reaction had progressed but it was becoming more complex so it was stopped and worked up. The crude mixture was diluted with EtOAc and washed three times with a 1 N solution of NaOH. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 100 mg of a crude material that will be purified by HPLC_sep (basic pH) to give 1-(4-chlorobenzyl)-2-((4-methylpiperazin-1-yl)methyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole (**4.90**) (20 mg, 12% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.30-7.34 (m, 2H), 6.87 (d, *J*=8.6 Hz, 2H), 5.24 (s, 2H), 3.77 (s, 2H), 3.09 (s, 3H), 2.73-2.79 (m, 2H), 2.50 (br. s., 4H), 2.37 (t, *J*=4.8 Hz, 2H), 2.30 (br. s., 4H), 2.25 (s, 3H), 1.72-1.88 ppm (m, 4H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.29 min, $[M+H]^+$ 436 (> 95% purity).

(1-(((1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methyl)amino)cyclopropyl)methanol (4.91)



To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-*N*-(1-(hydroxymethyl)cyclopropyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxamide (**4.82**) (75 mg, 0.172 mmol) in dry THF (10 mL), borane-methyl sulfide complex (0.08 mL, 0.9 mmol) was added. The reaction mixture was vigorously refluxed under an argon atmosphere for 6 h. After this time HPLC (basic pH) showed some starting material remaining. More THF (10 mL) and borane-methyl sulfide complex

(0.08 mL, 0.9 mmol) were added and the reaction was refluxed for an additional 3 h and at room temperature overnight. After this time, no SM was observed in the HPLC (basic pH), but additional impurities appeared. The reaction mixture was cooled to room temperature and quenched with methanol at 0 ºC. The solvent was removed under vacuum, and the resultant solid redissolved in methanol (5 mL) and heated to 90 ºC for 3 h to hydrolyse the borates. The solvent was removed under vacuum to give 60 mg of a crude material that was purified by semi-preparative HPLC_sep (basic pH). The appropiate fraction was freeze-dried to give (1-(((1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)methyl)amino)cyclopropyl)methanol (**4.91**) (7 mg, 10% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.31-7.37 (m, 2H), 6.95 (d, *J*=8.3 Hz, 2H), 5.09 (s, 2H), 3.89 (s, 2H), 3.55 (s, 2H), 3.11 (s, 3H), 2.73 (t, *J*=5.1 Hz, 2H), 2.37 (t, *J*=5.2 Hz, 2H), 1.70-1.85 (m, 4H), 0.56-0.62 (m, 2H), 0.45-0.52 ppm (m, 2H). The most acidic protons are not visible under the spectrum conditions.

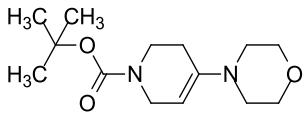
^{13}C NMR ($CDCl_3$, 101MHz): δ = 135.5, 133.5, 133.5, 129.7, 129.1, 127.0, 117.4, 117.4, 64.5, 46.2, 46.0, 40.2, 38.2, 26.9, 22.9, 22.6, 21.9, 21.8, 11.9 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.28 min, $[M+H]^+$ 423 (> 95% purity).

9.2.4.3.3. Reducing lipophilicity of the tetrahydroindole core:

- 6,7-Dihydro-1*H*-pyrrolo[3,2-*c*] pyridine system.

tert-Butyl 4-morpholino-5,6-dihydropyridine-1(2*H*)-carboxylate (4.97)



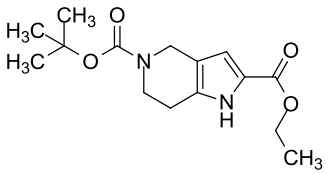
tert-Butyl 4-oxo-1-piperidinecarboxylate (**4.95**) (5 g, 25 mmol) and morpholine (**4.96**) (2.6 mL, 30 mmol) were mixed in toluene (25 mL) in a round bottomed flask equipped with a Dean Stark apparatus and a condenser (the Dean Stark apparatus was filled with toluene). The reaction was refluxed over 30 h and after that time the heating was stopped and the reaction was stirred at room temperature over the weekend. After that time, an aliquote was taken and the solvent was evaporated under vacuum to remove the toluene. ^{1}H NMR showed final product (**4.97**) and starting material (**4.95**) (3:2). The reaction was heated to vigorous reflux for a further 24 h and ^{1}H NMR after that time showed that the reaction had progressed to 4:1 (**4.97**:**4.95**). The reflux continued for one more day. The solvent was evaporated under vacuum to give an unseparable mixture of *tert*-butyl 4-morpholino-5,6-dihydropyridine-1(2*H*)-carboxylate (6.26 g, 23.3 mmol, 89 % yield) along with starting material (**4.95**) in a proportion of 9:1, and traces of toluene and morpholine, as a brown oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.52 (br. s., 1H), 3.83 (br. s., 2H), 3.61 (t, *J*=4.8 Hz, 4H), 3.41 (t, *J*=5.7 Hz, 2H), 2.67-2.77 (m, 4H), 2.08 (t, *J*=5.4 Hz, 2H), 1.40 ppm (s, 9H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 154.2, 144.4, 96.3, 79.1, 66.4, 48.1, 46.4, 40.8, 28.6, 28.5 ppm.

This reaction was initially performed with refluxing for a shorter time using the same amounts of substrates, to afford an unseparable mixture of *tert*-butyl 4-morpholino-5,6-dihydropyridine-1(2*H*)-carboxylate (3.4 g, 12.7 mmol, 50 % yield) (**4.97**) along with starting material (**4.95**) in a proportion of 9:1, and traces of toluene and morpholine, as a brown oil.

5-*tert*-Butyl-2-ethyl-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (4.98)



To a solution of *tert*-butyl 4-morpholino-5,6-dihydropyridine-1(2*H*)-carboxylate (**4.97**) (6.9 g, 25.7 mmol) in toluene (80 mL) was added the compound mixture of (*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate with (*Z*/*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (1: 0.15) (**4.105**) (2.7 g, 12.9 mmol), and the resulting mixture was stirred at room temperature for 3 h. Water (40 mL) was added to the mixture, and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to give 7.9 g of a brownish oil that corresponded to oxazine **4.106** and traces of toluene, morpholine, and *tert*-butyl 4-oxo-1-piperidinecarboxylate **4.95** (**^{1}H NMR** (DMSO-d_6, 400MHz): δ = 4.22 (dd, *J*=7.1, 2.8 Hz, 2H), 3.73 (br. s., 2H), 3.61 (t, *J*=6.2 Hz, 4H), 3.43-3.56 (m, 4H), 2.57 (br. s., 3H), 2.35 (t, *J*=6.2 Hz, 4H), 1.43 (s, 9H), 1.25 ppm (t, *J*=7.1 Hz, 3H)).

The yield was considered quantitative for the next step of the conversion.

To a solution of oxazine **4.106** (5.1 g, 12.9 mmol) in DCE (80 mL), triirondodecacarbonyl (9.71 g, 19.28 mmol) and TFA (2.95 mL, 38.6 mmol) were added and the resulting mixture was stirred at 80 ºC for 15 h. LCMS Neutral after that time, showed that the reaction had finished affording the desired product. After cooling to room temperature, the reaction was filtered through a celite pad, the pad was washed with DCM, and the filtrate was concentrated under vacuum. The obtained residue was purified by silica gel on two 100 g cartridges (Cy/EtOAc 0-30%) to give 5-*tert*-butyl 2-ethyl 6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.98**) (1.1 g, 29% yield) with *tert*-butyl 4-oxo-1-piperidinecarboxylate (**4.95**) (9:1).

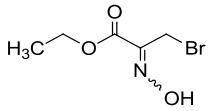
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.56 (br. s., 1H), 6.57 (d, *J*=1.8 Hz, 1H), 4.28 (s, 2H), 4.20 (q, *J*=7.0 Hz, 2H), 3.55-3.64 (m, 2H), 2.59 (t, *J*=5.4 Hz, 2H), 1.43 (s, 9H), 1.25 ppm (t, *J*=7.1 Hz, 3H).

The first time this reaction was carried out, the starting material **4.97** contained *tert*-butyl 4-oxo-1-piperidinecarboxylate (**4.95**) in a proportion of 1:1. The reaction was performed as a *one-pot* process (the oxazine was not isolated), to give 5-*tert*-butyl 2-ethyl 6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.98**) (750 mg,

18% yield), along with *tert*-butyl 4-oxo-1-piperidinecarboxylate (**4.95**) (1:1). The amounts of the reagents were:

- *tert*-butyl 4-morpholino-5,6-dihydropyridine-1(2*H*)-carboxylate (**4.97**) (6.8 g, 25.3 mmol) in toluene (80 mL)
- (*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate compound with (*Z*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (1: 0.15) (**4.105**) (2.9 g, 13.8 mmol)
- triirondodecacarbonyl (10.4 g, 20.7 mmol)
- TFA (3.2 mL, 41.4 mmol)

(*E*)-Ethyl 3-bromo-2-(hydroxyimino)propanoate compound with (*Z*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (1: 0.15) (4.105)

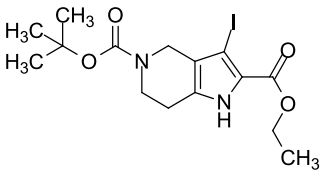


To a solution of ethyl 3-bromo-2-oxopropanoate (**4.104**) (5 mL, 36.2 mmol) in a mixture of chloroform (10 mL), and water (10 mL), hydroxylamine hydrochloride (2.52 g, 36.2 mmol) was added, and the mixture was stirred at room temperature for 18 h. DCM and water were added to the reaction mixture and the phases were separated, the organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under vacuum to give (*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate compound with (*Z*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (**4.105**) (6.5 g, 85% yield) as a pale yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.18 (s, 1H), 13.13 (s, 0.15H), 4.21-4.28 (m, 0.3H), 4.19 (s, 0.3H), 4.21 (s, 2H), 4.17-4.22 (m, 2H), 1.22-1.26 ppm (m, 0.45H), 1.24-1.28 ppm (m, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 162.6, 147.2, 62.1, 17.6, 14.1 ppm.

5-*tert*-Butyl 2-ethyl 3-iodo-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (4.99)



NIS (1 g, 4.5 mmol) was added to a solution of 5-*tert*-butyl 2-ethyl 6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.98**) (1.1 g, 3.7 mmol) in DCM (35 mL) and the mixture stirred for 90 min at 0 °C. LCMS Neutral showed that the reaction had reached completion. $Na_2S_2O_5$ solution (20% wt) and DCM were added to the reaction, and phases were separated in a funnel, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to afford 5-*tert*-butyl 2-ethyl 3-iodo-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.99**) along with *tert*-butyl 4-oxo-1-piperidinecarboxylate (**4.95**) in a proportion of 9:1 (1.55 g, 99% yield)

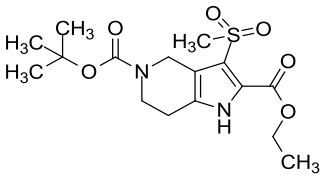
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.99 (s, 1H), 4.24 (q, *J*=7.1 Hz, 2H), 4.10 (s, 2H), 3.56-3.60 (m, 2H), 2.59 (t, *J*=5.4 Hz, 2H), 1.42 (s, 9H), 1.30 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 159.9, 154.7, 138.5, 132.9, 128.7, 120.6, 79.6, 60.1, 47.9, 46.5, 28.6, 22.2, 14.8 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.32 min, $[M-H]^-$ 419 (> 95% purity).

The first time this reaction was carried out, the starting material **4.98** contained *tert*-butyl 4-oxo-1-piperidinecarboxylate (**4.95**) in a proportion of 1:1. This reaction was performed using 5-*tert*-butyl 2-ethyl 6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.98**) (700 mg, 2.4 mmol) and NIS (642 mg, 2.8 mmol) in DCM (25 mL) to obtain 5-*tert*-butyl 2-ethyl 3-iodo-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.99**) (680 mg, 68% yield).

5-*tert*-Butyl 2-ethyl 3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (4.100)



To a solution of 5-*tert*-butyl 2-ethyl 3-iodo-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.99**) (1.55 g, 3.7 mmol) in dry NMP (40 mL) was added CuI (0.7 g, 3.7 mmol) and methanesulfinic acid, sodium salt (0.9 g, 9.2 mmol). The reaction mixture was stirred at 110 ºC for 20 h, and LCMS Neutral after that time showed starting material remaining. More CuI (0.7 g, 3.7 mmol) and methanesulfinic acid, sodium salt (1:1) (0.9 g, 9.2 mmol) were added to the reaction mixture and heated continued at 110 ºC for two hours. LCMS Neutral showed reaction completion. EtOAc was added and a beige solid precipitated, it was filtered, and the filtrate was washed with a saturated solution of NH_4Cl, and phases were separated in a funnel. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give a crude product that was purified on a silica gel cartridge to give 5-*tert*-butyl 2-ethyl 3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.100**) (1.25 g, 91% yield) as a yellow oil.

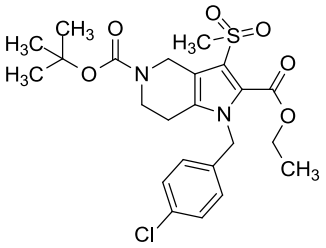
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.63 (br. s., 1H), 4.51 (s, 2H), 4.31 (q, *J*=7.1 Hz, 2H), 3.58 (t, *J*=5.7 Hz, 2H), 3.31 (s, 3H), 2.62 (t, *J*=5.6 Hz, 2H), 1.41 (s, 9H), 1.32 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 159.0, 154.8, 138.16, 130.9, 120.6, 118.3, 79.6, 61.3, 49.0, 44.4, 30.6, 28.4, 17.7, 14.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.10 min, $[M+H]^+$ 373 (> 95% purity).

The first time this reaction was carried out, the starting material **4.99** contained *tert*-butyl 4-oxo-1-piperidinecarboxylate (**4.95**) in a proportion of 1:1. This reaction was performed using 5-*tert*-butyl 2-ethyl 6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.99**) (580 mg, 1.4 mmol) and CuI (0.3 + 0.13 g, 1.4 + 0.5 mmol) and methanesulfinic acid, sodium salt (1:1) (0.35 + 0.17 g, 3.4 + 1.7 mmol) in NMP (30 mL) to obtain 5-*tert*-butyl 2-ethyl 3-iodo-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.99**) (317 mg, 62% yield).

5-*tert*-Butyl 2-ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (4.101)



To a solution of 5-*tert*-butyl 2-ethyl 3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.100**) (1.25 g, 3.4 mmol) in dry ACN (40 mL) was added K_2CO_3 (0.93 g, 6.7 mmol), then 1-(bromomethyl)-4-chlorobenzene (**4.2**) (0.83g, 4 mmol) was added and the reaction was refluxed for 2 h. LCMS Neutral showed reaction completion. Solvent was evaporated under vacuum to give a crude product that was dissolved in water, extracted with EtOAc (x 3), and the combined organic layers were dried over $MgSO_4$, filtered, and evaporated to afford a crude material that was triturated with cyclohexane for 36 h. The solid was filtered to give 5-*tert*-butyl 2-ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.101**) (945 mg, 57% yield) as a white solid.

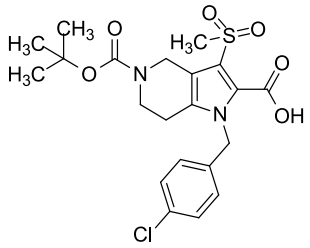
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.42 (d, *J*=8.6 Hz, 2H), 7.05 (d, *J*=8.3 Hz, 2H), 5.39 (s, 2H), 4.53 (s, 2H), 4.23 (q, *J*=7.2 Hz, 2H), 3.58 (t, *J*=5.7 Hz, 2H), 3.30 (s, 3H), 2.51-2.57 (m, 2H), 1.40 (s, 9H), 1.21 ppm (t, *J*=7.1 Hz, 3H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.34 min, $[M+H]^+$ 497 (> 95% purity).

This reaction was performed the first time on a smaller scale, using the same procedure and the amounts depicted below to obtain 5-*tert*-butyl 2-ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.101**) (277 mg, 67% yield) as a beige solid.

- 5-*tert*-butyl 2-ethyl 3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.100**) (308 mg, 0.83 mmol) in ACN (15 mL)
- K_2CO_3 (229 mg, 1.6 mmol),
- 1-(bromomethyl)-4-chlorobenzene (**4.2**) (0.2 g, 1 mmol)

5-(*tert*-Butoxycarbonyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (4.102)



5-*tert*-Butyl 2-ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.101**) (445 mg, 0.9 mmol) was dissolved in a mixture of THF (5 mL) and water (5 mL). LiOH.H_2O (150 mg, 3.6 mmol) was added and the mixture was heated at 40 ºC for 5 h. LCMS Neutral after that time showed reaction completion. A 2 N HCl solution was added to the reaction mixture until acid pH (4-5), EtOAc was added to the reaction mixture, and the phases were separated in a funnel, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 5-(*tert*-butoxycarbonyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (**4.102**) (400 mg, 95% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.08-14.48 (br.s, 1H), 7.41 (d, *J*=8.3 Hz, 2H), 7.06 (d, *J*=8.3 Hz, 2H), 5.44 (s, 2H), 4.52 (s, 2H), 3.52-3.60 (m, 2H), 3.32 (s, 3H), 2.50 (br. s., 2H), 1.39 ppm (s, 9H).

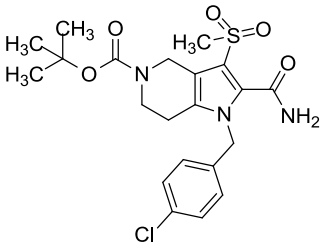
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.6, 154.4, 136.4, 132.7, 131.5, 129.2, 128.7, 117.4, 79.7, 60.2, 48.1, 45.1, 40.5, 40.3, 40.1, 39.9, 28.5 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.03 min, $[M+H]^+$ 469 (> 95% purity).

This reaction was performed the first time on a smaller scale, using the same procedure and the amounts depicted below to obtain 5-(*tert*-butoxycarbonyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (**4.102**) (229 mg, 90% yield).

- 2-ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.101**) (270 mg, 0.54 mmol) in THF and water (5 & 5 mL)
- LiOH.H_2O (91 mg, 2.2 mmol)

***tert*-Butyl 2-carbamoyl-1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-5(4*H*)-carboxylate (4.103)**

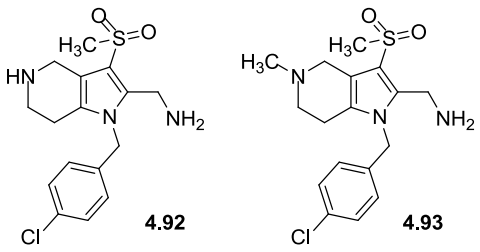


5-(*tert*-Butoxycarbonyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-c]pyridine-2-carboxylic acid (**4.102**) (217 mg, 0.46 mmol) was dissolved in 1,4-dioxane (6 mL) and pyridine (0.03 mL, 0.32 mmol) was added dropwise. Boc_2O (151 mg, 0.69 mmol) was added, and the reaction was stirred for 15 min. After this time, ammonium bicarbonate (44 mg, 0.56 mmol) was added, and the reaction was stirred at room temperature for 19 h. The solvent was evaporated under vacuum to give *tert*-butyl 2-carbamoyl-1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-c]pyridine-5(4*H*)-carboxylate (**4.103**) (217 mg, 100% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.02 (br. s., 1H), 7.93 (br. s., 1H), 7.41 (d, *J*=8.3 Hz, 2H), 7.16 (d, *J*=8.3 Hz, 2H), 5.20 (s, 2H), 4.46 (br. s., 2H), 3.54 (t, *J*=5.6 Hz, 2H), 3.20 (s, 3H), 2.42 (br. s., 2H), 1.39 ppm (s, 9H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.21 min, $[M+H]^+$ 468 (> 95% purity).

(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)methanamine (4.92) and (1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)methanamine (4.93)



To a solution of *tert*-butyl 2-carbamoyl-1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-5(4*H*)-carboxylate (**4.103**) (55 mg, 0.118 mmol) in dry THF (5 mL) stirred at 0 ºC, dimethylsulfide borane complex (44.6 mg, 0.588 mmol) was added. The reaction mixture was vigorously refluxed under argon atmosphere for 1 h. LCMS Neutral after that time mainly showed starting material. A second addition of $BH_3.SMe_2$ (44.6 mg, 0.588 mmol) was made and the reaction was refluxed for 3 h more. LCMS Neutral and HPLC (basic pH) showed progression but there was still some starting material remaining. The reaction mixture was refluxed overnight (19 h) and HPLC (basic pH) showed reaction completion. The reaction mixture was cooled to 0 ºC in an ice-bath and then MeOH was added, it was evaporated under vacuum, HCl in MeOH 3N was added, and the mixture was refluxed for 1 h, with HPLC (basic pH) showing that the borate complexes were broken. MeOH and HCl were evaporated under vacuum to give a colorless oil (100 mg) that was loaded in a SCX cartridge (1 g), previously washed with MeOH, MeOH was added to elute the non-protonated products, and then a 7 M solution of NH_3 in MeOH was added to elute a mixture of **4.92** and **4.93** (25 mg). The mixture was separated by HPLC_sep (basic pH)

Two fractions were obtained:

- (1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)methanamine (3 mg, 7% yield) (**4.92**)

  ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.24 (d, *J*=8.3 Hz, 2H), 6.82 (d, *J*=8.3 Hz, 2H), 5.05 (s, 2H), 3.94 (s, 2H), 3.80 (s, 2H), 3.27-3.32 (m, 1H), 3.04 (t, *J*=5.8 Hz, 2H), 3.01 (s, 3H), 2.38-2.45 ppm (m, 2H). (NH_2 not observed).
- (1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-yl)methanamine (7.3 mg, 17% yield) (**4.93**)

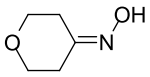
  ^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.33 (d, *J*=8.6 Hz, 2H), 6.93 (d, *J*=8.3 Hz,

2H), 5.17 (s, 2H), 3.96 (s, 2H), 3.67 (s, 2H), 3.09 (s, 3H), 2.71-2.80 (m, 2H), 2.58 (t, *J*=5.7 Hz, 2H), 2.50-2.55 (m, 2H), 2.13 ppm (br. s., 3H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$0.92 min, $[M+H]^+$ 368 (90% purity).

- 1,4,6,7-Tetrahydropyrano[4,3-*b*] pyrrole system.

Dihydro-2*H*-pyran-4(3*H*)-one oxime (4.111)[132]



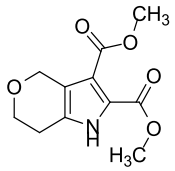
To a solution of dihydro-2*H*-pyran-4(3*H*)-one (**4.109**) (4.6 mL, 50 mmol) in ethanol were added hydroxylamine hydrochloride (14.6 g, 210 mmol) and sodium acetate (14.3 g, 175 mmol). The mixture was heated at reflux for 20 h. The precipitate was filtered, and discarded. The filtrate was evaporated to give a crude material that was treated with ^{t}BuOMe but no solution was obtained even under reflux. The insoluble solid was decanted and the solvent was evaporated under vacuum to give dihydro-2*H*-pyran-4(3*H*)-one oxime (**4.111**) (2.9 g, 50% yield) as a white solid. The product contained around 10% of acetic acid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.38 (br. s., 1H), 3.67 (t, *J*=5.7 Hz, 2H), 3.60 (t, *J*=5.9 Hz, 2H), 2.45-2.50 (t, *J*=5.8 Hz, 2H), 2.23 ppm (t, *J*=5.6 Hz, 2H).

The solid from the previous decantation was triturated with MeOH and filtered, salts were removed by filtration and the mother liquors were evaporated to give dihydro-2*H*-pyran-4(3*H*)-one oxime (3.1 g, 54% yield) as a yellowish oil.

Total yield >100 % (presence of acetic acid).

Dimethyl 1,4,6,7-tetrahydropyrano[4,3-*b*]pyrrole-2,3-dicarboxylate (4.113)



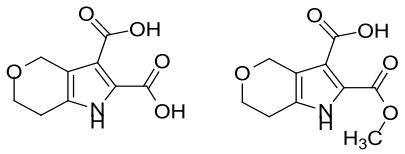
A suspension of dihydro-2*H*-pyran-4(3*H*)-one oxime (**4.111**) (1.8 g, 15.6 mmol), DMAD (**4.112**) (2.5 mL, 20.3 mmol) and DABCO (0.5 mL, 4.7 mmol) in toluene (8 mL) was heated at 165 ºC. After 2 h, the solvent was evaporated under vacuum and the crude material was purified on a silica gel cartridge (Cy/EtOAc 0-100%) to give

dimethyl 1,4,6,7-tetrahydropyrano[4,3-*b*]pyrrole-2,3-dicarboxylate (**4.113**) (620 mg, 17% yield) as an orange solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.08-12.36 (m, 1H), 4.58 (s, 2H), 3.81 (t, *J*=5.6 Hz, 2H), 3.77 (s, 3H), 3.70 (s, 3H), 2.61 ppm (t, *J*=5.4 Hz, 2H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$ 0.91 min, $[M-H]^-$ 238 (>85% purity).

1,4,6,7-Tetrahydropyrano[4,3-*b*]pyrrole-2,3-dicarboxylic acid compound with 2-(methoxycarbonyl)-1,4,6,7-tetrahydropyrano[4,3-*b*]pyrrole-3-carboxylic acid (1:1) (4.114)

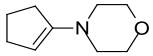


Dimethyl 1,4,6,7-tetrahydropyrano[4,3-*b*]pyrrole-2,3-dicarboxylate (**4.113**) (110 mg, 0.5 mmol) was dissolved in a mixture of THF (3 mL) and water (3 mL) and $LiOH.H_2O$ (77 mg, 1.8 mmol) was added. The mixture was stirred at room temperature for 20 h and, after this time, no SM was detected in the LCMS Neutral but two peaks were observed. 2 N HCl solution was added to the reaction mixture to reach acidic pH, EtOAc was added to the reaction mixture and the phases were separated in a funnel, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 80 mg a mixture of two products that were tentatively identified as the dicarboxylic acid and the ester and acid moiety (position 2,3 or 3,2) (**4.114**).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.58-13.91 (m, 1H), 12.58 (br. s., 1H), 12.41 (br. s., 1H), 4.67 (s, 1H), 4.64 (s, 2H), 3.87 (s, 3H), 3.78-3.84 (m, 4H), 2.63 ppm (d, *J*=3.5 Hz, 4H).

9.2.4.3.4. Reducing lipophilicity by reducing the size of the homoaryl part of the tetrahydroindole core: 1,4,5,6-tetrahydrocyclopentane[*b*]pyrrole system

4-(Cyclopent-1-en-1-yl)morpholine (4.125)



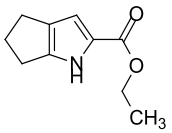
Cyclopentanone (5.3 mL, 59.4 mmol) and morpholine (5.2 mL, 59.4 mmol) were mixed in toluene (25 mL) in a round bottomed flask equipped with a Dean Stark apparatus, and a condenser (the Dean Stark apparatus was also filled with toluene). The reaction was refluxed over 4 h and, after that time, the heating was stopped, and toluene was removed under vacuum to give 4-(cyclopent-1-en-1-yl)morpholine (**4.125**) (7.8 g, 85% yield) as an orange oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 4.36 (s, 1H), 3.56-3.66 (m, 4H), 2.73-2.89 (m, 4H), 2.17-2.36 (m, 4H), 1.76-1.83 ppm (m, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 152.0, 96.4, 66.5, 49.0, 31.3, 30.3, 22.5 ppm.

This reaction was performed twice before. The first time the reaction was carried out using 1 g of cyclopentanone (1 mL, 12 mmol) and morpholine (1 mL, 12 mmol) in toluene (5 mL) and refluxing over 4 h using a Dean-Stark apparatus and a condender to give 4-(cyclopent-1-en-1-yl)morpholine (**4.125**) (0.95 g, 6.2 mmol, 51 % yield) that contained cyclopentanone (10:1). The second time it was carried out using 5 g of cyclopentanone (5.3 mL, 59.4 mmol) and morpholine (5.2 mL, 59.4 mmol) in toluene (25 mL) and refluxing over 4 h using a Dean-Stark apparatus and a condenser to give 4-(cyclopent-1-en-1-yl)morpholine (**4.125**) (3.6 g, 23% yield) that contained cyclopentanone (2:1).

Ethyl 1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (4.126)



To a solution of 4-(cyclopent-1-en-1-yl)morpholine (**4.125**) (4.38 g, 28.6 mmol) in toluene (80 mL) was added the product mixture of (*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate with (*Z*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (1: 0.15) (**4.105**) (3 g, 14.3 mmol), and the resulting mixture was stirred at room

temperature for 3.5 h. Water (40 mL) was added to the mixture, the phases were separated in a funnel, and the organic layer was dried over anhydrous $MgSO_4$, filtered, and evaporated to give a crude product that was dissolved in DCE (80 mL). Triirondodecacarbonyl (10.8 g, 21.4 mmol) and TFA (3.3 mL, 43 mmol) were added to the organic layer at room temperature, the resulting mixture was stirred at 80 ºC for 3 h. After cooling to room temperature, the reaction was filtered through a celite pad, and the filtrate was concentrated under vacuum. The residue obtained was purified by silica gel on two 100 g cartridges (Cy/EtOAc 0-30%) to give ethyl 1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.126**) (820 mg, 32% yield) as a pale yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.45 (br. s., 1H), 6.49 (s, 1H), 4.18 (q, *J*=7.1 Hz, 2H), 2.57-2.67 (m, 2H), 2.47-2.54 (m, 2H), 2.27-2.38 (m, 2H), 1.25 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.9, 143.8, 127.8, 124.9, 110.5, 59.5, 29.1, 25.1, 24.9, 14.8 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.41 min, $[M+H]^+$ 180 (> 95% purity).

The product was used with a previous batch obtained from a test reaction that yielded ethyl 1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.126**) (140 mg, 23% yield) as an orangish solid. The amounts used are depicted below:

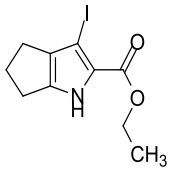
- 4-(cyclopent-1-en-1-yl)morpholine (**4.125**) (0.7 g, 3.3 mmol)
- (*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate compound with (*Z*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (1: 0.15) (**4.105**) (1021 mg, 6.67 mmol)
- triirondodecacarbonyl (2.5 g, 5 mmol)
- TFA (0.25 mL, 3.3 mmol)

This reaction was repeated in a similar scale, but isolating the oxazine **4.132** and using the same procedure and the amounts depicted below to obtain ethyl 1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.126**) (410 mg, 24% yield) as a pale yellow solid.

- 4-(cyclopent-1-en-1-yl)morpholine (**4.125**) (2.9 g, 19 mmol)
- (*E*)-ethyl 3-bromo-2-(hydroxyimino)propanoate compound with (*Z*)-ethyl 3-bromo-2-(hydroxyimino)propanoate (1: 0.15) (**4.105**) (4.3 g, 26.7 mmol)

- triirondodecacarbonyl (7.2 g, 14.3 mmol)
- TFA (2.2 mL, 28.7 mmol)

Ethyl 3-iodo-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (4.127)



NIS (1.2 g, 5.3 mmol) was added to a solution of ethyl 1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.126**) (950 mg, 5.3 mmol) in DCM (50 mL), and the mixture was stirred for 90 min at room temperature. LCMS Neutral after that time, showed that the reaction had almost reached completion. A 20% Wt. $Na_2S_2O_5$ solution and DCM were added to the reaction mixture. The phases were separated in a funnel, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated under vacuum to afford ethyl 3-iodo-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.127**) (1.67 g, 95% yield). The product contained around 8% of SM (**4.126**) by 1H NMR.

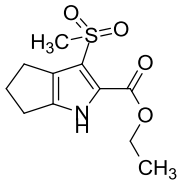
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.79 (br. s., 1H), 4.18-4.25 (m, 2H), 2.71 (t, *J*=7.2 Hz, 2H), 2.37-2.45 (m, 2H), 2.26-2.37 (m, 2H), 1.29 ppm (t, *J*=7.1 Hz, 3H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.33 min, $[M+H]^+$ 306 (> 95% purity).

This reaction was repeated in a similar scale, using the same procedure, and the amounts depicted below to obtain ethyl 3-iodo-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.134**) (1.3 g, 98% yield)

- ethyl 1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.126**) (780 mg, 4.3 mmol) in DCM (40 mL)
- NIS (979 mg, 4.3 mmol)

Ethyl 3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (4.128)



To a solution of ethyl 3-iodo-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.127**) (1.67 g, 5.5 mmol) in dry NMP (50 mL) was added CuI (1 g, 5.5 mmol) and methanesulfinic acid, sodium salt (1.4 g, 13.68 mmol) and reaction was stirred at 80 ºC. LCMS Neutral after 18 h showed desired product, and a little of the starting material remaining **4.127**. The temperature was increased to 110 ºC and 6 h later, LCMS Neutral showed reaction completion. EtOAc and a saturated solution of NH_4Cl were added to the reaction mixture and phases were separated in a funnel. The organic layer was extracted two more times with NH_4Cl to remove the NMP as much as possible. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give a crude material that was purified on a silica gel cartridge to give ethyl 3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.128**) (560 mg, 40% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 12.24-12.60 (m, 1H), 4.28 (q, *J*=7.1 Hz, 2H), 3.26 (s, 3H), 2.72 (t, *J*=7.2 Hz, 2H), 2.65 (t, *J*=7.3 Hz, 2H), 2.34 (quin, *J*=7.2 Hz, 2H), 1.31 ppm (t, *J*=7.1 Hz, 3H).

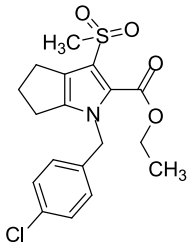
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 158.9, 141.6, 131.0, 124.4, 122.5, 61.0, 44.2, 28.5, 26.0, 24.9, 14.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.05 min, $[M+H]^+$ 258 (> 95% purity).

This reaction was repeated in a similar scale, using the same procedure at 80 ºC, and the amounts depicted below to obtain ethyl 3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.128**) (720 mg, 66% yield) as a beige solid.

- ethyl 3-iodo-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.127**) (1.3 g, 4.3 mmol) in NMP (50 mL)
- CuI (0.8 g, 4.3 mmol)
- methanesulfinic acid, sodium salt (1.1 g, 10.6 mmol)

Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (4.129)



To a solution of ethyl 3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.128**) (560 mg, 2.176 mmol) in dry ACN (25 mL), was added K_2CO_3 (602 mg, 4.35 mmol), then 1-(bromomethyl)-4-chlorobenzene (**4.2**) (537 mg, 2.61 mmol) was added, and reaction was refluxed for 17 h. LCMS Neutral, after this time, showed that the reaction had reached completion. The solvent was evaporated under vacuum, the crude mixture was dissolved in water, extracted with EtOAc (x 3) and then the combined organic layers were dried over $MgSO_4$, filtered, and evaporated affording ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.129**) (814 mg, 98% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.39-7.48 (m, 2H), 7.11 (d, *J*=8.6 Hz, 2H), 5.42 (s, 2H), 4.21 (q, *J*=7.1 Hz, 2H), 3.25 (s, 3H), 2.76 (t, *J*=7.1 Hz, 2H), 2.64 (t, *J*=7.3 Hz, 2H), 2.31 (t, *J*=7.1 Hz, 2H), 1.21 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 159.4, 144.8, 136.6, 132.5, 129.3, 129.1, 129.0, 125.3, 123.5, 61.5, 50.5, 44.6, 27.7, 26.2, 24.8, 14.0 ppm

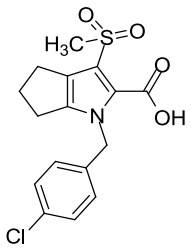
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.35 min, $[M+H]^+$ 382 (> 95% purity).

This reaction was repeated in a similar scale, using the same procedure and the amounts depicted below to obtain ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.136**) (1 g, 97% yield).

as a beige solid.

- ethyl 3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.128**) (560 mg, 2.176 mmol) in ACN (25 mL)
- K_2CO_3 (763 mg, 5.5 mmol)
- 1-(bromomethyl)-4-chlorobenzene (**4.2**) (680 mg, 3.3 mmol)

1-(4-Chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (4.130)



Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylate (**4.129**) (400 mg, 1.047 mmol) was dissolved in a 10 mL mixture of THF/Water (1:1) and $LiOH.H_2O$ (176 mg, 4.19 mmol) was added. The mixture was stirred at room temperature for 20 h and, after that time, no SM was detected by LCMS Neutral. A 2 N aqueous solution of HCl was added to the reaction mixture to acid pH. EtOAc was added to the reaction mixture and the phases were separated in a funnel. The organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum to give 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (**4.130**) (221 mg, 60% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.48 (br. s., 1H), 7.42 (d, *J*=8.3 Hz, 2H), 7.12 (d, *J*=8.1 Hz, 2H), 5.46 (s, 2H), 3.27 (s, 3H), 2.75 (t, *J*=6.8 Hz, 2H), 2.62 (t, *J*=6.9 Hz, 2H), 2.24-2.38 ppm (m, 2H).

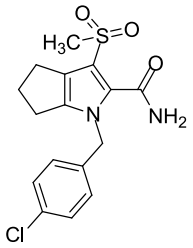
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.0, 144.1, 136.8, 132.6, 129.3, 129.1, 129.2, 128.7, 124.8, 50.3, 44.5, 27.7, 26.4, 24.8 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$0.96 min, $[M+H]^+$ 354 (> 95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (**4.130**) (730 mg, 78% yield) as a beige solid.

- Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-. tetrahydrocyclopenta[*b*] pyrrole-2-carboxylate (**4.129**) (1 g, 2.7 mmol) in 10 mL in THF/Water (1:1)
- $LiOH.H_2O$ (448 mg, 10.7 mmol)

1-(4-Chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxamide (4.131)



1-(4-Chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (**4.130**) (220 mg, 0.6 mmol) was dissolved in 1,4-dioxane (15 mL) and pyridine (0.025 mL, 0.3 mmol) was added dropwise. Di-*tert*-butyl dicarbonate (176 mg, 0.8 mmol) was added and the reaction was stirred for 15 min at room temperature. After that time, ammonium bicarbonate (49 mg, 0.62 mmol) was added and reaction was stirred 60 h at room temperature. LCMS Neutral after that time showed starting material. Pyridine (0.025 mL, 0.3 mmol) and di-*tert*-butyl dicarbonate (176 mg, 0.8 mmol) were added and 15 min later ammonium bicarbonate (49 mg, 0.62 mmol) was added too. The mixture was stirred at room temperature for 4 h and LCMS Neutral after that time showed reaction completion. The solvent was evaporated under vacuum and MeOH (HPLC quality) was added, a solid precipitated, and it was filtered to give 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxamide (**4.131**) (125 mg, 57% yield) as a white solid.

Mother liquors were evaporated under vacuum and MeOH (HPLC quality) was added again, and the white solid was filtered to give 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxamide (**4.131**) (33 mg, 15% yield).

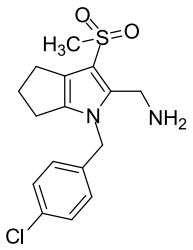
Total yield: 72%

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.82 (br. d, 2H), 7.41 (d, *J*=8.3 Hz, 2H), 7.19 (d, *J*=8.3 Hz, 2H), 5.28 (s, 2H), 3.18 (s, 3H), 2.70 (t, *J*=6.8 Hz, 2H), 2.52-2.61 (m, 2H), 2.25-2.35 ppm (m, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 164.5, 162.0, 155.8, 154.2, 149.4, 140.1, 129.7, 129.1, 127.0, 49.8, 46.2, 28.0, 25.7, 24.8 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.16 min, $[M+H]^+$ 353 (> 95% purity).

(1-(4-Chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)methanamine (4.123)



To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxamide (**4.131**) (139 mg, 0.394 mmol) in dry THF (6 mL), borane dimethyl sulfide complex (0.19 mL, 1.97 mmol) was added. The reaction mixture was vigorously refluxed under an argon atmosphere for 2 h and then stirred at room temperature for 14 h. HPLC (basic pH) after that time showed SM remaining. The reaction was refluxed for a further 1.5 h and HPLC (basic pH) showed starting material remaining. More borane dimethyl sulfide complex (0.19 mL, 1.97 mmol) was added and the reaction was refluxed 2.5 h. HPLC (basic pH) after that time showed a little of SM remaining. The reaction mixture was cooled to 0 ºC and MeOH was added. The solvent was removed under vacuum, and the resultant solid was redissolved in 5 mL of 3 N HCl in MeOH and heated to 90 ºC for 1 h to hydrolyse the borates. The solvent was removed under vacuum to give 190 mg of a crude material that was purified by HPLC_sep (basic pH). Appropiate fractions were evaporated together to give (1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)methanamine (**4.123**) (25 mg, 19% yield) as a yellow sticky solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.42 (d, *J*=8.3 Hz, 2H), 7.16 (d, *J*=8.3 Hz, 2H), 5.20 (s, 2H), 3.82 (s, 2H), 3.08 (s, 3H), 2.66 (t, *J*=6.8 Hz, 2H), 2.43-2.50 (m, 2H), 2.21-2.36 (m, 2H), 1.81-2.19 ppm (m, 2H).

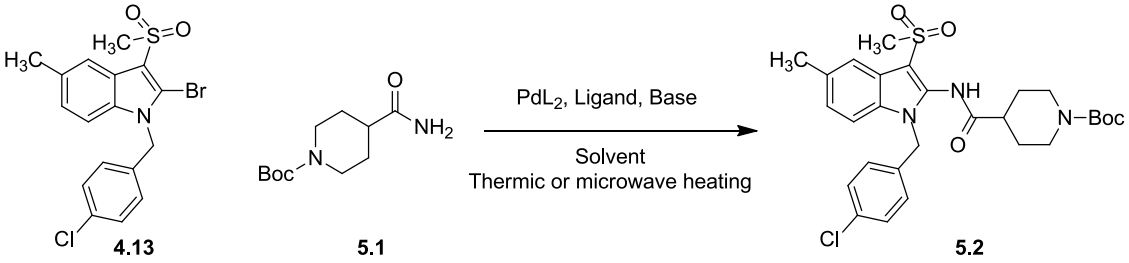
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 138.9, 138.8, 135.2, 133.8, 129.2, 127.8, 125.7, 115.9, 49.2, 45.5, 35.8, 27.5, 25.6, 24.8 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.08 min, $[M+H]^+$ 339 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3271.9, 2929.3, 2860.2, 1682.7, 1518.5, 1284.5, 1092.8, 768.5.

9.3. LIBRARY OF AMIDES

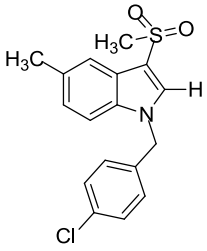
Buchwald reaction conditions optimisation



Entry 1

In a round bottom flask, were added $Pd_2(dba)_3$ (22.2 mg, 0.02 mmol), Xantphos (28 mg, 0.05 mmol), and Cs_2CO_3 (237 mg, 0.7 mmol) under nitrogen, and the flask purged a few minutes with nitrogen. Then, a solution of *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (111 mg, 0.5 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (200 mg, 0.5 mmol) in 1,4-dioxane (10 mL) was added and the reaction was refluxed for 2 h. HPLC (basic pH) after that time showed starting material remaining, some final product and more dehalogenated starting material (**5.8**) than final product. The reaction was refluxed overnight and then HPLC (basic pH) showed that the reaction had progressed but there was some starting material remaining. More $Pd_2(dba)_3$ (22.2 mg, 0.02 mmol) and Xantphos (28 mg, 0.05 mmol) were added and the reaction was refluxed 4 h more (total reaction time = 20 h). HPLC (basic pH), after that time did not show any changes, so the reaction was stopped. The reaction mixture was filtered through a celite cartridge and solvent was evaporated to give a dark green crude that was purified on a 12 g silica gel cartridge (0-60% Cy/EtOAc) to give three fractions:

- F1: complex mixture of products by ^{1}H NMR.
- F2: dehalogenated starting material (**5.8**) by ^{1}H NMR



^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.14 (s, 1H), 7.51 (s, 1H), 7.39 (d, *J*=8.6 Hz, 1H), 7.30-7.34 (m, 2H), 7.19-7.24 (m, 2H), 7.03 (d, *J*=8.6 Hz, 1H), 5.41 (s, 2H), 3.11 (s, 3H), 2.32 ppm (s, 3H)

- F3: impure desired product by ^{1}H NMR and LCMS Neutral

Entry 2

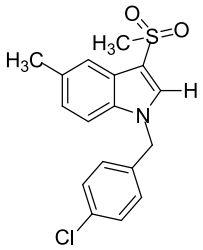
In a microwave vial, were placed $Pd_2(dba)_3$ (9.4 mg, 10.3 µmol), Xantphos (11.9 mg, 0.02 mmol), Cs_2CO_3 (201 mg, 0.6 mmol), the vial was sealed and the mixture was dissolved in 2 mL of dioxane and degassed. In a round bottomed flask were placed *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (70.5 mg, 0.3 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (2 mL) and degassed. This mixture was added to the microwave vial and the final mixture was degassed again and then capped with a teflon cap. The reaction was irradiated in the microwave at 130 ºC for 30 min and, after this time, HPLC (basic pH) and LCMS Neutral showed that the reaction had reached completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated to give 150 mg of a crude product that was purified on a 12 g silica gel cartridge to give the desired *tert*-butyl 4-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)piperidine-1-carboxylate (30 mg, 26% yield) as an off-white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.36 (s, 1H), 7.73 (s, 1H), 7.22-7.26 (m, 2H), 7.01-7.10 (m, 2H), 6.94 (d, *J*=8.3 Hz, 2H), 5.20 (s, 2H), 4.13 (br.s, *J*=7.3 Hz, 2H), 3.12 (s, 3H), 2.65-2.78 (m, 2H), 2.42-2.52 (s, 3H), 1.86 (d, *J*=11.4 Hz, 2H), 1.58-1.73 (m, 3H), 1.45-1.50 ppm (m, 9H).

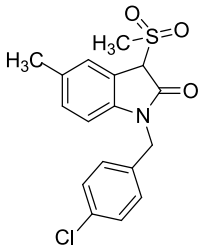
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.39 min, $[M+H]^+$ 560 (> 95% purity).

Entry 3

In a microwave vial, were placed $Pd_2(dba)_3$ (2.8 mg, 3 µmol), Xantphos (3.6 mg, 6.2 µmol), and Cs_2CO_3 (60.4 mg, 0.2 mmol), the vial was sealed and the mixture was dissolved in 2 mL of 1,4-dioxane and degassed. In a round bottomed flask were placed *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (21.2 mg, 0.09 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (30 mg, 0.062 mmol) in 1,4-dioxane (2 mL) and degassed. This mixture was added to the microwave vial and the final reaction mixture was degassed again and capped with a teflon screw cap. The reaction mixture was irradiated for 30 min at 90 ºC, after this time no reaction was observed by LCMS Neutral. The reaction was irradiated 30 min more at 110 ºC and, after this time, some desired product was detected by LCMS Neutral. The reaction was irradiated one more hour at 110 ºC and HPLC (basic pH) showed some progression. The dehalogenated (**5.8**) and lactam **5.39** were formed but in a small proportion. The reaction was not worked-up and purified as it was just a test reaction to study the effect of decreasing the temperature of the reaction.



5.8



5.39

Entry 4

In a microwave vial, were placed $Pd_2(dba)_3$ (9.4 mg, 10.3 µmol), Xantphos (11.9 mg, 0.02 mmol), and Cs_2CO_3 (201 mg, 0.6 mmol), the vial was sealed and the mixture was dissolved in 2 mL of THF and degassed. In a round bottomed flask were placed *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (70.5 mg, 0.3 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in THF (2 mL) and degassed. This mixture was added to the microwave vial and the final mixture was degassed again and then capped with a teflon cap. The reaction was irradiated in the microwave at 130 ºC for 30 min and, HPLC (basic pH) and LCMS Neutral, after this time showed that the reaction had reached completion. EtOAc and water were added to the reaction mixture and the phases were separated, the organic

layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated to give 150 mg of a crude product that was purified on a 12 g silica gel cartridge to give the desired *tert*-butyl 4-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)piperidine-1-carboxylate (39 mg, 34% yield) as a yellow solid.

This same conditions were repeated and the yield was 7 %.

Entry 5

In a microwave vial, were placed $Pd_2(dba)_3$ (4.7 mg, 5.1 µmol), BINAP (6 mg, 0.01 µmol), and Cs_2CO_3 (118 mg, 0.4 mmol), the vial was sealed and the mixture was dissolved in 1 mL of THF and degassed. In a round bottomed flask were placed *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (33.2 mg, 0.15 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) in THF (1 mL) and degassed. This mixture was added to the microwave vial and the final reaction mixture was degassed again and capped with a teflon cap. The reaction mixture was irradiated in the microwave at 130 ºC for 30 min and, after this time, HPLC (basic pH) showed reaction completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine, dried over Na_2SO_4, filtered, and the solvent was evaporated to give 90 mg of a crude product that was purified on a 5 g silica gel cartridge to give the desired *tert*-butyl 4-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)piperidine-1-carboxylate (14 mg, 25% yield) as a yellow solid.

Entry 6

In a microwave vial, were placed $Pd_2(dba)_3$ (4.7mg, 5.1 µmol), BINAP (6 mg, 0.01 µmol), and Cs_2CO_3 (118 mg, 0.4 mmol), the vial was sealed and the mixture was dissolved in 1 mL of THF and degassed. In a round bottomed flask were placed *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (33.2 mg, 0.15 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) in THF (1 mL) and degassed. This mixture was added to the microwave vial and the final mixture was degassed again and then capped with a teflon cap. The reaction was

irradiated in the microwave at 100 ºC for 30 min and after this time HPLC (basic pH) & LCMS Neutral just showed SM. The reaction was irradiated at 115 ºC for 30 min (total reaction time = 1 h) and LCMS Neutral showed that dehalogenated product (**5.8**) was forming in the same proportion to the final product so these conditions were discarded.

Entry 7

In a microwave vial were placed $Pd_2(dba)_3$ (6 mg, 6 µmol), BINAP (8 mg, 0.01 mmol), and Cs_2CO_3 (118 mg, 0.4 mmol), *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (33.2 mg, 0.15 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) in toluene (2 mL) and then capped with a teflon cap. The reaction was degassed and heated at 110 ºC over 16 h and after that time HPLC (basic pH) showed SM and an unknown product. The reaction was evaporated under vacuum to give a crude mixture that was dissolved in DCM and filtered using a syringe filter. This was evaporated under vacuum to give a crude product that precipitated when MeOH (HPLC quality) was added. The solid was filtered and solid and filtrate were analyzed by HPLC (basic pH). The desired product was present in the filtrate but there was insufficient material to allow purification and analysis.

Entry 8

In a microwave vial, were placed $Pd(OAc)_2$ (2.5 mg, 10.3 µmol), ^{t}*Bu*BrettPhos (10 mg, 0.02 mmol), and Cs_2CO_3 (47 mg, 0.15 mmol) and water (one drop), the vial was sealed and the mixture was dissolved in 2 mL of *tert*-butanol (2 mL) and degassed. In a round bottomed flask were placed *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (32.9 mg, 0.15 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) in *tert*-butanol (2 mL) and degassed. This mixture was added to the microwave vial and the final mixture was degassed again and then capped with a teflon cap. The reaction was heated at 100 ºC overnight (16 h) but no reaction was observed after this time by HPLC (basic pH). The reaction mixture was discarded.

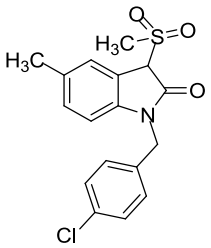
Entry 9

A microwave flask was charged with $Pd(OAc)_2$ (6 mg, 0.03 mmol) and *tBu*BrettPhos (6 mg, 0.01 mmol). The tube was capped and evacuated and backfilled with argon (three times) and *tert*-butanol (0.2 mL) and degassed water (2 µL, 0.1 mmol) were added via syringe. After addition of the water the solution was heated to 110 ºC for 2 min. A second microwave tube charged with *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (33.2 mg, 0.15 mmol), K_3PO_4 (36.0 mg, 0.2 mmol), and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) was capped and evacuated and backfilled with argon (three times). The activated catalyst solution was transferred from the first reaction tube into the second *via* syringue. The reaction mixture was heated at 110 ºC and checked by HPLC (basic pH) at 40 min and 3 h and no changes were observed. The reaction was considered failed and it was discarded.

Entry 10

A microwave flask was charged with BrettPhos (7 mg, 0.01 mmol) and (BrettPhos) palladium(II) phenethylamine chloride (11 mg, 0.01 mmol). The tube was capped and evacuated and backfilled with argon (three times) and THF (1 mL) was added and the flask degassed further. LHMDS (1M THF) (0.4 mL, 0.4 mmol) was added *via* syringe. After addition of two drops of water the solution was heated to 110 ºC for 2 min. A second microwave tube charged with *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (**5.1**) (41.5 mg, 0.2 mmol), and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) was capped and evacuated and backfilled with argon (three times). The activated catalyst solution was transferred from the first reaction tube into the second *via* syringe. The reaction was heated at 110 ºC for 1 h and, after this time, an unknown product was formed and no starting material remained. The solvent was evaporated under vacuum to give a crude mixture that did not correspond to desired product by ^{1}H NMR. The crude mixture was discarded and the reaction was considered failed.

1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)indolin-2-one (5.39)



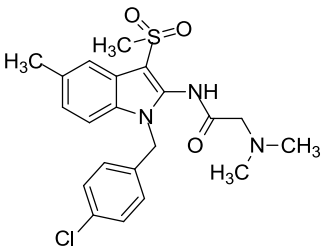
This product was isolated from a failed Buchwald reaction (**Entry 3**) that gave this product instead of that expected.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.37-7.43 (m, 2H), 7.33 (m, 3H), 7.18 (d, *J*=8.1 Hz, 1H), 6.87 (d, *J*=8.1 Hz, 1H), 5.79 (s, 1H), 4.82-5.08 (m, 2H), 3.36 (s, 3H), 2.30 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 168.3*, 142.0, 135.2, 132.6, 132.5, 130.8, 129.5, 129.0, 127.5, 118.2, 110.0, 80.8, 66.3, 42.7, 21.1 ppm. *This signal indicated that the isolated form was the ketone instead of the alcohol.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.23 min, $[M+H]^+$ 350 (> 95% purity).

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-_1H_-indol-2-yl)-2-(dimethylamino)acetamide (5.14)



In a microwave vial, were placed $Pd_2(dba)_3$ (14.1 mg, 0.02 mmol), Xantphos (18 mg, 0.03 mmol), and Cs_2CO_3 (302 mg, 0.9 mmol), the vial was sealed, and evacuated and filled with N_2 several times. In a round bottomed flask, were placed 2-(dimethylamino)acetamide (47.3 mg, 0.5 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (150 mg, 0.3 mmol) in 1,4-dioxane (5 mL) and degassed. This mixture was added to the microwave vial *via* syringe, and the final mixture was degassed for 5 min. The reaction was irradiated in the microwave at 130 ºC for 30 min and, after this time, LCMS Neutral showed that the reaction had reached completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine and dried over Na_2SO_4, filtered, and the solvent was evaporated to give 120 mg of a crude product that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-60%) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-(dimethylamino)acetamide (**5.14**) (36 mg, 27% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.03-10.26 (m, 1H), 7.64 (s, 1H), 7.34-7.38 (m, 2H), 7.32 (d, *J*=8.3 Hz, 1H), 7.17 (d, *J*=8.3 Hz, 2H), 7.07 (dd, *J*=8.5, 1.4 Hz, 1H), 5.33 (s, 2H), 3.16 (s, 3H), 3.10 (s, 2H), 2.40 (s, 3H), 2.29 ppm (s, 6H).

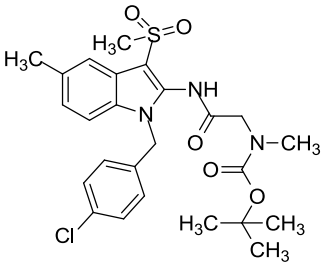
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 171.8, 136.4, 136.1, 132.5, 131.6, 129.3, 129.9, 128.9, 125.1, 124.1, 119.2, 111.6, 107.4, 63.0, 46.0, 45.7, 44.3, 21.7 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.34 min, $[M+H]^+$ 434 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3304.6, 2931.0, 2827.4, 2778.4, 1689.7, 1399.6, 1117.8.

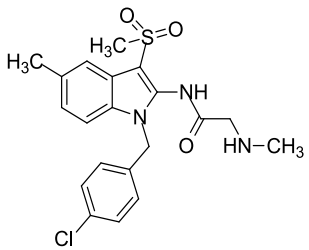
HRMS (ES) calcd for $C_{21}H_{25}{}^{35}ClN_3O_3S$, $(M + H)^+$ 434.1300 found 434.1293.

***tert*-Butyl (2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl)(methyl)carbamate (5.15)**



In a round bottom flask were added $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol), and Cs_2CO_3 (118 mg, 0.4 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of *tert*-butyl (2-amino-2-oxoethyl)(methyl)carbamate (91 mg, 0.5 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (100 mg, 0.2 mmol) (**4.13**) in 1,4-dioxane (5 mL) was added and the reaction was degassed for 5 min. The reaction mixture was irradiated in the microwave at 130 ºC for 1 h. LCMS Neutral showed reaction completion. The reaction mixture was filtered through a celite cartridge and rinsed with DCM. The solvent was evaporated to give 206 mg of a crude that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-60%). Appropiate fractions were evaporated under vacuum to give *tert*-butyl (2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl)(methyl)carbamate (**5.15**) (15 mg, 12% yield) as a beige solid. The product was identified by LCMS Neutral and used in the next step of the synthesis.

***N*-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-(methylamino)acetamide (5.30)**



tert-Butyl (2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl)(methyl)carbamate (**5.15**) (15 mg, 0.03 mmol) was dissolved in DCM (3 mL) and TFA (0.1 mL) was added. The reaction mixture was stirred at room temperature for 1 h and, after this time, LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded in a 1 g SCX cartridge that had been previously washed with

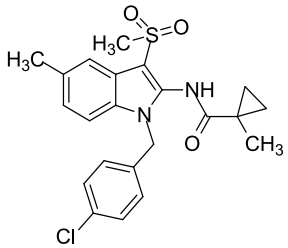
MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of NH_3 in MeOH to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-(methylamino)acetamide (**5.30**) (10 mg, 83% yield) as a beige solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.80 (br. s., 1H), 7.30 (br. s., 2H), 7.05-7.18 (m, 3H), 7.00 (d, *J*=8.1 Hz, 2H), 5.33 (br. s., 2H), 3.52-3.64 (m, 1H), 3.47 (br. s., 2H), 3.07-3.27 (m, 3H), 2.43-2.61 ppm (m, 6H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=1.17 min, $[M+H]^+$ 420 (> 85% purity).

HRMS (ES) calcd for $C_{20}H_{23}{}^{35}ClN_3O_3S$, $(M + H)^+$ 420.1143 found 420.1140.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-1-methylcyclopropanecarboxamide (5.16)

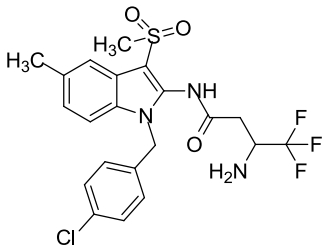


In a microwave vial, were placed $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol), and Cs_2CO_3 (237 mg, 0.7 mmol), the vial was sealed, and evacuated and filled with N_2 several times. In a round bottomed flask were placed 1-methylcyclopropanecarboxamide (36 mg, 0.4 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (4 mL) and degassed. This mixture was added to the microwave vial and the final mixture was degassed for 5 min. The reaction mixture was irradiated in the microwave at 130 ºC for 30 min and, after this time, LCMS Neutral showed that the reaction had reached completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine and dried over Na_2SO_4, filtered, and the solvent was evaporated to give 120 mg of a crude product that was purified on a 4 g silica gel cartridge (Cy/EtOAc 0-60%) to give 50 mg of a brownish solid that contained desired product, dehalogenated by-product, and unknown product by LCMS Neutral. The crude was triturated with MeOH (HPLC quality) and *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1-methylcyclopropanecarboxamide (**5.16**) (10 mg, 10% yield) was filtered as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.65 (s, 1H), 7.63 (br. s., 1H), 7.30-7.44 (m, 3H), 7.21 (d, *J*=8.1 Hz, 2H), 7.08 (d, *J*=8.3 Hz, 1H), 5.29 (br. s., 2H), 3.10 (s, 3H), 2.39 (s, 3H), 1.38 (s, 3H), 1.10 (br. s., 2H), 0.68 ppm (br. s., 2H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.44 min, $[M+H]^+$ 431 (> 90% purity).

3-Amino-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-4,4,4-trifluorobutanamide (5.17)



In a microwave vial were placed $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol), and Cs_2CO_3 (118 mg, 0.4 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of 3-amino-4,4,4-trifluorobutanamide (41.6 mg, 0.3 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (5 mL) was added to the microwave vial and the reaction was degassed and irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral, after this time, showed reaction completion. The reaction mixture was filtered through a celite cartridge, and the cartridge was rinsed with DCM. The solvent was evaporated to give 220 mg of a crude that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-60%) to give 3-amino-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-4,4,4-trifluorobutanamide (**5.17**) (20 mg, 17% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.57 (s, 1H), 7.24-7.35 (m, 3H), 7.12 (d, *J*=8.6 Hz, 2H), 6.96-7.03 (m, 1H), 5.27 (s, 2H), 3.59-3.77 (m, 1H), 3.06 (s, 3H), 2.45-2.67 (m, 2H), 2.31 ppm (s, 3H). NH units not observed in this experiment conditions.

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.2, 135.9, 135.2, 134.3, 133.8, 132.9, 132.0, 129.1, 127.9, 127.0, 125.7, 123.9, 119.0, 110.7, 50.4 (q, *J*=30.7 Hz), 46.9, 44.7, 36.2, 21.6 ppm.

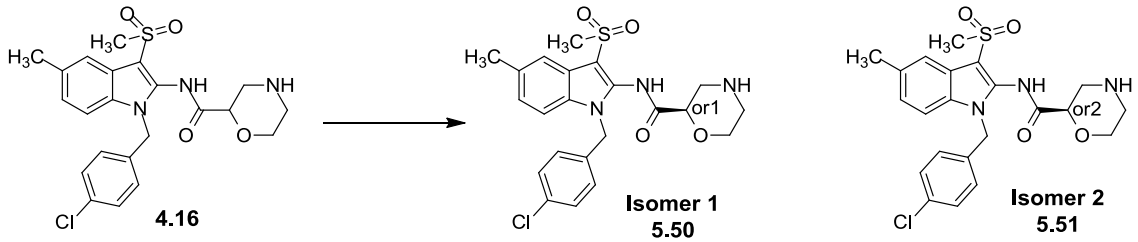
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$0.97 min, $[M+H]^+$ 488 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3258.9, 2920.5, 1692.8, 1300.9, 1106.4, 773.4.

M.P. 214.7 ºC

HRMS (ES) calcd for $C_{21}H_{22}{}^{35}ClF_3N_3O_3S$, $(M + H)^+$ 488.1017 found 488.1026.

Chiral separation of 4.16 enantiomers by supercritical fluid chromatography (SFC)[133]



Racemic compound **4.16** was separated in preparative SFC Waters 100 with CHIRALPAK column: IC 20 x 250 mm; Method: CO_2: Methanol (0.2%DEA) 70:30. 80 mL/min in 30 minutes.

Isomer 1 (5.50)

Retention time in chiral conditions: 4.5 min

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.35-10.49 (m, 1H), 7.63 (s, 1H), 7.36 (d, *J*=8.6 Hz, 2H), 7.32 (d, *J*=8.3 Hz, 1H), 7.18 (d, *J*=8.6 Hz, 2H), 7.07 (d, *J*=8.3 Hz, 1H), 5.30 (s, 2H), 4.02 (dd, *J*=9.6, 2.5 Hz, 1H), 3.89 (d, *J*=11.1 Hz, 1H), 3.50-3.62 (m, 1H), 3.15 (s, 3H), 2.99 (d, *J*=12.9 Hz, 1H), 2.59-2.79 (m, 3H), 2.40 ppm (s, 3H). NH unit is not visible in the spectrum conditions.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.19 min, $[M+H]^+$ 462 (> 95% purity).

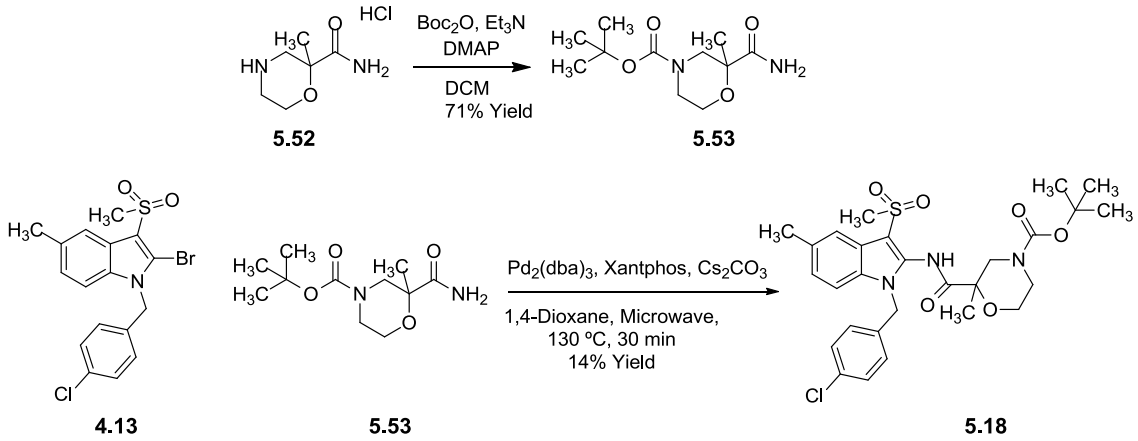
Isomer 2 (5.51)

Retention time in chiral conditions: 7.1 min

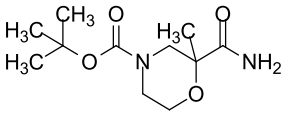
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.35-10.49 (m, 1H), 7.63 (s, 1H), 7.36 (d, *J*=8.6 Hz, 2H), 7.32 (d, *J*=8.3 Hz, 1H), 7.18 (d, *J*=8.6 Hz, 2H), 7.07 (d, *J*=8.3 Hz, 1H), 5.30 (s, 2H), 4.02 (dd, *J*=9.6, 2.5 Hz, 1H), 3.89 (d, *J*=11.1 Hz, 1H), 3.50-3.62 (m, 1H), 3.15 (s,

3H), 2.99 (d, *J*=12.9 Hz, 1H), 2.59-2.79 (m, 3H), 2.40 ppm (s, 3H). NH unit is not visible in the spectrum conditions.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.19 min, $[M+H]^+$ 462 (> 95% purity).



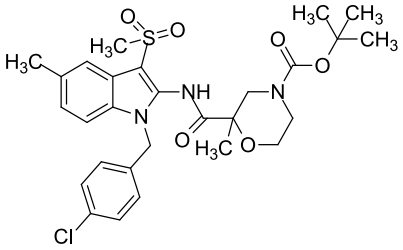
***tert*-Butyl 2-carbamoyl-2-methylmorpholine-4-carboxylate (5.53)**



2-Methylmorpholine-2-carboxamide, 2 hydrochloride (**5.52**) (0.3 g, 1.4 mmol), Et_3N (1 mL, 6.9 mmol) and DMAP (0.03 g, 0.3 mmol) were dissolved in DCM (5 mL) and Boc_2O (0.33 g, 1.5 mmol) was added. The reaction was stirred at room temperature for 16 h. TLC (Cy/EtOAc 1:1) showed the consumption of the starting material and the formation of a new apolar spot. The reaction was washed with a saturated solution of sodium bicarbonate and brine, dried over $MgSO_4$, filtered, and evaporated to give *tert*-butyl 2-carbamoyl-2-methylmorpholine-4-carboxylate (**5.53**) (240 mg, 71% yield) as a white solid.

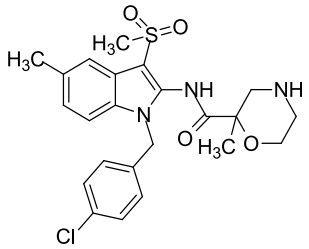
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.15-7.32 (m, 2H), 3.73-3.85 (m, 1H), 3.56-3.72 (m, 2H), 3.35-3.40 (m, 1H), 3.13-3.23 (m, 1H), 3.06-3.11 (m, 1H), 1.39 (s, 9H), 1.22 ppm (s, 3H).

tert-Butyl 2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)carbamoyl)-2-methylmorpholine-4-carboxylate (5.18)



In a microwave vial were placed $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol), and Cs_2CO_3 (118 mg, 0.4 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of *tert*-butyl 2-carbamoyl-2-methylmorpholine-4-carboxylate (**5.53**) (89 mg, 0.4 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (5 mL) was added and the reaction was degassed and then irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral showed reaction completion. The reaction mixture was filtered through a celite cartridge, and the cartridge was rinsed with DCM. The solvent was evaporated to give *tert*-butyl 2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-2-methylmorpholine-4-carboxylate (**5.18**) (20 mg, 14% yield). LCMS Neutral showed the product and small impurities but the product was used in the next step without any further purification.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-methylmorpholine-2-carboxamide (5.31)



tert-Butyl 2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-2-methylmorpholine-4-carboxylate (**5.18**) (15 mg, 0.03 mmol) was dissolved in DCM (3 mL) and TFA (0.1 mL) was added. The reaction was stirred at room temperature for 2 h and, after this time, LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude material that was loaded on a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M

solution of NH_3 in MeOH to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-methylmorpholine-2-carboxamide (**5.31**) (11 mg, 89% yield) as a beige solid.

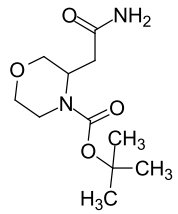
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.70-9.65 (br.s, 1H), 7.78 (s, 1H), 7.25 (d, *J*=8.3 Hz, 2H), 7.10 (s, 2H), 6.96 (d, *J*=8.3 Hz, 2H), 5.23-5.38 (m, 2H), 3.67-3.83 (m, 2H), 3.49 (s, 1H), 3.46 (d, *J*=12.6 Hz, 1H), 3.15 (s, 3H), 2.80-2.93 (m, 2H), 2.75 (d, *J*=12.6 Hz, 1H), 2.47 (s, 3H). 1.41 ppm (s, 3H).

^{13}C NMR ($CDCl_3$, 101 MHz): Low resolution data were obtained.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.20 min, $[M+H]^+$ 476 (> 95% purity).

When the product was recovered from our products storage center, ^{1}HNMR showed an almost complete decomposition so no further studies were possible.

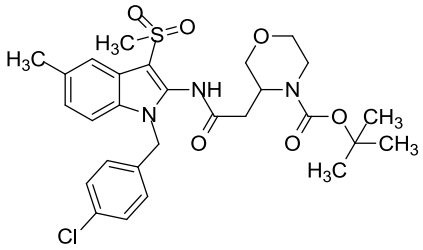
***tert*-Butyl 3-(2-amino-2-oxoethyl)morpholine-4-carboxylate (5.9)**



2-(Morpholin-3-yl)acetic acid, hydrochloride (0.5 g, 2.8 mmol) was dissolved in 1,4-dioxane (13 mL), DIPEA (0.3 mL, 1.7 mmol) was added and the reaction was stirred for 10 min and pyridine (0.7 mL, 8.3 mmol) was added dropwise. Boc_2O (1.5 g, 7 mmol) was added and the reaction mixture was stirred for 15 min. After that time, ammonium bicarbonate (0.43 g, 5.5 mmol) was added and the reaction was stirred for 16 h. After this time, EtOAc and water were added, the phases were separated and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum affording *tert*-butyl 3-(2-amino-2-oxoethyl)morpholine-4-carboxylate (**5.9**) (126 mg, 47% yield) as a yellow oil.

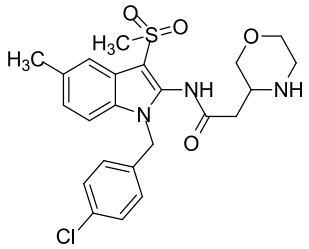
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.28-7.37 (br.s., 1H), 6.74-6.89 (br.s., 1H), 4.14-4.24 (m, 1H), 3.73-3.85 (m, 1H), 3.60-3.73 (m, 1H), 3.57 (s, 2H), 3.43 (br. s., 1H), 3.30 (br. s., 1H), 2.93-3.07 (m, 1H), 2.53-2.66 (m, 1H), 1.39 ppm (s, 9H).

tert-Butyl 3-(2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsufonyl)-1_H_-indol-2-yl)amino)-2-oxoethyl)morpholine-4-carboxylate (5.19)



In a microwave vial were placed $Pd_2(dba)_3$ (17 mg, 0.02 mmol), Xantphos (21 mg, 0.04 mmol), and Cs_2CO_3 (355 mg, 1.1 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of *tert*-butyl 3-(2-amino-2-oxoethyl)morpholine-4-carboxylate (**5.9**) (124 mg, 0.5 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (150 mg, 0.4 mmol) in 1,4-dioxane (5 mL) was added and the reaction was degassed and, irradiated in the microwave at 120 ºC for 30 min. LCMS Neutral showed that the reaction had finished and some desired product was identified. The reaction mixture was filtered thorugh a celite cartridge and solvent was evaporated to give 105 mg of an orange crude that was purified on a 4 g silica gel cartridge (0-60% Cy/EtOAc) to give 20 mg of a crude material that was analyzed by TLC (Cy/EtOAc 0-60%) and a main spot was observed with small impurities. This crude product was used in the next step without any further purification.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-(morpholin-3-yl)acetamide (5.32)



tert-Butyl-3-(2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl)morpholine-4-carboxylate (**5.19**) (20 mg, 0.04 mmol) was dissolved in DCM (3 mL) and TFA (0.2 mL) was added. The reaction was stirred at room temperature for 90 min and, after this time, LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with

NH_3 (7 M in MeOH) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-(morpholin-3-yl)acetamide (**5.32**) (13 mg, 79% yield) as a beige solid.

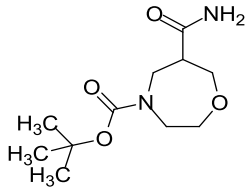
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.56 (s, 1H), 7.25-7.33 (m, 3H), 7.06 (d, *J*=8.3 Hz, 2H), 6.97-7.02 (m, 1H), 5.27 (s, 2H), 3.49-3.64 (m, 3H), 3.06 (s, 3H), 2.97-3.03 (m, 2H), 2.57-2.68 (m, 2H), 2.31 (s, 3H), 2.24-2.27 ppm (m, 2H). The NH units are not visible in these spectrum conditions.

^{13}C NMR (CDCl3, 101 MHz): δ = 171.1, 135.4, 134.4, 133.8, 132.8, 131.9, 131.7, 129.1, 127.9, 125.6, 124.2, 119.3, 110.5, 70.4, 66.4, 51.6, 46.6, 44.2, 44.1, 37.1, 21.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.15 min, $[M+H]^+$ 476 (> 95% purity).

HRMS (ES) calcd for $C_{23}H_{27}{}^{35}ClN_3O_4S$, $(M + H)^+$ 476.1405 found 476.1403.

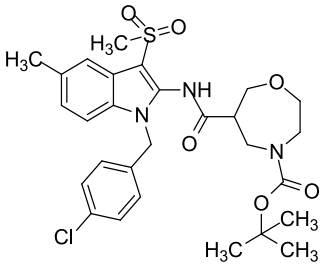
***tert*-Butyl 6-carbamoyl-1,4-oxazepane-4-carboxylate (5.10)**



4-(*tert*-Butoxycarbonyl)-1,4-oxazepane-6-carboxylic acid (0.5 g, 2 mmol) was dissolved in 1,4-dioxane (13 mL) and pyridine (0.08 mL, 1 mmol) was added dropwise. Boc_2O (0.58 g, 2.6 mmol) was added and the reaction was stirred for 15 min. After that time, ammonium bicarbonate (0.161 g, 2 mmol) was added and the stirring continued for 16 h. EtOAc and water were added, the phases were separated and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum affording *tert*-butyl 6-carbamoyl-1,4-oxazepane-4-carboxylate (**5.10**) (300 mg, 60% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.18-7.37 (m, 1H), 6.54-7.05 (m, 1H), 4.03-4.15 (m, 1H), 3.64-3.73 (m, 1H), 3.51-3.64 (m, 1H), 3.48 (s, 1H), 3.31-3.39 (m, 1H), 3.21 (br. s., 1H), 2.83-2.96 (m, 1H), 2.45-2.53 (m, 1H), 2.03-2.20 (m, 1H), 1.30 ppm (s, 9H).

tert-Butyl 6-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate (5.20)

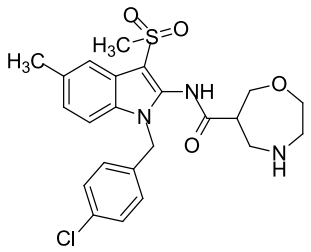


In a microwave vial, were placed $Pd_2(dba)_3$ (9.5 mg, 10.3 µmol), Xantphos (12 mg, 0.02 mmol), and Cs_2CO_3 (201 mg, 0.6 mmol), the vial was sealed, and evacuated and filled with N_2 several times. In a round bottomed flask were placed *tert*-butyl 6-carbamoyl-1,4-oxazepane-4-carboxylate (**5.10**) (75 mg, 0.3 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (4 mL) and degassed. This mixture was added to the microwave vial and the final mixture was degassed again. The reaction mixture was irradiated in the microwave at 130 ºC for 30 min and, after this time, LCMS Neutral showed that the reaction had reached completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine and dried over Na_2SO_4, filtered, and the solvent was evaporated to give a dark green crude product that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-60%) to give *tert*-butyl 6-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate (**5.20**) (38 mg, 32% yield) as a beige solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.56-9.05 (m, 1H), 7.74 (s, 1H), 7.25 (d, *J*=8.6 Hz, 2H), 7.06 (s, 2H), 6.99 (d, *J*=8.3 Hz, 2H), 5.26 (s, 2H), 4.42-4.52 (m, 1H), 3.80 (dd, *J*=11.4, 4.0 Hz, 4H), 3.60 (br. s., 1H), 3.44 (d, *J*=3.0 Hz, 1H), 3.17 (s, 3H), 2.81-3.00 (m, 1H), 2.62-2.82 (m, 1H), 2.46 (s, 3H), 1.43 ppm (s, 9H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.49 min, $[M+H]^+$ 576 (95% purity).

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-1,4-oxazepane-6-carboxamide (5.33)



tert-Butyl 6-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-1,4-oxazepane-4-carboxylate (**5.20**) (35 mg, 0.06 mmol) was dissolved in DCM (3 mL) and TFA (0.2 mL) was added. The reaction was stirred at room temperature for 1 h. LCMS Neutral, after this time, showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give 60 mg of a crude that was loaded in a 1 g SCX cartridge had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of 7 M in MeOH, *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1,4-oxazepane-6-carboxamide (**5.33**) (22 mg, 76% yield) was eluted as free base.

^{1}H NMR (DMSO-d_6 + TFA, 400 MHz): δ = 10.51 (s, 1H), 8.65-9.09 (m, 2H), 7.56 (s, 1H), 7.26 (d, *J*=8.3 Hz, 3H), 7.06 (d, *J*=8.1 Hz, 2H), 6.93-7.03 (m, 1H), 5.28 (s, 2H), 3.74-3.92 (m, 2H), 3.50-3.63 (m, 2H), 3.32-3.42 (m, 1H), 3.09-3.23 (m, 1H), 3.06 (s, 3H), 2.67 (s, 2H), 2.30 ppm (s, 3H).

^{13}C NMR ($CDCl_3$, 101MHz): δ = 171.8, 135.7, 134.5, 134.1, 133.8, 132.7, 132.0, 128.9, 127.8, 125.5, 124.0, 119.3, 110.4, 71.3, 67.5, 50.9, 46.8, 44.7, 44.2, 37.5, 21.6 ppm.

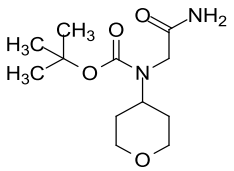
LCMS (Acetate NH_4 20 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.1 min 100: 0; 0.1 – 3.0 min 10:90; 3.0 – 3.5 min 10:90; 3.51 – 4.5 min 100:0; Flow: 1 mL/min): $t_{R=}$3.62 min, $[M+H]^+$ 476 (> 90% purity).

IR ν_{max} (neat) cm^{-1}: 2921.4, 2852.4, 1691.8, 1290.1, 1119.6, 801.7, 762.9.

M.P. 205.3 ºC

HRMS (ES) calcd for $C_{23}H_{27}{}^{35}ClN_3O_4S$, $(M + H)^+$ 476.1411 found 476.1406.

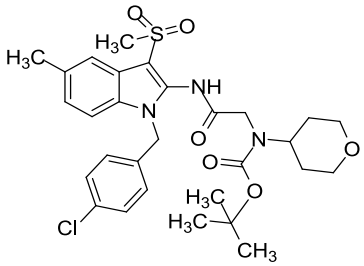
tert-Butyl (2-amino-2-oxoethyl)(tetrahydro-2*H*-pyran-4-yl)carbamate (5.11)



2-((Tetrahydro-2*H*-pyran-4-yl)amino)acetic acid (300 mg, 1.9 mmol) was dissolved in 1,4-dioxane (10 mL) and pyridine (0.08 mL, 0.9 mmol) was added dropwise. Boc_2O (1234 mg, 5.6 mmol) was added, and the reaction was stirred for 15 min. After this time, ammonium bicarbonate (149 mg, 1.9 mmol) was added and reaction was stirred for 16 h. EtOAc and water were added, phases were separated, and organic phase was washed with brine, dried over $MgSO_4$, filtered, and evaporated under vacuum affording *tert*-butyl (2-amino-2-oxoethyl)(tetrahydro-2*H*-pyran-4-yl)carbamate (**5.11**) (450 mg, 92% yield) as a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.12-7.31 (m, 1H), 6.76-7.00 (m, 1H), 3.95-4.15 (m, 2H), 3.79-3.92 (m, 4H), 3.54-3.73 (m, 3H), 3.31 (s, 2H), 1.37 ppm (s, 9H).

tert-Butyl (2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl)(tetrahydro-2*H*-pyran-4-yl)carbamate (5.21)

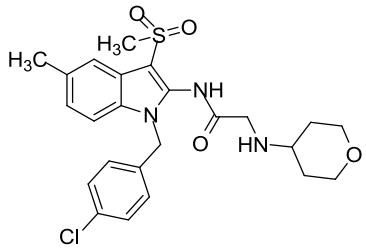


In a microwave vial were placed $Pd_2(dba)_3$ (22 mg, 0.05 mmol), Xantphos (28 mg, 0.05 mmol) and Cs_2CO_3 (237 mg, 0.7 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of *tert*-butyl (2-amino-2-oxoethyl)(tetrahydro-2*H*-pyran-4-yl)carbamate (**5.11**) (225 mg, 0.9 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (200 mg, 0.5 mmol) in 1,4-dioxane (5 mL) was added and the reaction was degassed and then irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral showed reaction completion. The reaction mixture was filtered through a celite cartridge that was rinsed with DCM. The solvent was evaporated to give 200 mg of a crude that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-60%) to give two fractions:

- Fraction 1 : 27 mg of desired product (**5.21**) with small quantities of impurities by LCMS Neutral. This product was used in the next step of the synthesis without any further purification.
- Fraction 2: 100 mg of starting material (**4.13**) by ^{1}H NMR. ^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.68 (s, 1H), 7.47 (d, *J*=8.3 Hz, 1H), 7.27-7.37 (m, 2H), 6.97-7.10 (m, 3H), 5.53 (s, 2H), 3.13 (s, 3H), 2.33 ppm (s, 3H)

The LCMS Neutral at 30 min did not detect the SM and the reaction was stopped before it had reached completion.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-((tetrahydro-2_H_-pyran-4-yl)amino)acetamide (5.34)

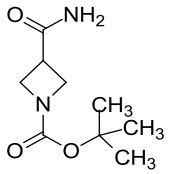


tert-Butyl (2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl)(tetrahydro-2*H*-pyran-4-yl)carbamate (**5.21**) (20 mg, 0.03 mmol) was dissolved in DCM (3 mL) and TFA (0.3 mL) was added. The reaction was stirred at room temperature for 2 h and, after this time, the LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude material that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with NH_3 (7 M in MeOH) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-((tetrahydro-2*H*-pyran-4-yl)amino)acetamide (**5.34**) (5 mg, 30% yield) as a beige solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 9.30-10.43 (m, 1H), 7.77 (s, 1H), 7.27 (m, 2H), 7.08-7.16 (m, 2H), 6.96 (d, *J*=8.3 Hz, 2H), 5.32 (s, 2H), 3.91-3.99 (m, 2H), 3.47 (s, 2H), 3.35 (td, *J*=11.7, 1.9 Hz, 2H), 3.14 (s, 3H), 2.57-2.69 (m, 1H), 2.47 (s, 3H), 1.82 (dd, *J*=12.6, 1.8 Hz, 2H), 1.36 ppm (qd, *J*=11.8, 4.5 Hz, 2H). NH unit is not visible in the spectrum conditions.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.22 min, $[M+H]^+$ 490 (> 95% purity).

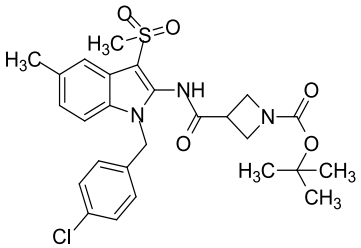
***tert*-Butyl 3-carbamoylazetidine-1-carboxylate (5.12)**



1-(*tert*-Butoxycarbonyl)azetidine-3-carboxylic acid (1 g, 5 mmol) was dissolved in 1,4-dioxane (13 mL), and pyridine (0.2 mL, 2.5 mmol) was added dropwise. Boc_2O (1.2 g, 5.5 mmol) was added, and the reaction was stirred for 15 min. After that time, ammonium bicarbonate (0.4 g, 5 mmol) was added and reaction was stirred for 22 h. EtOAc and water were added, the phases were separated, and the organic phase was washed with a 5% solution of H_2SO_4, dried over anh. Na_2SO_4, filtered, and evaporated under vacuum affording 100 mg of *tert*-butyl 3-carbamoylazetidine-1-carboxylate (**5.12**) (100 mg, 10 % yield) as a beige solid. The aqueous phase was neutralized with a 1 M solution of NaOH and then EtOAc was added, the phases were separated, and the organic layer was dried over $MgSO_4$, filtered, and the solvent was evaporated to give *tert*-butyl 3-carbamoylazetidine-1-carboxylate (**5.12**) (406 mg, 41% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.31-7.58 (m, 1H), 6.90-7.15 (m, 1H), 3.90 (br. s., 4H), 3.21 (s, 1H), 1.37 ppm (s, 9H).

***tert*-Butyl 3-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)azetidine-1-carboxylate (5.22)**



In a microwave vial, were placed $Pd_2(dba)_3$ (9.5 mg, 10.3 µmol), Xantphos (12 mg, 0.02 mmol), and Cs_2CO_3 (201 mg, 0.6 mmol), the vial was sealed, and evacuated and filled with N_2 several times. In a round bottomed flask, were placed *tert*-butyl 3-carbamoylazetidine-1-carboxylate (**5.12**) (62 mg, 0.3 mmol) and 2-bromo-1-(4-

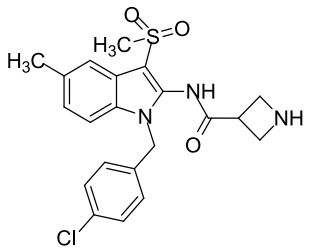
chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (4 mL), and degassed. This mixture was added to the microwave vial and the final mixture was degassed again. The reaction was irradiated in the microwave at 130 ºC for 30 min and, after this time LCMS Neutral showed that the reaction had reached completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine, and dried over Na_2SO_4, filtered, and the solvent was evaporated to give 260 mg of a crude material that was purified on a 12 g silica gel cartridge (Cy/EtOAc 0-60%) to give *tert*-butyl-3-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)azetidine-1-carboxylate (**5.22**) (64 mg, 58% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.30 (s, 1H), 7.56 (s, 1H), 7.20-7.34 (m, 3H), 7.05 (d, *J*=8.6 Hz, 3H), 5.26 (s, 2H), 3.84-3.98 (m, 4H), 3.41-3.54 (m, 1H), 3.06 (s, 3H), 2.32 (s, 3H), 1.30 ppm (s, 9H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.5, 156.1, 135.1, 134.0, 133.8, 133.0, 132.0, 128.9, 127.9, 125.7, 123.6, 118.9, 110.8, 105.0, 80.0, 51.4, 47.1, 44.8, 33.7, 28.3, 21.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=1.47 min, $[M-H]^-$ 530 (> 95% purity).

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)azetidine-3-carboxamide (5.35)



tert-Butyl-3-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)azetidine-1-carboxylate (**5.22**) (58 mg, 0.1 mmol) was dissolved in DCM (3 mL) and TFA (0.3 mL) was added. The reaction mixture was stirred at room temperature for 2 h and, after this time, LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give 70 mg of a crude that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution

of NH_3 in MeOH. *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)azetidine-3-carboxamide (**5.35**) (41 mg, 87% yield) was eluted as free base as a beige oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.54 (s, 1H), 7.27 (d, *J*=8.3 Hz, 2H), 7.22 (d, *J*=8.6 Hz, 1H), 7.07 (d, *J*=8.3 Hz, 2H), 6.96 (d, *J*=7.6 Hz, 1H), 5.22 (s, 2H), 3.65-3.74 (m, 2H), 3.43-3.58 (m, 3H), 2.98-3.11 (m, 3H), 2.30 ppm (s, 3H). The NH units are not visible in the spectrum conditions.

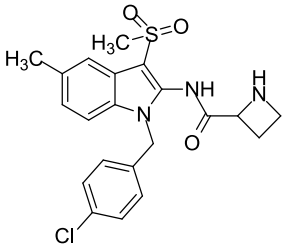
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 161.7, 143.8, 134.3, 133.8, 132.9, 132.1, 129.1, 128.2, 125.6, 123.9, 119.1, 110.7, 66.6, 50.0, 47.2, 44.9, 31.2, 21.5 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.24 min, $[M+H]^+$ 432 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3315.64, 2881.34, 1689.58, 1477.61, 1120.65, 805.16, 760.98.

HRMS (ES) calcd for $C_{21}H_{23}{}^{35}ClN_3O_3S$, $(M + H)^+$ 432.1143 found 432.1135.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)azetidine-2-carboxamide (5.23)



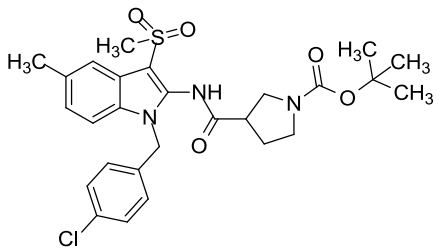
In a microwave vial were placed Cs_2CO_3 (59.2 mg, 0.182 mmol), Xantphos (7.01 mg, 0.012 mmol) and $Pd_2(dba)_3$ (5.55 mg, 6.06 µmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of azetidine-2-carboxamide (13 mg, 0.1 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (50 mg, 0.1 mmol) in 1,4-dioxane (2.5 mL) was added and the reaction was degassed and then irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral just showed starting material. The reaction was irradiated 60 min more at the same temperature and no changes were observed by LCMS Neutral. A second addition of $Pd_2(dba)_3$ (6 mg, 6 µmol) was done and the reaction was heated for 1 h more, after this time no big changes were observed by LCMS Neutral. More azetidine-2-carboxamide (24.3 mg, 0.2 mmol), Cs_2CO_3 (59.2 mg, 0.2 mmol), Xantphos (7 mg, 0.01 mmol), and $Pd_2(dba)_3$ (6 mg, 6 µmol) were added and the reaction was irradiated 30 min more at 130 ºC. LCMS

Neutral after this time showed that the reaction had finished. The reaction mixture was filtered through celite and rinsed with DCM. The solvent was evaporated to give a complex crude that was purified by HPLC_sep (basic pH). Appropiate fractions were evaporated under vacuum to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)azetidine-2-carboxamide (**5.24**) (5.6 mg, 11% yield) as a beige solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.79 (s, 1H), 7.29 (d, *J*=8.3 Hz, 2H), 7.00-7.10 (m, 2H), 6.97 (d, *J*=8.3 Hz, 2H), 6.62 (br. s., 1H), 5.62 (d, *J*=16.7 Hz, 1H), 5.32 (d, *J*=6.3 Hz, 1H), 5.21-5.30 (m, 2H), 3.99-4.09 (m, 2H), 3.23 (s, 3H), 2.64-2.75 (m, 1H), 2.46 (s, 3H), 2.36-2.44 ppm (m, 1H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.23 min, $[M+H]^+$ 432 (> 95% purity).

tert-Butyl 3-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)pyrrolidine-1-carboxylate (5.24)



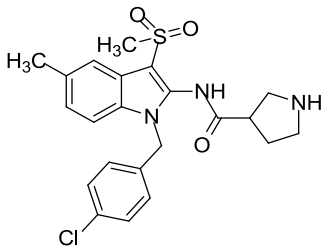
In a microwave vial, were placed $Pd_2(dba)_3$ (14 mg, 0.01 mmol), Xantphos (18 mg, 0.03 mmol), and Cs_2CO_3 (302 mg, 0.9 mmol), the vial was sealed, and evacuated and filled with N_2 several times. In a round bottomed flask were placed 3-carbamoyl-pyrrolidine-1-carboxylic acid *tert*-butyl ester (99 mg, 0.5 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (150 mg, 0.3 mmol) in 1,4-dioxane (4 mL) and degassed. This mixture was added to the microwave vial and the final reaction mixture was degassed again. The reaction was irradiated in the microwave at 130 ºC for 30 min and, after this time, LCMS Neutral showed that the reaction had reached completion. EtOAc and water were added and the phases were separated, the organic layer was washed with brine and dried over Na_2SO_4, filtered, and the solvent was evaporated to give 150 mg of a crude product that was purified on a 12 g silica gel cartridge to give *tert*-butyl 3-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-

indol-2-yl)carbamoyl)pyrrolidine-1-carboxylate (**5.24**) (63 mg, 37% yield) as an off-white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.30 (br. s., 1H), 7.73 (s, 1H), 7.27 (s, 2H), 7.09 (s, 2H), 6.94 (d, *J*=8.3 Hz, 2H), 5.24 (s, 2H), 3.46-3.78 (m, 4H), 3.29-3.43 (m, 1H), 3.04-3.18 (m, 3H), 2.47 (s, 3H), 2.20 (d, *J*=6.1 Hz, 2H), 1.47 ppm (s, 9H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=1.37 min, $[M+H]^+$ 546 (> 90% purity).

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)pyrrolidine-3-carboxamide (5.36)



tert-Butyl 3-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)pyrrolidine-1-carboxylate (**5.24**) (60 mg, 0.1 mmol) was dissolved in DCM (3 mL) and TFA (0.3 mL) was added. The reaction mixture was stirred at room temperature for 1 h, and after this time, HPLC (basic pH) showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give 70 mg of a crude product that was loaded on a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of NH_3 in MeOH. *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)pyrrolidine-3-carboxamide (**5.36**) (35 mg, 71% yield) was eluted as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.55 (s, 1H), 7.22-7.34 (m, 3H), 7.07 (d, *J*=8.3 Hz, 2H), 6.94-7.03 (m, 1H), 5.23 (s, 2H), 3.04 (s, 3H), 2.81-2.95 (m, 3H), 2.71 (d, *J*=17.9 Hz, 2H), 2.31 (s, 3H), 1.80 ppm (d, *J*=6.1 Hz, 2H). NHs units are not visible in the spectrum conditions.

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 176.4, 135.9, 134.5, 133.9, 133.7, 132.7, 132.0, 129.1, 127.8, 125.4, 124.0, 119.2, 110.5, 50.7, 46.9, 45.9, 44.9, 44.6, 29.5, 21.6 ppm.

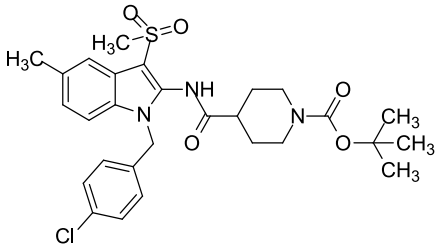
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.11 min, $[M+H]^+$ 446 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3261.34, 2966.51, 1682.63, 1284.26, 1104.17, 962.09, 792.68.

M.P. 216 ºC

HRMS (ES) calcd for $C_{22}H_{25}{}^{35}ClN_3O_3S$, $(M + H)^+$ 446.1300 found 446.1310.

***tert*-Butyl 4-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)piperidine-1-carboxylate (5.25)**

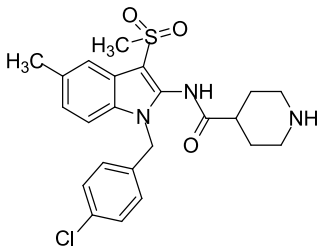


In a microwave vial were placed $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol), and Cs_2CO_3 (237 mg, 0.7 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of *tert*-butyl 4-carbamoylpiperidine-1-carboxylate (55.3 mg, 0.2 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (5 mL) was added and the reaction was degassed and then irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral, after this time, showed that the reaction had reached completion. The impure mixture was filtered through a celite cartridge, and the solvent was evaporated to give 380 mg of an orange crude material that was purified on a 24 g silica gel cartridge (0-60% Cy/EtOAc) to give *tert*-butyl 4-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)piperidine-1-carboxylate (**5.25**) (45 mg, 33% yield) as a pale yellow solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.36 (s, 1H), 7.73 (s, 1H), 7.22-7.26 (m, 2H), 7.01-7.10 (m, 2H), 6.94 (d, *J*=8.3 Hz, 2H), 5.20 (s, 2H), 4.13 (br.s, *J*=7.3 Hz, 2H), 3.12 (s, 3H), 2.65-2.78 (m, 2H), 2.42-2.52 (s, 3H), 1.86 (d, *J*=11.4 Hz, 2H), 1.58-1.73 (m, 3H), 1.45-1.50 ppm (m, 9H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.39 min, $[M+H]^+$ 560 (> 95% purity).

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)piperidine-4-carboxamide (5.37)



tert-Butyl 4-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)piperidine-1-carboxylate (**5.25**) (35 mg, 0.06 mmol) was dissolved in DCM (3 mL) and TFA (0.3 mL) was added. The reaction was stirred at room temperature for 2 h and LCMS Neutral, after this time, showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded in a 1 g SCX cartridge that was previously washed with MeOH. The column was rinsed with MeOH several times and then with NH_3 (7 M in MeOH) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)piperidine-4-carboxamide (**5.37**) (28 mg, 97% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.97-8.84 (m, 1H), 7.73 (s, 1H), 7.24 (d, *J*=8.3 Hz, 2H), 7.06 (s, 2H), 6.95 (d, *J*=8.3 Hz, 2H), 5.23 (s, 2H), 3.15 (br. s., 1H), 3.11 (s, 3H), 2.63 (td, *J*=12.2, 2.1 Hz, 2H), 2.43-2.51 (m, 4H), 1.90 (d, *J*=12.6 Hz, 2H), 1.65 ppm (qd, *J*=12.2, 4.0 Hz, 4H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 175.0, 136.8, 134.3, 133.8, 133.0, 132.1, 129.1, 127.9, 125.5, 123.7, 119.0, 110.6, 104.3, 47.5, 45.8, 44.9, 43.7, 29.5, 21.6 ppm.

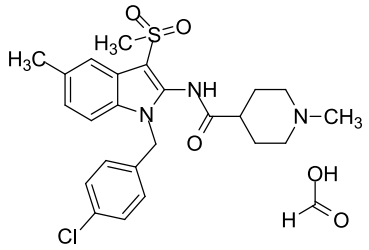
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.07 min, $[M+H]^+$ 460 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3267.1, 2922.0, 1691.4, 1282.6, 1106.4, 797.7, 770.8.

M.P. 224.5 ºC

HRMS (ES) calcd for $C_{23}H_{27}{}^{35}ClN_3O_3S$, $(M + H)^+$ 460.1456 found 460.1468.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-1-methylpiperidine-4-carboxamide formic acid salt (5.26)



In a microwave vial were placed $Pd_2(dba)_3$ (9.5 mg, 10.3 µmol), Xantphos (12 mg, 0.02 mmol), and Cs_2CO_3 (201 mg, 0.6 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of 1-methylpiperidine-4-carboxamide (44 mg, 0.3 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (4 mL) was added and the reaction was degassed and then irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral after this time showed that the reaction had reached completion. The reaction mixture was filtered through a celite cartridge and the solvent was evaporated to give 380 mg of an orange crude product that was purified on a silica gel cartridge (0-60% Cy/EtOAc). None of the fractions corresponded to the desired product. The gradient was increased to 100% EtOAc but no product eluted. A 7 M solution of NH_3 in MeOH was added and 90 mg of impure product was eluted. The crude was purified by HPLC_sep (acid pH) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1-methylpiperidine-4-carboxamide, formic acid salt (**5.26**) (15 mg, 14% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.20 (br. s., 1H), 8.18 (br. s., 1H), 7.65 (s, 1H), 7.31-7.41 (m, 3H), 7.15 (d, *J*=8.3 Hz, 2H), 7.08 (d, *J*=7.8 Hz, 1H), 5.30 (s, 2H), 3.11 (s, 3H), 2.84 (d, *J*=11.1 Hz, 2H), 2.40 (s, 3H), 2.37 (br. s., 1H), 2.19 (s, 3H), 1.96 (t, *J*=11.1 Hz, 2H), 1.81 (d, *J*=11.6 Hz, 2H), 1.63 ppm (d, *J*=9.6 Hz, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 174.9, 137.6, 135.8, 134.1, 133.8, 132.7, 131.9, 129.1, 127.9, 125.6, 123.1, 119.1, 110.6, 60.5, 60.0, 53.5, 47.0, 44.6, 44.5, 21.8 ppm

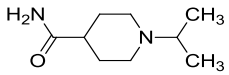
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.09 min, $[M+H]^+$ 474 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3270.5, 2920.8, 2853.6, 1687.3, 1106.0, 796.6.

M.P. 259 ºC

HRMS (ES) calcd for $C_{24}H_{29}{}^{35}ClN_3O_3S$, $(M + H)^+$ 474.1613 found 474.1600.

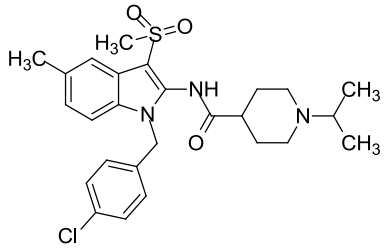
1-*iso*-Propylpiperidine-4-carboxamide (5.13)



1-*iso*-Propylpiperidine-4-carboxylic acid (500 mg, 2.9 mmol) was dissolved in 1,4-dioxane (15 mL) and pyridine (0.1 mL, 1.5 mmol) was added dropwise. Boc_2O (828 mg, 3.8 mmol) was added, and the reaction was stirred for 15 min. After this time, ammonium bicarbonate (231 mg, 2.9 mmol) was added and reaction was stirred 16 h at room temperature. EtOAc and water were added, the phases were separated, and the organic phase was washed with brine, dried over anh. $MgSO_4$, filtered, and evaporated under vacuum affording 80 mg of a yellow oil that did not correspond to the expected product. The aqueous phase was basified with 1 N sodium hydroxide, EtOAc was added and phases were separated in a funnel. The organic phase was washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated to give 1-*iso*-propylpiperidine-4-carboxamide (**5.13**) (300 mg, 60% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.19 (br. s., 1H), 6.68 (br. s., 1H), 2.77 (d, *J*=11.1 Hz, 2H), 2.59-2.72 (m, 1H), 1.93-2.18 (m, 3H), 1.66 (d, *J*=11.4 Hz, 2H), 1.48 (qd, *J*=12.1, 3.4 Hz, 2H), 0.94 ppm (d, *J*=6.6 Hz, 6H).

***N*-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1-*iso*-propylpiperidine-4-carboxamide (5.27)**



In a microwave vial were placed $Pd_2(dba)_3$ (11.09 mg, 0.012 mmol), Xantphos (14.02 mg, 0.024 mmol), and Cs_2CO_3 (103 mg, 0.315 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of 1-isopropylpiperidine-4-carboxamide (**5.13**) (41.3 mg, 0.242 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.242 mmol) in 1,4-dioxane (5 mL) was added, and the reaction was degassed and irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral showed reaction completion. The reaction mixture was filtered through a celite cartridge and rinsed with DCM. The solvent was evaporated to give 200 mg of a crude material that was purified on a 12 g silica gel cartridge to give *N*-(1-(4-

chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1-isopropylpiperidine-4-carboxamide (**5.27**) (27 mg, 22% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.04-10.23 (m, 1H), 7.65 (s, 1H), 7.36 (d, *J*=8.3 Hz, 3H), 7.15 (d, *J*=8.6 Hz, 2H), 7.05-7.10 (m, 1H), 5.29 (s, 2H), 3.11 (s, 3H), 2.75-2.84 (m, 2H), 2.62-2.72 (m, 1H), 2.39 (s, 3H), 2.28-2.37 (m, 1H), 2.08 (s, 2H), 1.76-1.89 (m, 2H), 1.52-1.70 (m, 2H), 0.95 ppm (d, *J*=6.6 Hz, 6H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.4, 141.9, 139.8, 134.4, 132.9, 129.8, 129.1, 127.9, 125.5, 123.6, 122.3, 119.1, 110.6, 48.1, 47.6, 45.1, 38.9, 21.5, 20.4, 19.6, 18.1 ppm.

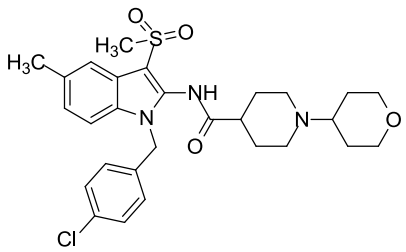
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.12 min, $[M+H]^+$ 502 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3295.4, 2970.9, 1670.7, 1337.8, 1117.6, 793.8, 765.8.

M.P. 190.2 ºC

HRMS (ES) calcd for $C_{26}H_{33}{}^{35}ClN_3O_3S$, $(M + H)^+$ 502.1926 found 502.1933.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1-(tetrahydro-2*H*-pyran-4-yl)piperidine-4-carboxamide (5.28)



In a microwave vial were placed $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol), and Cs_2CO_3 (118 mg, 0.4 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of 1-(tetrahydro-2*H*-pyran-4-yl)piperidine-4-carboxamide, hydrochloride (60.3 mg, 0.2 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (100 mg, 0.2 mmol) in 1,4-dioxane (5 mL) was added and the reaction was degassed and irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral just showed starting material. The reaction was irradiated 30 min more at the same temperature and no changes were observed. The mixture was irradiated 30 min at 150 ºC and no variation was observed. More Cs_2CO_3 (158 mg, 0.5 mmol), $Pd_2(dba)_3$ (11 mg, 0.01 mmol), Xantphos (14 mg, 0.02 mmol) and 1-(tetrahydro-2*H*-pyran-4-yl)piperidine-4-carboxamide, hydrochloride (60.3 mg, 0.2

mmol) were added to the mixture and this was irradiated again at 130 ºC for 30 min. After this time, LCMS Neutral showed reaction completion. The reaction mixture was filtered through celite and the solvent was evaporated under vacuum to give 350 mg of a crude material that was purified by HPLC_sep (basic pH). Appropiate fractions were evaporated together to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-1-(tetrahydro-2*H*-pyran-4-yl)piperidine-4-carboxamide (**5.28**) (22 mg, 17% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.14 (br. s., 1H), 7.65 (s, 1H), 7.36 (d, *J*=8.1 Hz, 3H), 7.15 (d, *J*=7.8 Hz, 2H), 7.08 (d, *J*=7.6 Hz, 1H), 5.30 (br. s., 2H), 3.87 (d, *J*=9.1 Hz, 2H), 3.19-3.30 (m, 2H), 3.11 (s, 2H), 2.90 (br. s., 3H), 2.40 (br. s., 5H), 2.12 (br. s., 2H), 1.82 (d, *J*=10.9 Hz, 2H), 1.52-1.73 (m, 4H), 1.41 ppm (d, *J*=8.6 Hz, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 175.4, 143.2, 136.2, 134.3, 133.7, 132.8, 132.1, 129.1, 128.0, 125.4, 123.8, 119.1, 110.5, 71.6, 67.6, 61.2, 48.4, 47.4, 44.8, 29.3, 28.7, 21.6 ppm.

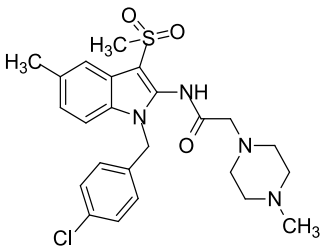
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.14 min, $[M+H]^+$ 544 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3262.57, 2966.96, 2919.69, 1680.98, 1287.32, 1092.18, 984.66, 836.42.

M.P. 236 ºC

HRMS (ES) calcd for $C_{28}H_{35}{}^{35}ClN_3O_4S$, $(M + H)^+$ 544.2031 found 544.2043.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (5.29)



In a microwave vial were placed $Pd_2(dba)_3$ (17 mg, 0.02 mmol), Xantphos (21 mg, 0.04 mmol), and Cs_2CO_3 (178 mg, 0.5 mmol), the vial was sealed, and evacuated and filled with N_2 several times. A solution of 2-(4-methylpiperazin-1-yl)acetamide (63 mg, 0.4 mmol) and 2-bromo-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indole (**4.13**) (150 mg, 0.4 mmol) in 1,4-dioxane (5 mL) was added, and the reaction was degassed

and irradiated in the microwave at 130 ºC for 30 min. LCMS Neutral after this time showed reaction completion. The reaction mixture was filtered through a celite cartridge and rinsed with DCM. The solvent was evaporated to give 320 mg of a crude material that was purified by HPLC_sep (basic pH) to obtain *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-(4-methylpiperazin-1-yl)acetamide (**5.29**) (22 mg, 12% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.79-10.04 (m, 1H), 7.56 (s, 1H), 7.23-7.33 (m, 3H), 7.05 (d, *J*=8.3 Hz, 2H), 7.00 (d, *J*=8.3 Hz, 1H), 5.26 (s, 2H), 3.07 (s, 3H), 3.05 (s, 2H), 2.40 (br. s., 4H), 2.31 (s, 3H), 2.27 (br. s., 4H), 2.08 ppm (s, 3H).

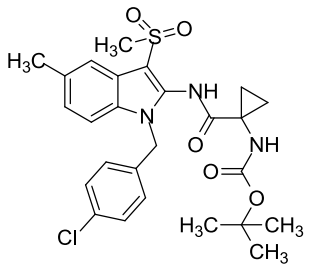
^{13}C NMR ($CDCl_3$, 101 MHz): δ = 170.8, 135.5, 134.4, 133.8, 132.8, 132.3, 129.1, 127.7, 125.5, 124.0, 119.2, 110.4, 105.0, 61.5, 54.9, 53.5, 47.4, 45.9, 45.0, 21.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.17 min, $[M+H]^+$ 489 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3306.8, 2941.6, 2793.8, 1705.7, 1293.9, 1117.5, 959.1, 758.3.

HRMS (ES) calcd for $C_{24}H_{30}{}^{35}ClN_4O_3S$, $(M + H)^+$ 489.1722 found 489.1703.

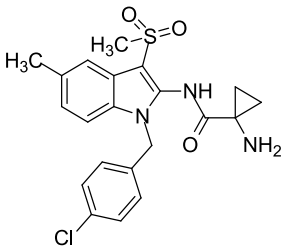
tert-Butyl (1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)cyclopropyl)carbamate (5.40)



To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-amine (**4.12**) (70 mg, 0.2 mmol) in DMF (4 mL) under N_2 atmosphere and cooled at 0 ˚C, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a solution of 1-[(*tert*-butoxycarbonyl)amino]cyclopropanecarboxylic acid (121 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in anhydrous DMF (1.5 mL) which was stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 6 h. After this time, LCMS Neutral, showed SM remaining. The reaction mixture was stirred for a further 15 h and LCMS Neutral did not show any progress. The reaction mixture was cooled to 0 ºC in an ice-bath and more NaH (60%

dispersion in mineral oil) (12.04 mg, 0.301 mmol) was added. A solution of 1-[(*tert*-butoxycarbonyl)amino]cyclopropanecarboxylic acid (121 mg, 0.602 mmol) and CDI (98 mg, 0.602 mmol) were dissolved in 1.5 mL of DMF and stirred for 1 h, after this time, the suspension was added to the reaction mixture and the reaction was allowed to reach room temperature. After 4 h LCMS Neutral showed that the reaction had progressed but it was not complete so it was stirred further at room temperature. LCMS Neutral after this time (16 h) did not show any progress. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in a funnel. The organic phase was washed again with sat. NH_4Cl solution, brine, and dried over $MgSO_4$, filtered and evaporated under vacuum to give a yellowish oil that was purified using a 12 g silica gel cartridge (Cy/EtOAc 0-60%) to give *tert*-butyl (1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)cyclopropyl)carbamate (**5.40**) (73 mg, 51% yield) as a beige solid. LCMS Neutral showed the product and starting material (3:1) but the product was used in the following step without any further purification.

1-Amino-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)cyclopropanecarboxamide (5.46)



tert-Butyl (1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)cyclopropyl)carbamate (**5.40**) (70 mg, 0.09 mmol) was dissolved in DCM (3 mL) and TFA (0.4 mL) was added. The reaction was stirred at room temperature for 2 h and, after this time, the LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded in a 1 g SCX cartridge previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of NH_3 in MeOH to give 1-amino-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)cyclopropanecarboxamide (**5.46**) (14 mg, 35% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.63 (s, 1H), 7.29-7.40 (m, 3H), 7.20 (d, *J*=8.3 Hz, 2H), 7.07 (dd, *J*=8.6, 1.3 Hz, 1H), 5.35 (s, 2H), 5.11-5.27 (m, 2H), 3.13 (s, 3H), 2.40 (s,

3H), 1.17 (q, *J*=3.3 Hz, 2H), 0.93 ppm (q, *J*=3.3 Hz, 2H). NH unit is not visible in the spectrum conditions.

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 176.1, 136.9, 134.6, 133.7, 132.7, 132.1, 129.0, 127.9, 125.3, 124.1, 119.0, 110.7, 100.0, 47.5, 44.9, 36.1, 21.7, 20.8 ppm.

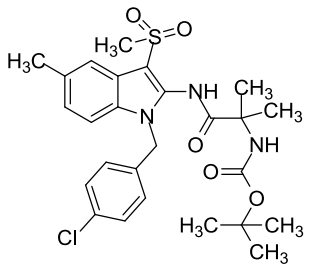
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.28 min, $[M+H]^+$ 432 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3327.1, 2925.7, 1698.0, 1550.7, 1115.0, 797.8, 772.7.

M.P. 233 ºC

HRMS (ES) calcd for $C_{21}H_{23}{}^{35}ClN_3O_3S$, $(M + H)^+$ 432.1143 found 432.1146.

***tert*-Butyl (1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (5.41)**

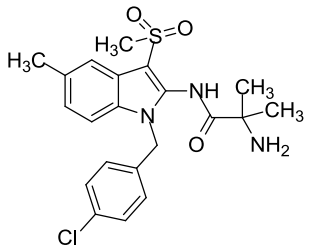


To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-amine (**4.12**) (70 mg, 0.2 mmol) in DMF (4 mL) under N_2 atmosphere and cooled at 0˚C, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a mixture of 2-((*tert*-butoxycarbonyl)amino)-2-methylpropanoic acid (122 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) previously dissolved in anhydrous DMF (1.5 mL) and stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 68 h. LCMS Neutral, after this time, showed that the reaction had reached completion. EtOAc was added and the mixture was washed with a saturated solution of NH_4Cl (x 2), and brine, and dried over $MgSO_4$, filtered, and evaporated under vacuum to give a yellowish oil that was purified in a 4 g silica gel cartridge (Cy/EtOAc 0-60%) to give *tert*-butyl (1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (**5.41**) (73 mg, 68% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.97-10.17 (m, 1H), 7.60-7.65 (m, 1H), 7.28 (d, *J*=18.4 Hz, 5H), 7.08-7.15 (m, 1H), 6.96-7.05 (m, 1H), 5.35 (s, 2H), 3.12 (s, 3H), 2.36 (s, 3H), 1.41 (s, 6H), 1.20 ppm (s, 9H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.39 min, $[M+H]^+$ 534 (> 95% purity).

2-Amino-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-methylpropanamide (5.47)



tert-Butyl (1-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-methyl-1-oxopropan-2-yl)carbamate (**5.41**) (63 mg, 0.1 mmol) was dissolved in DCM (3 mL) and TFA (0.4 mL) was added. The reaction was stirred at room temperature for 2 h and kept cold in the fridge overnight. Solvent and TFA were evaporated under vacuum to give a crude material that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of NH_3 in MeOH to give 2-amino-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-methylpropanamide (**5.47**) (50 mg, 98% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.59 (s, 1H), 7.31-7.38 (m, 2H), 7.17-7.29 (m, 3H), 6.98 (dd, *J*=8.5, 1.1 Hz, 1H), 5.76 (s, 2H), 5.28 (s, 2H), 3.13 (s, 3H), 2.37 (s, 3H), 1.32 ppm (s, 6H). NH unit not visible in the spectrum conditions.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 177.4, 141.6, 136.7, 132.4, 131.5, 130.8, 129.5, 128.8, 124.8, 123.9, 118.7, 110.8, 104.6, 55.9, 45.4, 43.9, 27.6, 21.7 ppm.

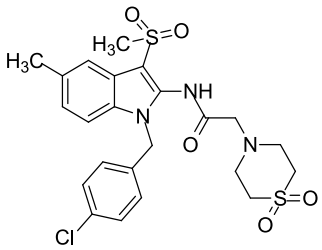
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.26 min, $[M+H]^+$ 434 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3250.8, 2697.9, 1697.3, 1493.8, 1436.3, 1119.5, 933.3, 792.1.

M.P. 153 ºC

HRMS (ES) calcd for $C_{21}H_{25}{}^{35}ClN_3O_3S$, $(M + H)^+$ 434.1305 found 434.1312.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-(1,1-dioxidothiomorpholino)acetamide (5.42)



To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-amine (**4.12**) (70 mg, 0.2 mmol) in DMF (4 mL) under N_2 atmosphere and cooled at 0 ˚C, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a mixture of 2-(1,1-dioxidothiomorpholino)acetic acid (116 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol), previously dissolved in anhydrous DMF (1.5 mL) and stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 6 h. After this time, LCMS Neutral showed SM remaining so it was stirred 15 h more and LCMS Neutral did not show any progress. The reaction mixture was cooled down to 0 ºC in an ice-bath and more NaH (60% dispersion in mineral oil) (12 mg, 0.301 mmol) was added. A solution of 2-(1,1-dioxidothiomorpholino)acetic acid (116 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in 1.5 mL of DMF and stirred for 1 h, was added to the reaction mixture and the reaction mixture was allowed to reach room temperature. LCMS Neutral, after 4 h, showed that the reaction was almost complete. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over $MgSO_4$, filtered, and evaporated under vacuum to give a beige solid that showed a little impurity by ^{1}HNMR (DMSO-d_6). This product was triturated with MeOH (HPLC quality) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-(1,1-dioxidothiomorpholino)acetamide (**5.42**) (35 mg, 33% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.11 (br. s., 1H), 7.56 (br. s., 1H), 7.28 (d, *J*=7.6 Hz, 3H), 7.10 (d, *J*=8.1 Hz, 2H), 7.00 (d, *J*=8.1 Hz, 1H), 5.26 (br. s., 2H), 3.31 (br. s., 2H), 3.11 (br. s., 4H), 3.07 (br. s., 3H), 2.99 (br. s., 4H), 2.32 ppm (s, 3H)

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.1, 135.3, 134.3, 133.8, 133.0, 132.2, 129.1, 127.8, 125.7, 123.7, 118.9, 110.7, 104.1, 60.8, 52.1, 51.3, 47.7, 45.2, 21.6 ppm.

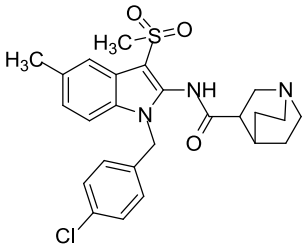
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.24 min, $[M+H]^+$ 524 , (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 3239.5, 2922.5, 2854.1, 1681.9, 1314.5, 1292.7, 1121.4, 951.1.

M.P. 276 ºC

HRMS (ES) calcd for $C_{23}H_{26}{}^{35}ClF_3N_3NaO_5S_2$, $(M + Na)^+$ 546.0895 found 546.0896.

***N*-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)quinuclidine-3-carboxamide (5.43)**



To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-amine (**4.12**) (70 mg, 0.2 mmol) in DMF (4 mL) under N_2 atmosphere and cooled at 0 ˚C, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a mixture of quinuclidine-3-carboxylic acid (93 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in anhydrous DMF (1.5 mL) and which was stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 6 h. After this time, LCMS Neutral showed SM, so it was stirred for a further 15 h and LCMS Neutral did not show any progress. The reaction mixture was cooled to 0 ºC in an ice-bath and more NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. A solution of quinuclidine-3-carboxylic acid (93 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in 1.5 mL of DMF previously stirred for 1 h was added to the reaction mixture and the reaction was allowed to reach room temperature. LCMS Neutral after 4 h showed that the reaction had progressed but it was not complete by LCMS Neutral. The mixture was stirred overnight at room temperature. No progress was observed by LCMS Neutral so the reaction was stopped. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over $MgSO_4$, filtered, and evaporated under vacuum to give a yellowish solid that was triturated with ACN (HPLC quality). A white solid precipitated and this was

filtered and dried under vacuum to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)quinuclidine-3-carboxamide (**5.43**) (55 mg, 56% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.23-10.47 (m, 1H), 7.56 (s, 1H), 7.26 (d, *J*=8.1 Hz, 3H), 7.06 (d, *J*=7.8 Hz, 2H), 6.98 (d, *J*=8.1 Hz, 1H), 5.26 (br. s., 2H), 3.06 (s, 3H), 2.94-3.03 (m, 1H), 2.74 (br. s., 1H), 2.60 (br. s., 4H), 2.31 (s, 3H), 2.06 (br. s., 1H), 1.46 (br. s., 3H), 1.14 ppm (br. s., 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 175.6, 136.3, 134.5, 134.3, 133.7, 132.9, 132.1, 129.0, 127.6, 125.5, 123.8, 118.7, 110.6, 49.0, 47.4, 47.1, 46.9, 45.1, 43.1, 26.6, 25.3, 22.0, 21.5 ppm.

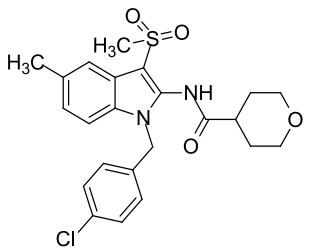
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.14 min, $[M+H]^+$ 486 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2983.5, 1689.2, 1293.1, 1119.4, 767.5.

M.P. 150.2 ºC

HRMS (ES) calcd for $C_{25}H_{29}{}^{35}ClN_3O_3S$, $(M + H)^+$ 486.1613 found 486.1627.

_N_-(1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1_H_-indol-2-yl)tetrahydro-2_H_-pyran-4-carboxamide (5.44)



To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-amine (**4.12**) (70 mg, 0.2 mmol) in DMF (4 mL) under N_2 atmosphere and cooled at 0 ˚C, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a mixture of tetrahydro-2*H*-pyran-4-carboxylic acid (78 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in anhydrous DMF (1.5 mL) and which was stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 68 h. After this time, LCMS Neutral showed that the reaction had reached completion. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in a extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over $MgSO_4$, filtered, and evaporated under vacuum to give a yellowish oil that was purified using a 4 g silica gel

cartridge (Cy/EtOAc 0-60%) to give *N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)tetrahydro-2*H*-pyran-4-carboxamide (**5.44**) (40 mg, 43% yield) as a yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.02-10.31 (m, 1H), 7.49-7.64 (m, 1H), 7.28 (d, *J*=8.3 Hz, 3H), 7.08 (s, 2H), 6.96-7.03 (m, 1H), 5.22 (s, 2H), 3.75-3.85 (m, 2H), 3.26-3.32 (m, 2H), 3.03 (s, 3H), 2.52-2.62 (m, 1H), 2.31 (s, 3H), 1.63-1.72 (m, 2H), 1.48-1.61 ppm (m, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 175.4, 136.0, 132.5, 131.6, 131.5, 129.2, 128.9, 125.2, 123.9, 119.1, 111.5, 107.7, 100.0, 66.6, 45.5, 44.1, 41.1, 28.9, 21.6 ppm.

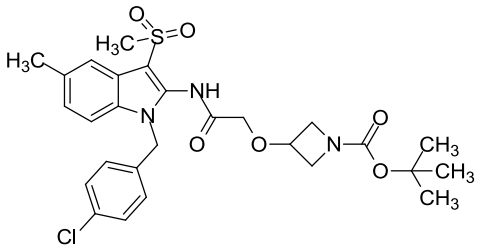
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.27 min, $[M+H]^+$ 461 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2841.6, 1689.5, 1107.0, 797.8, 769.8.

M.P. 275.7 ºC

HRMS (ES) calcd for $C_{23}H_{25}{}^{35}ClN_2NaO_4S$, $(M + Na)^+$ 483.1160 found 483.1190.

***tert*-Butyl 3-(2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethoxy)azetidine-1-carboxylate (5.45)**



To a solution of 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-amine (**4.12**) (70 mg, 0.2 mmol) in DMF (4 mL) under N_2 atmosphere and cooled at 0 ˚C, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a mixture of 2-((1-(*tert*-butoxycarbonyl)azetidin-3-yl)oxy)acetic acid (139 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in anhydrous DMF (1.5 mL) and previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 20 h. After this time, LCMS Neutral showed SM remaining. NaH (60% dispersion in mineral oil) (12.04 mg, 0.301 mmol) was added to the reaction mixture. A solution of 2-((1-(*tert*-butoxycarbonyl)azetidin-3-yl)oxy)acetic acid (139 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in 1.5 mL of DMF and which was stirred for 1 h, was added to the reaction mixture and the reaction was allowed to reach room

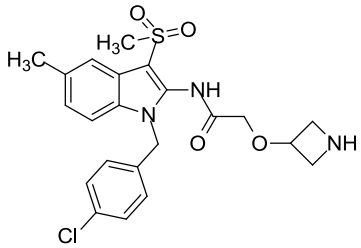
temperature. LCMS Neutral, after 4 h, showed that the reaction had progressed but it was not complete so it was stirred overnight at room temperature. LCMS Neutral after this time did not show any progress. The reaction was cooled to 0 ºC in an ice-bath and NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added to the reaction mixture. A solution of 2-((1-(*tert*-butoxycarbonyl)azetidin-3-yl)oxy)acetic acid (139 mg, 0.6 mmol) and CDI (98 mg, 0.6 mmol) in 1.5 mL of DMF and which was stirred for 1 h, was added to the reaction mixture and the reaction was allowed to reach room temperature. LCMS Neutral, after 4 h, showed that the reaction had almost reached completion. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over $MgSO_4$, filtered, and evaporated under vacuum to give a yellowish oil that was purified using a 4 g silica gel cartridge (Cy/EtOAc 0-60%) to give *tert*-butyl 3-(2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethoxy)azetidine-1-carboxylate (**5.45**) (60 mg, 53% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.14 (s, 1H), 7.56 (s, 1H), 7.23-7.31 (m, 3H), 7.10 (d, *J*=8.6 Hz, 2H), 7.01 (dd, *J*=8.6, 1.3 Hz, 1H), 5.26 (s, 2H), 4.25-4.36 (m, 1H), 4.03 (s, 2H), 3.93 (br. s., 2H), 3.71 (br. s., 2H), 3.06 (s, 3H), 2.31 (s, 3H), 1.29 ppm (s, 9H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 170.9, 156.0, 148.4, 135.9, 132.5, 131.7, 131.5, 129.4, 128.9, 125.2, 123.9, 122.0, 119.3, 111.3, 79.1, 68.7, 67.9, 45.6, 44.0, 28.4, 26.7, 21.5 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.35 min, $[M+H]^+$ 562 (> 95% purity).

2-(Azetidin-3-yloxy)-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)acetamide (5.48)



tert-Butyl 3-(2-((1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethoxy)azetidine-1-carboxylate (**5.45**) (48 mg, 0.09 mmol) was dissolved in DCM (3 mL) and TFA (0.3 mL) was added. The reaction was stirred at room temperature for 2 h and, after this time, the LCMS Neutral showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of NH_3 in MeOH to give 48 mg of a white solid that corresponded to desired product with small impurities by ^{1}H NMR (DMSO-d_6). This product was purified by HPLC_sep (basic pH) to give 2-(azetidin-3-yloxy)-*N*-(1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-1*H*-indol-2-yl)acetamide (**5.48**) (15 mg, 38% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.73 (s, 1H), 7.30-7.43 (m, 3H), 7.20 (td, *J*=13.1, 8.6 Hz, 3H), 5.19-5.50 (m, 2H), 4.19-4.47 (m, 2H), 3.85-4.02 (m, 1H), 3.70-3.79 (m, 1H), 3.64 (d, *J*=11.4 Hz, 1H), 3.46 (d, *J*=10.4 Hz, 1H), 3.35 (d, *J*=11.4 Hz, 1H), 3.12 (s, 3H), 2.61-2.78 (m, 2H), 2.45 ppm (s, 3H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 169.0, 138.3, 134.1, 133.8, 133.0, 131.9, 129.4, 128.0, 126.4, 124.0, 119.7, 110.8, 107.6, 68.4, 54.4, 46.4, 44.7, 43.6, 21.6 ppm.

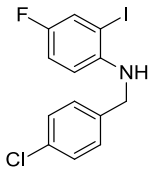
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.13 min, $[M+H]^+$ 462 (> 95% purity).

IR ν_{max} (neat) cm^{-1}: 2920.9, 1677.5, 1293.2, 1118.2, 804.5, 767.8, 747.9.

HRMS (ES) calcd for $C_{22}H_{25}{}^{35}ClN_3O_4S$, $(M + H)^+$ 462.1249 found 462.1257.

9.3.2. Synthesis of GSK3531659A (5.53)

N-(4-Chlorobenzyl)-4-fluoro-2-iodoaniline (5.56)



To a solution of 4-fluoro-2-iodoaniline (**5.55**) (5 g, 21.1 mmol) in isopropyl alcohol (92 mL), *p*-chlorobenzaldehyde (**5.54**) (5.93g, 42.2 mmol) and acetic acid (2.4 mL, 42.2 mmol) were added and the reaction was stirred at room temperature for 22 h. Sodium borohydride (1.6 g, 42.2 mmol) was added and the reaction was stirred at room temperature. Reaction was monitorised by HPLC (basic pH), and after 24 h the reaction had reached completion. Water was added to the reaction mixture and 1 N NaOH was added to adjust pH~8, the organic solvent was extracted with EtOAc, washed with water and brine, dried over $MgSO_4$, filtered, and concentrated to obtain an orange oil. The crude material (22 g) was purified on 3 silica gel cartridges (3 x 125 g) and was eluted with Cy/EtOAc (5-30%). Collected fractions were evaporated under reduced pressure to give *N*-(4-chlorobenzyl)-4-fluoro-2-iodoaniline (**5.56**) (4.36 g, 57% yield) of a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.53 (dd, *J*=8.1, 3.0 Hz, 1H), 7.35 (q, *J*=8.6 Hz, 4H), 6.98 (td, *J*=8.7, 2.9 Hz, 1H), 6.36 (dd, *J*=9.1, 5.1 Hz, 1H), 5.54 (t, *J*=6.1 Hz, 1H), 4.37 ppm (d, *J*=6.1 Hz, 2H).

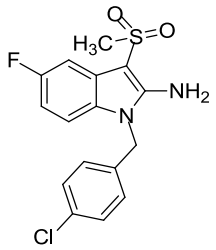
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 155.2, 153.0, 144.7, 139.1, 131.7, 129.0 (d, *J*=38 Hz), 125.5 (d, *J*=24 Hz), 116.0 (d, *J*=21 Hz), 111.3 (d, *J*=8 Hz), 84.0, 46.8 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.49 min, $[M+H]^+$ 362 (>95% purity).

This reaction was repeated in a bigger scale, using the same procedure and the amounts depicted below to obtain *N*-(4-chlorobenzyl)-4-fluoro-2-iodoaniline (**5.56**) (9.6 g, 63% yield) as a yellow oil:

- 4-Chlorobenzaldehyde (11.9 g mL, 84 mmol).
- 4-Fluoro-2-iodoaniline (10 g, 42.2 mmol) in IPA (183 mL)
- Acetic acid (4.8 mL, 84 mmol)
- Sodium borohydride (3.2 g, 84 mmol)

1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (5.59)



N-(4-Chlorobenzyl)-4-fluoro-2-iodoaniline (**5.56**) (4.36 g, 12.1 mmol), *trans*-4-hydroxy-L-proline (**5.58**) (0.32 g, 2.4 mmol), CuI (0.23 g, 1.2 mmol), and K_2CO_3 (5 g, 36.2 mmol) were placed in a 100 mL round bottomed flask containing a magnetic stirring bar. The flask was evacuated and backfilled with Ar for three times, then the flask was capped with a septum under a stream of argon. 2-(Methylsulfonyl)acetonitrile (**5.57**) (2.16 g, 18.1 mmol) dissolved in DMF (30 mL) was injected via a syringe, the resulting mixture was divided in two oven dried 20 mL microwave flasks, sealed, kept under Ar and heated at 90 ºC. LCMS Neutral after 3 h showed starting material remaining in both vials, so the reactions were heated for 16 h more and no progress was observed by LCMS Neutral. The heating was stopped, and when the temperature reached room temperature, EtOAc was added and a solid precipitated. The solid was filtered and the mother liquors were washed twice with a saturated solution of NH_4Cl, then with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated to give a brown solid that was purified on silica gel cartridge to give 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (1.3 g, 30% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.40 (d, *J*=8.3 Hz, 2H), 7.12-7.19 (m, 4H), 6.83 (s, 2H), 6.77 (td, *J*=9.1, 2.5 Hz, 1H), 5.37 (s, 2H), 3.08 ppm (s, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.1, 157.7, 150.0, 135.9, 132.3, 129.5, 129.1, 129.0, 126.7, 110.4, 107.1, 102.4, 44.8, 44.4 ppm.

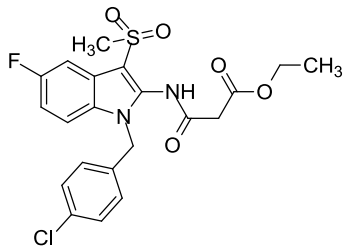
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.22 min, $[M+H]^+$ 353 (>95% purity).

A second fraction was isolated corresponding to SM: *N*-(4-chlorobenzyl)-4-fluoro-2-iodoaniline (**5.56**) (0.8 g).

This reaction was repeated in a similar scale, using the same procedure and the amounts depicted below to obtain 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (1.3 g, 27% yield) as a beige solid:

- *N*-(4-chlorobenzyl)-4-fluoro-2-iodoaniline (**5.56**) (5 g, 13.8 mmol)
- 2-(methylsulfonyl)acetonitrile (2.47 g, 20.7 mmol)
- *Trans*-4-hydroxy-*L*-proline (0.36 g, 2.8 mmol)
- CuI (0.26 g, 1.4 mmol)
- Potassium carbonate (5.7 g, 41.5 mmol)

Ethyl 3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-3-oxopropanoate (5.61)



(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (1.26 g, 3.57 mmol) was dissolved in DCM (36 mL) and pyridine (0.3 mL) was added, the mixture was stirred for 20 min and ethyl malonyl chloride (**5.60**) (0.5 mL, 3.9 mmol) was added after that time. The reaction mixture was stirred for 2 h and LCMS Neutral after that time showed a little of SM. Ethyl malonyl chloride (0.09 mL) was added and the reaction was stirred for overnight. LCMS Neutral after that time showed reaction completion. The solvent was evaporated under vacuum and the resulting crude material was purified on a silica gel cartridge to give a product that corresponded to desired product but contained small impurities. This was triturated with DCM to give ethyl 3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-3-oxopropanoate (**5.61**) (625 mg, 37% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.76 (s, 1H), 7.57 (d, *J*=9.3 Hz, 1H), 7.51 (dd, *J*=8.8, 4.0 Hz, 1H), 7.39 (d, *J*=8.3 Hz, 2H), 7.22 (d, *J*=8.1 Hz, 2H), 7.12-7.19 (m, 1H), 5.41 (br. s., 2H), 4.10 (q, *J*=7.0 Hz, 2H), 3.56 (s, 2H), 3.18 (s, 3H), 1.15 ppm (t, *J*=7.1 Hz, 3H).

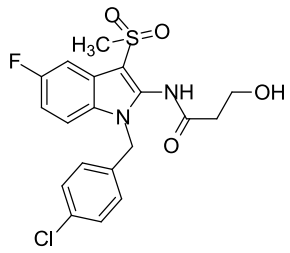
^{13}C NMR (DMSO-d_6, 101 MHz): δ = 167.6, 166.9, 160.0, 157.6, 136.9, 135.6, 132.6, 129.9, 129.5, 129.1, 124.3, 113.7, 112.4, 105.1, 61.4, 45.8, 44.3, 43.0, 14.4 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.23 min, $[M+H]^+$ 467 (>95% purity).

This reaction was repeated in a similar scale, using the same procedure and the amounts depicted below to obtain ethyl 3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-3-oxopropanoate (**5.61**) (592 mg, 35% yield) as a beige solid:

- 1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (1.3 g, 3.7 mmol)
- Ethyl malonyl chloride (0.37 + 0.09 mL)
- Pyridine (0.3 mL)

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-3-hydroxypropanamide (5.53)



To a previously cooled to 0 ºC solution of ethyl 3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-3-oxopropanoate (**5.61**) (625 mg, 1.4 mmol) in toluene (15 mL), was added $LiBH_4$ (43.7 mg, 2 mmol), and the reaction was stirred at 0 ºC for 2 h. LCMS Neutral after this time showed mainly starting material and some desired product. $LiBH_4$ (73 mg, 3.3 mmol) was added and the reaction was stirred at 4 ºC over 1 h. LCMS Neutral showed some progression but there was still some starting material remaining. The reaction was stirred between 0-4 ºC in the fridge (using an IKA® topolino stirrer) for 16 h. LCMS Neutral, after that time showed an appreciable progression, but there was starting material remaining. $LiBH_4$ (43.7 mg, 2 mmol) was added and the reaction mixture was stirred in the fridge between 0-4 ºC for 19 h. After that time the reaction was stopped by adding 2 N HCl aqueous solution. EtOAc was added, phases were separated in a funnel, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give a crude material that was purified by HPLC_sep (basic pH) to give *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-3-hydroxypropanamide (**5.53**) (190 mg, 33% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.47-10.94 (m, 1H), 7.49-7.59 (m, 2H), 7.38 (d, *J*=8.3 Hz, 2H), 7.23 (d, *J*=8.3 Hz, 2H), 7.15 (td, *J*=9.2, 2.5 Hz, 1H), 5.37 (s, 2H), 4.60-4.88 (m, 1H), 3.72 (t, *J*=6.1 Hz, 2H), 3.17 (s, 3H), 2.55 ppm (t, *J*=6.4 Hz, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 172.6, 160.0, 157.6, 137.8, 135.7, 132.8, 129.8, 129.6, 129.1, 113.6, 112.1, 105.0, 57.8, 45.8, 44.4, 40.3, 39.7 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.12 min, $[M+H]^+$ 425 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3283.93, 1639.38, 1478.44, 1126.07, 797.54.

M.P. 186.4 ºC

HRMS (ES) calcd for $C_{19}H_{18}{}^{35}ClFN_2NaO_4S$, $(M + Na)^+$ 447.0552 found 447.0545.

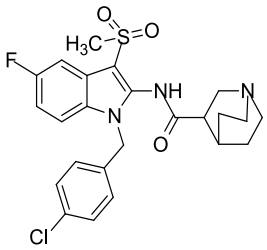
This reaction was repeated in a similar scale, using the same procedure and the amounts depicted below to obtain *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-3-hydroxypropanamide (**5.53**) (310 mg, 58% yield) as a white solid:

- Ethyl-3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)3-oxopropanoate (**5.61**) (592 mg, 1.27 mmol)
- $LiBH_4$ (69 + 28 + 28 mg)

9.4. ATTEMPTS AT IMPROVING GSK3531659A (5.53)

9.4.2. Replacement of 3-hydroxypropanamideside side chain from 5.53

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)quinuclidine-3-carboxamide (6.1)



To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (70 mg, 0.2 mmol) in DMF (4 mL), under N_2 atmosphere, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a solution of quinuclidine-3-carboxylic acid (**6.2**) (92 mg, 0.6 mmol) and CDI (97 mg, 0.6 mmol), in anhydrous DMF (1.5 mL), previously stirred for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 16 h. After this time, LCMS Neutral showed SM and no desired product. The reaction mixture was cooled to 0 ºC in an ice-bath and more NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. A solution of quinuclidine-3-carboxylic acid (**6.2**) (92 mg, 0.6 mmol) and CDI (97 mg, 0.6 mmol) were dissolved in 1.5 mL of DMF and stirred for 1 h. After this time, the suspension was added to the reaction mixture and the reaction was allowed to reach room temperature. LCMS Neutral, after 6 h, showed that the reaction had progressed but it was not complete. The reaction mixture was stirred for 4 additional days. LCMS Neutral, after that time did not show any evolution. The reaction mixture was heated at 65 ºC for 22 h but no progress was observed by LCMS Neutral. A saturated solution of NH_4Cl and EtOAc were added, and the mixture was separated in a separative funnel. The organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a yellow solid that corresponded to SM and a little amount of final product. The crude material was dissolved in DMF (4 mL), cooled at 0 ºC and NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. Quinuclidine-3-carboxylic acid (**6.2**) (92 mg, 0.6 mmol) and CDI (97 mg, 0.6 mmol) were dissolved in DMF (1.5 mL) and stirred at room temperature for 1 h, and then

added over the previous mixture. The mixture was then heated at 70 ºC for 16 h. LCMS Neutral, after that time, showed traces of SM remaining but the reaction was stopped. A saturated aqueous solution of NH_4Cl, and EtOAc were added, and the mixture was separated in a separative funnel, the organic phase was washed a second time with a saturated aqueous solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a yellow oil. The crude material was loaded in a silica gel cartridge and Cy/EtOAc 0-100% was added but the product did not elute. 7 M NH_3 solution in MeOH was added and *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)quinuclidine-3-carboxamide (**6.1**) (41 mg, 42% yield) was eluted from the column as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.52-7.58 (m, 3H), 7.38 (d, *J*=8.3 Hz, 2H), 7.12-7.21 (m, 3H), 5.45 (s, 2H), 3.22 (s, 3H), 3.17 (m, 1H), 2.99-3.09 (m, 3H), 2.95 (t, *J*=7.8 Hz, 2H), 1.74-1.82 (m, 3H), 2.40 (br. s., 1H), 1.69 (m, 1H), 1.51 ppm (m, 1H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 172.0, 158.1, 155.0, 151.4, 136.4, 134.1, 133.8, 129.8, 129.1, 128.0, 124.5, 112.4, 105.2, 48.2, 46.6, 46.2, 43.3, 39.5, 23.4, 23.0, 22.9, 19.4 ppm.

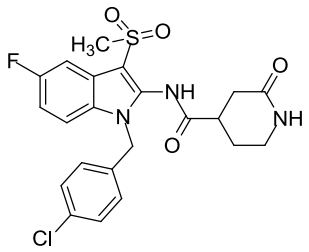
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.07 min, $[M+H]^+$ 490 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3118.1, 2920.9, 1694.0, 1403.3, 1127.9, 858.7.

M.P. 130.2 ºC

HRMS (ES) calcd for $C_{24}H_{26}{}^{35}ClFN_3O_3S$, $(M + H)^+$ 490.1362 found 490.1376.

_N_-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-oxopiperidine-4-carboxamide (6.4)



To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (70 mg, 0.2 mmol) in DMF (4 mL), under N_2 atmosphere, NaH (60% dispersion in mineral oil) (12 mg, 0.3 mmol) was added. After stirring for 30 min, a mixture of 2-oxopiperidine-4-carboxylic acid (**6.3**) (85 mg, 0.6 mmol), and CDI (97 mg, 0.6 mmol), in dry DMF (1.5 mL), and previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature, and stirred for 16 h, after this time, LCMS Neutral showed SM so the mixture was heated at 80 ºC for 16 h and monitored by LCMS Neutral until no evolution was observed. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in a separative funnel, and the organic phase was washed a second time with a saturated solution of NH_4Cl. The organic layer was washed with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give an orangish solid that was triturated with ACN (HPLC quality). The white solid precipitated was filtered, and dried to give *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-oxopiperidine-4-carboxamide (**6.4**) (14 mg, 15% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.48 (s, 1H), 7.47-7.61 (m, 3H), 7.39 (d, *J*=8.6 Hz, 2H), 7.18 (d, *J*=8.3 Hz, 3H), 5.39 (s, 2H), 3.18 (s, 5H), 2.90-3.00 (m, 1H), 2.22-2.41 (m, 2H), 1.97-2.07 (m, 1H), 1.68-1.86 ppm (m, 1H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 174.8, 169.5, 160.0, 157.6, 137.6, 135.8, 132.7, 129.9, 129.4, 129.0, 124.4, 113.6, 112.2, 105.1, 45.9, 44.1, 40.1, 38.9, 33.7, 25.4 ppm.

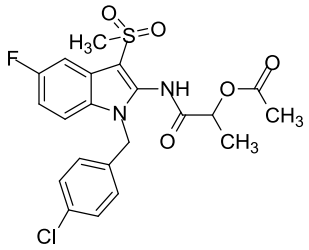
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.09 min, $[M+H]^+$ 478 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3272.6, 1679.3, 1478.2, 1285.4, 1106.6, 800.1.

M.P. 258.7 ºC

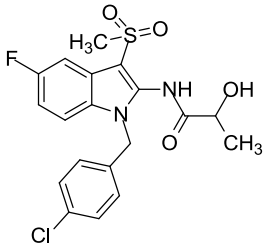
HRMS (ES) calcd for $C_{22}H_{21}{}^{35}ClFN_3NaO_4S$, $(M + Na)^+$ 500.0818 found 500.0828.

1-((1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-1-oxopropan-2-yl acetate (6.6)



To previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (135 mg, 0.38 mmol) in DMF (6 mL) under N_2 atmosphere, NaH (60% dispersion in mineral oil) (23 mg, 0.6 mmol) was added. After stirring for 30 min, a mixture of 2-acetoxypropanoic acid (**6.6**) (0.13 mL, 1.15 mmol) and CDI (186 mg, 1.15 mmol), in anhydrous DMF (1.5 mL), and previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 40 h. After this time, LCMS Neutral showed reaction completion. A saturated solution of NH_4Cl and EtOAc were added and the mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give 166 mg an orangish oil that corresponded to 1-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-1-oxopropan-2-yl acetate (**6.6**) (166 mg, 93% yield) with small impurities by LCMS Neutral.

***N*-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-hydroxypropanamide (6.7)**



A round-bottomed flask was charged with 1-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-1-oxopropan-2-yl acetate (**6.6**) (166 mg, 0.36 mmol) in ACN (5 mL) and water (2.5 mL). K_2CO_3 (98 mg, 0.7 mmol) was added and the reaction mixture was stirred at r.t. overnight. LCMS Neutral after that time did not show any progress. K_2CO_3 (260 mg, 1.9 mmol) was added and 4 h later LCMS Neutral showed some progression. The reaction was stirred at r.t. during 60 h, and no variation

was observed by LCMS Neutral after that time. The reaction was heated at 60 ºC for 6 h and LCMS Neutral after this time showed progression but there was still SM remaining in a proportion of 1:1. K_2CO_3 (200 mg, 1.5 mmol) was added and the reaction was heated at 60 ºC for 16 h. LCMS Neutral showed progression (3:2 desired product *vs.* SM). K_2CO_3 (500 mg, 3.6 mmol) was added and the reaction was heated at 60 ºC for 24 h. LCMS Neutral after this time did not show any advance. EtOAc and a saturated solution of NH_4Cl were added to the reaction mixture, and the phases were separated, the organic layer was washed with brine, dried over $MgSO_4$, filtered, and dried under vacuum to give a yellow solid that was triturated with ACN (HPLC quality) to give *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-hydroxypropanamide (**6.7**) (40 mg, 26% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.23 (s, 1H), 7.55 (dd, *J*=9.6, 2.3 Hz, 1H), 7.49 (dd, *J*=8.8, 4.3 Hz, 1H), 7.38 (d, *J*=8.6 Hz, 2H), 7.22 (d, *J*=8.6 Hz, 2H), 7.14 (td, *J*=9.2, 2.3 Hz, 1H), 5.92 (d, *J*=4.8 Hz, 1H), 5.38 (s, 2H), 4.15-4.27 (m, 1H), 3.16-3.23 (m, 3H), 1.31 ppm (d, *J*=6.6 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 176.4, 160.0, 157.6, 137.7, 135.8, 132.7, 129.9, 129.5, 129.0, 124.5, 113.4, 112.0, 105.0, 68.0, 45.9, 44.1, 21.0 ppm.

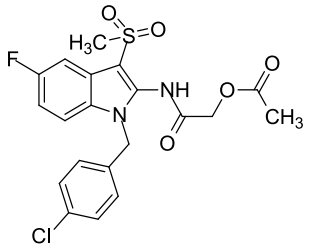
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.15 min, $[M+H]^+$ 425 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3258.8, 1668.3, 1478.9, 1284.5, 1109.8, 774.6.

M.P. 195.6 ºC

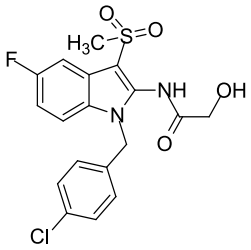
HRMS (ES) calcd for $C_{19}H_{18}{}^{35}ClFN_2NaO_4S$, $(M + Na)^+$ 447.0552 found 447.0559.

2-((1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl acetate (6.9)



To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (155 mg, 0.44 mmol) in DMF (6 mL), under N_2 atmosphere, NaH (60% dispersion in mineral oil) (26.4 mg, 0.7 mmol) was added. After stirring for 30 min, a mixture of 2-acetoxyacetic acid (**6.8**) (156 mg, 1.3 mmol) and CDI (214 mg, 1.3 mmol) in anhydrous DMF (2.5 mL), previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 18 h, after this time, LCMS Neutral showed starting material remaining. The reaction was stirred at r.t. for a further 30 h. LCMS Neutral after this time did not show any change. A saturated solution of NH_4Cl and EtOAc were added, and the reaction mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a brownish oil. ACN (HPLC quality) was added and a white solid precipitated but it did not correspond with desired product by ^1H NMR. The filtrate was evaporated under vacuum to give 200 mg of a crude material that was purified on a silica gel cartridge (Cy/EtOAc 0-60%) to give 2-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl acetate (**6.9**) (145 mg) along with SM by LCMS Neutral. The product was used in the next step of the synthesis without any further purification.

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-hydroxyacetamide (6.10)



To a solution of 2-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)amino)-2-oxoethyl acetate (**6.9**) (145 mg, 0.32 mmol) in THF (2 mL), a 6 M solution of NaOH (2 mL, 12 mmol) was added. After 3 h stirring at r.t., LCMS Neutral showed reaction completion. A saturated solution of NH_4Cl and EtOAc were added, and the mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a brownish oil. MeOH (HPLC quality) was added and a *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-hydroxyacetamide (**6.10**) (63 mg, 35% two steps overall yield) precipitated as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.26 (br. s., 1H), 7.45-7.64 (m, 2H), 7.38 (d, *J*=8.1 Hz, 2H), 7.22 (d, *J*=7.8 Hz, 2H), 7.14 (t, *J*=8.1 Hz, 1H), 5.88 (br. s., 1H), 5.39 (br. s., 2H), 4.06 (d, *J*=4.0 Hz, 2H), 3.20 ppm (br. s., 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 174.0, 159.9, 157.4, 137.9, 135.7, 132.7, 129.8, 129.5, 129.1, 124.5, 113.5, 112.1, 105.0, 62.1, 45.9, 44.1 ppm.

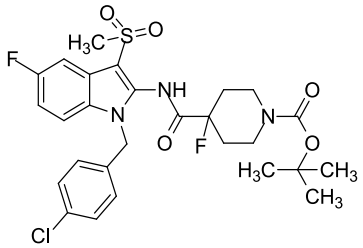
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.11 min, $[M+H]^+$ 411 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3224.8, 1670.5, 1478.5, 1288.2, 1086.8, 770.3.

M.P. 196.1 ºC

HRMS (ES) calcd for $C_{18}H_{16}{}^{35}ClFN_2NaO_4S$, $(M + Na)^+$ 433.0396 found 433.0401.

tert-Butyl 4-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-4-fluoropiperidine-1-carboxylate (6.12)

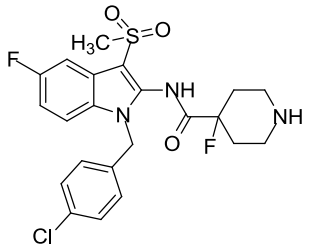


To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (88 mg, 0.25 mmol) in DMF (6 mL) under N_2 atmosphere, NaH (60% dispersion in mineral oil) (15 mg, 0.4 mmol) was added. After stirring for 30 min, a mixture of 1-(*tert*-butoxycarbonyl)-4-fluoropiperidine-4-carboxylic acid (**6.11**) (185 mg, 0.75 mmol) and CDI (121 mg, 0.75 mmol) in anhydrous DMF (1.5 mL), previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach r.t. and stirred for 16 h. After this time, LCMS Neutral showed SM remaining. The reaction mixture was heated 3 h at 80 ºC and no changes were observed by LCMS Neutral. A saturated solution of NH_4Cl, and EtOAc were added, and the reaction mixture was separated in a funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a greenish solid that was triturated with ACN (HPLC quality) to give *tert*-butyl 4-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-4-fluoropiperidine-1-carboxylate (**6.12**) (30 mg, 21% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.61-10.81 (m, 1H), 7.49-7.69 (m, 2H), 7.39 (d, *J*=8.3 Hz, 2H), 7.19 (d, *J*=8.3 Hz, 3H), 5.40 (s, 2H), 3.81-4.04 (m, 2H), 3.18 (s, 3H), 2.96-3.12 (m, 2H), 1.92 (br. s., 4H), 1.41 ppm (s, 9H)

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.36 min, $[M-H]^-$ 580 (>95% purity).

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-4-fluoropiperidine-4-carboxamide (6.13)



tert-Butyl-4-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-4-fluoropiperidine-1-carboxylate (**6.12**) (28 mg, 0.05 mmol) was dissolved in DCM (1 mL), and TFA (0.1 mL) was added. The reaction was stirred at room temperature for 30 min. After that time, HPLC (basic pH), showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude material that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with NH_3 (7 M in MeOH) to give *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-4-fluoropiperidine-4-carboxamide (**6.13**) (10 mg, 43% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.50 (dd, *J*=10.0, 2.4 Hz, 1H), 7.42-7.47 (m, 1H), 7.37 (d, *J*=8.3 Hz, 2H), 7.23 (d, *J*=8.3 Hz, 2H), 7.02-7.11 (m, 1H), 5.33 (s, 2H), 3.18 (s, 3H), 3.01 (d, *J*=12.4 Hz, 2H), 2.83 (t, *J*=11.0 Hz, 2H), 1.93-2.15 (m, 2H), 1.79-1.91 ppm (m, 2H). NH units are not visible in the spectrum conditions.

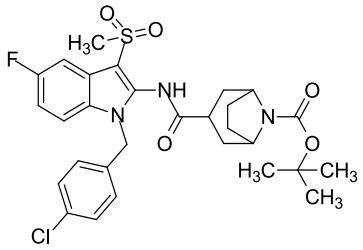
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.06 min, $[M+H]^+$ 482 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 2927.1, 1692.6, 1478.3, 1284.4, 1128.7, 764.7.

M.P. 246.4 ºC

HRMS (ES) no mass available.

tert-Butyl 3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (6.15)



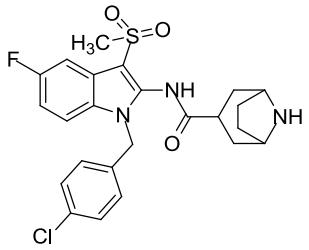
To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (100 mg, 0.3 mmol) in DMF (6 mL), under N_2 atmosphere, NaH (60% dispersion in mineral oil) (17 mg, 0.4 mmol) was added. After stirring for 30 min, a mixture of 8-(*tert*-butoxycarbonyl)-8-azabicyclo[3.2.1]octane-3-carboxylic acid (**6.14**) (217 mg, 0.85 mmol) and CDI (138 mg, 0.85 mmol) in anhydrous DMF (1.5 mL), and previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 16 h. After this time, LCMS Neutral, showed SM remaining. The reaction mixture was heated at 80 ºC. LCMS Neutral after 16 h showed that the reaction had almost reached completion. A saturated solution of NH_4Cl and EtOAc were added and the phases were separated in a separative funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a yellowish solid that was triturated with ACN (HPLC quality). A white solid precipitated, this was filtered, and dried under vacuum to give *tert*-butyl 3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**6.15**) (110 mg, 66% yield).

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.29 (s, 1H), 7.53-7.58 (m, 1H), 7.46-7.52 (m, 1H), 7.38 (d, *J*=8.3 Hz, 2H), 7.10-7.22 (m, 3H), 5.35 (s, 2H), 4.11 (br. s., 2H), 3.14 (s, 3H), 2.94-3.09 (m, 1H), 1.84-1.98 (m, 2H), 1.69 (d, *J*=5.3 Hz, 6H), 1.40 ppm (s, 9H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 175.8, 160.1, 157.5, 152.9, 137.6, 135.7, 132.7, 129.8, 129.2, 129.1, 124.4, 113.5, 112.1, 105.0, 78.9, 45.8, 44.0, 40.5, 40.3, 39.9, 35.4, 28.5 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.35 min, $[M-H]^-$ 588 (>95% purity).

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-8-azabicyclo[3.2.1]octane-3-carboxamide (6.16)



tert-Butyl-3-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-8-azabicyclo[3.2.1]octane-8-carboxylate (**6.15**) (105 mg, 0.18 mmol) was dissolved in DCM (3 mL) and TFA (1 mL, 13 mmol) was added. The reaction was stirred at room temperature for 1 h, and HPLC (basic pH) after that time showed that the reaction had reached completion. Solvent and TFA were evaporated under vacuum to give a crude material that was loaded onto a 1g SCX cartridge that had been previously washed with MeOH. The column was rinsed with MeOH several times and then with a 7 M solution of NH_3 in MeOH to give *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-8-azabicyclo[3.2.1]octane-3-carboxamide (**6.16**) (80 mg, 92% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.53 (dd, *J*=9.9, 2.5 Hz, 1H), 7.47 (dd, *J*=9.0, 4.4 Hz, 1H), 7.37 (d, *J*=8.6 Hz, 2H), 7.16 (d, *J*=8.6 Hz, 2H), 7.11 (td, *J*=9.2, 2.5 Hz, 1H), 5.32 (s, 2H), 3.47 (br. s., 2H), 3.14 (s, 3H), 2.80 (dt, *J*=11.4, 5.8 Hz, 1H), 1.55-1.81 ppm (m, 8H). NH units are not visible in the spectrum conditions.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 176.1, 160.6, 159.9, 157.6, 135.9, 132.9, 129.8, 129.4, 128.9, 124.7, 113.2, 111.6, 104.8, 54.0, 45.7, 43.6, 36.4, 34.8, 29.1 ppm.

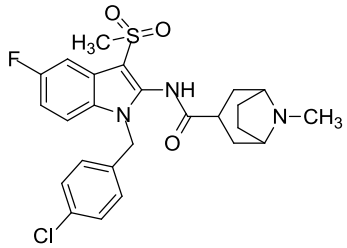
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.06 min, $[M-H]^-$ 488 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 2926.6, 1693.7, 1478.9, 1288.3, 1129.1, 803.4.

M.P. 126.2 ºC

HRMS (ES) calcd for $C_{24}H_{26}{}^{35}ClFN_3O_3S$, $(M + H)^+$ 490.1362 found 490.1343.

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane-3-carboxamide (6.17)



_N_-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-8-azabicyclo[3.2.1]octane-3-carboxamide (**6.16**) (77 mg, 0.2 mmol) was dissolved in water (3 mL), and formic acid (0.15mL, 3.5 mmol) (90% Wt. solution in H_2O) and formaldehyde (0.13 mL, 1.6 mmol) (37% solution in H_2O) were added in sequence at r.t. The resulting mixture was heated to reflux for 2 h. LCMS Neutral, after this time, showed that reaction had reached completion. Solvent was evaporated under vacuum to give a white viscous product. This crude product was loaded onto a 1 g SCX cartridge, which had previously been washed with MeOH, the column was then washed several times with MeOH, and finally _N_-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane-3-carboxamide (**6.17**) (60 mg, 76% yield) was eluted when the column was rinsed with a 7 M solution of NH_3 in MeOH, and evaporated to give the product as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.59 (dd, _J_=9.0, 1.9 Hz, 1H), 7.27 (s, 2H), 7.01-7.08 (m, 1H), 6.95 (d, _J_=8.3 Hz, 3H), 5.21 (s, 2H), 3.62 (br. s., 1H), 3.24 (br. s., 1H), 3.10 (s, 3H), 2.62-2.85 (m, 1H), 1.95-2.14 (m, 2H), 1.78-1.95 (m, 4H), 1.65-1.78 (m, 2H), 1.56-1.65 (m, 1H), 1.43 ppm (s, 3H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 182.3, 175.8, 160.5, 158.2, 156.5, 133.8, 130.1, 129.1, 127.9, 123.6, 112.3, 105.0, 100.0, 60.5, 53.9, 47.5, 44.9, 35.2, 28.8, 26.9 ppm.

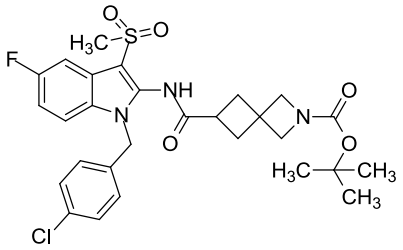
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.06 min, $[M+H]^+$ 504 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 2926.42, 1693.51, 1478.56, 1287.85, 1128.47, 802.93.

M.P. 124.1 ºC

HRMS (ES) calcd for $C_{25}H_{28}{}^{35}ClFN_3O_3S$, $(M + H)^+$ 504.1518 found 504.1502.

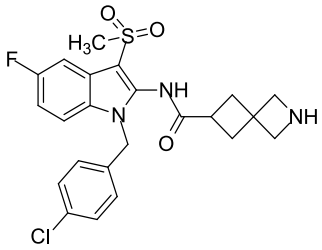
tert-Butyl 6-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-2-azaspiro[3.3]heptane-2-carboxylate (6.19)



To a previously cooled at 0 ºC solution of 1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**5.59**) (150 mg, 0.42 mmol) in DMF (6 mL) under N_2 atmosphere, NaH (60% dispersion in mineral oil) (26 mg, 0.64 mmol) was added. After stirring for 30 min, a mixture of 2-(*tert*-butoxycarbonyl)-2-azaspiro[3.3]heptane-6-carboxylic acid (**6.18**) (308 mg, 1.3 mmol) and CDI (207 mg, 1.3 mmol) in anhydrous DMF (2.5 mL), and previously stirred at r.t. for 1 h, was added dropwise. The reaction mixture was allowed to reach room temperature and stirred for 3 days. After this time, LCMS Neutral showed reaction completion. A saturated solution of NH_4Cl and EtOAc were added, and the mixture was separated in an extraction funnel, the organic phase was washed a second time with a saturated solution of NH_4Cl, then with brine, and dried over anh. $MgSO_4$, filtered, and evaporated under vacuum to give a brownish oil. ACN (HPLC quality) was added and *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-8-methyl-8-azabicyclo[3.2.1]octane-3-carboxamide (**6.19**) (182 mg, 75% yield) was isolated as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.24 (s, 1H), 7.47-7.66 (m, 2H), 7.37 (d, *J*=8.3 Hz, 2H), 7.15 (d, *J*=8.1 Hz, 3H), 5.36 (s, 2H), 3.86 (br. s., 2H), 3.74 (br. s., 2H), 3.18 (s, 3H), 3.07-3.16 (m, 1H), 2.36 (d, *J*=7.8 Hz, 4H), 1.36 ppm (s, 9H).

_N_-(1-(4-Chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1_H_-indol-2-yl)-2-azaspiro[3.3]heptane-6-carboxamide (6.20)



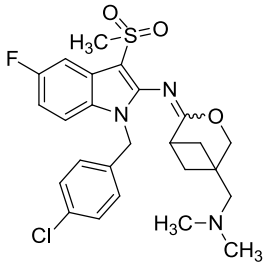
tert-Butyl-6-((1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)carbamoyl)-2-azaspiro[3.3]heptane-2-carboxylate (**6.19**) (120 mg, 0.2 mmol) was dissolved in DCM (5 mL) and TFA (0.5 mL, 6.5 mmol) was added. The reaction was stirred at room temperature for 2 h and LCMS Neutral after this time showed reaction completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded in a 1 g SCX cartridge that had been previously washed with MeOH. The column was washed with methanol and then *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-azaspiro[3.3]heptane-6-carboxamide (**6.20**) (90 mg, 91% yield) was freed with a 7 M solution of NH_3 in MeOH, and evaporated to give the desired product as a white solid.

^{1}H NMR at 25 ºC showed rotamers but when the NMR sample was heated at 80 ºC the signals collapsed into a single set consistent with *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-azaspiro[3.3]heptane-6-carboxamide (**6.20**).

^{1}H NMR (DMSO-d_6, 80 ºC, 400 MHz): δ = 7.65 (dd, *J*=9.6, 2.3 Hz, 1H), 7.48 (dd, *J*=9.0, 4.4 Hz, 1H), 7.36 (d, *J*=8.3 Hz, 2H), 7.10-7.27 (m, 3H), 5.29-5.52 (m, 2H), 3.37-3.71 (m, 2H), 3.15 (s, 3H), 2.73 (t, *J*=5.4 Hz, 1H), 2.58-2.63 (m, 2H), 2.21 (dd, *J*=9.2, 5.9 Hz, 1H), 2.12-2.17 (m, 1H), 2.05 (s, 1H), 1.79 ppm (dd, *J*=9.1, 7.1 Hz, 1H). NH units are not visible in the spectrum conditions.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=0.96 min, $[M+H]^+$ 476 (>95% purity).

1-(4-Chlorobenzyl)-*N*-(5-((dimethylamino)methyl)-3-oxabicyclo[3.1.1]heptan-2-ylidene)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (6.22)



A round-bottomed flask was charged with *N*-(1-(4-chlorobenzyl)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-yl)-2-azaspiro[3.3]heptane-6-carboxamide (**6.20**) (48 mg, 0.1 mmol) in water (3 mL). 37% wt. Formaldehyde (82 mg, 1 mmol) and 90% wt. formic acid (113 mg, 2.2 mmol) were added and 16 h later LCMS Neutral showed an unknown by-product along with some desired product and some starting material. Formaldehyde (82 mg, 1 mmol) and formic acid (113 mg, 2.2 mmol) were added and the reaction was refluxed for 5 h. LCMS Neutral after that time showed reaction completion but the unknown by-product was the major component. The solvent was evaporated under vacuum to give a crude material that was purified by HPLC_sep (basic pH) to give two fractions:

- Fraction 1: contained the desired product (LCMS Neutral), but when the solvent was removed under vacuum just 3 mg were obtained and the purity by HPLC (basic pH) was not sufficient for biological analysis.
- Fraction 2: A structural study (HMBC, HSQC & COSY) of this compound suggested that the structure was that corresponding to 1-(4-chlorobenzyl)-*N*-(5-((dimethylamino)methyl)-3-oxabicyclo[3.1.1]heptan-2-ylidene)-5-fluoro-3-(methylsulfonyl)-1*H*-indol-2-amine (**6.22**) (15 mg, 29% yield) as a white solid.

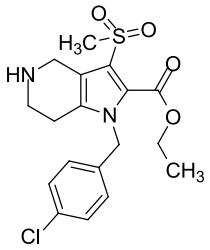
^{1}H NMR ($CDCl_3$, 400 MHz): δ = 7.81 (dd, *J*=9.2, 2.4 Hz, 1H), 7.34 (d, *J*=8.3 Hz, 2H), 7.13-7.20 (m, 1H), 7.03-7.12 (m, 3H), 5.12-5.40 (m, 2H), 3.93 (d, *J*=10.4 Hz, 1H), 3.35 (d, *J*=10.6 Hz, 1H), 3.16 (s, 3H), 2.94 (t, *J*=5.8 Hz, 1H), 2.48 (t, *J*=8.6 Hz, 1H), 2.32-2.43 (m, 2H), 2.23 (dd, *J*=9.3, 5.8 Hz, 1H), 2.16 (dd, *J*=9.1, 6.1 Hz, 1H), 2.11 (s, 6H), 1.84 ppm (t, *J*=8.7 Hz, 1H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.27 min, $[M+H]^+$ 504 (>95% purity).

9.4.3. Replacement of the 5-F-Indole core from 5.51

9.4.3.2. Synthesis of the compound 6.26

Ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (6.29)

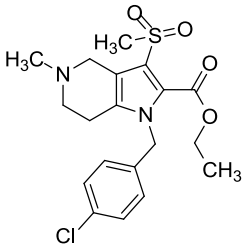


5-*tert*-Butyl 2-ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-6,7-dihydro-1*H*-pyrrolo[3,2-*c*]pyridine-2,5(4*H*)-dicarboxylate (**4.101**) (500 mg, 1 mmol) was dissolved in DCM (5 mL) and TFA (2.5 mL, 1 mmol) was added. The reaction was stirred at room temperature for 1 h and LCMS Neutral after that time showed reaction completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded onto a 10 g SCX cartridge that had been previously washed with MeOH. The column was washed with methanol and then ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (**6.29**) (396 mg, 99% yield) was freed with 7 N solution of ammonia in methanol and evaporated to give the desired product as a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.42 (d, *J*=8.6 Hz, 2H), 7.05 (d, *J*=8.3 Hz, 2H), 5.37 (s, 2H), 4.22 (q, *J*=7.1 Hz, 2H), 3.84 (s, 2H), 3.26 (s, 4H), 2.90 (t, *J*=5.7 Hz, 2H), 2.42 (t, *J*=5.3 Hz, 2H), 1.21 ppm (t, *J*=7.2 Hz, 3H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.03 min, $[M+H]^+$ 397 (>95% purity).

Ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (6.30)



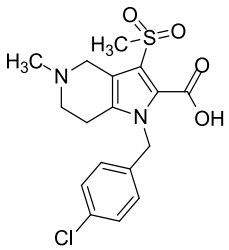
A round-bottomed flask was charged with ethyl 1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (**6.29**) (390 mg, 0.98 mmol) in water (10 mL). 37 % wt. Formaldehyde (0.8 mL, 9.8 mmol) and 90% wt. formic acid (0.9 mL, 21.6 mmol) were added, and the reaction mixture was stirred at r.t. for 16 h. LCMS Neutral after this time showed reaction completion. The solvent and excess of reactants were removed under vacuum to give a crude that was evaporated under vacuum to give a beige oil that was loaded onto a 1 g SCX cartridge that had been previously washed with MeOH. The column was washed with methanol and then ethyl 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (**6.30**) (300 mg, 74% yield) was freed with 20 mL of a 7 N solution of ammonia in methanol and evaporated to give the desired product as a yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.42 (d, *J*=8.3 Hz, 2H), 7.02 (d, *J*=8.3 Hz, 2H), 5.38 (s, 2H), 4.22 (q, *J*=7.1 Hz, 2H), 3.50 (s, 2H), 3.27 (s, 3H), 2.52-2.63 (m, 4H), 2.34 (s, 3H), 1.21 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.1, 136.4, 132.5, 129.2, 128.5, 124.1, 123.1, 118.9, 62.0, 52.1, 51.3, 48.2, 45.5, 45.1, 26.8, 22.7, 14.3 ppm.

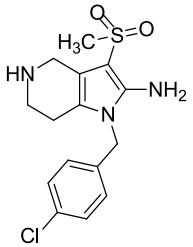
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.15 min, $[M+H]^+$ 411 (>95% purity).

1-(4-Chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (6.31)



Ethyl-1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylate (**6.30**) (50 mg, 0.1 mmol) was dissolved in a mixture of THF (1.5 mL) and water (1.5 mL) and LiOH.H_2O (110 mg, 2.6 mmol) was added. The mixture was stirred at room temperature for 16 h and, after this time, LCMS Neutral showed reaction completion. A 2 N aqueous solution of HCl was added to the reaction mixture to reach neutral pH and the solvents were evaporated under vacuum to give 620 mg of a crude material corresponded with desired product along with appreciable amounts of salts. The crude material was charged into a diaion resin cartridge (previously stabilised with MeOH), MeOH was removed using compressed air and water was added (several column volumes). The crude was added dissolved in water and the salts were eluted with water from the cartridge. After several column volumes, MeOH was added to elute the compound. The solvent was evaporated to give 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (**6.31**) (97 mg, 42% yield) also containing appreciable quantities of salts. It was used as such in the next step, without success.

1-(4-Chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-c]pyridin-2-amine (6.33)



5-(*tert*-Butoxycarbonyl)-1-(4-chlorobenzyl)-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridine-2-carboxylic acid (**4.102**) (266 mg, 0.6 mmol) was dissolved in dry toluene (10 mL) and Et_3N (0.16 mL, 1.1 mmol) was added. After 15 min, DPPA (0.24 mL, 1.1 mmol) was added and the reaction mixture was refluxed for 3 h. HPLC

(basic pH) after this time did not show starting material. Water (10 mL) was added, and the mixture was refluxed for 2 h. Phases were separated in a funnel and the organic phase was washed with brine, dried over Na_2SO_4, filtered, and evaporated under vacuum to give a crude material that seemed to contain some desired product and Boc-deprotected product by LCMS Neutral. The crude was purified by HPLC_sep (basic pH) and the appropiate fractions were evaporated together to give 1-(4-chlorobenzyl)-5-methyl-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-pyrrolo[3,2-*c*]pyridin-2-amine (**6.33**) (28 mg, 7% yield) as a yellow solid.

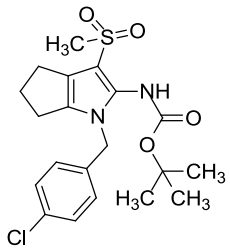
^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.41 (d, *J*=8.3 Hz, 2H), 7.10 (d, *J*=8.3 Hz, 2H), 5.59 (s, 2H), 4.97 (s, 2H), 3.64 (br. s., 2H), 2.94 (s, 3H), 2.86 (br. s., 2H), 2.21 ppm (br. s., 2H). The NH unit is not visible in the spectrum conditions.

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 142.6, 137.0, 132.2, 129.0, 128.9, 120.0, 113.2, 95.0, 45.8, 44.2, 43.0, 42.0, 22.1 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=0.89 min, $[M+H]^+$ 340 (>95% purity).

9.4.3.3. Synthesis of the compound 6.27

tert-Butyl (1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)carbamate (6.34)



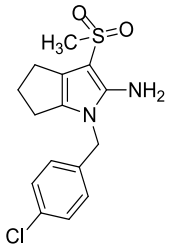
1-(4-Chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrole-2-carboxylic acid (**4.130**) (632 mg, 1.8 mmol), DPPA (0.4 mL, 2 mmol), and Et_3N (0.27 mL, 2 mmol) were added to a round-bottomed flask with *tert*-butanol (10 mL) previously warmed at 25 ºC - 30 ºC (to avoid the solidification of the *tert*-butanol). After the addition, the reaction mixture was warmed to 90 ºC. LCMS Neutral after 3 h, showed some starting material. The reaction mixture was heated at this temperature for another 18 h and LCMS Neutral after that time showed no evolution and the solvent was completely evaporated to give a brown syrup, that was dissolved in a little amount of methanol. Water was added and a solid precipitated. This was triturated, and filtered to give a purple solid that was purified by flash chromatography (Cy-EtOAc 0-60%). Appropiate fractions were collected to give *tert*-butyl-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)carbamate (**6.34**) (326 mg, 43% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 8.98 (br. s., 1H), 7.40 (d, *J*=8.3 Hz, 2H), 7.20 (d, *J*=7.8 Hz, 2H), 4.92 (s, 2H), 3.00 (s, 3H), 2.66 (t, *J*=6.3 Hz, 2H), 2.46 (t, *J*=6.4 Hz, 2H), 2.15-2.30 (m, 2H), 1.15-1.59 ppm (m, 9H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.5, 136.2, 134.5, 132.8, 129.9, 129.3, 129.0 (2C), 123.7, 80.0, 46.9, 44.4, 28.4, 27.1, 25.8, 25.3 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.30 min, $[M+H]^+$ 425 (>95% purity).

1-(4-Chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-amine (6.35)

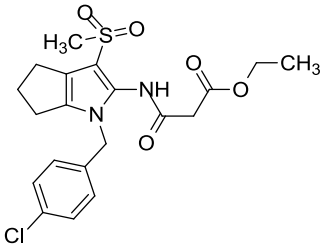


tert-Butyl-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*] pyrrol-2-yl)carbamate (**6.34**) (160 mg, 0.38 mmol) was dissolved in DCM (5 mL) and TFA (1 mL, 13 mmol) was added. The reaction was stirred at room temperature for 17 h and LCMS Neutral after that time showed reaction completion. Solvent and TFA were evaporated under vacuum to give a crude product that was loaded onto 1 g SCX cartridge that had been previously washed with MeOH. The column was washed with methanol and then 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-amine (**6.35**) (114 mg, 93% yield) was freed with 20 mL of a 7 N solution of ammonia in methanol and evaporated to give the desired product as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.42 (d, *J*=8.3 Hz, 2H), 7.17 (d, *J*=8.3 Hz, 2H), 5.55 (s, 2H), 4.94 (s, 2H), 2.94 (s, 3H), 2.53-2.61 (m, 2H), 2.37-2.46 (m, 2H), 2.14-2.25 ppm (m, 2H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.20 min, $[M+H]^+$ 325 (>95% purity).

Ethyl 3-((1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)amino)-3-oxopropanoate (6.36)



To a solution of 1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-amine (**6.35**) (100 mg, 0.31 mmol) in 2-MeTHF (8 mL) at 0 ºC were added anhydrous pyridine (0.025 mL, 0.31 mmol) and ethyl malonyl

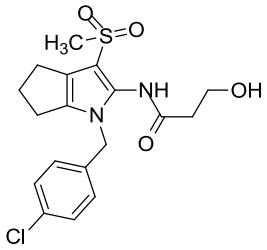
chloride (**5.58**) (0.04 mL, 0.34 mmol), and the mixture was stirred at 0 ºC for 20 min and LCMS Neutral showed reaction completion. The reaction mixture was partitioned between EtOAc and water, the aqueous phase was extracted with EtOAc (x 2). The organic phases were combined and washed with water and brine, dried over Na_2SO_4, filtered, and concentrated *in vacuo*. The crude product was triturated with ^{t}BuOMe, filtered, and rinsed with ^{t}BuOMe to obtain ethyl 3-((1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)amino)-3-oxopropanoate (**6.36**) (78 mg, 57% yield) as a yellow solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.12 (s, 1H), 7.42 (d, *J*=8.6 Hz, 2H), 7.23 (d, *J*=8.3 Hz, 2H), 4.93 (s, 2H), 4.09 (q, *J*=7.1 Hz, 2H), 3.46 (s, 2H), 2.98 (s, 3H), 2.66 (t, *J*=6.8 Hz, 2H), 2.43 (t, *J*=6.8 Hz, 2H), 2.12-2.34 (m, 2H), 1.14 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 167.8, 167.3, 136.1, 135.1, 132.8, 130.1, 129.1, 128.0, 124.1, 113.8, 61.2, 47.1, 44.8, 42.7, 27.2, 25.8, 25.4, 14.4 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.18 min, $[M+H]^+$ 439 (>95% purity).

_N_-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)-3-hydroxypropanamide (6.27)



Ethyl 3-((1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)amino)-3-oxopropanoate (**6.36**) (60 mg, 0.14 mmol) was dissolved in THF (1.5 mL) and MeOH (0.02 mL, 0.55 mmol), and cooled at 0 ºC. To this cooled solution, $LiBH_4$ (12 mg, 0.55 mmol) was added, and the resulting solution was stirred at 0 ºC for 3 h. LCMS Neutral, showed that the reaction had reached completion along with an unknown by-product. The reaction mixture was treated with 1 N HCl at 0 ºC, EtOAc was added, the layers were separated, and the aqueous phase was extracted with EtOAc. The combined organic layers were washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to give a solid. The solid was triturated with ^{t}BuOMe, filtered, and rinsed with ^{t}BuOMe to give 79 mg of a crude material that

corresponded to desired product and the by-product observed previously by LCMS Neutral. The mixture was purified by HPLC_sep (basic pH) to give *N*-(1-(4-chlorobenzyl)-3-(methylsulfonyl)-1,4,5,6-tetrahydrocyclopenta[*b*]pyrrol-2-yl)-3-hydroxypropanamide (**6.27**) (27 mg, 49% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.45-10.66 (m, 1H), 7.41 (d, *J*=8.3 Hz, 2H), 7.23 (d, *J*=8.3 Hz, 2H), 4.89 (s, 2H), 4.65 (br. s., 1H), 3.69 (t, *J*=6.4 Hz, 2H), 2.97 (s, 3H), 2.66 (t, *J*=6.8 Hz, 2H), 2.45 (t, *J*=6.4 Hz, 4H), 2.22 ppm (quin, *J*=6.9 Hz, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.7, 136.0, 134.5, 133.9, 129.1, 128.6, 127.9, 124.5, 111.8, 58.5, 48.3, 44.4, 39.2, 27.3, 25.6, 25.5 ppm.

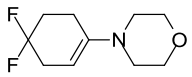
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.06 min, $[M+H]^+$ 397 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3271.9, 2929.3, 2860.2, 1682.7, 1284.5, 1092.8, 801.0.

HRMS (ES) calcd for $C_{18}H_{22}{}^{35}ClN_2O_4S$, $(M + H)^+$ 397.0983 found 397.0988.

9.4.3.4. Synthesis of the compound 6.28

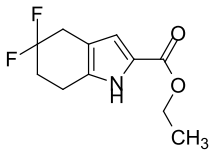
4-(4,4-Difluorocyclohex-1-en-1-yl)morpholine (6.38)



4,4-Difluorocyclohexan-1-one (**6.37**) (5 g, 37 mmol) and morpholine (**4.96**) (3.2 mL, 37 mmol) were mixed in toluene (25 mL) in a round bottomed flask equipped with a Dean Stark and a refrigerant (Dean Stark was filled with toluene). The reaction was refluxed vigorously during 4 days. After this time, toluene was removed under vacuum to give 4-(4,4-difluorocyclohex-1-en-1-yl)morpholine (**6.38**) (7.3 g, 96% yield) as a dark orange liquid.

^{1}H NMR (DMSO-d_6, 400MHz): δ = 4.42 (br. s., 1H), 3.56-3.66 (m, 4H), 2.69-2.77 (m, 4H), 2.51-2.59 (m, 2H), 2.27 (t, *J*=6.4 Hz, 2H), 2.05 ppm (tt, *J*=13.8, 6.8 Hz, 2H).

Ethyl 5,5-difluoro-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (6.39)



To a solution of 4-(4,4-difluorocyclohex-1-en-1-yl)morpholine (**6.38**) (7.3 g, 35.8 mmol) in toluene (60 mL) was added an *E*/*Z* mixture of 3-bromo-2-(hydroxyimino)propanoate (**4.105**) (3.7 g, 17.6 mmol), and the resulting mixture was stirred at room temperature for 2 h. LCMS Neutral after this time showed desired product . Water (60 mL) was added to the mixture. Phases were separated in a funnel and the organic layer was dried over anhydrous magnesium sulfate, filtered, and dried to obtain ethyl 6,6-difluoro-8a-morpholino-4a,5,6,7,8,8a-hexahydro-4*H*-benzo[*e*][1,2]oxazine-3-carboxylate (7.8 g, 133 % yield) with some impurities. This intermediate was used in the next step of the reaction without any further purication. The yield was considered quantitative for the next step (Theo mass: 5.85 g)

To a solution of ethyl 6,6-difluoro-8a-morpholino-4a,5,6,7,8,8a-hexahydro-4*H*-benzo[*e*][1,2]oxazine-3-carboxylate (5.85 g, 17.6 mmol) in DCE (200 mL) was added TFA (4 mL, 17.6 mmol) and $Fe_3(CO)_{12}$ (13.3 g, 26.4 mmol) and the resulting mixture was stirred at 80 ºC for 3 h. LCMS Neutral after that time, showed that the reaction had reached completion. After cooling to room temperature, the reaction was filtered, washed with DCM, and the filtrate was concentrated under vacuum. The residue

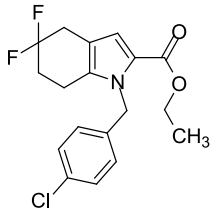
obtained was purified by silica gel on a 100 g cartridge (Cy/EtOAc 0-60%) to give ethyl 5,5-difluoro-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.39**) (1.7 g, 42% yield) as a beige solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 11.59 (br. s., 1H), 6.55 (d, *J*=2.3 Hz, 1H), 4.20 (q, *J*=7.1 Hz, 2H), 2.99 (t, *J*=14.5 Hz, 2H), 2.74 (t, *J*=6.6 Hz, 2H), 2.22 (tt, *J*=13.9, 6.8 Hz, 2H), 1.25 ppm (t, *J*=7.1 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.7, 131.1, 127.3, 125.0 (t, *J*=240.5 Hz), 122.0, 114.0, 59.8, 32.8 (t, *J*=26.3 Hz), 30.5(t, *J*=24.9 Hz), 20.1(t, *J*=5.9 Hz), 14.9 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.16 min, $[M+H]^+$ 230 (>95% purity).

Ethyl 1-(4-chlorobenzyl)-5,5-difluoro-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (6.40)



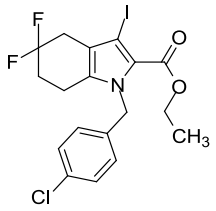
To a solution of ethyl 5,5-difluoro-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.39**) (1.7 g, 7.4 mmol) in dry ACN (40 mL) was added K_2CO_3 (2 g, 14.8 mmol), and the reaction mixture was stirred for 30 min, then 1-(bromomethyl)-4-chlorobenzene (1.83 g, 8.9 mmol) was added and reaction was refluxed for 20 h. LCMS Neutral after that time showed reaction completion. The heating was stopped, and EtOAc and water were added to the reaction mixture, phases were separated in a funnel, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and evaporated to give 4 g of a dark brown crude material that was loaded on a silica gel cartridge and purified using Cy/EtOAc 0-60% to give ethyl 1-(4-chlorobenzyl)-5,5-difluoro-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.40**) (2.43 g, 93% yield) as a yellow oil.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.38 (d, *J*=8.6 Hz, 2H), 6.97 (d, *J*=8.6 Hz, 2H), 6.80 (s, 1H), 5.52 (s, 2H), 4.14 (q, *J*=7.1 Hz, 2H), 3.03 (t, *J*=14.4 Hz, 2H), 2.66 (t, *J*=6.6 Hz, 2H), 2.23 (tt, *J*=13.8, 6.7 Hz, 2H), 1.14-1.28 ppm (m, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.7, 137.7, 134.1, 132.2, 129.0, 128.3, 124.5 (t, *J*=240.8 Hz), 121.8, 116.7, 113.9, 59.9, 47.4, 32.6 (t, *J*=27.1 Hz), 30.1(t, *J*=24.9 Hz), 19.5(t, *J*=5.9 Hz), 14.6 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.43 min, $[M+H]^+$ 354 (>95% purity).

Ethyl 1-(4-chlorobenzyl)-5,5-difluoro-3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (6.41)

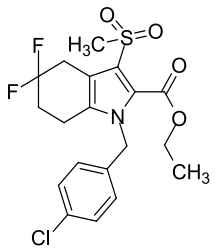


To a mixture of ethyl 1-(4-chlorobenzyl)-5,5-difluoro-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.40**) (2.4 g, 6.8 mmol) and silver sulfate (2.54 g, 8.1mmol) in H_2O (80 mL) and ethanol (80 mL), was added a solution of iodine (1.9 g, 7.5 mmol) in THF (40 mL). The reaction mixture was stirred at r.t. for 30 min. LCMS Neutral after this time showed reaction completion. A saturated solution of aq. $Na_2S_2O_3$ (50 mL) and a 20% wt. aqueous solution of Na_2CO_3 (50 mL) were added to the reaction mixture. The resulting suspension was filtered. The residue was washed with DCM (30 mL). The aqueous phase was extracted three times with DCM. The combined organic layers were washed with brine, dried over Na_2SO_4, filtered, and evaporated under vacuum to give a crude product that was purified on a silica gel cartridge using Cy/EtOAc 0-60% to give ethyl 1-(4-chlorobenzyl)-5,5-difluoro-3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.41**) (2.4 g, 74% yield) as a brownish solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.34-7.43 (m, 2H), 6.96 (d, *J*=8.6 Hz, 2H), 5.53 (s, 2H), 4.18 (q, *J*=7.1 Hz, 2H), 2.86 (t, *J*=14.0 Hz, 2H), 2.69 (t, *J*=6.6 Hz, 2H), 2.16-2.37 (m, 2H), 1.23 ppm (t, *J*=7.1 Hz, 3H).

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.51 min, $[M+H]^+$ 480 (>85% purity).

Ethyl 1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (6.42)



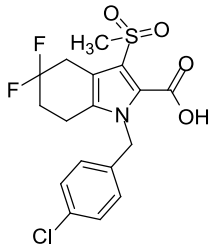
To a solution of ethyl 1-(4-chlorobenzyl)-5,5-difluoro-3-iodo-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.41**) (2.4 g, 5 mmol) in dry NMP (50 mL) was added CuI (0.95 g, 5 mmol) and methanesulfinic acid, sodium salt (1.3 g, 12.5 mmol) and reaction was stirred at 125 ºC. LCMS Neutral after 60 min showed that reaction had reached completion. EtOAc was added and a beige solid precipitated. This was filtered, the filtrate was washed with a saturated solution of NH_4Cl, and phases were separated in a funnel. The organic layer was washed with brine, dried over Na_2SO_4, filtered, and evaporated to give a crude material that was purified on a silica gel cartridge (Cy/EtOAc 0-60%) to give ethyl 1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.42**) (1.4 g, 76% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 7.43 (d, *J*=8.3 Hz, 2H), 7.06 (d, *J*=8.3 Hz, 2H), 5.37 (s, 2H), 4.23 (q, *J*=7.1 Hz, 2H), 3.29 (s, 3H), 3.21-3.28 (m, 2H), 2.67 (t, *J*=6.6 Hz, 2H), 2.24 (tt, *J*=13.7, 6.6 Hz, 2H), 1.21 ppm (t, *J*=7.2 Hz, 3H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 160.1, 136.0, 132.6, 131.3, 129.2, 128.7, 125.2, 124.5, 124.2 (t, *J*=240.8 Hz), 115.3, 62.2, 48.5, 45.4, 32.5 (t, *J*=28.5 Hz), 29.5(t, *J*=26.3 Hz), 19.3(t, *J*=5.9 Hz), 13.9 ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.28 min, $[M+H]^+$ 432 (>95% purity).

1-(4-Chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (6.43)



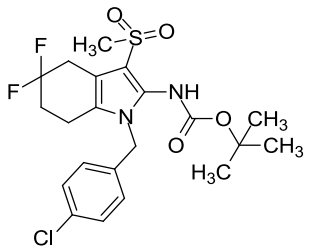
To a solution of ethyl 1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylate (**6.42**) (1.4 g, 3.2 mmol) in a mixture of THF/Water 50/50 mL, was added LiOH.H_2O (0.54 g, 13 mmol) and the reaction mixture was stirred for 16 h. LCMS Neutral after that time showed reaction completion. A 1N aqueous solution of HCl and EtOAc were added to the reaction mixture, phases were separated, and the organic layer was washed with brine, dried over $MgSO_4$, filtered, and the solvent was evaporated under vacuum to give 1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**6.43**) (1.3 g, 99% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 13.89 (br. s., 1H), 7.43 (d, *J*=8.3 Hz, 2H), 7.06 (d, *J*=8.6 Hz, 2H), 5.42 (s, 2H), 3.30 (s, 3H), 3.19-3.30 (m, 2H), 2.64 (t, *J*=6.4 Hz, 2H), 2.14-2.33 ppm (m, 2H).

^{13}C NMR (DMSO-d_6, 101 MHz): δ = 161.5, 136.1, 132.4, 130.7, 129.2, 128.7, 126.6, 124.1(t, *J*=239.4 Hz), 117.2, 115.0, 48.4, 45.2, 32.7 (t, *J*=27.1 Hz), 29.6 (t, *J*=25.6 Hz), 19.3 (t, *J*=5.8 Hz) ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): t_R=0.96 min, $[M-H]^-$ 402 (>95% purity).

***tert*-Butyl (1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)carbamate (6.44)**

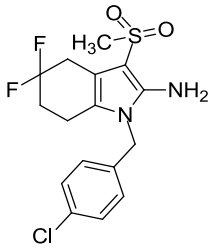


1-(4-Chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indole-2-carboxylic acid (**6.43**) (1.3 g, 3.2 mmol), diphenyl phosphorazidate (0.8 mL, 3.5 mmol), and Et_3N (0.5 mL, 3.5 mmol) were added to a round-bottomed flask with *tert*-butanol (35 mL) previously warmed at 25-30 ºC (to avoid the solidification of the *tert*-butanol). After the addition, the reaction mixture was warmed to 90 ºC, and stirred for 21 h at this temperature, after which time LCMS Neutral showed reaction completion. The heating was stopped and the reaction mixture was cooled to room temperature. The reaction mixture was concentrated to give a pale orange syrup. The syrup was dissolved in a little amount of methanol, water was added, and a solid precipitated, which was triturated, and filtered to give a mixture of *t*ert-butyl (1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)carbamate (**6.44**) (1.6 g) and the boc-deprotected derivative (**6.45**) as a pale pink solid. This crude product was used in the next step as a mixture with **6.45**.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 9.01 (br. s., 1H), 7.40 (d, *J*=8.3 Hz, 2H), 7.15 (d, *J*=8.1 Hz, 2H), 4.99 (s, 2H), 3.11-3.23 (m, 2H), 3.06 (s, 3H), 2.53 (br. s., 2H), 2.10-2.28 (m, 2H), 1.40 ppm (br. s., 9H).

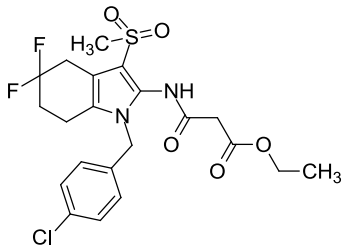
LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.30 min, $[M-H]^-$ 473 (>85% purity).

1-(4-Chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-amine (6.45)



tert-Butyl (1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)carbamate (**6.44**) in a mixture with **6.45** (1.6 g, 3.37 mmol) was dissolved in DCM (10 mL) and TFA (2 mL) was added. The reaction mixture was stirred at room temperature for 2 h and LCMS Neutral showed starting material remaining. More TFA (2 mL) was added and the reaction mixture was stirred at room temperature for 1 more hour. LCMS Neutral after that time showed that the reaction had almost reached completion. The solvent and TFA were evaporated under vacuum to give a crude material that was loaded into a SCX column, previously washed with MeOH. The column wast rinsed with MeOH to give 1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-amine (**6.45**) (850 mg, 67% yield) with some impurities (by LCMS Neutral) as an orange sticky oil. The product was used in the next step without any further purification.

Ethyl 3-((1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)amino)-3-oxopropanoate (6.46)

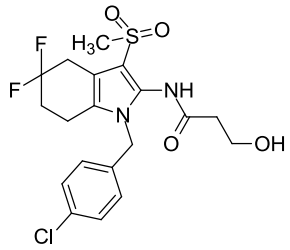


To a solution of 1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-amine (**6.45**) (100 mg, 0.27 mmol) in 2-MeTHF (10 mL) at 0 ºC were added anhydrous pyridine (0.022 mL, 0.27 mmol) and ethyl malonyl chloride (0.034 mL, 0.27 mmol). The reaction mixture was stirred at 0 ºC for 5 min. LCMS Neutral showed reaction completion. The reaction was partitioned between EtOAc and H_2O, the aqueous phase was extracted with EtOAc (x2), The organic phases were put together and washed with H_2O, brine, and dried over Na_2SO_4, filtered, and concentrated under

vacuum to give a crude material that corresponded to desired product with some impurities. This crude material was purified on a silica gel cartridge (0-50% Cy/EtOAc) to give ethyl 3-((1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)amino)-3-oxopropanoate (**6.46**) (38 mg, 29% yield) as a white solid.

^{1}H NMR (DMSO-d_6, 400 MHz): δ = 10.14 (s, 1H), 7.42 (d, *J*=8.3 Hz, 2H), 7.15 (d, *J*=8.3 Hz, 2H), 5.02 (s, 2H), 4.06 (q, *J*=7.1 Hz, 2H), 3.45 (s, 2H), 3.10-3.23 (m, 2H), 3.04 (s, 3H), 2.51-2.57 (m, 2H), 2.18 (br. s., 2H), 1.12 ppm (t, *J*=7.2 Hz, 3H).

_N_-(1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1_H_-indol-2-yl)-3-hydroxypropanamide (6.28)



Ethyl 3-((1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)amino)-3-oxopropanoate (**6.46**) (35 mg, 0.07 mmol) was dissolved in 2-MeTHF (1 mL) and MeOH (0.01 mL, 0.3 mmol). To this solution $LiBH_4$ (6.2 mg, 0.3 mmol) was added and the resulting solution was stirred at r.t. overnight. After this time, LCMS Neutral showed reaction completion. The reaction was treated with a 1N aqueous solution of HCl at 0 ºC, EtOAc was added, layers were separated, and the aqueous layer was extracted with EtOAc. The combination of the organic layers was washed with brine, dried over anhydrous $MgSO_4$, filtered, and concentrated under vacuum to give a crude material that was purified on a silica gel cartridge (Cy/EtOAc 0-65%) to give *N*-(1-(4-chlorobenzyl)-5,5-difluoro-3-(methylsulfonyl)-4,5,6,7-tetrahydro-1*H*-indol-2-yl)-3-hydroxypropanamide (15 mg, 47% yield) as a white solid.

^{1}H NMR ($CDCl_3$, 400 MHz): δ = 8.27 (s, 1H), 7.33 (d, *J*=8.3 Hz, 2H), 6.99 (d, *J*=8.6 Hz, 2H), 4.97 (s, 2H), 3.95 (q, *J*=5.6 Hz, 2H), 3.12-3.25 (m, 3H), 3.09 (s, 3H), 2.53-2.63 (m, 4H), 2.20 ppm (tt, *J*=13.3, 6.5 Hz, 2H).

^{13}C NMR ($CDCl_3$, 101 MHz): δ = 173.5, 134.1, 133.9, 129.3, 127.9, 127.8, 124.9 (t, *J*=242.3 Hz), 122.6, 112.9, 112.3, 59.2, 46.9, 44.5, 38.8, 32.2 (t, *J*=29.2 Hz), 30.2 (t, *J*=25.6 Hz), 19.1 (t, *J*=5.8 Hz) ppm.

LCMS (Acetate NH_4 25 mM + 10% ACN at pH 6.6 /ACN 0.0 – 0.2 min 99.9: 0.1; 0.2 – 1.0 min 10:90; 1.0 – 1.8 min 10:90; 1.9 – 2.0 min 99.9:0.1; Flow: 0.8 mL/min): $t_{R=}$1.08 min, $[M-H]^-$ 445 (>95% purity).

IR ν_{max} (neat) cm^{-1}: 3567.1, 3271.5, 2924.8, 1686.4, 1513.2, 1495.3, 1280.4, 1103.6, 973.4, 800.0.

M.P. Decomposes at 71.3 ºC

HRMS (ES) calcd for $C_{19}H_{21}{}^{35}ClF_2N_2NaO_4S$, $(M + Na)^+$ 469.0771 found 469.0769.

10. BIBLIOGRAPHIC REFERENCES

(1) Wells, T. N. C.; Alonso, P. L.; Gutteridge, W. E. *Nat. Rev. Drug Discov.* **2009**, *8*, 879–891.

(2) WHO. World Malaria Report 2017 http://www.who.int/malaria/publications/world_malaria_report_2017/en/ (accessed Apr 26, 2018).

(3) MMV.Parasite cycle /www.mmv.org/aboutus/ (accessed Oct 6, 2014).

(4) Dondorp, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithatpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; Shunmay, Y.; An, S. S.; Singhasivanom, P.; Day, N. P. J.; Lindegardh, N.; Socheat, D.; White, N. J. *N. Engl. J. Med.* **2009**, *361*, 455–467.

(5) Calderón, F.; Wilson, D.; Gamo, F. J. In *Progress in Medicinal Chemistry, Vol 52*; Elsevier B.V.: Amsterdam, 2013; pp 97–143.

(6) Achan, J.; Talisuna, A. O.; Erhart, A.; Yeka, A.; Tibenderana, J. K.; Baliraine, F. N.; Rosenthal, P. J.; D'Alessandro, U. *Malar. J.* **2011**, *10*, 144.

(7) Castelli, F.; Odolini, S.; Autino, B.; Foca, E.; Russo, R. *Pharmaceuticals* **2010**, *3*, 3212–3239.

(8) Foley, M.; Tilley, L. *Pharmacol. Ther.* **1998**, *79*, 55–87.

(9) Visser, B. J.; van Vugt, M.; Grobusch, M. P. *Expert Opin. Pharmacother.* **2014**, 1–36.

(10) Tafenoquine-Clinical trials Phase III https://us.gsk.com/en-us/media/press-releases/2014/gsk-and-mmv-announce-start-of-phase-iii-programme-of-tafenoquine-for-plasmodium-vivax-malaria/ (accessed Jan 9, 2016).

(11) Barnett, D. S.; Guy, R. K. *Chem. Rev.* **2014**, *114*, 11221–11241.

(12) Omodeo-Salè, F.; Cortelezzi, L.; Basilico, N.; Casagrande, M.; Sparatore, A.; Taramelli, D. *Antimicrob. Agents Chemother.* **2009**, *53*, 4339–4344.

(13) Nidhi, K.; Indrajeet, S.; Khushboo, M.; Gauri, K.; Sen, D. J. *Int. J. Drug Dev. Res.* **2011**, *3*, 26–33.

(14) GSK source http://www.gsksource.com/gskprm/en/US/adirect/gskprm?cmd=ProductDetailPa g e&product_id=1244172404936&featureKey=600601 (accessed Feb 14, 2015).

(15) Färnert, A.; Lindberg, J.; Gil, P.; Swedberg, G.; Berqvist, Y.; Thapar, M. M.; Lindegårdh, N.; Berezcky, S.; Björkman, A. *Brit. Med. J.* **2003**, *326*, 628–629.

(16) Schwartz, E.; Bujanover, S.; Kain, K. C. *Clin. Infect. Dis.* **2003**, *37*, 450–451.

(17) Barton, V.; Fisher, N.; Biagine, G. A.; Ward, S. A.; O'Neill, P. M. *Sci. Direct* **2010**, *14*, 440–446.

(18) Zara, V.; Conte, L.; Trumpower, B. L. *FEBS J.* **2009**, *276*, 1900–1914.

(19) Olliaro, P. *Pharmacol. Ther.* **2001**, *89*, 207–219.

(20) Hyde, J. E. *Acta Trop.* **2005**, *94*, 191–206.

(21) Chulay, J. D.; Atkins, W. M.; Sixsmith, D. G. *Am. J. Trop. Med. Hyg.* **1984**, *33*, 325–330.

(22) Zheng, W.; Jiang, H.; Xiong, Z.; Jiang, Z.; Chen, H. *Iran J Parasitol* **2013**, *8*, 1–17.

(23) Neill, P. M. O.; Barton, V. E.; Ward, S. A. *Molecules* **2010**, *15*, 1705–1721.

(24) Posner, G. H.; Wang, D.; Cumming, J. N.; Oh, C. H.; French, A. N.; Bodley, A. L.; Shapiro, T. A. *J. Med. Chem.* **1995**, *38*, 2273–2275.

(25) Posner, G. H.; Oh, C. H. *J. Am. Chem. Soc.* **1992**, *114*, 8328–8329.

(26) Posner, G. H.; Oh, C. H.; Wang, D.; Gerena, L.; Milhous, W. K.; Meshnick, S. R.; Asawamahasadka, W. *J. Med. Chem.* **1994**, *37*, 1256–1258.

(27) Jefford, C. W.; Favarger, F.; Vicente, M. G. H.; Jacquier, Y. *Helv. Chim. Acta* **1995**, *78*, 452–458.

(28) Jefford, C. W.; Vicente, M. G. H.; Jacquier, Y.; Favarger, F.; Mareda, J.; Millasson-Schmidt, P.; Brunner, G.; Burger, U. *Helv. Chim. Acta* **1996**, *79*, 1475–1487.

(29) Butler, A. R.; Gilbert, B. C.; Hulme, P.; Irvine, L. R.; Whitwood, A. C. *Free Radic. Res.* **1998**, *28*, 471–476.

(30) Neill, P. M. O.; Bishop, L. P. D.; Searle, N. L.; Maggs, J. L.; Storr, R. C.; Ward, S. A.; Park, B. K.; Mabbs, F. *J. Org. Chem.* **2000**, *65*, 1578–1582.

(31) Wu, W.-M.; Wu, Y.; Wu, Y.-L.; Yao, Z.-J.; Zhou, C.-M.; Li, Y.; Shan, F. *J. Am. Chem. Soc.* **1998**, *120*, 3316–3325.

(32) Haynes, R. K.; Chan, C.; Lung, C.; Uhlemann, C.; Eckstein, U.; Taramelli, D.; Parapini, S.; Monti, D.; Krishna, S. *ChemMedChem* **2007**, *2*, 1480–1497.

(33) Haynes, R. K.; Vonwiller, S. C. *Tetrahedron Lett.* **1996**, *37*, 253–256.

(34) Haynes, R. K.; Hung-On Pai, H.; Voerste, A. *Tetrahedron* **1999**, *40*, 4715–4718.

(35) Haynes, R. K.; Vonwiller, S. C. *Tetrahedron Lett.* **1996**, *37*, 257–260.

(36) Ma, J.; Song, W.; Chen, C.; Ma, W.; Zhao, J.; Tang, Y. *Environ. Sci. Technol.* **2005**, *39*, 5810–5815.

(37) Uhlemann, A.; Fidock, D. *Lancet* **2012**, *379*, 1928–1930.

(38) Trenholme, C. M.; Williams, R. L.; Desjardins, R. E.; Frischer, H.; Carson, P. E.; Rieckmann, K. H.; Canfield, C. J. *Science (80-.).* **1975**, *190*, 792–794.

(39) Ridley, R. G. *Nature* **2002**, *415*, 686–693.

(40) Clinical trials of malaria worldwide © Clinicaltrials.gov https://clinicaltrials.gov/ (accessed Apr 18, 2018).

(41) NIAID http://www.niaid.nih.gov/Pages/default.aspx (accessed Feb 14, 2015).

(42) LSHTM http://malaria.lshtm.ac.uk/about-us (accessed Feb 14, 2015).

(43) University of Oxford http://www.tropicalmedicine.ox.ac.uk/home (accessed Feb 14, 2015).

(44) Burrows, J. N.; Burlot, E.; Campo, B.; Cherbuin, S.; Jeanneret, S.; Leroy, D.; Spangenberg, T.; Waterson, D.; Wells, T. N.; Willis, P. *Parasitology* **2014**, *141*, 128–139.

(45) NITD609 http://www.malaria.com/tag/nitd609 (accessed Jun 28, 2015).

(46) van Pelt-Koops, J. C.; Pett, H. E.; Graumans, W.; van Der Vegte-Bolmer, M.; van Gemert, G. J.; Rottmann, M.; Yeung, B. K. S.; Diagana, T. T.; Sauerwein, R. W. *Antimicrob. Agents Chemother.* **2012**, *56*, 3544–3548.

(47) NITD609_MMV http://www.mmv.org/sites/default/files/uploads/docs/events/2012/Stakeholder_meeting_presentations/Diagana_NITD609.pdf (accessed Jun 28, 2015).

(48) Flannery, E. L.; Chatterjee, A. K.; Winzeler, E. A. *Nat. Rev. Microbiol.* **2013**, *11*, 849–862.

(49) Coteron, J. M.; Marco, M.; Esquivias, J.; Deng, X.; White, K. L.; White, J.; Koltun, M.; El Mazouni, F.; Kokkonda, S.; Katneni, K.; Bhamidipati, R.; Shackleford, D. M.; Angulo-Barturen, I.; Ferrer, S. B.; Jiménez-Díaz, M. B.; Gamo, F. J.; Goldsmith, E. J.; Charman, W. N.; Bathurst, I.; Floyd, D.; Matthews, D.; Burrows, J. N.; Rathod, P. K.; Charman, S. A.; Phillips, M. A. *J. Med. Chem.* **2011**, *54*, 5540–5561.

(50) P218_MMV http://www.mmv.org/research-development/project-portfolio/p218 (accessed Jun 28, 2015).

(51) Yuthavong, Y.; Tarnchompoo, B.; Vilaivan, T.; Chitnumsub, P.; Kamchonwongpaisan, S.; Charman, S. A.; McLennan, D. N.; White, K. L.;

Vivas, L.; Bongard, E.; Thongphanchang, C.; Taweechai, S.; Vanichtanankul, J.; Rattanajak, R.; Arwon, U.; Fantauzzi, P.; Yuvaniyama, J.; Charman, W. N.; Matthews, D. *Proc. Natl. Acad. Sci.* **2012**, *109*, 16823–16828.

(52) GSK Malaria Vaccine https://connect.gsk.com/sites/gskglobal/Pages/oc-our-stories-malaria.aspx (accessed Jan 9, 2016).

(53) Biernaux, S. *GSK, Malaria Vaccine Franchise*.

(54) Malaria Vaccine Initiative (MVI) http://www.malariavaccine.org/ (accessed Apr 18, 2018).

(55) Trends in reported malaria incidence ©World Health Organization http://www.who.int/en/ (accessed Apr 18, 2018).

(56) Kola, I.; Landis, J. *Nat. Rev. Drug Discov.* **2004**, *3*, 711–715.

(57) Lipinski, C. A.; Lombardo, F.; Dominy, B. W.; Feeney, P. J. *Adv. Drug Deliv. Rev.* **2001**, *46*, 3–26.

(58) Veber, D. F.; Johnson, S. R.; Cheng, H.; Smith, B. R.; Ward, K. W.; Kopple, K. D. *J. Med. Chem.* **2002**, *45*, 2615–2623.

(59) Ghose, A. K.; Viswanadhan, V. N.; Wendoloski, J. J. *J. Comb. Chem.* **1999**, *1*, 55–68.

(60) Gleeson, M. P. *J. Med. Chem.* **2008**, *51*, 817–834.

(61) Johnson, T. W.; Dress, K. R.; Edwards, M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 5560–5564.

(62) Hughes, J. D.; Blagg, J.; Price, D. A.; Bailey, S.; DeCrescenzo, G. A.; Devraj, R. V.; Ellsworth, E.; Fobian, Y. M.; Gibbs, M. E.; Gilles, R. W.; Greene, N.; Huang, E.; Krieger-Burke, T.; Loesel, J.; Wager, T.; Whiteley, L.; Zhang, Y. *Bioorganic Med. Chem. Lett.* **2008**, *18*, 4872–4875.

(63) Price, D. A.; Blagg, J.; Jones, L.; Greene, N.; Wager, T. *Expert Opin. Drug Metab. Toxicol.* **2009**, *5*, 921–931.

(64) Muthas, D.; Boyer, S.; Hasselgren, C. *Medchemcomm* **2013**, *4*, 1058–1065.

(65) Lipinski, C. *Nat. Rev. Drug Discov.* **2005**, *4*, 184.

(66) Varma, M. V. S.; Obach, R. S.; Rotter, C.; Miller, H. R.; Chang, G.; Steyn, S. J.; El-Kattan, A.; Troutman, M. D. *J. Med. Chem.* **2010**, *53*, 1098–1108.

(67) Sugano, K.; Kansy, M.; Artursson, P.; Avdeef, A.; Bendels, S.; Di, L.; Ecker, G. F.; Faller, B.; Fischer, H.; Gerebtzoff, G.; Lennernaes, H.; Senner, F. *Nat. Rev. Drug Discov.* **2010**, *9*, 597–614.

(68) Hopkins, A. L.; Groom, C. R.; Alex, A. *Drug Discov. Today* **2004**, *9*, 430–431.

(69) Leeson, P. D.; Springthorpe, B. *Nat. Rev. Drug Discov.* **2007**, *6*, 881–890.

(70) Young, R. J.; Green, D. V. S.; Luscombe, C. N.; Hill, A. P. *Drug Discov. Today* **2011**, *16*, 822–830.

(71) Ritchie, T. J.; MacDonald, S. J. F.; Young, R. J.; Pickett, S. D. *Drug Discov. Today* **2011**, *16*, 164–171.

(72) Lin, J. H.; Lu, A. Y. H. *Pharmacol. Rev.* **1997**, *49*, 403–449.

(73) Vertzoni, M.; Dressman, J.; Butler, J.; Hempenstall, J.; Reppas, C. *Eur. J. Pharm. Biopharm.* **2005**, *60*, 413–417.

(74) Kestranek, A.; Chervenak, A.; Longenberger, J.; Placko, S. *Curr. Protoc. Chem. Biol.* **2013**, *5*, 269–280.

(75) Balimane, P. V; Han, Y. H.; Chong, S. *AAPS J.* **2006**, *8*, E1–E13.

(76) Obach, R. S. *Drug Metab. Dispos.* **1999**, *27*, 1350–1359.

(77) Chiba, M.; Ishii, Y.; Sugiyama, Y. *AAPS J.* **2009**, *11*, 262–276.

(78) Duffus, J. H.; Nordberg, M.; Templeton, D. M. *Pure Appl. Chem.* **2007**, *79*, 1153–1344.

(79) Urso, R.; Blardi, P.; Giorgi, G. *Eur. Rev. Med. Pharmacol. Sci.* **2002**, *6*, 33–44.

(80) Stella, V. J.; Charman, W. N. A.; Naringrekar, V. H. **1985**, *473*, 455–473.

(81) Alley, S. C.; Okeley, N. M.; Senter, P. D. *Curr. Opin. Chem. Biol.* **2010**, *14*, 529–537.

(82) Esterase sensitive motif technology http://www.life-sciences-europe.com/product/esm-technology-esterase-sensitive-macrophage-pharma-ltd-therapeutics-2001-19162.html (accessed Oct 29, 2017).

(83) Stevenage. *GSK, Unpublished results. Internal guideline for candidate selection.*

(84) *GSK, Unpublished results. Aminoindole project team.*

(85) Soci.org http://www.soci.org/~/media/Files/Conference Downloads/2013/Choosing the Right Target May 2013/Graeme_Walker_Presentation.ashx (accessed Apr 11, 2015).

(86) Gamo, F. J.; Sanz, L. M.; Vidal, J.; de Cozar, C.; Alvarez, E.; Lavandera, J. L.; Vanderwall, D. E.; Green, D. V. S.; Kumar, V.; Hasan, S.; Brown, J. R.; Peishoff, C. E.; Cardon, L. R.; Garcia-Bustos, J. F. *Nature* **2010**, *465*, 305–310.

(87) Fernández, E.; Gamo, F. J.; Lavandera, J. L.; León, M. L.; Macdonald, S. J. F.; Mallo, A.; Manzano, P.; Porras, E.; Fiandor, J. M.; Castro, J. *ACS Med. Chem. Lett.* **2011**, *2*, 741–746.

(88) ebi.ac www.ebi.ac.uk/chemblntd (accessed Feb 14, 2015).

(89) MMV http://www.mmv.org./ (accessed Apr 18, 2018).

(90) Spillman, N. J.; Allen, R. J. W.; McNamara, C. W.; Yeung, B. K. S.; Winzeler, E. A.; Diagana, T. T.; Kirk, K. *Cell Host Microbe* **2013**, *13*, 227–237.

(91) Jiménez-díaz, M. B.; Ebert, D.; Salinas, Y.; Pradhan, A.; Lehane, A. M.; Myrand-Lapierre, M.-E.; O'Loughlin, K. G.; Shackleford, D. M.; Almeida, M. J. De; Carrillo, A. K.; Clark, J. A.; Dennis, A. S. M.; Diep, J.; Deng, X.; Duffy, S.; Endsley, A. N.; Fedewa, G.; Guiguemde, A. W.; Gómez, M. G.; Holbrook, G.; Horst, J.; Kim, C. C.; Liu, J.; Lee, M. C. S.; Matheny, A.; Martínez, M. S.; Miller, G.; Rodríguez-Alejandre, A.; Sanz, L. M.; Sigal, M.; Spillman, N. J.; Stein, P. D.; Wang, Z.; Zhu, F.; Waterson, D.; Knapp, S.; Shelat, A.; Avery, V. M.; Fidock, D. A.; Gamo, F. J.; Charman, S. A.; Mirsalis, J. C.; Ma, H.; Ferrer, S. B.; Kirk, K.; Angulo-Barturen, I.; Kyle, D. E.; DeRisi, J. L.; Floyd, D. M.; Guy, R. K. *Proc. Natl. Acad. Sci.* **2015**, *112*, E5764–E5764.

(92) León, M. *GSK, Unpublished results*.

(93) FDA http://www.fda.gov/downloads/drugs/guidancecomplianceregulatoryinformation/guidances/ucm074929.pdf (accessed Apr 11, 2015).

(94) OECD http://www.oecd.org/chemicalsafety/risk-assessment/1948418.pdf (accessed Apr 11, 2015).

(95) Jiménez-Díaz, M. B.; Mulet, T.; Viera, S.; Gómez, V.; Garuti, H.; Ibáñez, J.; Alvarez-Doval, A.; Shultz, L. D.; Martínez, A.; Gargallo-Viola, D.; Angulo-Barturen, I. *Antimicrob. Agents Chemother.* **2009**, *53*, 4533–4536.

(96) Suzuki, H.; Abe, H. *Tetrahedron Lett.* **1995**, *36*, 6239–6242.

(97) García, M.; Gordo, M. *GSK, Unpublished results*.

(98) Fernández, J.; Gordo, M. *GSK, Unpublished results*.

(99) Surry, D. S.; Buchwald, S. L. *Chem. Sci.* **2011**, *2*, 27–50.

(100) Patil, S. M.; Kulkarni, S.; Mascarenhas, M.; Sharma, R.; Roopan, S. M.; Roychowdhury, A. *Tetrahedron* **2013**, *69* (38), 8255–8262.

(101) Gordo, M. *GSK, Unpublished results*.

(102) Balsamini, C.; Bedini, A.; Diamantini, G.; Spadoni, G.; Tontini, A.; Tarzia, G.; Fabio, R. Di; Feriani, A.; Reggiani, A.; Tedesco, G.; Valigi, R. *J. Med. Chem.* **1998**, *41*, 808–820.

(103) Miyaura, N.; Suzuki, A. *Chem. Rev.* **1995**, *95*, 2457–2483.

(104) Bioduro. *GSK, Unpublished results*.

(105) Fernández, J. *GSK, Unpublished Results*.

(106) Purser, S.; Moore, P. R.; Swallow, S.; Gouverneur, V. V. *Chem. Soc. Rev.* **2008**, *37*, 320–330.

(107) García, M. *GSK, Unpublished Results*.

(108) Nassar, A. E. F.; Kamel, A. M.; Clarimont, C. *Drug Discov. Today* **2004**, *9*, 1055–1064.

(109) Thompson, T. N. *Med. Res. Rev.* **2001**, *21*, 412–449.

(110) De la Rosa, J. C. *GSK, Unpublished Results*.

(111) Puente-Felipe, M. *GSK, Unpublished Results*.

(112) Nakanishi, S.; Otsuji, Y.; Itoh, K.; Hayashi, N. *Bull. Chem. Soc. Jpn.* **1990**, *63*, 3595–3600.

(113) Martín Hernando, J. I. *GSK, Unpublished Results*.

(114) Ngwerume, S.; Camp, J. E. *Chem. Commun.* **2011**, *47*, 1857–1859.

(115) Ngwerume, S.; Lewis, W.; Camp, J. E. *J. Org. Chem.* **2013**, *78*, 920–934.

(116) Gellibert, F.; de Gouville, A.-C.; Woolven, J.; Mathews, N.; Nguyen, V.-L.; Bertho-Ruault, C.; Patikis, A.; Grygielko, E. T.; Laping, N. J.; Huet, S. *J. Med. Chem.* **2006**, *49*, 2210–2221.

(117) Chakravorty, S. *GSK, Unpublished results*.

(118) Kumar, A. S.; Amulya Rao, P. V.; Nagarajan, R. *Org. Biomol. Chem.* **2012**, *10*, 5084.

(119) Fors, B. P.; Dooleweerdt, K.; Zeng, Q.; Buchwald, S. L. *Tetrahedron* **2009**, *65*, 6576–6583.

(120) Beattie, K. A.; Luscombe, C.; Williams, G.; Muñoz-Muriedas, J.; Gavaghan, D. J.; Cui, Y.; Mirams, G. R. *J. Pharmacol. Toxicol. Methods* **2013**, *68*, 88–96.

(121) Jiménez-Díaz, M. B.; Mulet, T.; Gómez, V.; Viera, S.; Alvarez, A.; Garuti, H.; Vázquez, Y.; Fernández, A.; Ibáñez, J.; Jiménez, M.; Gargallo-Viola, D.; Angulo-Barturen, I. *Cytometry. A* **2009**, *75*, 225–235.

(122) Gill, P.; Moghadam, T. T.; Ranjbar, B. *J. Biomol. Tech.* **2010**, *21*, 167–193.

(123) Desjardins, R. E.; Canfield, C. J.; Haynes, J. D.; Chulay, J. D. *Antimicrob. Agents Chemother.* **1979**, *16*, 710–718.

(124) Redfern, W. S.; Carlsson, L.; Davis, A. S.; Lynch, W. G.; Mackenzie, I.; Palethorpe, S. *Cardiovasc. Res.* **2003**, *58*, 32–45.

(125) Valentin, J. P. *Br. J. Pharmacol.* **2010**, *159*, 5–11.

(126) Viskin, S.; Rosovski, U. *Eur. Heart J.* **2005**, *26*, 536–537.

(127) Mathes, C.; Friis, S.; Finley, M.; Liu, Y. **2009**, *12*, 78–95.

(128) Schmalhofer, W. A.; Swensen, A. M.; Thomas, B. S.; Felix, J. P.; Haedo, R. J.; Solly, K.; Kiss, L.; Kaczorowski, G. J.; Garcia, M. L. *Assay Drug Dev. Technol.* **2010**, *8*, 714–726.

(129) Crouch, S. P. M.; Kozlowski, R.; Slater, K. J.; Fletcher, J. *Elsevier Sci. Publ.* **1993**, *160*, 81–88.

(130) Stappaerts, J.; Wuyts, B.; Tack, J.; Annaert, P.; Augustijns, P. *Eur. J. Pharm. Sci.* **2014**, *63*, 178–186.

(131) Valkó, K.; Bevan, C.; Reynolds, D. *Anal. Chem.* **1997**, *69* (11), 2022–2029.

(132) Bergeron, P.; Bodil Van Niel, M.; Dragovich, P.; Hurley, C.; Kulagowski, J.; Labadie, S.; McLean, N. J.; Mendonca, R.; Pulk, R.; Zak, M. WO 2013/007765 A1, 2013.

(133) Siles, J. M. *GSK, Unpublished results*.