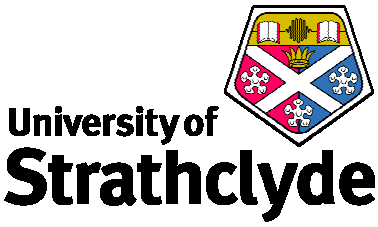
SYNTHESIS OF PYRROLO[2,3-*d*]PYRIMIDINES AND PYRAZINO[2,3-*d*]PYRIMIDINES AND THEIR BIOLOGICAL ACTIVITIES

By

JEONG-HWAN LEE



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ABSTRACT

Pyrrolo[2,3-*d*]pyrimidines and pyrazino[2,3-*d*]pyrimidines were synthesised to investigate their biological activities to eventually produce potential new antifolates. Funtionalised nitroalkenes were successfully added to the C5 of 2-thioalkyl-6-amino-4-oxopyrimidines *via* a Michael reaction. Subsequent cyclisation was accomplished by a Nef reaction using a titanium(III) chloride mediated system. Displacement of the thioalkyl moiety at C2 was also successful *via* a modified oxidation-substitution methodology, obtaining crude sulfone by oxidation followed by reaction in neat amine. 6,7-Functionalised pteridines were prepared by the Isay cyclocondensation. The thiobenzyl group at C4 of prepared pteridines was effectively displaced by five different amines without an oxidation step. Microwave assisted reaction reduced the reaction times and produced a library of 25 compounds in moderate to good yields. Cytotoxic, antibacterial and antiparasitic activities were screened for thirty pteridines and eight 7-deazapurines. In many cases, pteridines having 2-chlorophenyl groups at C6 and C7 exhibited cell growth inhibition for cancer/normal cells and bacterial cells. The majority of compounds had antiparasitic activity for *T. b. brucei* and four pyrrolo[2,3-*d*]pyrimidines had encouraging IC_{50}s (12.5 ~ 50 μM) depending on the functional group at the C2 position.

Through a modeling study, a deazaguanine was selected as an ideal framework to design potential inhibitors for trypanosomatid parasites' pteridine reductase 1 (PTR1). Two major hydrophobic pockets were found at the C5 and C6 positions of a deazaguanine scaffold. To occupy hydrophobic pockets in the active sites of trypanosomal PTR1, phenethyl and phenyl groups were introduced at the C5 and C6, respectively. Six 4-thiobenzyl pyrimidines were also synthesised to investigate for their inhibitory activities for PTR1. Two deazaguanines and six pyrimidines were examined for inhibitory activity for *L. major* and *T. brucei* PTR1. Two deazaguanines exhibited 70% inhibition at 10 μM for *T. brucei* but only less than 30% inhibitory activities for *L. major* were found. Six pyrimidines showed better inhibitory activities (45 ~ 61%) for *L. major* PTR1 than the two deazaguanines but lower activities (2 ~ 38%) were found for *T. brucei* PTR1.

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encouragement by two great women, my mother, Soon-Bok Hong, and elder sister, Cha-Sook Lee, this journey would not have been be easy.

ABBREVIATIONS

AMT	Aminopterin
AIDS	Acquired immune deficiency syndrome
BH_2	Dihydrobiopterin
BH_4	Tetrahydrobiopterin
CI	Chemical ionisation
CL	Cutaneous leishmaniasis
CNS	Central nervous system
DBU	1,8-Diazabicyclo[5.4.0]undec-7-ene
DCM	Dichloromethane
DHF	7,8-Dihydrofolate
DHFR	Dihydrofolate reductase
DHFS	Dihydrofolate synthase
DHNA	Dihydroneopterin aldolase
DHPS	Dihydropteorate synthase
DMDO	Dimethyldioxirane
DMF	Dimethylformamide
DMSO	Dimethyl sulfoxide
DNA	Deoxyribonucleic acid
dTMP	Deoxythymidine 5'-monophosphate
dUMP	Deoxyuridine 5'-monophosphate
E. coli	*Escherichia coli*
EI	Electron impact
ESI	Electron Spray Ionisation
FAB	Fast atom bombardment
FDA	Food and Drug Administration
FPGS	Folylpolyglutamate synthase
FtsZ	Filamenting temperature-sensitive mutant Z
GTP	Guanosine triphosphate
GTP-CH-I	Guanosine triphosphate cyclohydrolase I
HAT	Human African trypanosomiasis

HCV	Hepatitis C virus
HMBC	Heteronuclear multiple bond correlation
HPLC	High-performance liquid chromatography
HPPK	7,8-Dihydro-6-hydroxymethylpterin pyrophosphokinase
HRMS	High resolution mass spectrometry
IC_{50}	The half maximal (50%) inhibitory concentration (IC) of a substance
IPA	*iso*-Propyl alcohol
IR	Infrared
L. m.	*Leishmania major*
LRMS	Low resolution mass spectrometry
MCL	Mucocutaneous leishmaniasis
m-CPBA	*Meta*-Chloroperbenzoic acid
MDR	Multi-drug resistance
mp	Melting point
MTX	Methotrexate
NADPH	Reduced nicotinamide adenine dinucleotide phosphate
NMR	Nuclear magnetic resonance
pABA	*Para*-aminobenzoic acid
PCC	Pyridinium chlorochromate
ppm	Parts per million
PTC	Phase transfer catalyst
PTR1	Pteridine reductase 1
PTR2	Pteridine reductase 2
PTPS	6-Pyruvoyltetrahydropterin synthase
PTX	Piritrexim
RFC	Reduced folate carrier
RNA	Ribonucleic acid
rt	Room temperature
SHMT	Serine hydroxymethyltransferase
SR	Sepiapterin reductase
T. b.	*Trypanosoma brucei*
T. c.	*Trypanosoma cruzi*

TFA	Trifluoroacetic acid
THF	5,6,7,8-Tetrahydrofolate or Tetrahydrofuran
TLC	Thin layer chromatography
TMP	Trimethoprim
TS	Thymidylate synthase
VL	Visceral leishmaniasis

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1. INTRODUCTION

This thesis is focused on discovery of new potential antifolates to produce new drugs for treatment of cancer, bacterial and parasitic diseases.

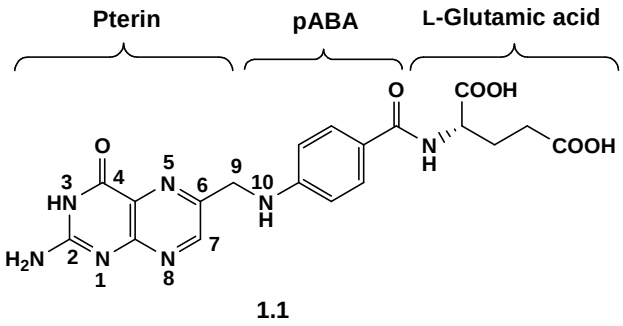
As an introduction of this thesis, coverage of the following will be given: biochemical background based on folate biosynthesis (Section 1.1), strategies for development of antifolates (Section 1.2), trypanosomal diseases, and pteridine reductase 1 (PTR1) as a main biological target (Section 1.3), synthetic review for pyrimidine based 6-5 and 6-6 heterocyclic antifolates (Section 1.4), and finally, a project overview (Section 1.5).

1.1. Biochemical outline

In this section, the importance of folic acid in living organisms, its biosynthetic pathway (Section 1.1.1), and enzymes involving its biosynthesis (Section 1.1.2) will be outlined.

1.1.1. Folic acid and its biosynthesis

Folic acid **1.1** consists of three building blocks: a pterin, *p*-aminobenzoic acid (pABA) and glutamic acid (Fig. 1.1).



1.1

Figure 1.1. Structure of folic acid.

Folic acid **1.1** is biosynthesised and is found in green plants and microorganisms but it was first discovered when some lactic acid bacteria (*Streptococcus lactis*) were found to require an essential growth factor isolated from green leaves,[1] and other sources, which was ultimately shown to be folic acid **1.1**.[2]

Folate is a generic name of folic acid derivatives. Mammalian cells do not synthesise folates which are acquired from dietary intake. The *de novo* biosynthetic pathway of pterins and folates is well known (Fig. 1.2). The folate biosynthesis pathway is a particular therapeutic target not only because of crucial biological role of folate but also mammalian inability of its biosynthesis.

Several microorganisms and higher eukaryotic cells have the ability to synthesise pterin derivatives *de novo* from GTP (guanosine triphosphate), although in mammals a reduced pterin in the form of tetrahydrobiopterin (BH_4) is formed.[3] The first and rate-limiting step is catalysed by GTP cyclohydrolase I (GTP-CH-I). G. Auerbach *et al.*[4] and M. J. Dufton *et al.*[5] proposed the detailed mechanism.

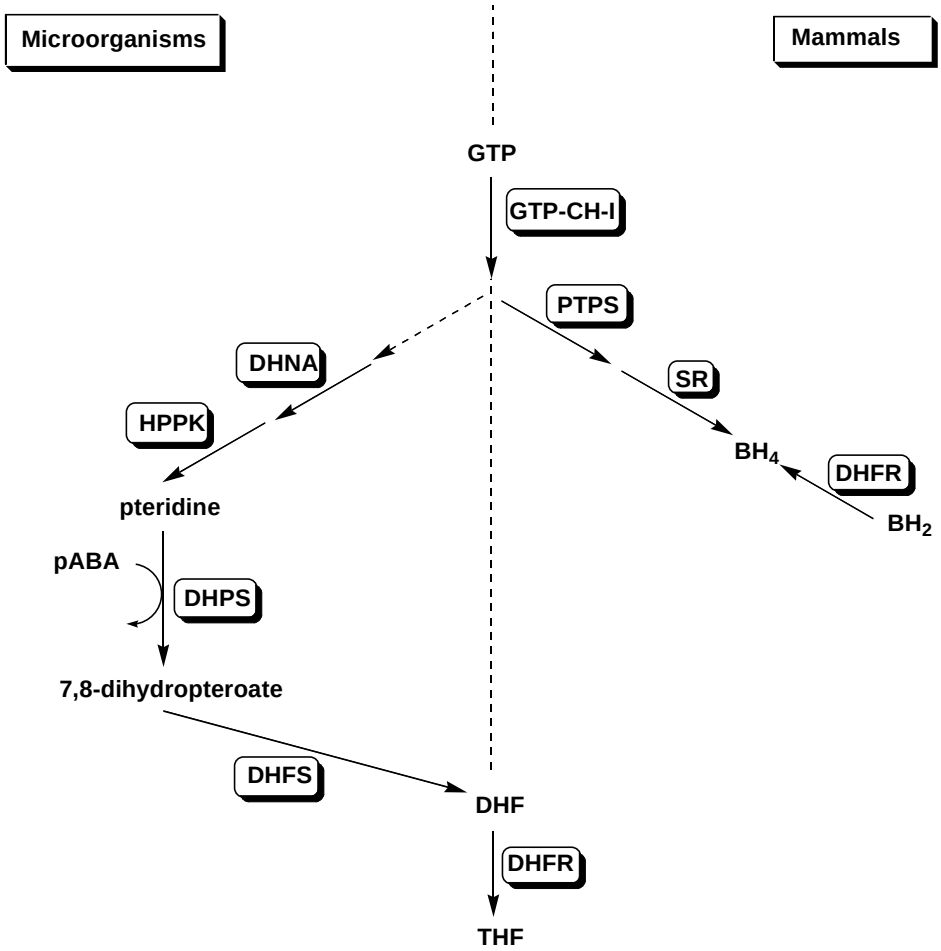


Figure 1.2. *De novo* biosynthesis of pterins and folates. GTP-CH-I (GTP cyclohydrolase I), DHNA (dihydroneopterin aldolase), HPPK (hydroxymethyl-dihydropterin pyrophosphokinase), DHPS (dihydropteroate synthase), DHFS (dihydrofolate synthase), DHFR (dihydrofolate reductase), PTPS (6-pyruvoyltetrahydropterin synthase), SR (sepiapterin reductase).[3]

Tetrahydrofolate (THF) **1.3**, a reduced form of two double bonds of folic acid, is a crucial cellular cofactor involved in supplying one-carbon units of three major metabolic pathways: the biosynthesis of (i) methionine, (ii) purines and (iii) pyrimidines. Pathways (ii) and (iii) are essential for DNA generation. For example, folate in the form of 5,10-methylenetetrahydrofolate **1.4** is necessary to provide the methyl group that converts dUMP (2′-deoxyuridine-5′-monophosphate) **1.5** into dTMP (2′-deoxythymidine-5′-monophosphate) **1.6**, whose triphosphate derivatives are used by DNA polymerase to add thymine nucleotides to growing DNA chains (Fig. 1.3).[6] All active folate cofactors are found in the tetrahydro form. They are also

subject to polyglutamation by folypolyglutamate synthase (FPGS) activity, whereby additional glutamate residues are linked *via* peptide bond formation at the gamma position.[7] It has been shown that this process is critical for the cellular retention of folates as well as enhancing their affinity for folate-dependent enzymes.[8]

From these facts, we know the enzymes which are involved in folate biosynthesis and metabolism, must be effective targets to make new inhibitors. We will look at the key enzymes involving folate metabolism in the following section.

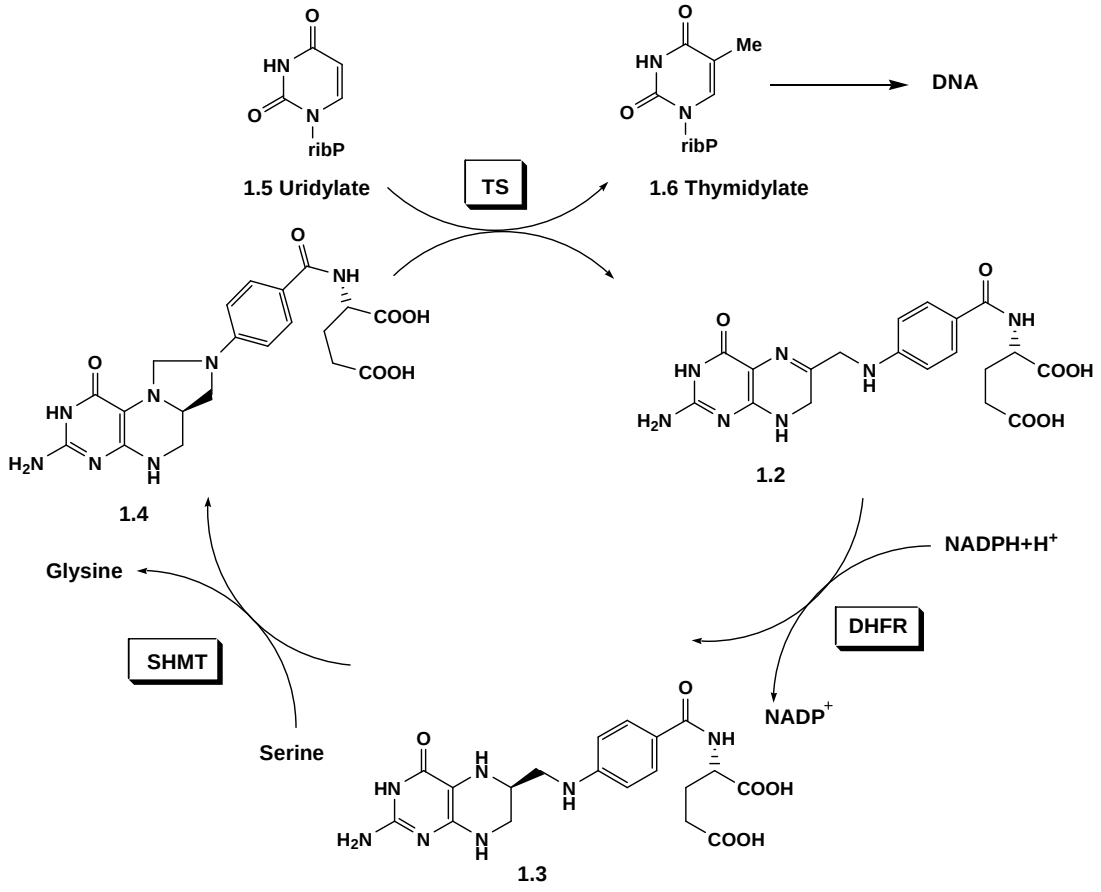


Figure 1.3. The thymidylate synthase-dihydrofolate reductase cycle. NADPH (nicotinamide adenine dinucleotide phosphate), DHFR (dihydrofolate reductase), SHMT (serine hydroxymethyl transferase), TS (thymidylate synthase).[6]

1.1.2. Folate pathway enzymes as drug targets

In this section, brief characteristics of six enzymes guanosine triphosphate cyclohydrolase I (GTP-CH-I), dihydroneopterin aldolase (DHNA), 7,8-dihydro-6-hydroxymethylpterin pyrophosphokinase (HPPK), dihydropteorate synthase (DHPS), dihydrofolate synthase (DHFS) and dihydrofolate reductase (DHFR) involved in the biosynthesis of tetrahydrofolic acid (Fig. 1.1) along with thymidylate synthase (TS), serine hydroxymethyltransferase (SHMT) and folylpolyglutamate synthase (FPGS) will be introduced. In a broad sense, all these enzymes are biological targets as inhibition of any of these can lead to disruption of folate metabolism which eventually results in cell death.

1.1.2.1. GTP-CH-I (Guanosine triphosphate cyclohydrolase I)

GTP cyclohydrolase I catalyses the conversion of GTP **1.7** into 7,8-dihydroneopterin triphosphate **1.8** (Fig. 1.4, A). In plants and certain microorganisms, the enzyme product serves as the first committed intermediate in the biosynthesis of tetrahydrofolate.[9] The structures of the GTP-CH-I from *E. coli* and humans have been solved. These studies identified the key role of a zinc ion in human and bacterial GTP-CH-I and provide a mechanistically complex ring expansion reaction (Fig. 1.4, B).[4]

The suggested mechanisms can be explained as follows. (Fig. 1.4, B)[4]

(a) The zinc-bound hydroxyl ligand could attack C8 of GTP, whereas His210 could act as a proton donor for N7 of the substrate.
(b) Protonation of the furanose ring oxygen atom by His143 could assist the opening of the imidazole ring *via* transient generation of a Schiff base from the *N*-glycoside.
(c) Deprotonation by His143 reforms the *N*-glycoside.
(d) Displacement of the formamide bond: zinc could simultaneously polarise the formamide carbonyl bond and activate a water molecule for nucelophilic

attack.

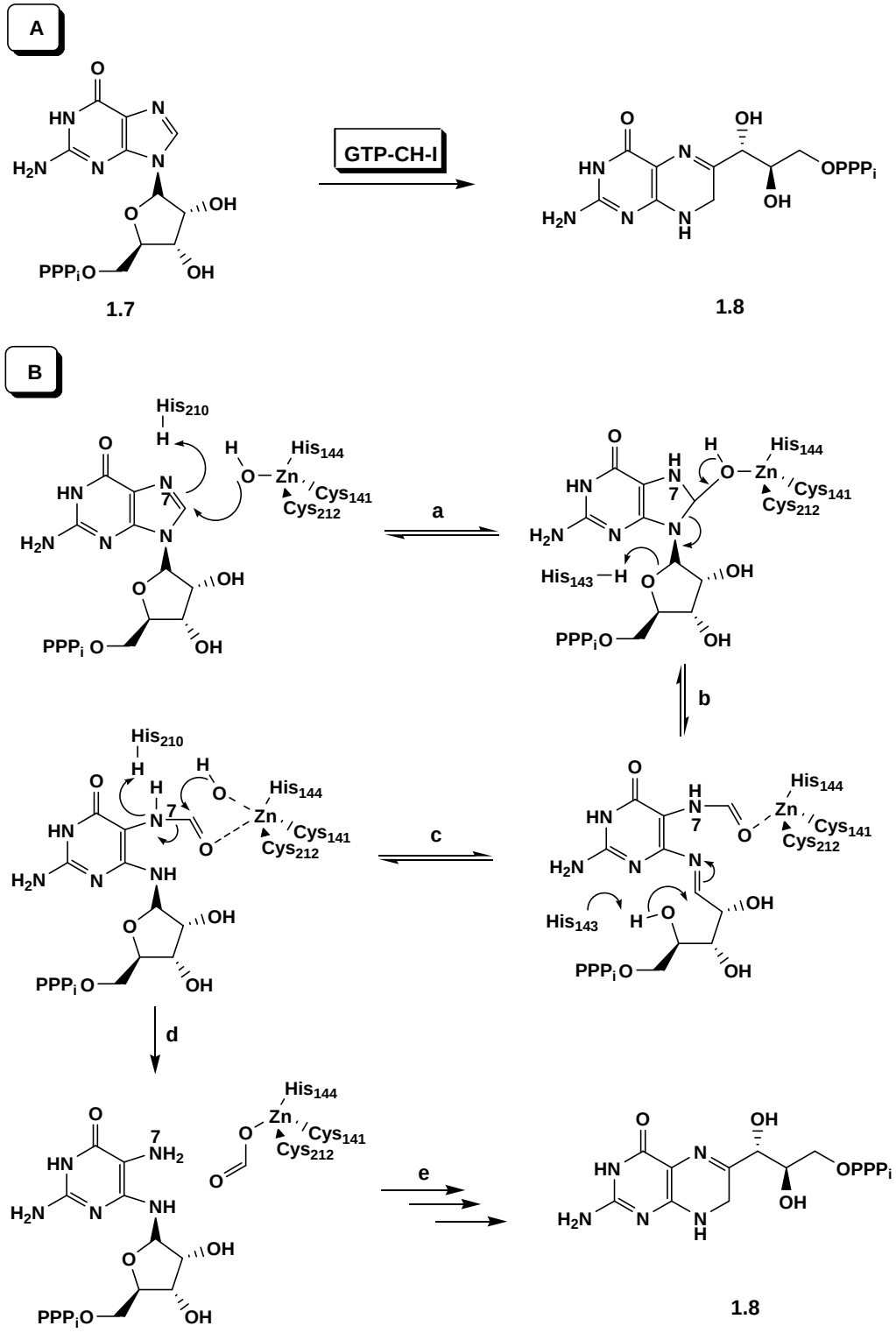


Figure 1.4. A: Conversion of GTP to dihydroneopterine triphosphate by GTP-CH-I. B: Hypothetical reaction mechanism for GTP-CH-I.[4]

(e) Amadori rearrangement involves in the final ring formation to give **1.8** but detailed mechanisms are unknown.

Although, catalytic mechanisms seem to be identical in the *E. coli* and the human enzymes, the sequence identity between the two enzymes is only 37%. In addition, superposition of human and bacterial GTP-CH-I indicates that the human enzyme lacks the *N*-terminal region.[4] These sufficient structural differences allow us to consider GTP-CH-I, the first enzyme in folate pathway, as an interesting target so that to exploit for the design of selective inhibitors.

1.1.2.2. DHNA (Dihydroneopterin aldolase)

A pyrophosphatase and a phosphatase have been proposed to convert dihydroneopterin triphosphate **1.8** into 7,8-dihydroneopterin **1.9** in two consecutive steps, but the details are still not fully understood (Fig. 1.5).[10]

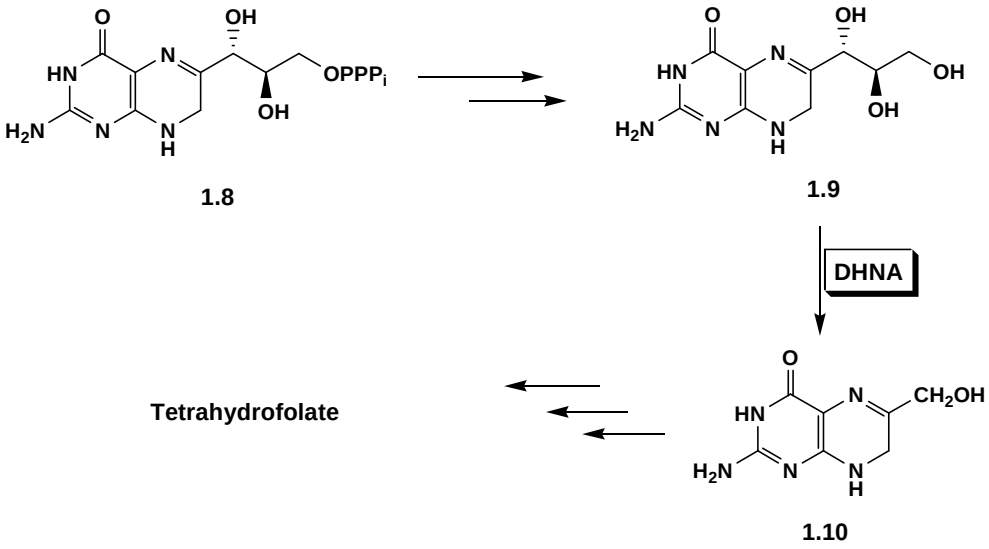


Figure 1.5. Conversion of 7,8-dihydroneopterin **1.9** to 6-hydroxymethyl-7,8-dihydropterin **1.10** by DHNA.

Dihydroneopterin aldolase (DHNA) catalyses the conversion of 7,8-dihydroneopterin **1.9** into 6-hydroxymethyl-7,8-dihydropterin **1.10** (Fig. 1.5). A hypothetical reaction mechanism is shown in Figure 1.6.[11] A retroaldol displacement of the C-C bond between C-1′ and C-2′ has been proposed to be the crucial reaction step for the enzyme catalysed reaction.

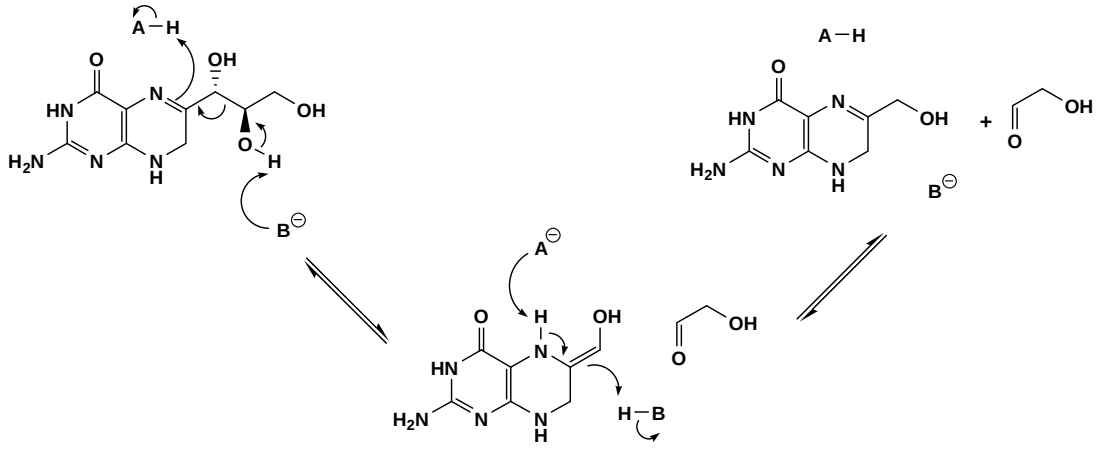


Figure 1.6. Hypothetical mechanism for retroaldol displacement of 7,8-dihydroneopterine.

The crystal structure of DHNA from *Staphylococcus aureus*, first reported by M. Hennig and coworkers, shows that this enzyme crystallises as an octamer.[12] The crystal structure of DHNA complexed with the product of the enzyme-catalysed reaction, 6-hydroxymethyl-7,8-dihydropterin **1.10**, was also reported by M. Hennig and confirmed the location of the enzyme active site.[12] DHNA is conserved across bacterial species and is nonexistent in humans.

However, only limited efforts have been directed toward the design of new DHNA inhibitors. As a relatively recent result, W. J. Sanders *et al.* reported inhibitors of DHNA for *Staphylococcus aureus* using CrystaLEAD X-ray crystallographic high-throughput screening followed by structure-directed optimisation.[13] Screening of a directed library in the enzymatic assay provided several hits with IC_{50} values on the order of 1 μM (Fig. 1.7).[13]

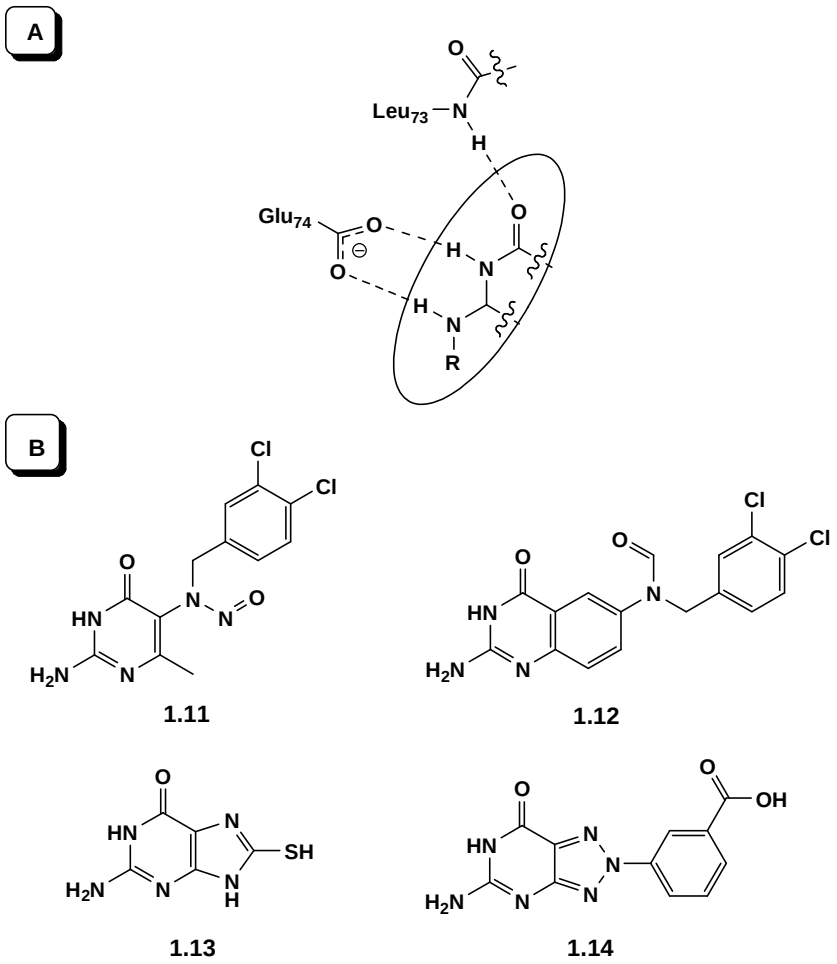


Figure 1.7. (A) Three-point hydrogen-bonding motif used in the construction of a directed library for enzyme inhibition screening. (B) Inhibitors identified in an enzyme inhibition assay of a directed library. IC_{50}: **1.11** (1.3 μM), **1.12** (0.6 μM), **1.13** (1.0 μM), **1.14** (1.5 μM).

1.1.2.3. HPPK (Hydromethyl-dihydropterin pyrophosphokinase)

HPPK catalyses the transfer of pyrophosphate from ATP (adenosine triphosphate) to 6-hydroxymethyl-7,8-dihydropterin **1.10** (Fig. 1.8). This enzyme is essential for microorganisms but absent in mammalian cells. To date, HPPK has not been the target for any existing antibiotics and is therefore an attractive target for the development of novel antibiotics that are urgently needed to fight the worldwide antibiotic resistance. The mechanism of HPPK-catalysed pyrophosphoryltransfer and

the crystal structure of the *E coli*. HPPK 3D-structure, complexed with one (HPPP, 6-hydroxymethyl-7,8-dihydropterinpyrophosphate **1.15**) and two product molecules (HPPP and AMP, adenosine monophosphate) have been recently described.[14]

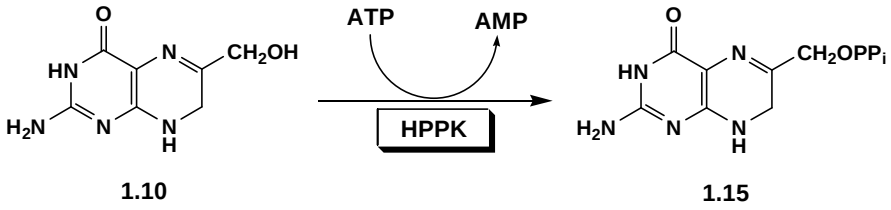


Figure 1.8. Reaction catalysed by HPPK.

1.1.2.4. DHPS (Dihydropteorate synthase)

DHPS is essential for the *de novo* synthesis of folate in prokaryotes, in lower eukaryotes such as protozoa and yeast, and in plants. DHPS is absent in mammals. This enzyme catalyses the condensation of *para*-aminobenzoic acid (pABA) with 6-hydromethyl-7,8-dihydropterinpyrophosphate (HPPP) **1.15** to form pyrophosphate and 7,8-dihydropteroate **1.16** (Fig. 1.9).[15, 16]

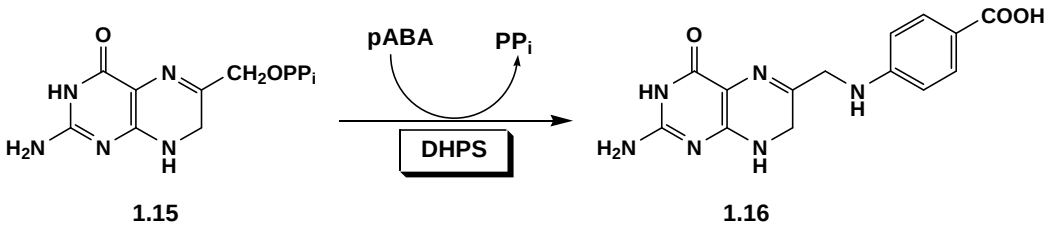


Figure 1.9. Reaction catalysed by DHPS.

The crystallographic structure of DHPS has been solved in many bacterial species, including *Staphylococcus aureus*[17] and *Mycobacterium tuberculosis*.[18] It is the only

enzyme of the *de novo* folate pathway that is used as a drug target in the clinic. Although this has two substrates: 6-hydromethyl-7,8-dihydropterinpyrophosphate (HPPP) and pABA, currently, only analogs of pABA (sulfur-based drug, **1.17** and **1.18**), act as competitive inhibitors of DHPS, are used clinically (Fig. 1.10).[18]

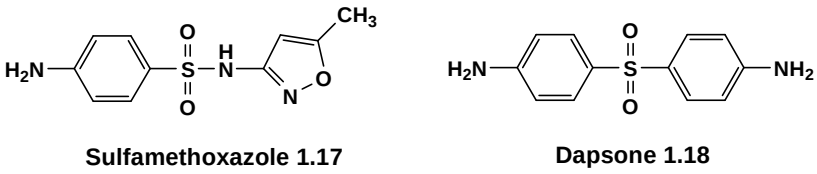


Figure 1.10. Two sulfa drugs used in the treatment of mycobacterial infections.

1.1.2.5. DHFS (Dihydrofolate synthase)

DHFS adds the first glutamate residue to the *p*-aminobenzoate component of dihydropteroate **1.16**, the product of DHPS catalysis, to form dihydrofolate **1.2**, the substrate of DHFR (Fig. 1.11); this reaction is the final step in *de novo* folate synthesis. In bacteria, DHFS and FPGS (folypolyglutamate synthase; the enzyme for adding further glutamate residues to the molecules) activities can be found on the same or different proteins, depending on the species.[19] The potential of this enzyme as a drug target has been demonstrated with the use of analogs of pteridine in bacteria.[20, 21] S. Pongsmart *et al.* tested dihydrofolate, the product of the reaction catalysed by DHFS, as an inhibitor and reported that 530μM dihydrofolate gave 50% inhibition against DHFS from *Neisseria gonorrhoeae*.[21]

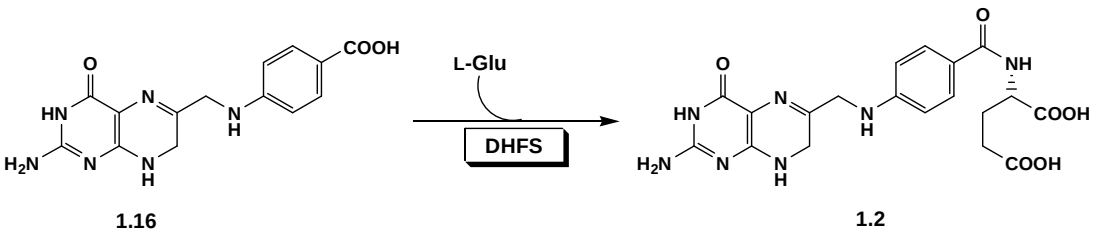


Figure 1.11. Reaction catalysed by DHFS.

1.1.2.6. DHFR (Dihydrofolate reductase)

DHFR catalyses the reduction of 7,8-DHF **1.2** to 5,6,7,8-THF **1.3** (Fig. 1.12) and has a pivotal role in two major folate-based reactions: (i) synthesis of endogenous THF **1.3** (in microorganisms that can produce folate *de novo*) and (ii) salvage of oxidised forms of folate, particularly the recycling of DHF **1.2** resulting from the synthesis of dTMP (Section 1.1.1, Fig. 1.3), and from exogenous sources.[22] The central role of this enzyme has made it a major target for drug development against cancer and a variety of infectious diseases caused by bacteria, protozoa, and fungi.[23]

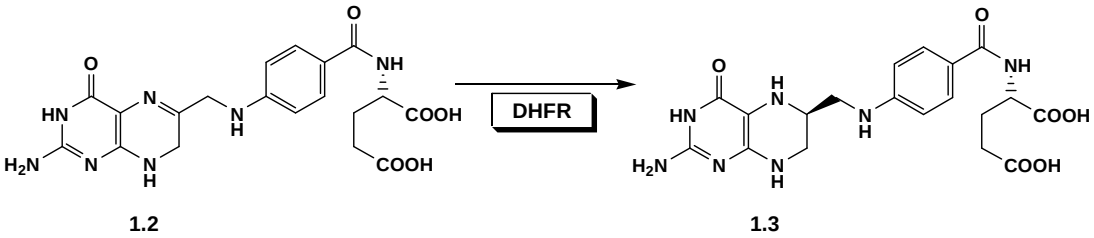


Figure 1.12. Reaction catalysed by DHFR.

The recognition of the importance of these enzymes and the routine availability of protein crystallography led to an explosion of information of 3D-structures of DHFRs from human, protozoal, fungal, and bacterial sources with numerous ligands and cofactors bound in their active centres.[23] Several examples of DHFR inhibitors are introduced in Section 1.2.1 (Fig. 1.14). Characteristics of trypanosomatid parasitic DHFR are presented in Section 1.3.4.2.

1.1.2.7. TS (Thymidylate synthase)

TS catalyses the reductive methylation of dUMP to give dTMP, using 5,10-methylene tetrahydrofolate as the one-carbon unit donor (Section 1.1.1, Fig. 1.3).

The TS cycle is the sole *de novo* pathway for the synthesis of dTMP. Complete blocking of TS ultimately leads to “thymineless death”. Mammalian cells can either synthesise thymidine *de novo* or salvage it exogenously. However, the demand for this DNA precursor is so great in rapidly dividing cells that the salvage pathway cannot supply enough nucleotides, making TS a particularly vulnerable target in tumour cells.[22] Recently approved anticacer drugs as TS inhibitors are introduced in Section 1.2.2. (Fig. 1.16 and 1.17)

The TSs studied exhibit striking structural homologies, and so far there are no appropriate selective inhibitors known for TS from bacteria or protozoa. However, this situation could alter based on the recent discovery of thymidylate synthase complementing proteins in a number of bacteria that exhibit a different kinetic and molecular mechanism. These new proteins could be interesting targets for selective inhibitors.[24, 25]

1.1.2.8. SHMT (Serine hydroxymethyl transferase)

SHMT, a pyridoxal-5′-phosphate (PLP) requiring enzyme, cleaves the C_α-C_β bond of serine to form glycine and formaldehyde and then immediately transfers formaldehyde to the coenzyme (THF) to produce 5,10-methylene-THF **1.4** (Fig. 1.13); this provides the primary source of the one-carbon unit for the THF-dependent reactions. In the first step of the mechanism for PLP-catalysed C_α-C_β bond displacement, a basic group at the active site of the enzyme removes a proton from a hydroxyl group bonded to the β-carbon of serine (Fig. 1.13). This causes the C_α-C_β bond to be cleaved.[24]

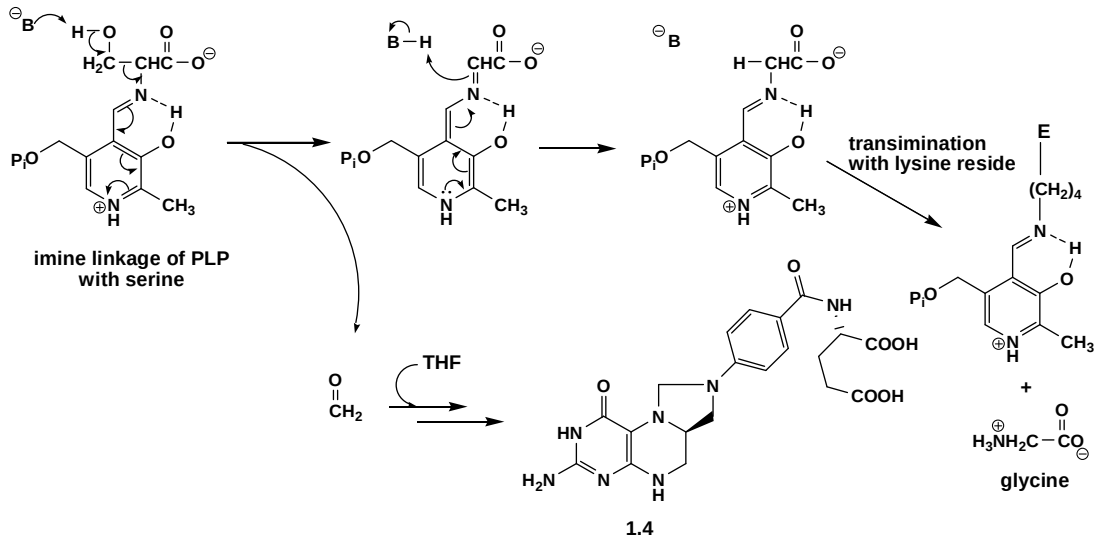


Figure 1.13. Reaction catalysed by SHMT.

There are about 10 entries in the protein data bank for X-ray structures of SHMTs isolated from different bacteria, animals, or man.[23] SHMT inhibition is expected to affect cell growth dramatically due to the decisive role for producing a one-carbon unit donor, especially because there is a considerable increase in its activity in proliferating cells. Thus, human SHMT is considered a target for anticancer drugs, but there are currently no useful potent and selective inhibitors known.[25]

1.1.2.9. FPGS (Folylpolyglutamate synthase)

The polyglutamylation reaction is catalysed by the enzyme FPGS, an MgATP-dependent enzyme present in all cells. FPGS forms a complex with MgATP, a folate derivative, and glutamate, in an ordered manner whereby the three substrates are added sequentially.[26] Polyglutamylated forms have been studied extensively in mammalian cells and consist of between one and nine glutamate residues attached to the *p*-aminobenzoate moiety of the folate molecule.[27]

Polyglutamated folates are better substrates than monoglutamated substrates for

folate-dependent enzymes, exhibiting more efficient kinetics and longer retention times, resulting in an increased concentration within cells.[27] Recognition of the significance of polyglutamylation for the cytotoxicity and theraputic efficacy of antifolates has led to a drug development strategy for agents that are both inhibitors of folate-dependent enzymes and are efficiently polyglutamylated.[26] A representative example is shown in Section 1.2.2.2 .

1.2. Strategies for development of antifolates

This section reviews the characteristics of methotrexate (MTX) **1.20** as a key antifolate and the strategies to produce novel antifolates based on overcoming drug resistance for MTX **1.20**.

1.2.1. Characteristics of methotrexate (MTX)

Aminopterin (AMT **1.19**, Fig. 1.14) was the first antifolate used to treat patients with lymphoblastic leukemia in 1947 ~ 1948. It was, however, soon displaced by its 10-methyl analogue, methotrexate (MTX **1.20**, Fig. 1.14), which proved to be therapeutically superior.[28] MTX **1.20** is a folate-based drug that primarily inhibits dihydrofolate reductase to block tetrahydrofolate regeneration from dihydrofolate. This inhibition induces cytotoxic effects by the ultimate suppression of *de novo* biosynthesis of purine nucleotides and thymidylate.[29, 30] MTX **1.20** is effectively transported across the cell membrane by the ubiquitously expressed reduced folate carrier (RFC). Upon entering the cell, MTX **1.20** undergoes polyglutamylation that decreases its efflux and enhances inhibition.[31]

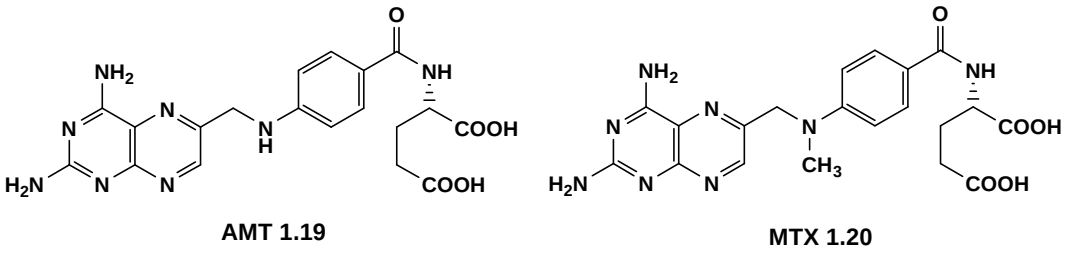


Figure 1.14. Chemical structures of AMT **1.19** and MTX **1.20**.

Since the late 1940s, MTX **1.20** remains one of the most widely used drugs in medical oncology for treatment of several types of cancer.[22] It is also used in the

treatment of inflammatory diseases such as rheumatoid arthritis.[28] However, several causes of cellular resistance against MTX **1.20** have been discovered. The major causes of resistance to MTX **1.20** are categorised as follows.

Cause A. Inefficient cellular accumulation:

A-1. Impaired polyglutamylation of MTX **1.20** due to alterations in the expression or function of FPGS[32]

A-2. Overexpression of multidrug-resistance (MDR) genes that encodes efflux pumps can lead to rapid drug excretion and consequently reduced exposure of the target protein (DHFR) to the inhibitor (MTX **1.20**)[33]

A-3. Impaired transport: In murine and human cell lines, mutations in the RFC have been associated with resistance to MTX **1.20** by virtue of alterations in the binding properties of the carrier and/or the mobility of the carrier loaded with its substrates[34]

Cause B. Qualitative and quantitative decrease of binding affinity of MTX **1.20** due to overexpression and mutation of DHFR[35]

To overcome resistance to MTX **1.20**, a great deal of effort has been put into the development of novel antifolates.

1.2.2. Strategies for development of new antifolates

This section will review different approaches to develop novel antifolates with examples.

1.2.2.1. Multi-targeted strategy (Strategy A)

As described above, in folate pathway, there are specific enzymes for each stage and all these enzymes can be an effective drug target. This fact means that the multi-

targeted strategy can be a solution to overcome drug resistance mainly derived from cause B (Section 1.2.1). The multi-target strategy can be divided into two different approaches.

Strategy A-1. Combination therapy

Combination therapy has been developed on the scientific basis that a cell might overcome inhibition of a single enzyme but not accumulate sufficient changes to become resistant to a combination of compounds that inhibit several enzymes.[36]

A major advantage of combination therapy is that it may enable mutual protection of one inhibition mechanism by the other thereby extending the useful therapeutic life of the compounds involved.[37] In many cases, the development of antifolates against malaria and bacteria is based on the combinations of DHFR inhibitors and sulfur-based drugs as DHPS inhibitors. Although sulfur-based drugs are weak antimicrobial agents on their own, they substantially increase the activity of the DHFR inhibitors, and are therefore used as a component of the antifolate combination.[38] Fansidar™ (pyrimethamine-sulfadoxine), Metakelfin® (pyrimethamine-sulfalene) and Lapdap® (chlorproguanil-dapsone) are well known as combination drugs for treatment of malaria (Fig. 1.15).

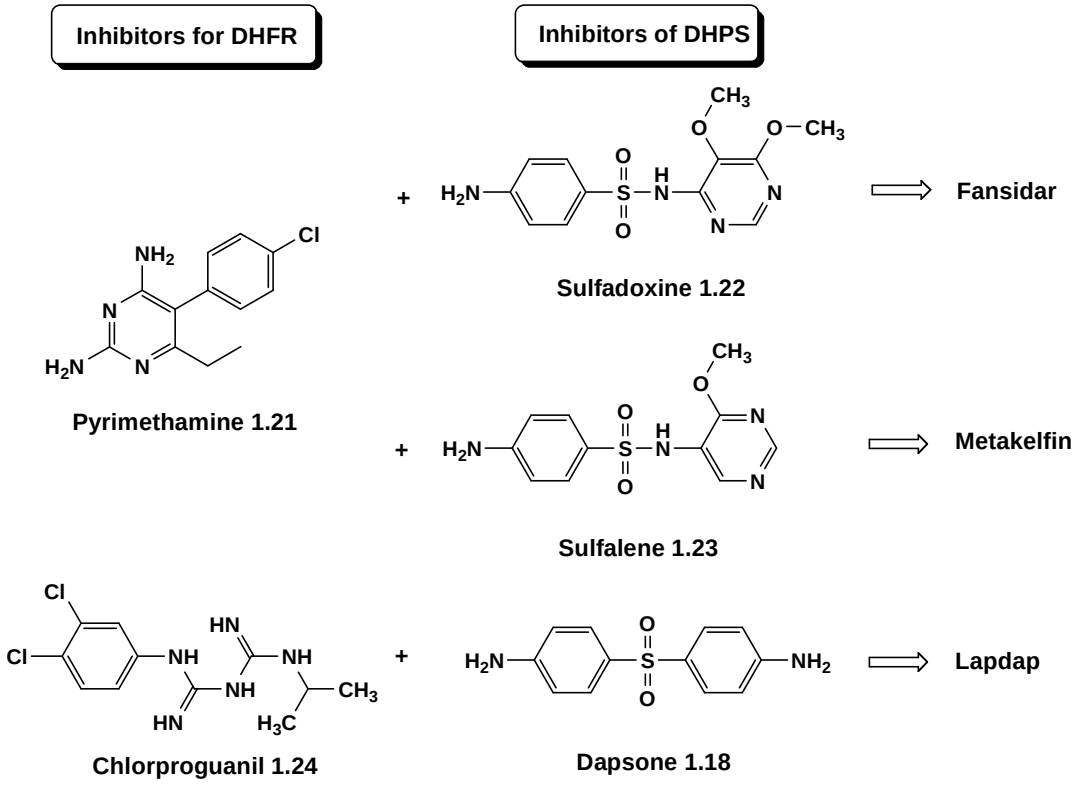
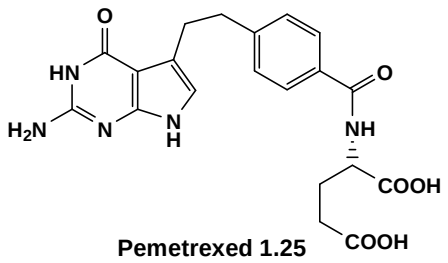


Figure 1.15. Examples of combinational drugs for treatment of malaria.

Strategy A-2. Single-agent multi-targeted strategy

The other approach in multi-targeted strategy is using a single agent having inhibitory ability toward more than one target enzymes. Pemetrexed (Alimta®, Fig 1. 16) **1.25** is a representative drug fulfilling the single-agent multi-targeted strategy. Although pemetrexed **1.25** is considered to be primarily an inhibitor of TS, it can also inhibit other enzymes including DHFR. The compound was developed by Eli Lilly and approved in February 2004 by the FDA (Food and Drug Administration) as the first treatment of malignant pleural mesothelioma, a condition usually associated with asbestos exposure.[23]

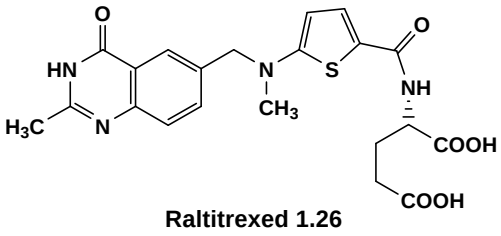


Pemetrexed 1.25

Figure 1.16. An example for a single-agent multi-targeted drug.

1.2.2.2. Increase on folypolyglutamate synthase (FPGS) activity (Strategy B)

FPGS is the enzyme involving polyglutamation of antifolates to give sufficient accumulation so that a drug can inhibit a target enzyme. Raltitrexed (Tomudex®, Fig. 1.17) **1.26** is an example for Strategy B. Raltitrexed **1.26** is an excellent substrate for FPGS hence rapidly and almost completely polyglutamated inside cells. It is a selective TS inhibitor developed by AstraZeneca.[23] Extensive polyglutamation has been confirmed in a number of human cell lines.[39]



Raltitrexed 1.26

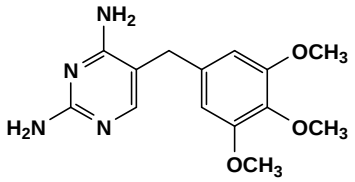
Figure 1.17. An antifolate having good FPGS activity.

Polyglutamation of raltitrexed **1.26** has several consequences. Firstly, it increases the potency of the drug through accumulation of chemical species which are significantly more active as TS inhibitors.[40] Secondly, it leads to intracellular drug retention. This property induces prolonged inhibition of TS and cytotoxic effects without the necessity for continuous drug exposure.[41]

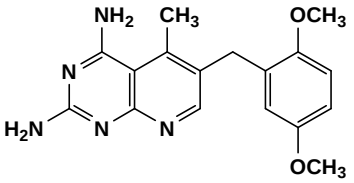
1.2.2.3. Non-classical antifolates (Strategy C)

The non-classical antifolates which do not carry the glutamate moiety, have been developed in an attempt to overcome the resistance caused by MTX **1.20**. These compounds are envisioned to be more lipophilic, thus can enter the cell *via* passive diffusion. This character of non-classical antifolates avoids low cellular accumulation which is found in classical antifolates derived from poor activities toward folate carrier (e.g. reduced folate carrier, RFC) and FPGS.[42]

Two exemplary compounds belonging to non-classical antifolates are trimethoprim (TMP) **1.27**, a pyrimidine derivative, and piritrexim (PTX) **1.28**, a pyrido[2,3-*d*]pyrimidine derivative (Fig. 1.18).



Trimethoprim 1.27



Piritrexim 1.28

Figure 1.18. Examples of non-classical antifolates.

While TMP **1.27** has excellent species selectivity in its binding to nonmammalian *vs.* mammalian DHFR, its potency as a DHFR inhibitor is relatively low (a weak but selective inhibitor). For this reason, it is clinically useful only when given in combination with a sulfur drug like sulfamethoxazole **1.17** or dapsone **1.18** to block *de novo* reduced folate synthesis.[43]

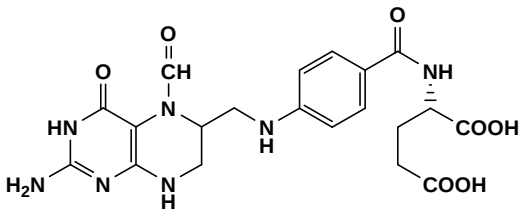
PTX **1.28** was first synthesised by E. M. Grivsky *et al.* as a lipophilic analogue of the MTX **1.20**.[44] Extensive *in vitro* and *in vivo* preclinical studies were subsequently carried out with a view to exploring the mechanism and scope of action of PTX **1.28**. The binding of PTX **1.28** to DHFR is orders of magnitude higher than that of TMP **1.27**, but unfortunately this increased potency is achieved at the cost of a dramatic

loss of species selectivity (Table 1.1).[45] Thus, PTX **1.28** requires coadministration of leucovorin **1.29** (Fig. 1.19), a mixture of the diastereoisomers of the 5-formyl derivative of tetrahydrofolic acid, to selectively protect the mammalian host from toxic effects.[46]

Table 1.1. Inhibition of *P. carinii*, *T. gondii*, *M. avium*, and rat DHFRs by TMP **1.27** and PTX **1.28**.[45]

	IC_{50} (nM)				selectivity		
Compounds	pc	tg	ma	rl	rl/pc	rl/tg	rl/ma
TMP **1.27**	12 000	2800	300	180 000	14	65	600
PTX **1.28**	13	4.3	0.61	3.3	0.26	0.76	5.4

pc (*Pneumocystis carinii*), tg (*Toxoplasma gondii*), ma (*Mycobacterium avium*), rl (rat liver)



Leucovorin 1.29

Figure 1.19. An antidote to drugs which act as folic acid antagonists.

Therefore, new lipophilic non-classical antifolates are required to enhance potency without assistance of sulfa drugs and to reduce host-cell toxicity without expensive leucovorin **1.29**. To tackle this challenging task, medicinal chemists have been designing, synthesising, and evaluating new pyrimidine based non-classical antifolates, mainly as DHFR inhibitors.[45, 47] A. Rosowsky *et al.* reported two remarkably improved compounds by structural modification of TMP **1.27,** with regard to selectivity as well as potency (Fig. 1.20). As an inhibitor of *Toxoplasma gondii* DHFR, **1.30** had IC_{50} of 5.5 nM (510-fold lower than that of TMP **1.27** and similar to that of PTX **1.28**) and selectivity value of 120 (2-fold better than TMP **1.27**

and vastly superior to PTX **1.28**). Compound **1.31** with the carboxy group at the *para* rather than the *meta* position, had a low IC_{50} of 3.7 nM against *Mycobacterium avium* DHFR and diplayed 2200-fold selectivity (IC_{50} against rat liver: 8200 nM).[45]

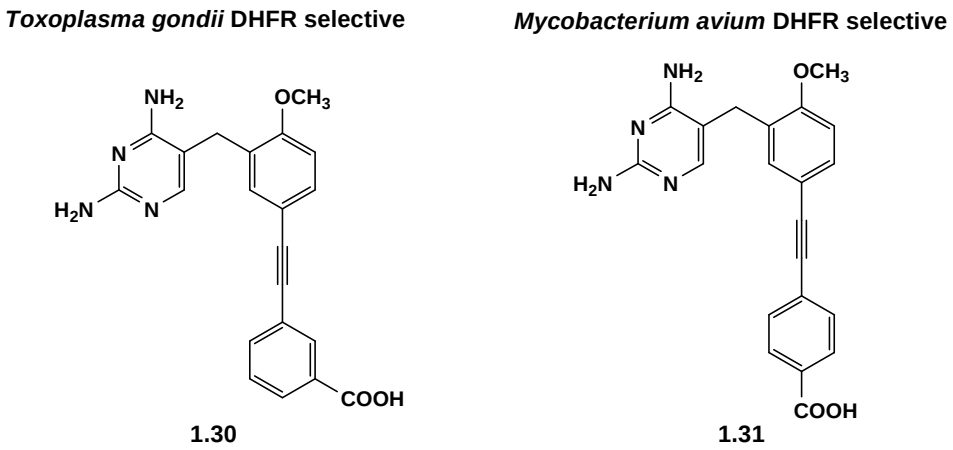


Figure 1.20. Two compounds with good inhibitory activity (potency/selectivity) against DHFRs.

1.3. Trypanosomatid parasites and PTR1

In this section, the research background of drug discovery for trypanosomatid parasitic diseases is introduced, part of this PhD research specifically belongs to this area. This review covers a general introduction (Section 1.3.1), past and present endeavours for treatment of the diseases (Section 1.3.2 and 1.3.3) and the key trypanosomatid parasitic enzymes (Section 1.3.4), DHFR (dihydrofolate reductase) and PTR1 (pteridine reductase 1).

1.3.1. Trypanosomatid parasites and related diseases

Trypanosomatid is the common name for a member of the family of Trypanosomatidae. Trypanosomatid parasites, a group of kinetoplastid protozoa, are distinguished by having only a single flagellum and are found primarily in insects. A few genera have life-cycles involving a secondary host, which may be a vertebrate or a plant. These include several species that cause major diseases in humans. The most notable trypanosomal diseases are sleeping sickness, Chagas disease, and Leishmaniasis.[48]

Sleeping sickness also known as Human African Trypanosomiasis (HAT), is caused by single-celled parasites, *Trypanosoma brucei*, which are transmitted to humans by infected tsetse flies. Two sub-species of *T. brucei* cause different forms of the disease. *T. b. gambiense* causes chronic infection, whereas *T. b. rhodesiense* generally causes a more acute infection. The parasites first develop in the blood, lymph and peripheral organs (early-stage), then spread to the central nervous system (late-stage), where they cause serious neurological disorders. Without treatment, the disease is fatal. HAT is known to occur in rural areas of 36 countries in sub-Saharan Africa, where tsetse flies are endemic. *T. b. gambiense* is found in central, west and some parts of eastern Africa, whereas *T. b. rhodesiense* is found in southern and eastern Africa.[49] In certain villages of many provinces of Angola, the Democratic Republic of Congo

and southern Sudan, the prevalence is between 20% and 50%. Now, sleeping sickness has become the first or second greatest cause of mortality, ahead of AIDS, in those provinces.[50]

Chagas disease (South American trypanosomiasis) is an infection caused by the parasite *Trypanosoma cruzi* and found mainly in Latin America. It is named after Carlos Chagas, a Brazilian doctor who first described the disease in 1909. The parasite is transmitted to humans in three ways. (i) The faeces of reduviid (known as assassin bug or kissing bug) contain parasites which can enter the wound left after the blood-meal, usually when it is scratched or rubbed. (ii) Transfusional transmission with infected blood is also possible by the insect vectors. (iii) In some cases, the parasite is transmitted from mother to foetus, congenitally.[51] After the initial acute phase, which has low mortality and is often asymptomatic, a chronic condition establishes which can lead in 30 ~ 40% of cases to irreversible lesions in the gastrointestinal tract and of the heart.[52] Currrently, there are 18 ~ 20 million people infected with *T. cruzi* and another 40 million people at risk of acquiring the disease.[52-54]

Transmitted by the bite of the infected female phlebotomine sandfly, leishmaniasis is a globally widespread group of parasitic diseases and remains a major public health problem throughout much of the tropical and subtropical world. The sandfly vector is usually infected with one species of flagellate protozoa belonging to the genus *Leishmania*.[55] The epidemiology is extremely diverse: (i) There are 24 infective *Leishmania* species, excluding the number of intra-species variants;[56] (ii) 30 sandfly species are known vectors.[57] Leishmaniasis has traditionally been classified in three different clinical forms, visceral (VL), cutaneous (CL) and mucocutaneous leishmaniasis (MCL), which have different immunopathologies and degrees of morbidity and mortality. Most VL caused by *Leishmania donovani* is fatal if untreated, whereas CL, caused by species such as *Leishmania major*, *Leishmania mexicana, Leishmania braziliensis* and *Leishmania panamensis*, frequently self-cures within 3 ~ 18 months, leaving disfiguring scars. The cutaneous forms of leishmaniasis are the most common and represent 50 ~ 75% of all new cases.[58]

1.3.2. Current treatments of trypanosomal diseases

1.3.2.1. Human African Trypanosomiasis (HAT)[59]

Research over the past century has yielded only four clinically approved drugs, three of which were introduced more than 50 years ago (Fig. 1.21) to treat HAT. These drugs arose as a result of research and development for veterinary or anticancer indications, and only one (eflornithine **1.35**) of these was destined for use against HAT.

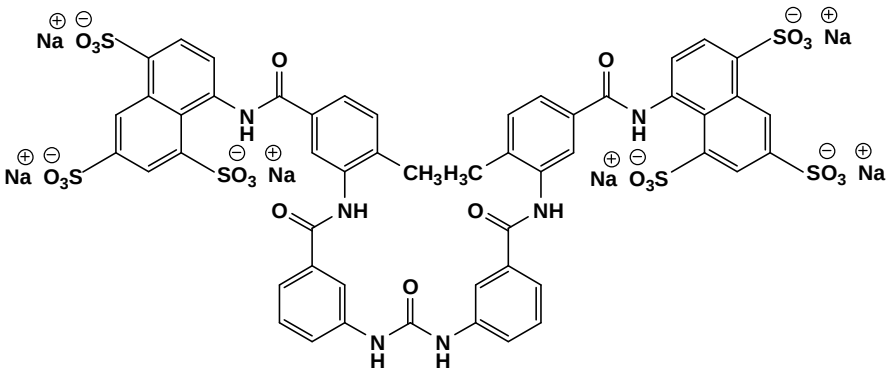
Sumarine **1.32** was introduced in the early 1920s and to this day remains the drug of choice for treatment of the early phase of *T. b. rhodesiense* infections.[59] Owing to its highly, ionic nature, the drug does not penetrate well into the central nervous system (CNS) and is therefore only effective in the primary stage of the disease. Immediate life-threatening side-effects include collapse, with nausea, vomiting and shock.[59]

Pentamidine **1.33**, an aromatic diamidine, was first introduced in the 1940s and remains the drug of the choice for early *T. b. gambiense* infections.[59] Efficacy is restricted to early-phase disease because the compound does not readily penetrate the CNS. Pentamidine can cause damage to the liver, kidneys and the pancreas.

Melarsoprol **1.34** was introduced in 1949 for the treatment of late-stage HAT caused by either *T. b. gambiense* or *T. b. rhodesiense*.[59] Melarsoprol **1.34** causes a serious reactive encephalopathy in 5 ~ 10% of cases, half of which are fatal.[60] Other common side-effects include vomiting, abdominal colic, peripheral neuropathy, arthralgia and thrombophlebitis.

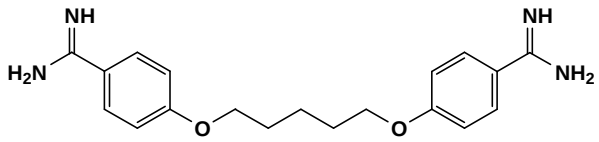
(a) Early-stage African trypanosomiasis

(i) Sumarin (1916)



1.32

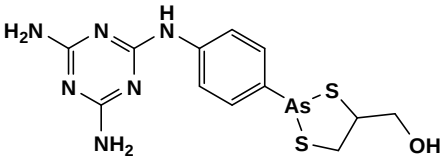
(ii) Pentamidine (1937)



1.33

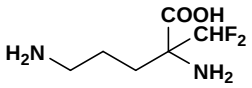
(b) Late-stage African trypanosomiasis

(i) Melarsoprol (1946)



1.34

(ii) Eflornithine (1977)

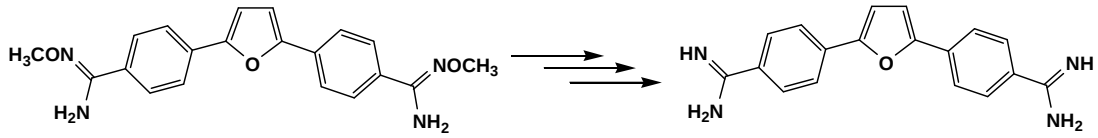


1.35

Figure 1.21. Structure of drugs used to treat (a) early stage (b) late stage human African trypanosomiasis and their date of synthesis.

Eflornithine **1.35** is the drug of choice for treatment of late-stage HAT caused by *T. b. gambiense*. The drug is far from ideal; it is costly and difficult to administer, requiring 400 mg kg^{-1} per day in four daily infusion over two hours for seven or fourteen days. Only one drug for treating HAT is currently undergoing clinical trials. The orally available prodrug DB289 **1.36** (Fig. 1.22) is converted systemically into

another diamidine **1.37** (DB75) that is active against early-stage disease.[61]

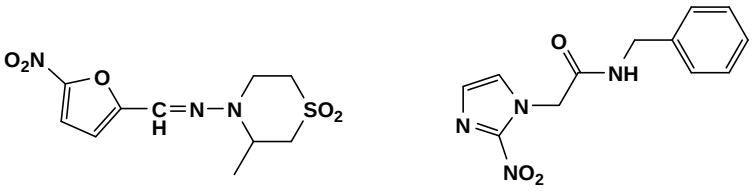


1.36 (DB289) **1.37 (DB75, furamidine)**

Figure 1.22. The diamidine DB289 **1.36** is a prodrug of 2,5-bis(4-aminodinophenyl)furan **1.37** (DB75) and is currently in Phase III clinical trials for treatment of stage 1 *T. b. gambiense* infection in Central Africa.

1.3.2.2. Chagas disease[62]

The drugs most frequently used for the treatment of Chagas disease are nitroheterocyclic compounds, a nitrofuran, nifurtimox **1.38**, and benznidazole **1.39**, a nitroimidazole derivative (Fig. 1.23), whose anti-*T. cruzi* activities were discovered empirically three decades ago.[62] Both drugs have significant side effects, including anorexia, vomiting, peripheral polyneropathy and allergic dermopathy,[63] can in some cases lead to treatment discontinuation. The major limitations of these compounds are, however, their very low antiparasitic activity in the chronic form of the disease because over 80% of treated patients are not parasitologically cured.[64]



1.38 (Nifurtimox) **1.39 (Benznidazole)**

Figure 1.23. Chemical structure of drugs currently available for the specific treatment of Chagas disease.

These clinical limitations could be related to unfavorable pharmacokinetic properties of the drugs in the chronic stages.[65] Novel approaches, rationally developed on the basis of increasing knowledge of the physiology and biochemistry of the etiological agent, are being advanced.[62] As a successful example, X. Du *et al.* reported new lead scaffolds for inhibitors of cruzipain, also known as cruzain, which is responsible for the major proteolytic activity of the parasite life cycle (Fig. 1.24).[66] Many of the compounds, derived from the lead scaffolds **1.40** and **1.41**, were active in the low nanomolar range against pure cruzipain and had trypanocidal activity against intracellular parasites *in vitro*.[62, 66] These results made cruzipain as an attractive anti-*T. cruzi* target in order to produce a potential new treatment for Chagas disease.

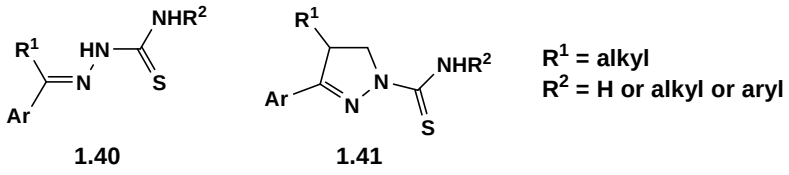
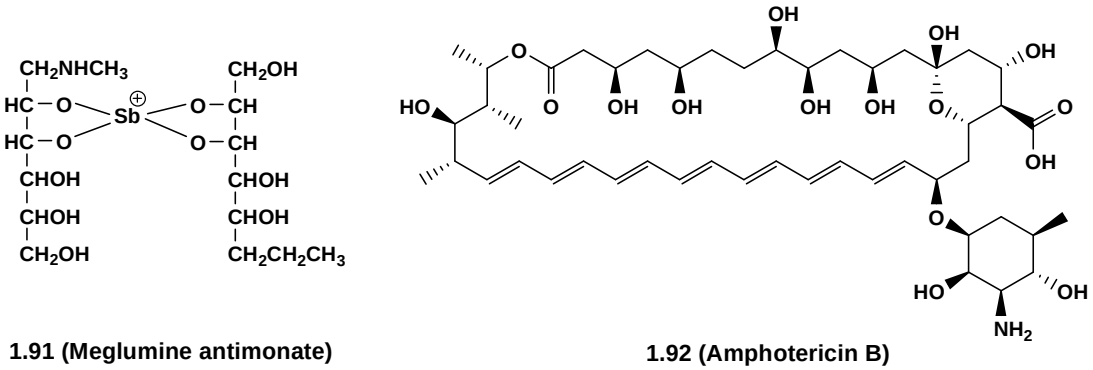


Figure 1.24. New scaffolds for inhibitors of cruzipain.[66]

1.3.2.3. Leishmaniasis[58]

The drugs currently recommended for the treatment of leishmaniasis include the pentavalent antimonials, sodium stibogluconate (Pentostam) and meglumine antimonate (Glucantime, Fig. 1.25, **1.42**), amphotericin B (Fungizone, Fig. 1.25, **1.43**) and its lipid formulation (AmBisome), and pentamidine (Fig. 1.21, **1.33**). The antimonials were first introduced in 1945 and remain effective treatments for some forms of leishmaniasis, but the requirement for up to 28 days of parenteral administration, the variable efficacy against VL and CL, and the emergence of significant resistance are all factors limiting the drugs' usefulness. The usefulness of the diamidine pentamidine **1.33** as an antileishmanial drug has been limited by its toxicity. It is normally used as a second-line drug when antimonials have proved ineffective.

The polyene antibiotic amphotericin B **1.42** has proved to be highly effective for the treatment of antimonial-resistant *L. donovani* VL and cases of MCL that have not responded to antimonials. However, it is an unpleasant drug because of its toxicity and the need for slow infusion parenteral administration over four hours. Fortunately, lipid-associated formulation of amphotericin B (AmBisome), which has reduced toxicity and an extended plasma half-life in comparison to the parent drug, has proved to be effective and has been approved by the Food and Drug Administration.[67] But high cost limits the wider use of AmBisome for treatment. Several new compounds are undergoing clinical trials, although most of these have been painfully slow and are based on studies and proof of principle established several decades ago.[58]



1.91 (Meglumine antimonate) **1.92 (Amphotericin B)**

Figure 1.25. Chemical structure of antileishmanial drugs.

1.3.3. Future prospects for treatments of trypanosomal diseases

As previously mentioned above, the currently used drugs for the treatment of the three trypanosomal diseases (HAT, Chagas disease, Leishmaniasis) were derived empirically, mainly decades ago and simply modified from already developed ones. Moreover, for the majority, severe adverse effects and resistance of the drugs have limited the real clinical usages, and the high cost has also prevented the wider spread

of the currently available drugs. Although, combinations of drugs can be alternatives which might be expected synergistic effects, dose reduction, fewer adverse events, and improved cost-effectiveness,[68] but these cannot be the final solutions. Thereby, these circumstances continually have spurred not only medicinal chemists but also molecular biologists to search new drugs to cure these diseases effectively.

Unprecedented advances in the understanding of the biology of the trypanosomatid parasites during the past years have been made and now allow scientists to access potential drug targets. Now, modeling based on the three-dimensional crystal structure of proteins from the parasites provides targets for structural-based lead discovery. Hence, it is now possible to synthesise potential inhibitors more easily to provide better possibility to produce new effective drugs for the treatment of trypanosomatid parasites in the future.

We are interested in making new potential inhibitors for specific target enzymes in biosynthetic and metabolic pathways of folate.[69-72] This thesis shows the partial results of our work in collaboration with Professor W. N. Hunter (University of Dundee, Dundee, UK). It concerns the design of inhibitors, their synthetic methods as well as *in vitro* results specific for trypanosomatid parasites PTR1 (pteridine reductase 1), which is a crucial enzyme in folate metabolism.

1.3.4. Target enzymes (DHFR and PTR1)

1.3.4.1. Metabolic pathway of pteridine (folate and pterin)

Once trypanosomatid parasites take up pterins (e.g. biopterin) or folic acid from the environment, these are reduced to their bioactive form by the appropriate enzymes, DHFR and PTR1 (Fig. 1.26), which will be discussed below in turn. Pterins are also essential for the growth of several species of trypanosomatid.[73] Although some of the roles of pterins are still putative, reduced pterins (e.g. 5,6,7,8-tetrahydrobiopterin, **1.46**) are involved in a number of metabolic and cellular functions.

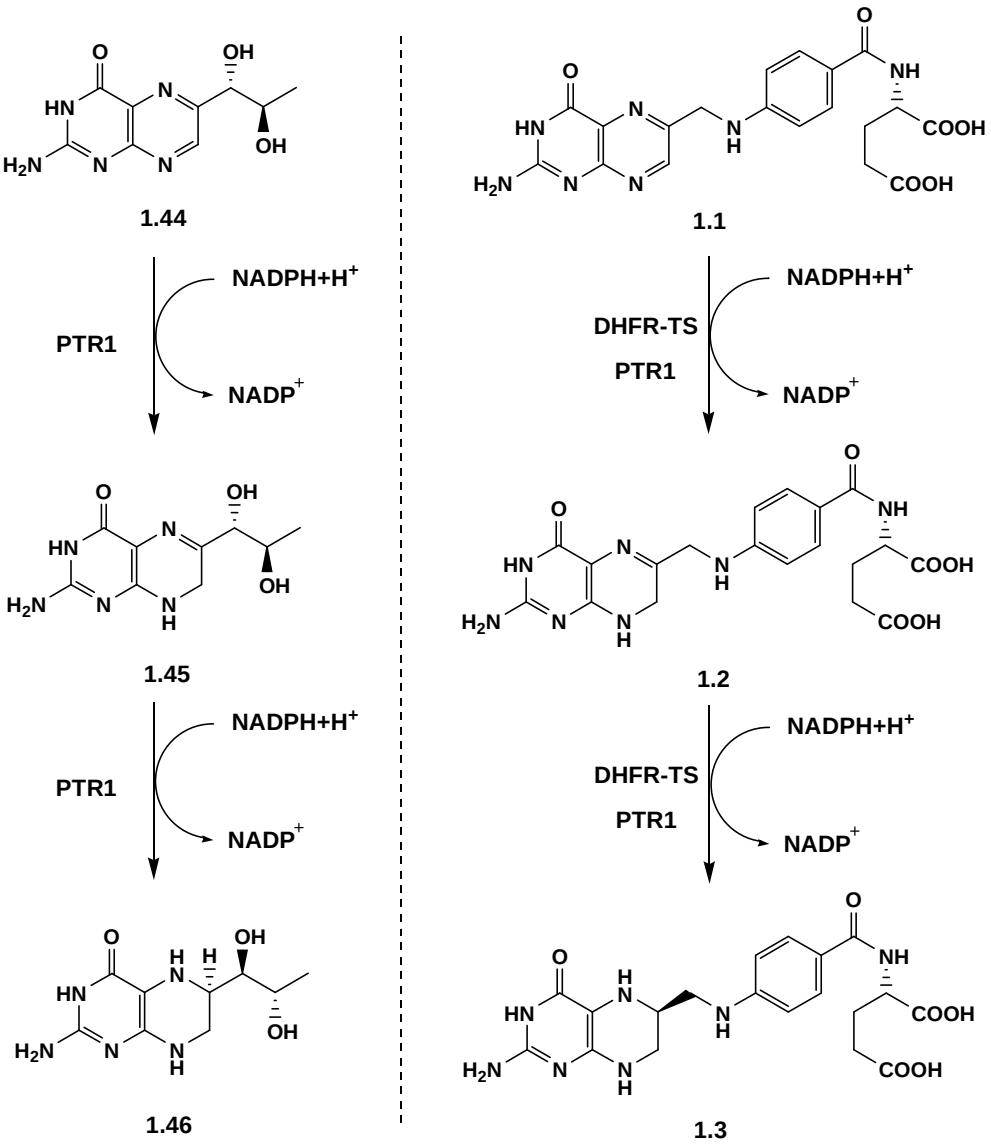


Figure 1.26. Reactions catalyzed by the DHFR domain of DHFR-TS and PTR1 from *Leishmania major*. The left side depicts the reduction of biopterin **1.44** into 5,6,7,8-tetrahydrobiopterin **1.46**. The right side depicts the reduction of folate **1.1** to 5,6,7,8-tetrahydrofloate **1.3**. NADPH (nicotinamide adenine dinucleotide phosphate), DHFR-TS (dihydrofolate reductase-thymidylate synthase), PTR1 (pteridine reductase 1).

The main known function of reduced folates in *Leishmania* is in the biosynthesis of thymidylate **1.6** (Section 1.1.1, Fig. 1.3). The enzyme dihydrofolate reductase (DHFR) reduces dihydrofolate **1.2** to tetrahydrofolate **1.3**. This reduced folate is

modified to 5,10-methylenetetrahydrofolate **1.4** to serve as a carbon donor for the synthesis of thymidylate – an essential component of DNA biosynthesis - in a reaction catalysed by thymidylate synthase (TS).

1.3.4.2. DHFR

Dihydrofolate reductase (DHFR) is a biologically universal housekeeping enzyme, which catalyzes the NADPH-linked reduction of 7,8-dihydrofolate (DHF) **1.2** to 5,6,7,8-tetrahydrofolate (THF) **1.3**. As reduction of DHF **1.2** to THF **1.3** is a starting and decisive step for the synthesis of thymidylate, a building block of DNA, DHFR can be a crucial drug target for trypanosomatid parasites. In trypanosomatid protozoan parasites, DHFR and TS (thymidylate synthase) are fused resulting in a bifunctional DHFR-TS protein.[74]

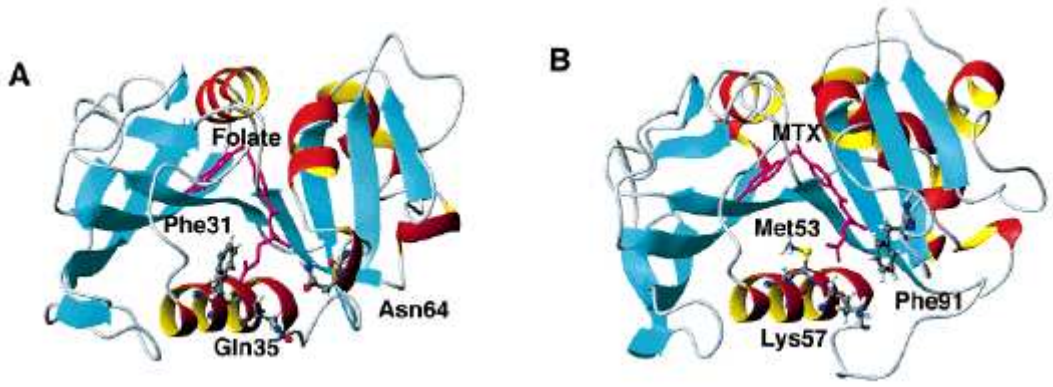


Figure 1.27. Tertiary structure of the active site of (A) human and (B) *L. major* DHFR. The human enzyme is co-crystallised with folate in the active site (shown in purple) and the *L. major* enzyme with methotrexate (shown in purple). Residues showing significant variation between the human and protozoan enzymes are also indicated human Phe31, Gln35, Asn64; *L. major* Met53, Lys57, Phe91.[76] The figures were reproduced under permission of American Chemical Society (Appendix V).

Structurally, the protozoan enzymes are closely related to one another, suggesting that it may be possible to design a compound which simultaneously inhibits the

enzymes from all three species (*Leishmania, T. b., T. c.*).[75] The active sites of the enzymes also show significant differences from the human enzymes, which suggest the possibility of designing compounds, which are selective for the protozoan DHFR (Fig. 1.27). These differences are found in the shapes of the active sites and the nature of residues present at the active site. Initially sequence alignment of the structures was undertaken. Alignments were found as follows: *L. major* and *T. cruzi*, 50%; *L. major* and *T. brucei*, 46%; *T. brucei* and *T. cruzi*, 58%; *L. major* and human, 26%; *T. cruzi* and human, 27%; *T. brucei* and human, 26%.[75] The leishmanial and trypanosomal enzymes showed much higher sequence identity to one another than to the human enzyme. This suggests that structurally the leishmanial and trypanosomal structures are more closely related to one another than to the human structure.

From this knowledge, we can predict that antifolates targeting DHFR, in principle, should provide an ideal treatment. However, DHFR inhibitors are largely ineffective for the control of trypanosomatid infections, partly due to the presence of pteridine reductase 1 (PTR1).[77]

1.3.4.3. PTR1

Pteridine reductase 1 (PTR1) is a short-chain reductase (SDR) responsible for the salvage of pterins in parasitic trypanosomatids. PTR1 is a NADPH dependent pterin reductase active as a tetramer.[78] In addition to reducing biopterin to dihydrobiopterin and tetrahydrobiopterin, PTR1 is also capable of reducing folate to dihydrofolate and tetrahydrofolate. W. N. Hunter *et al.*, our collaborators, have well described accurate structures of PTR1-ligand complexes of *Leishmania major*, and *Trypanosoma brucei*.[79, 80] Ribbon diagrams of the *Lm*PTR1 and the *Tb*PTR1 subunits are shown in Figure 1.28.

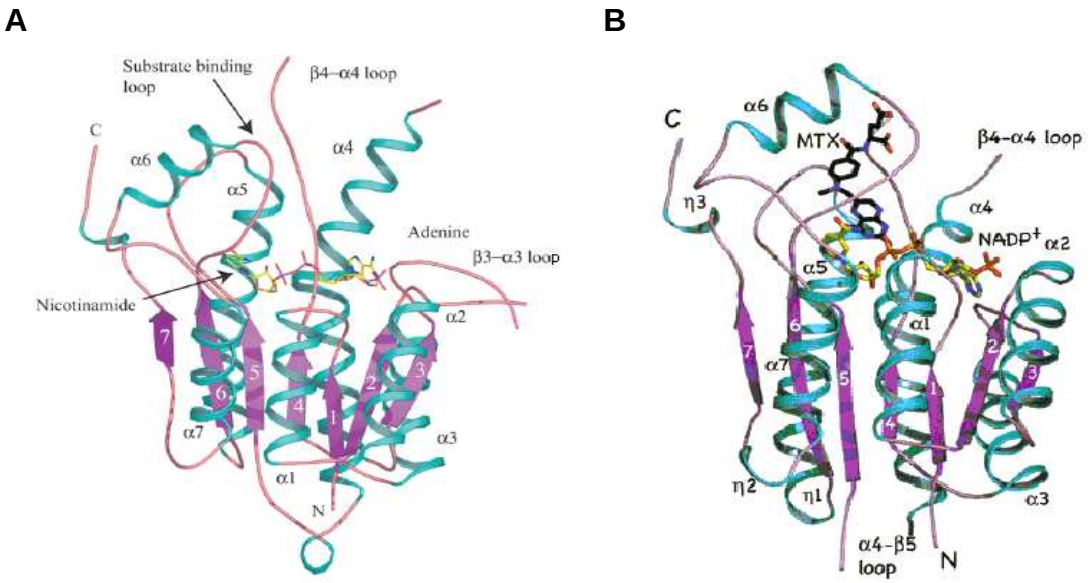


Figure 1.28. A ribbon diagram of the *Lm*PTR1 (A)[79] and the *Tb*PTR1 (B)[80] subunits. Helices are coloured cyan, b-strands are coloured purple. Cofactor bonds are drawn as sticks, coloured according to atom type: N, blue; P, purple; O, red; C, yellow. The figures were reproduced under permission of Elsevier Ltd. and John Wiley & Sons Ltd. (Appendix VI and VII).

PTR1 is the only enzyme known to reduce biopterin in *Leishmania* and has been proven to be essential for growth *in vivo* by gene knockout studies.[77] Since PTR1 is less sensitive to methotrexate (MTX) than DHFR (IC_{50} = 1.1 µM and 0.005 µM for *L. major* PTR1 and DHFR-TS, respectively)[81] but catalyses the reduction of DHF, it compromises drugs targeting of DHFR by acting as a metabolic bypass.[78] This, in part, explains why antifolate therapies focused on inhibition of DHFR have failed against trypanosomatid infections.[82] Any antifolate therapy targeting the DHFR of trypanosomatids must also target PTR1 to block the bypass and allow effective inhibition of essential folate metabolism. Therefore, it is necessary to develop two separate compounds (I-1 and I-2) inhibiting the individual enzymes or a single compound (I-3) with good inhibitory properties against both (Fig. 1.29).

A number of compounds active against both enzymes have been characterised but in these cases either PTR1 is less susceptible to inhibition than DHFR or the level of inhibition is poor.[79, 81] There are explanations why envisaging a single compound with the necessary inhibitory properties for use against both DHFR and PTR1 is extremely difficult. (1) DHFR presents distinct structural features compared with

PTR1 allowing it to bind cofactor and substrate or inhibitors in any order.[80] (2) DHFR also undergoes extensive conformational changes upon ternary complex formation,[84, 85] whereas PTR1 appears more rigid.[79]

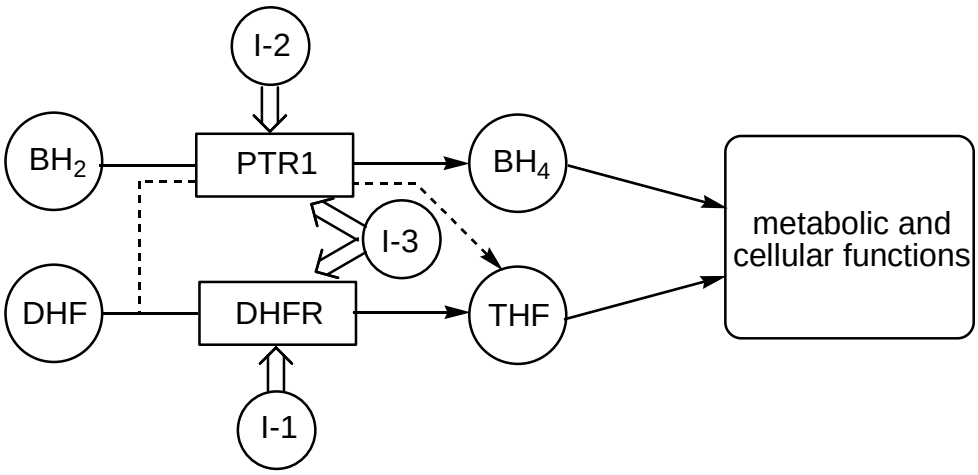


Figure 1.29. Role of PTR1 as a metabolic bypass (dotted line). BH_2 (dihydrobiopterin), BH_4 (tetrahydrobiopterin), DHF (dihydrofolate), THF (tetrahydrofolate), PTR1 (pterin reductase), DHFR (dihydrofolate reductase), I-1 (inhibitor of DHFR), I-2 (inhibitor of PTR1), I-3 (dual inhibitor of DHFR and PTR1).[83]

A structure-based sequence alignment of *Tb*PTR1 and *Lm*PTR1, the sequences share 51% identity. The *Tb*PTR1 topology is closely related to *Lm*PTR1; an overlay of one monomer of *Tb*PTR1 onto one subunit of *Lm*PTR1 matches 244 residues (Fig. 1.30). The *Tb*PTR1 sequence is shorter than *Lm*PTR1 due to two deletions and a truncation at the *N*-terminus. In *Tb*PTR1 a short β3-α3 loop is well ordered whereas in *Lm*PTR1, the loop is extended by 13 residues and generally disordered.[79] A second, smaller deletion of four residues occurs at the C-terminal segment of the loop linking β4 and α4 in *Tb*PTR1. This loop is also surface-exposed and disordered in both *Tb*PTR1 and *Lm*PTR1.[80]

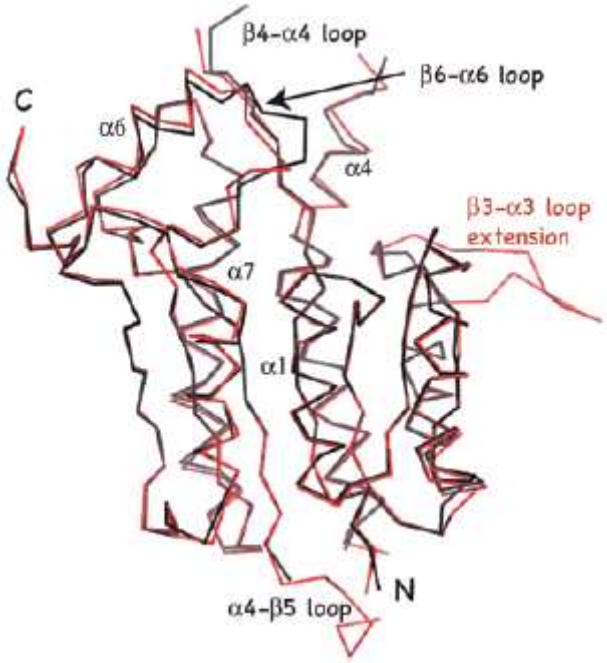


Figure 1.30. The overlapping view of a subunit of *Tb*PTR1 (black) and *Lm*PTR1 (red).[80] The figure was reproduced under permission of John Wiley & Sons Ltd. (Appendix VII).

There are already potent DHFR inhibitors with well characterized pharmacokinetics.[86] Therefore, based on this information we are now focusing on development of PTR1 inhibitors to complement existing drugs though dual inhibitors for both PTR1 and DHFR can be found in a fortuitous case.

Recently, N. Schormann *et al.* have expressed a recombinant *T. cruzi* enzyme, *Tc*PTR2[87] which can also reduce dihydropteridines.[88] However, PTR2 cannot reduce oxidised pteridines[104] so if we find an effective PTR1 inhibitor which can reduce oxidised pteridines thus block the formation of dihydropteridines, then the role of PTR2 to reduce dihydropteridines might be neglected. Therefore, we are not considering PTR2 as a target enzyme at this stage.

1.4. Synthetic review

This section proposes a couple of synthetic models for 7-deazapurine and pteridine analogues based on their bicyclic ring formation.

1.4.1. Structural classification

Pyrimidine based 6-5 and 6-6 membered heterocycles have been synthesised continually not only for synthetic interests but also for biological applications. Pyrimidine **1.47** (Fig 1.31) is a heterocyclic aromatic compound containing two nitrogen atoms at position 1 and 3 of the six-membered ring.

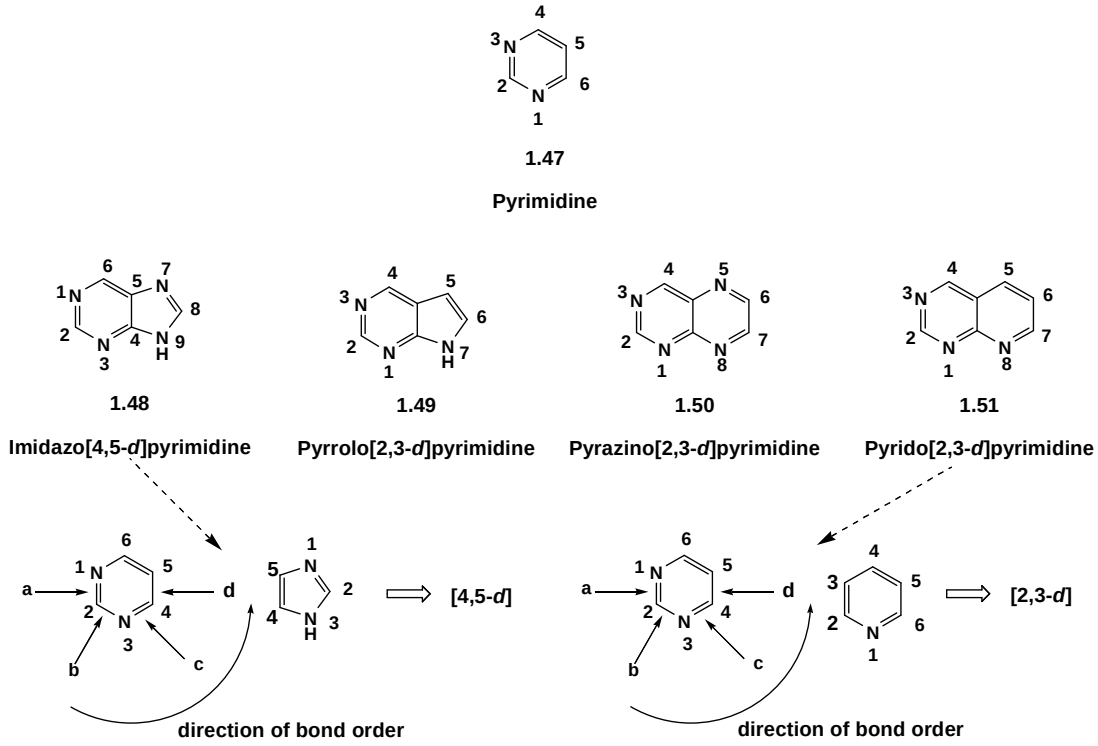


Figure 1.31. Structures and the naming method of pyrimidine and its fused rings.

Although pyrimidine **1.47** itself has a great deal of possibilities to be functionalised for biomedical applications, pyrimidine based fused ring compounds are becoming a main stream for development of novel antifolates due to their structural similarities with folic acid **1.1** with regard to the main scaffold, and its diversification potentials at annular carbons and nitrogens. In pyrimidine based bicyclic fused ring systems, imidazo[4,5-*d*]pyrimidine **1.48**, pyrrolo[2,3-*d*]pyrimidine **1.49**, pyrazino[2,3-*d*]pyrimidine **1.50**, and pyrido[2,3-*d*]pyrimidine **1.51** are main scaffolds (Fig. 1.31). And these fused rings can be funtionalised depending on specific biological activities to produce new medicinal compounds.

In the following sections, we will see some synthetic examples of pyrrolo[2,3-*d*]pyrimidines and pyrazino[2,3-*d*]pyrimidines based on their ring formation.

1.4.2. Synthesis of pyrrolo[2,3-*d*]pyrimidines

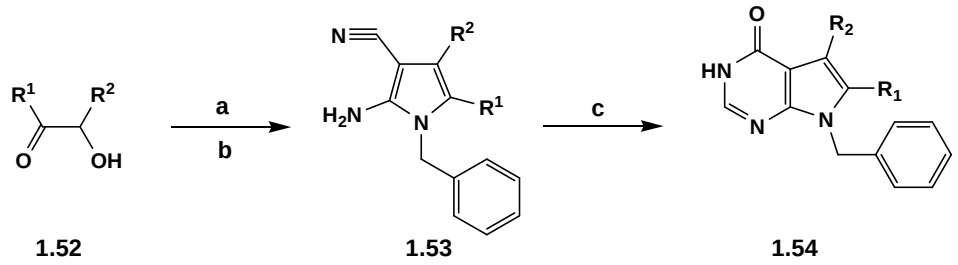
The synthetic methods for pyrrolo[2,3-*d*]pyrimidines can be divided into three major approaches. Firstly, a pyrimidine can be annulated onto a pyrrole intermediate. Secondly, a ring transformation-annulation involving guanidine is also known. Thirdly, a pyrrole ring can be fused onto a pyrimdine scaffold. The examples of the three synthetic strategies are introduced in the next sections.

1.4.2.1. Synthesis from pyrrole intermediates

This strategy is based on formation of a 2-amino-3-cyano pyrrole from a reaction of an appropriate starting material with malononitrile. This intermediate can easily provide a fused pyrimidine ring in the following step. Detailed reaction conditions and numbers of reaction steps can be different depending on starting materials and desired positions of functional groups. Two examples are shown as follows.

Synthesis from an α-hydroxy ketone with malononitrile

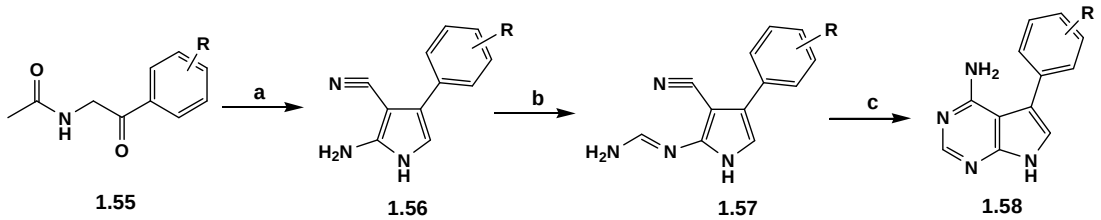
Substituted 1-benzyl-2-amino-3-cyanopyrroles **1.53** can be obtained by reaction of α-hydroxy ketones **1.52** with benzylamine and malononitrile. Reflux of the cyanoaminopyrroles **1.53** with 85% formic acid afforded the 5,6-substituted 4-hydroxy-7-benzylpyrrolo[2,3-*d*]pyrimidines **1.54** (Scheme 1.1).[89]



Scheme 1.1. Reagents and conditions: (a) benzylamine, toluene, HCl, reflux. (b) malononitrile, toluene, reflux. (c) formic acid (85%), 110 °C, 5 h.

Synthesis from a substituted acetamide with malononitrile

Condensation of substituted amides **1.55** with malononitrile furnishes 2-amino-3-cyano-4-phenyl pyrroles **1.56**, which are then converted into the amidine **1.57** by reaction with triethyl orthoformate followed by ammonolysis of the resulting iminoester. Cyclization to the 4-amino-5-aryl-7*H*-pyrrolo[2,3-*d*]pyrimidine **1.58** can be achieved by treatment with sodium ethoxide (Scheme 1.2).[90]



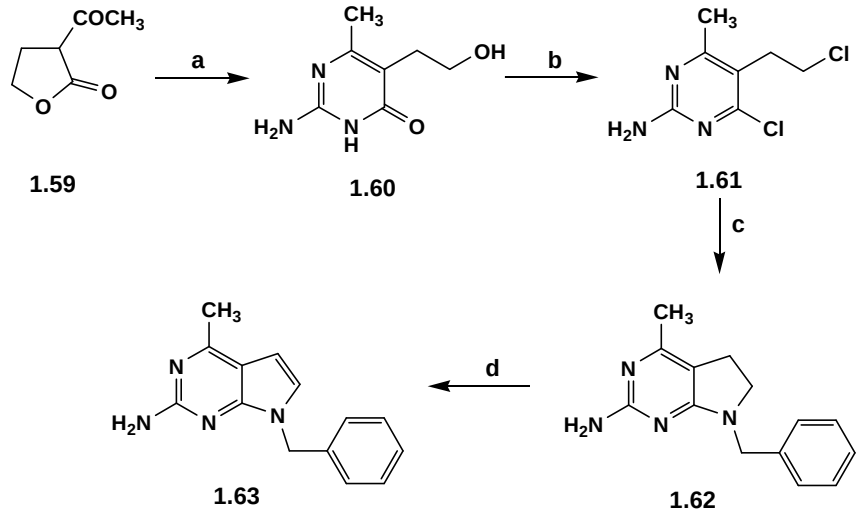
Scheme 1.2. Reagents and conditions: (a) $CH_2(CN)_2$, NaOEt, EtOH, 55 °C. (b) (i) $HC(OEt)_3$, 80 °C. (ii) NH_3, MeOH, rt. (c) NaOEt, EtOH, 80 °C.

1.4.2.2. Ring transformation/ring annulation approach

This method is based on a ring transformation/ring annulation by a reaction of an appropriate cyclic intermediate with guanidine. Two examples are shown as follows.

Synthesis from 2-acetylbutyrolactone

Refluxing 2-acetylbutyrolactone **1.59** and guanidine carbonate with absolute ethanol in the presence of triethylamine or sodium methoxide affords the intermediate compound **1.60**. Compound **1.60** can be converted into **1.61** by refluxing with phosphorus oxychloride. Condensation of benzylamine with compound **1.61** in the presence of triethylamine under reflux in *n*-butanol affords the bicyclic compound **1.62**. Oxidation of compound **1.62** by manganese dioxide produced 2-amino-4-methyl-7-benzylpyrrolo[2,3-*d*]pyrimidine **1.63** (Scheme 1.3).[91]



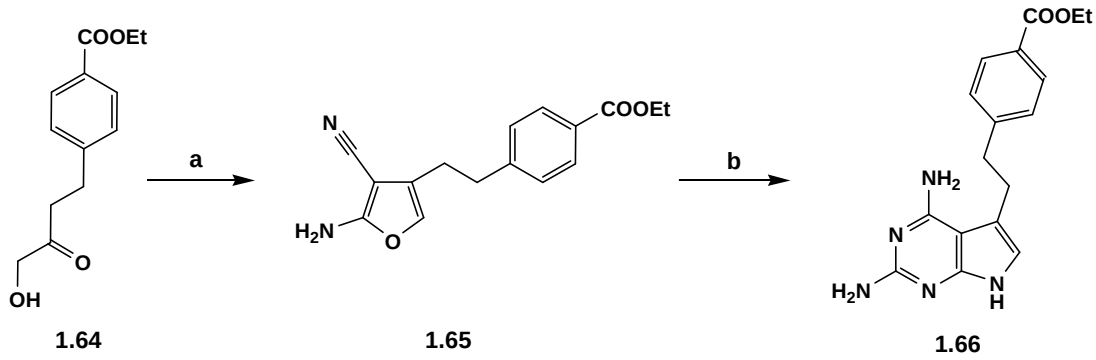
1.59 1.60 1.61 1.62 1.63

Scheme 1.3. Reagents and conditions: (a) guanidine carbonate, EtOH, triethylamine, reflux. (b) $POCl_3$, reflux. (c) benzylamine, triethylamine, *n*-BuOH, 90 °C. (d) MnO_2, 1,4-dioxane, reflux.

Synthesis from 2-amino-3-cyano-4-alkyl furan

The requisite furan *o*-aminonitrile precursor **1.65** can be obtained by the condensation of a suitable α-hydroxy ketone **1.64** with malononitrile in the presence of triethylamine. Reaction with guanidine in refluxing ethanol then yielded 2,3-diamino-5-alkyl-7*H*-pyrrolo[2,3-*d*]pyrimidine **1.66** (Scheme 1.4).[92-94]

Plausible mechanisms for the amidine-mediated ring transformation/ring annulation reactions were suggested by E. C. Taylor *et al*.[92]



1.64 **1.65** **1.66**

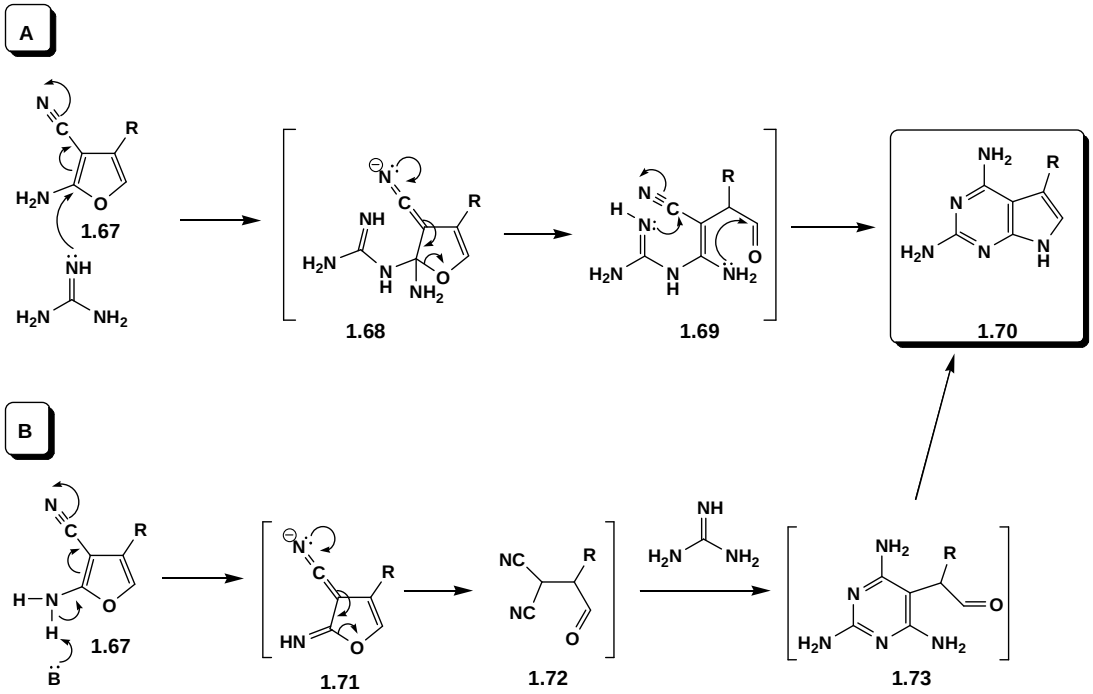
Scheme 1.4. Reagents and conditions: (a) malononitrile, triethylamine, MeOH, 25 °C. (b) guanidine, EtOH, reflux.

Mechanism A:

Initial Michael addition of the guanidine to the 2-position of the furan *o*-aminonitrile **1.67** could be followed by furan ring displacement to generate an open-chain carbonyl derivative **1.69**, which was then recyclises to an intermediate pyrrole. Subsequent intramolecular addition completes the pyrimidine ring annulation to give compound **1.70** (Scheme 1.5, A).[92]

Mechanism B:

Deprotonation of the acidic 2-amino group of the furan *o*-aminonitrile by the strongly basic guanidine could be followed by C-O bond displacement to generate an intermediate ketemine which, by prototropic rearrangement, would lead to the substituted malononitrile **1.72**. Further reaction with the guanidine in the usual way followed by ring closure would form the fused pyrrole ring (Scheme 1.5, B).[92]



Scheme 1.5. Two plausible mechanisms for syntheses of pyrrolo[2,3-*d*]pyrimidines from 2-amino-3-cyano-4-alkyl furan.

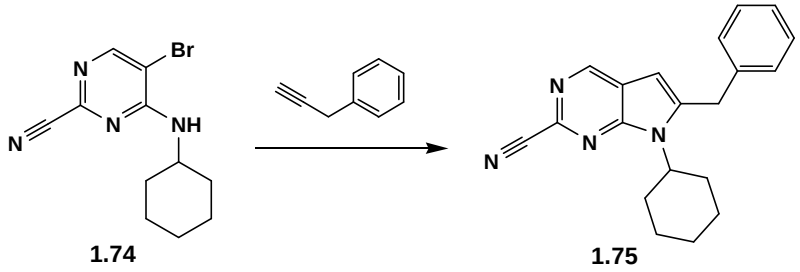
1.4.2.3. Synthesis from pyrimidine intermediates

This method is related to the reactivities of substituted pyrimidines. Halopyrimidines with a halogen atom at position 5 or 6 can be substituted by a suitable nucleophile, these can undergo ring closure to form a fused pyrrole.[95-97] In the case of 2,6-diamino-4-pyrimidinones, reaction of C5 and the 6-amino group with electrophilic

partners, eventually to form pyrrolo[2,3-*d*]pyrimidines have been reported. Some related examples are shown as follows.[98]

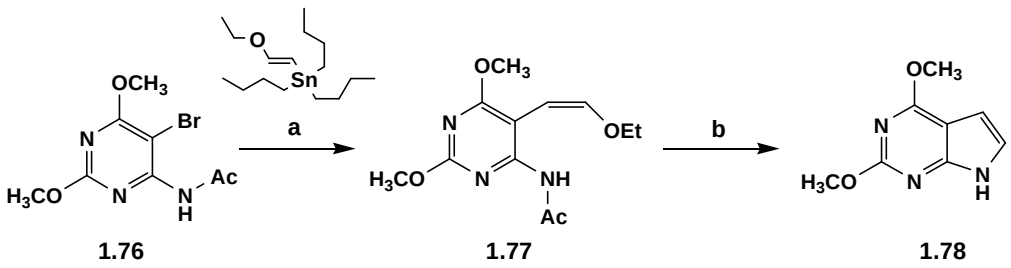
Synthesis from 5-halopyrimidine

Typical Sonogashira coupling with a suitable terminal alkyne followed by immediate ring closure can afford the 6-substituted pyrrolo[2,3-*d*]pyrimidine **1.75** (Scheme 1.6).[95]



Scheme 1.6. Reagents and condition: $Pd(PPh_3)_2Cl_2$, CuI, Et_3N, DMF, 80 °C.

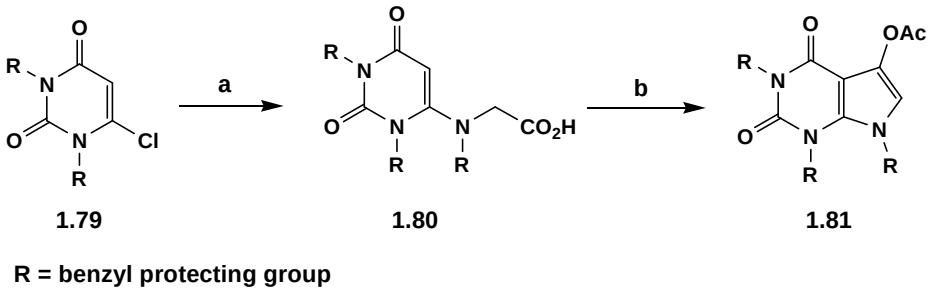
A Stille cross-coupling reaction of a 5-bromopyrimidine **1.76** with a vinylstannane produced an intermediate **1.77** that was deprotected and cyclised under acidic conditions to yield the pyrrolo[2,3-*d*]pyrimidine **1.78** (Scheme 1.7).[96]



Scheme 1.7. Reagents and conditions: (a) $Pd(PPh_3)_2Cl_2$, Et_4NCl. (b) HCl, MeOH.

Synthesis from 6-halopyrimidines

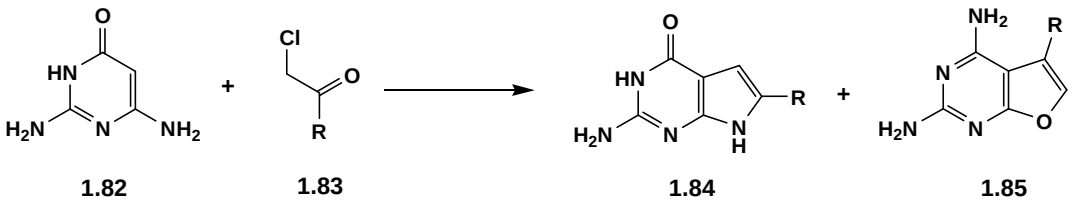
The displacement of a 6-chloro group from a 1,3-dibenzyl protected uracil **1.79** by an *N*-benzyl protected glycine yielded the corresponding aminouracil **1.80**. Heating this compound with acetic acid anhydride caused cyclisation to the acetoxypyrrolo[2,3-*d*]pyrimidine **1.81** (Scheme 1.8).[97]



Scheme 1.8. Reagents and conditions: (a) sodium 2-(benzylamino)acetate, EtOH, water, reflux. (b) Ac_2O, 80 °C.

Cyclocondensation with α-halo ketones

Although, this reaction can produce mixtures of pyrrolo[2,3-*d*]pyrimidines and furo[2,3-*d*]pyrimidines (Scheme 1.9), separation of these two products is not difficult. In addition, this synthetic strategy provides facile one-step annulations of substituted pyrroles or furans.[98-100]



Scheme 1.9. Reagent and condition: DMF, 50 ~ 60 °C.

The mechanisms leading to **1.84** and **1.85** must involve at least three major steps: (i) bond formation between a heteroatom (O or N) of **1.82**, and a carbon of the α-halo ketone, (ii) bond formation between C-5 of **1.82** and a carbon of the α-halo ketone, and (iii) loss of water.[98]

1.4.3. Synthesis of pyrazino[2,3-*d*]pyrimidines (pteridines)

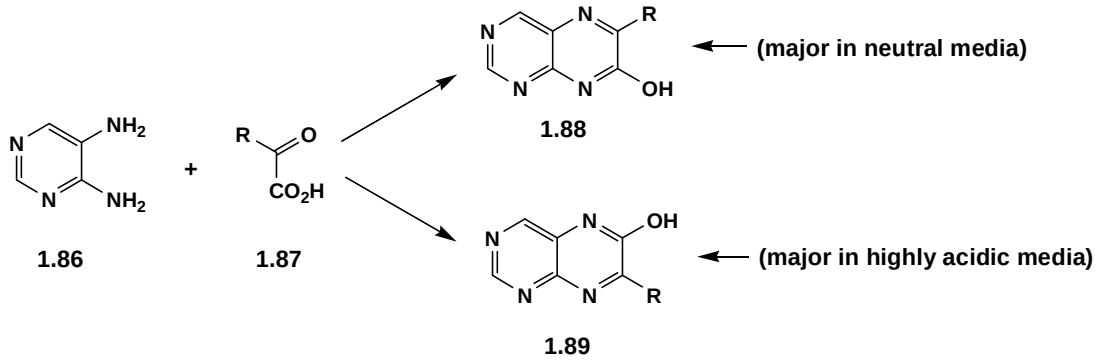
Pteridines have been synthesised continually due to their significant possibilities with regard to bioactivities, which are mainly derived from the structural similarities of the main scaffolds compared to folic acids and their metabolic derivatives. The synthesis of the ring system has been approached by two obvious routes: one is a classical method involving condensation of a 4,5-diaminopyrimidine with an α,β-dicarbonyl compounds (Isay synthesis),[101] and the second, the elaboration of the pyrimidine ring on a preformed pyrazine (Taylor synthesis).[102]

The disadvantage of the Isay-type synthesis is the low selectivity of condensation of the 5,6-diaminopyrimidine with an unsymmetrical 1,2-dicarbonyl compound. Since separation of a mixture of 5/6-substitutued isomers is often extremely difficult, this approach is only used when selectivity can be achieved. Syntheses are sought which selectively produce a single isomer.[103] Representative examples of Isay and Taylor synthesis are introduced in the following sections.

1.4.3.1. Condensation of 5,6-diaminopyrazines (Isay synthesis)

Most pyrimidines possesing 5,6-diamino groups seem to undergo this reaction with appropriate doubly activated two-carbon fragments. The types of such compound which may be used include; 1,2-diketones, aldehydoketones, dialdehydes, α-keto acids (or esters), α-aldehydoacids (or esters), 1,2-dicarboxylic acids (or esters).[104]

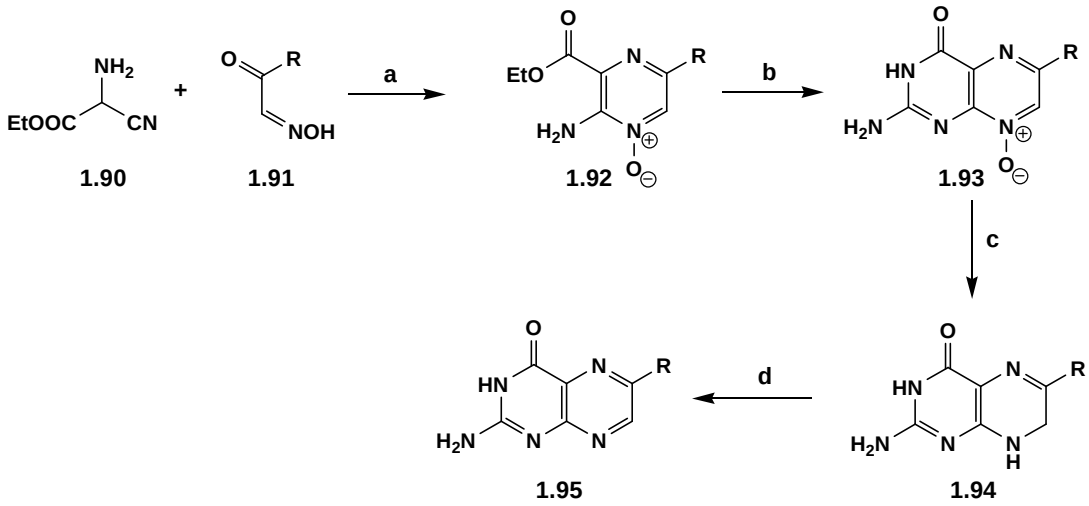
Generally, it is observed that in reaction with aldehyde or keto acids in neutral or weakly acidic media (pH ~ 5), the primary condensation between the 5-amino and the keto (or aldehydo) groups produces 7-hydroxypteridines **1.88**, whereas in a highly acidic midium (pH ~ 0.25), protonation of the more strongly basic 5-amino group leads to the formation of 6-hydroxypteridines **1.89** (Scheme 1.10).[103]



Scheme 1.10. Preferential orientation at different pH.

1.4.3.2. Synthesis from pyrazine intermediates (Taylor synthesis)

This method was first exploited by E. C. Taylor *et al.*[102] This approach has an advantage that because the pyrazine ring is pre-synthesised, it eventually produces, regioselectively, 6-substituted pteridines. The condensation of ethyl α-aminocyanoacetate **1.90** with oximinoacetone **1.91** gave 2-amino-3-carbethoxy-5-methylpyrazine 1-oxide **1.92**, which was cyclised with guanidine in the presence of sodium methoxide to 6-methylpterin 8-oxide **1.93**. This latter compound can be reduced with aqueous sodium dithionite to 6-methyl-7,8-dihydropterin **1.94**. Potassium permanganate oxidation of **1.94** proceeded to give 6-methylpterin **1.95** (Scheme 1.11).[102]



Scheme 1.11. Reagents and conditions: (a) MeOH, 35 °C. (b) guanidine, DMF, reflux. (c) sodium dithionite, hot water. (d) $KMnO_4$ (aq), rt.

1.5. Project overview

1.5.1. Aim of this project

As described in Section 1.2, 6-6 membered pyrimidine based small molecules have been a main stream of the antifolate discovery field. MTX **1.20** is a representative classical pyrazino[2,3-*d*]pyrimidine antifolate as a folate derivative. Raltitrexed **1.26** and Piritrexim **1.28** are relatively new antifolates with different fused rings, phenyl and pyridine rings, respectively. In addition, as a 6-5 membered fused pyrimidine, pemitrexed **1.25**, a pyrrolo[2,3-*d*]pyrimidine antifolate has stimulated medicinal chemists to synthesise derivatives of this scaffold to produce new antifolate drugs.

From the medicinal chemistry view point, this research is based on folate metabolism (Section 1.1), syntheses of new analogs of pyrrolo[2,3-*d*]pyrimidine and pyrazino[2,3-*d*]pyrimidine (Section 1.4), and development of effective antifolates (Section 1.2) eventually.

The main aims of this study can be summarised as follows.

(i) Development of effective synthetic methods for analogs of pyrrolo[2,3-*d*]pyrimidine and pyrazino[2,3-*d*]pyrimidine based on diversity oriented strategy to produce library compounds.

(ii) Target oriented synthesis for development of new inhibitors against trypanosomatid parasitic enzyme PTR1.

(iii) Evaluation of biological activities for the synthesised compounds for further synthesis and assays eventually for clinical tests.

Further details and backgrounds of this research are given in the following sections.

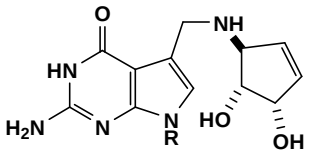
1.5.1.1. Diversity oriented strategy

This strategy involves screening analogs of pyrrolo[2,3-*d*]pyrimidine and pyrazino[2,3-*d*] pyrimidine for their abilities to inhibit a biological pathway in cells or organisms without regard for any particular protein target. For this, we explored effective synthetic routes to synthesise efficiently a collection of small molecules capable of perturbing any disease-related biological pathway, mainly, folate metabolism, leading eventually to the identification of the therapeutic protein targets.

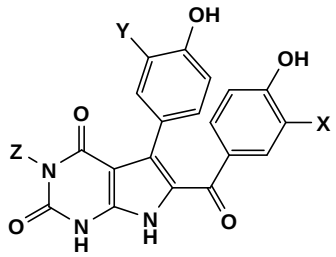
Pyrrolo[2,3-*d*]pyrimidines

The pyrrolo[2,3-*d*]pyrimidine ring system has aroused considerable interest as this skeleton has been often encountered in important pharmacologically active substances. A couple of examples are as follows.

As natural antibiotics, Q base **1.96a** and nucleoside Q **1.96b** are well known examples with the pyrrolo[2,3-*d*]pyrimidine ring.[105] Rigidins **1.97a ~ 1.97e**, marine natural products containing a pyrrolo[2,3-*d*]pyrimidine ring, have been shown to exhibit very significant calmodulin antagonist activity. As a synthetic compound, multitargeted antifolate, Pemetrexed **1.25** (Section 1.2.2.1) is a representative example of a clinically approved drug to treat some types of cancer.



1.96a Q base (R = H)
1.96b Nucleoside Q (R = ribose)



1.97a X = Y = Z = H Rigidin
1.97b X = OCH_3 Y = Z = H Rigidin B
1.97c Y = OCH_3 X = Z = H Rigidin C
1.97d X = Y = OCH_3 Z = H Rigdin D
1.97e X = Y = H Z = CH_3 Rigidin E

Figure 1.32. Structures of Q base, Nucleoside Q, and Rigidins.

Due to the significant success of Pemetrexed **1.25**, the pyrrolo[2,3-*d*]pyrimidine structure has become an attractive hetero-bicyclic scaffold for drug discovery. This thesis details effective synthetic methods not only for the ring formation of pyrrolo[2,3-*d*]pyrimidines but also for incorporation of diverse functional groups at annular positions to produce a library of pyrrolo[2,3-*d*]pyrimidines.

Pyrazino[2,3-*d*]pyrimidines (Pteridines)

Because of multi-functional bioactivities, pteridine compounds have been actively synthesised and screened to produce new drugs. Anticancer activities of pteridines have been reported by many research groups.[107-110] Antiparasitic activity of 2,4-diamino pteridine analogues **1.98** with a bridged diarylamine side chain has also been reported.[111] Recently, Y. Ding *et al*[112] and M. Ślusarczyk *et al.*[113] reported 4,6,7-functionalised pteridines **1.99** as potent inhibitors of hepatitis C virus (HCV) NS5B, an RNA-dependent RNA polymerase. This viral enzyme is crucial to HCV replication in the host liver. In addition, R. C. Reynolds *et al.* reported a new 2-carbamoyl pteridine **1.100** as an inhibitor for mycobacterial FtsZ, named after filamenting temperature-sensitive mutant Z and is an essential protein for bacterial cell division.[114]

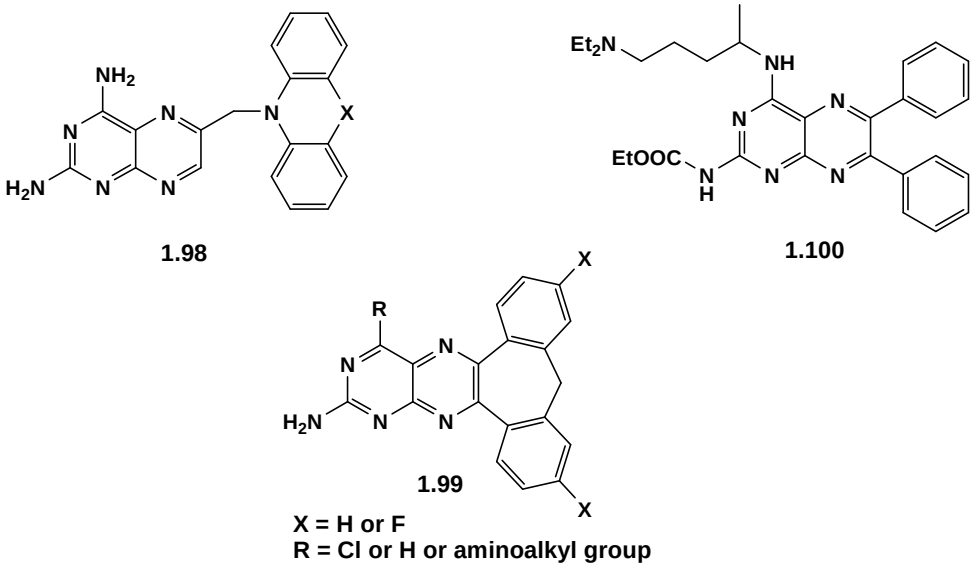


Figure 1.33. Bioactive pteridines.

These multifunctional bioactivities of pteridines have attracted us to synthesise novel library compounds to produce new possibilities of more effective drugs for related diseases. For these reasons, we planned to synthesise pteridine analogues funtionalised at C4, C6, and C7 positions (Fig. 1.34). The synthetic (Section 2.1.2) and biological results (Section 2.2.2) of these compounds will be described in this thesis as a part of this research.

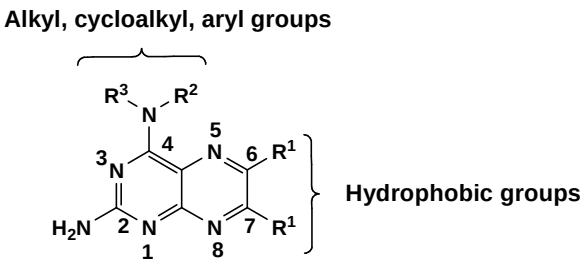


Figure 1.34. 4,6,7-functionalised pteridines.

1.5.1.2. Target oriented strategy (Inhibitor for PTR1)

Modern drug discovery often involves screening small molecules for their ability to bind to a pre-selected protein target.[115] As mentioned before, a part of this study was focused on synthesising new inhibitors of trypanosomatid parasites. PTR1 was selected as a target enzyme because of its crucial and peculiar action in pteridine (folate and pterin) metabolism (Section 1.3.4.3). The active site of *L. major* was well defined by Professor W. N. Hunter (University of Dundee), our collaborator, thereby we have discussed potential inhibitors based on crystal structure of the active site.

From the study of the active site from the X-ray strcture of *L. major* PTR1, we observed that the 7-deazaguanine (2-amino-4-oxopyrrolo[2,3-*d*]pyrimidine) framework fits into the active site (Section 2.1.3.1). Thus we designed several potential inhibitors and synthesised them. Accordingly, this report contains design of new inhibitors for PTR1, synthetic methods of designed compounds and their *in vitro*

results against trypanosomatids. We expect this study can give an inspiration to make new effective drugs for treatment of leishmanial and trypanosomal diseases. Indeed, finding new effective pro-drugs for these diseases is the goal of this research.

1.5.2. Synthetic plans

1.5.2.1. 2,5-Functionalised pyrrolo[2,3-*d*]pyrimidines

Michael reaction of 2,6-diamino-4-pyrimidinones with nitroalkenes followed by subsequent ring closure can provide 5-functionalised pyrrolo[2,3-*d*]pyrimidine (Fig. 1.35, A).[116] However, 2-thioalkyl-6-amino 4-pyrimidinones and 2-alkylhydrazino-6-amino 4-pyrimidinones have not been exploited in this route (Fig. 1.35, B and C). In addition, we were interested in diversification at the C2 position *via* substitutions by suitable amine nucleophiles. Therefore, we decided to exploit routes B and C (Fig. 1.34) to find out effective reaction conditions in order to provide 2,5-difunctionalised pyrrolo[2,3-*d*]pyrimidines for biological screening.

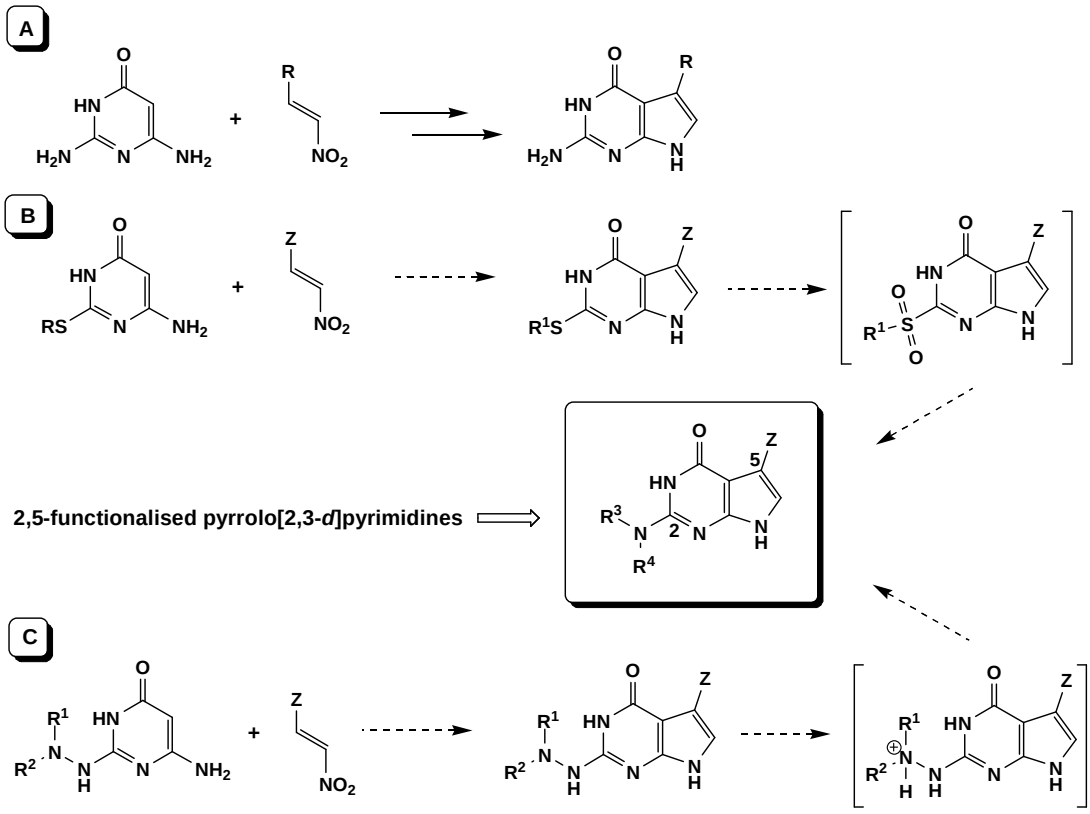


Figure 1.35. Michael reaction route for the formation of pyrrolo[2,3-*d*]pyrimidine.

1.5.2.2. 4,6,7-Functionalised pyrazino[2,3-*d*]pyrimidines

This work was based on our published synthetic work.[88] At the outset of this work, this had not been accomplished, mainly due to the inconvenient displacement stage using DMDO (dimethyldioxirane), which is used for oxidation of the sulfide.

To produce a library compounds for high-throughput screening, we selected five different symmetrical biacetyls to form pteridine rings and five different amines were chosen for final C4 substitution. Finding simple and convenient C4 displacement method was a challenging task. The proposed synthetic pathway is summarised in Figure 1.36.

The first step required benzylation of **1.101** with benzyl bromide. Then nitrosation at

C5 followed by reduction will produce compound **1.104**. The pteridine scaffold can be formed by condensation of **1.104** with biacetyls, subsequent oxidation of the sulfide moiety followed by displacement with amines should give final library compounds, 4,6,7-funtionalised pteridines **1.106**.

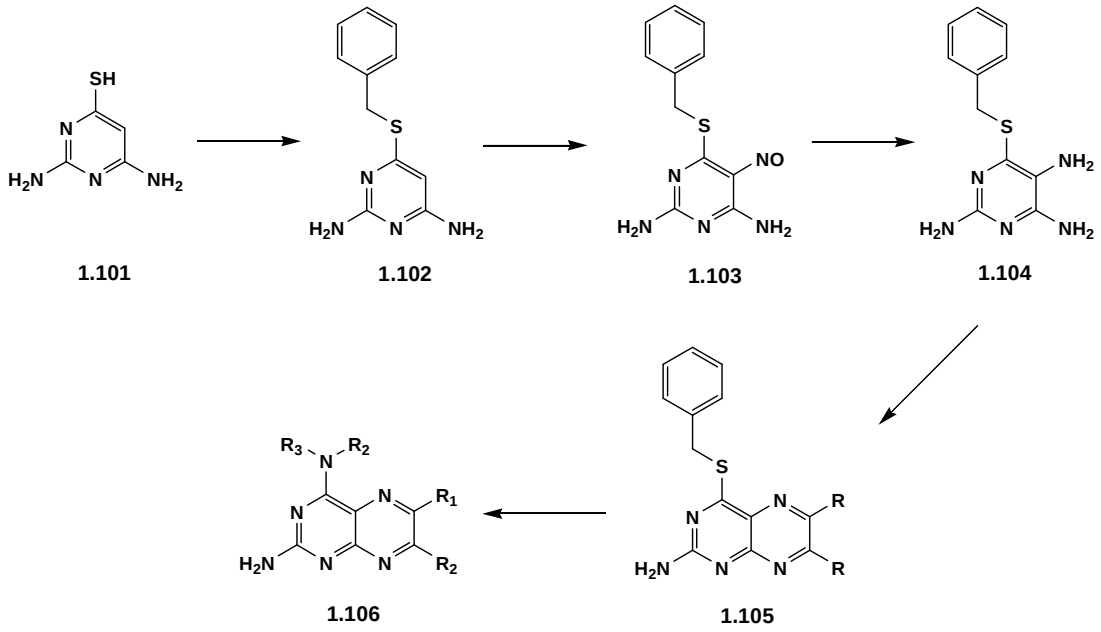


Figure 1.36. A synthetic pathway of 4,6,7-substituted pteridines **1.106**.

1.5.2.3. Inhibitors for PTR1 (5,6-funtionalised deazaguanine)

We have reached two important conclusions from the molecular modeling study of PTR1-ligand of *L. major* (Section 2.1.3.1): (i) deazaguanine is a reasonable framework to assemble into the active site, (ii) there are three accessible pockets, two major hydrophobic pockets (Fig. 1.36, A and B), and one minor pocket (Fig. 1.37, C) in the active site for deazaguanine.

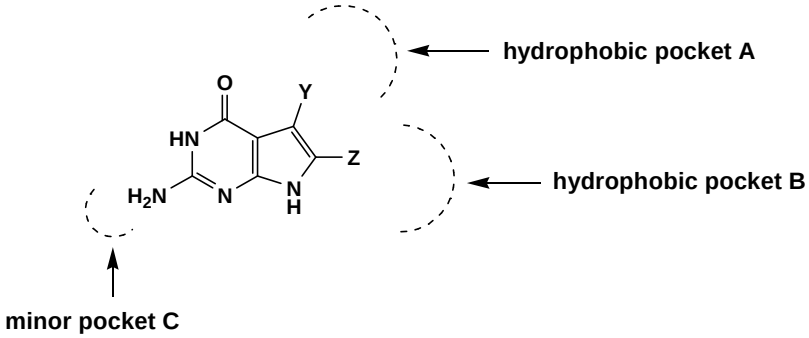


Figure 1.37. Deazaguanine framework and its three potential regions (A, B, and C) to diversify.

Thus we selected four different forms of potential inhibitors in order to diversify on the two regions of deazaguanine. Figure 1.38 shows the four selected categories of inhibitor candidates in this project. The first three categories were based on the deazaguanine framework. Though the inhibitor in category 1 (R = 4-COOH) has already reported by E. C. Taylor *et al.*[116] as an intermediate molecule for Pemetrexed **1.25**, an anticancer agent, we are interested in this molecule because of the diversifying possibilities (e.g. length of alkane moiety and substituent on the phenyl ring) at the C5 position. Category 2 shows representative model compounds in order to vary the substitutes at C6. Category 3 was designed as a structural hybrid of the categories 1 and 2, to accommodate the two major hydrophobic pockets at the same time.

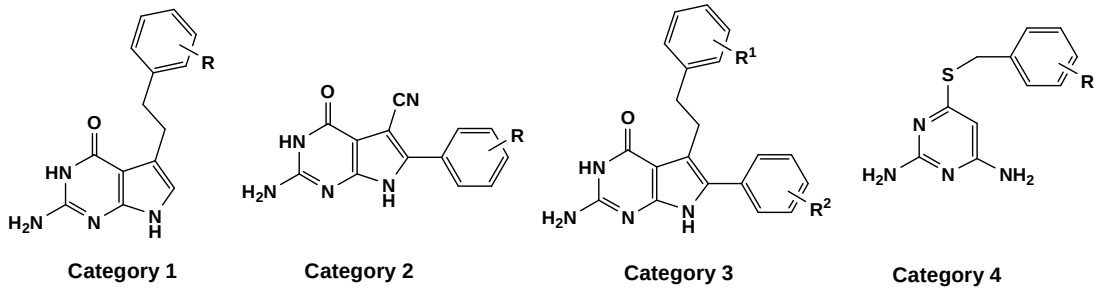
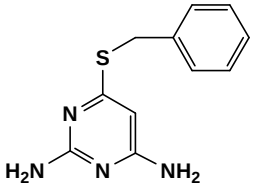


Figure 1.38. Selected potential inhibitors for trypanosomatid parasites' PTR1.

Category 4 (pyrimidine framework) was targeted based on previous biological results from our group where the compound **1.102** displayed an IC_{50} of 10 μM for *L. major* PTR1.[117] From this result, we decided to synthesise further analogues by varying the substitutes on phenyl ring in order to compare the inhibitory activity against PTR1 with compound **1.102**. Then, we might have more knowledge to modify this structure to gain stronger inhibitory activity. This can be supported by computer aided modeling study as X-ray crystal structures of PTR1 are available.



1.102

Figure 1.39. A potential lead compound **1.102** (IC_{50} = 10 μM for *L. major* PTR1) that was reported by J. K. Huggan.[117]

Routes to synthesise each group of potential inhibitors are outlined in Figure 1.40. Route 1-1 and 1-2 are well described by E. C. Taylor and B. Liu.[116] In this study, producing of the known compound 1.112 is a major concern for a preliminary biological screening hence the reported method[116] will be adopted without a significant change. In brief, route 1-1 is related to the synthesis of nitroalkene **1.109** through nitroaldol (Henry) reaction followed by mesylation. Then the synthesised compound **1.109** could be employed in a Michael addition with diaminopyrimidine **1.110** followed by conventional Nef reaction which should give the desired product **1.112**.

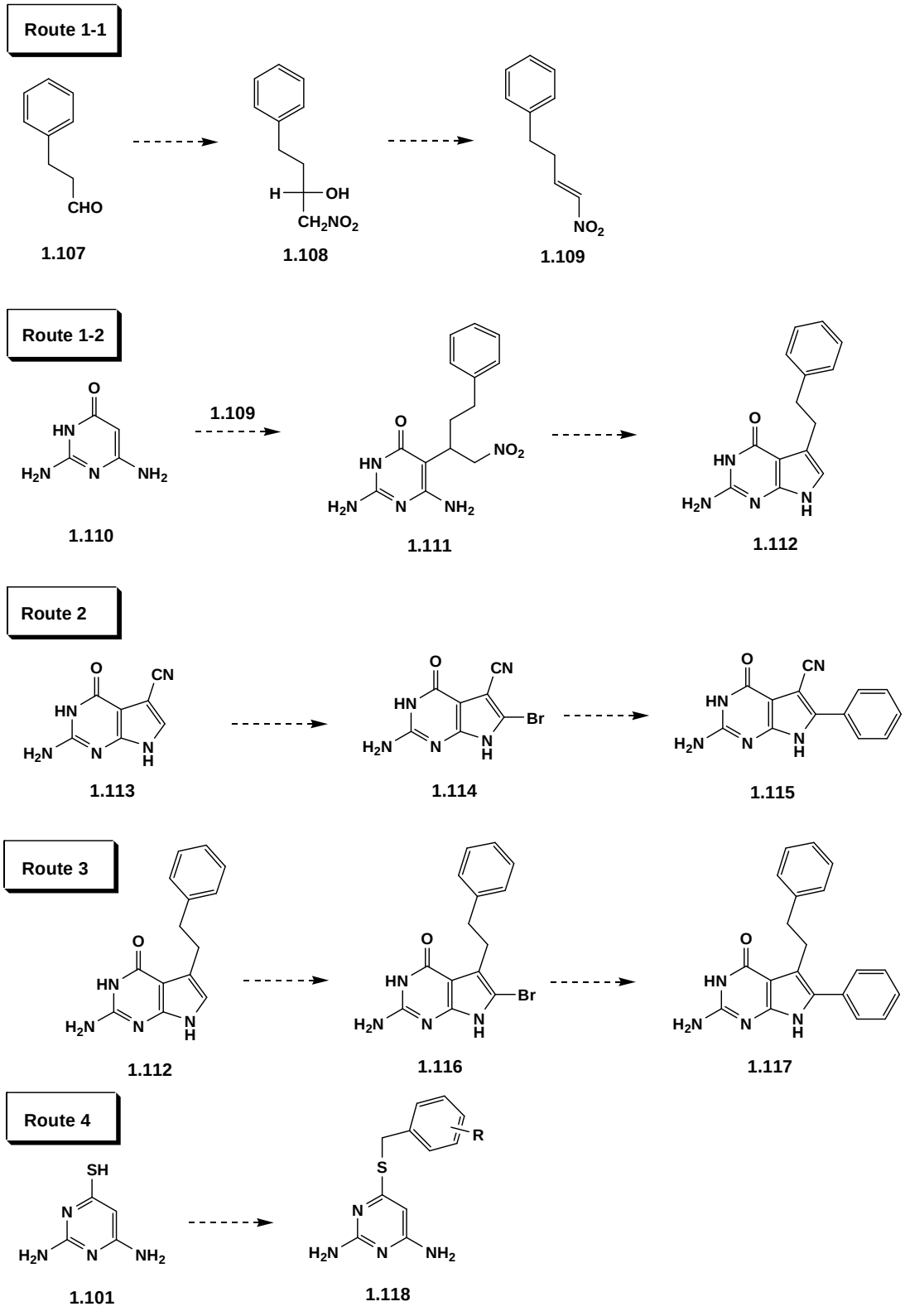


Figure 1.40. Synthetic routes for each five categories.

In route 2, bromination of **1.113** at C6 followed by Suzuki coupling can allow variation at the C6 position. This step has been exploited in our research group but poor yields due to the ineffectiveness of this coupling reaction made us improve the reaction method. To do this we will adopt a modified method by G. A. Molander *et al*.[118] Once we achieve successful improvement on the C6 extension *via* Suzuki coupling, in route 3, compound **1.112** will be treated with the same methodology of route 2 to synthesise a hybrid compound **1.117**. Route 4 is simple benzylation of commercially available **1.101** with different benzyl halides.

2. RESULTS AND DISCUSSION

2.1. Synthetic results

2.1.1. Pyrrolo[2,3-*d*]pyrimidine framework

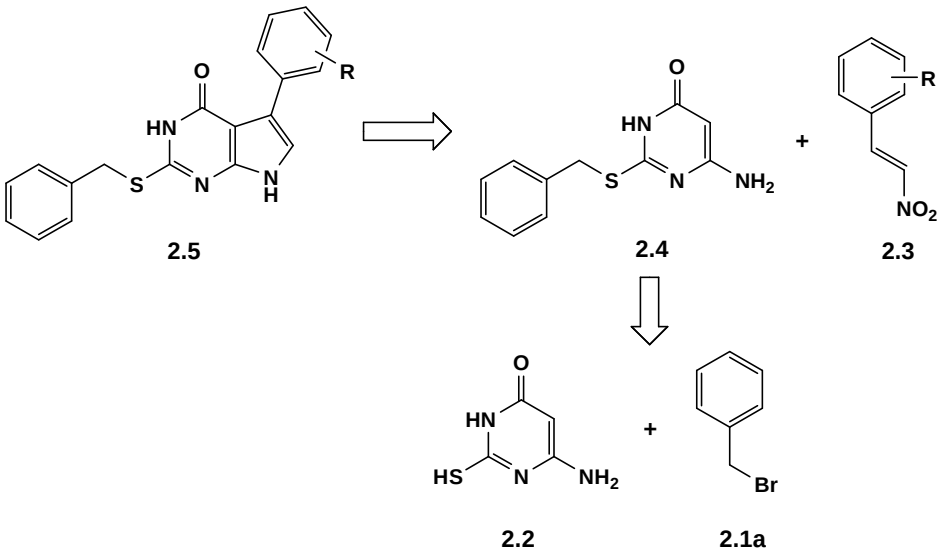
In the following sections, the formation of pyrrolo[2,3-*d*]pyrimidines categorised by C2 connected moieties; 2-thiobenzyl (Section 2.1.1.1.), 2-thiomethyl (Section 2.1.1.2), and 2-alkylhydrazino (Section 2.1.1.3) pyrrolo[2,3-*d*]pyrimidines will be delineated. Then, in Section 2.1.1.4, results of C2 displacement will be discussed.

2.1.1.1. 2-Thiobenzyl pyrrolo[2,3-*d*]pyrimidine

2-Thiobenzyl-5-substituted pyrrolo[2,3-*d*]pyrimidines **2.5** are key precursors to produce a library of 2,6-functionalised library compounds *via* C2 displacement by amine nucleophiles. In this section, synthetic results of analogs of 2-thiobenzyl pyrrolo[2,3-*d*]pyrimidine are introduced.

Retrosynthetic analysis (Scheme 2.1)

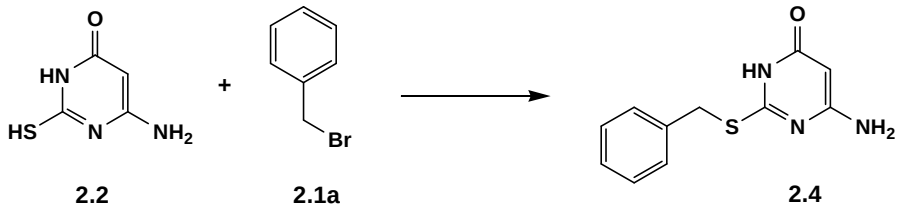
2-Thiobenzyl-5-substituted pyrrolo[2,3-*d*]pyrimidines **2.5** can be formed by a reaction of a 2-thiobenzyl-6-aminopyrimidine **2.4** with a nitroalkene **2.3** *via* Michael reaction followed by Nef reaction. To obtain the main intermediate pyrimidine **2.4**, benzyl bromide **2.1a** was used for benzylation of commercially available 2-mercapto-6-amino-4-pyrimidinone **2.2**.



Scheme 2.1. Retrosynthetic analysis of 2-thiobenzyl-5-substituted pyrrolo[2,3-*d*]pyrimidines **2.5**.

Benzylation

Benzylation was performed by a method developed in our group.[88] Compound **2.4** was obtained as a white solid (Scheme 2.2).



Scheme 2.2. Reagents, condition and yield: H_2O/EtOH (3:5), Et_3N, rt, 88%.

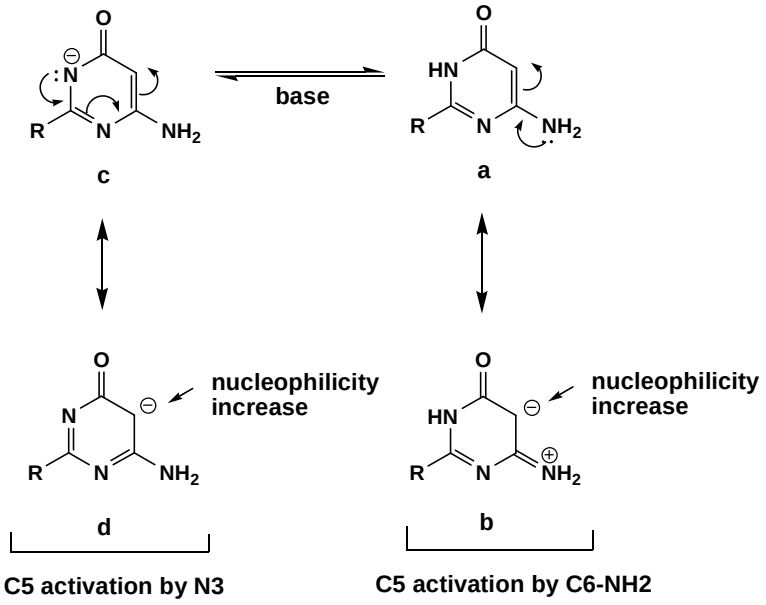
Michael addition

The Michael reaction, the addition of a stabilised carbon nucleophile (Michael donor) to an electrophilic C=C bond (Michael acceptor), has attracted enormous attention as one of the most important carbon-carbon bond forming reactions in

organic synthesis.[119]

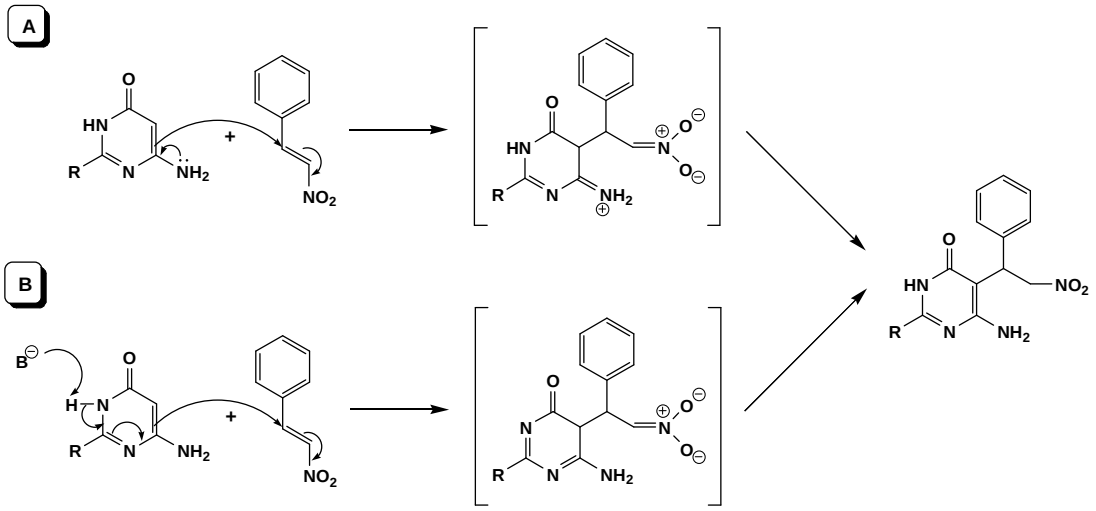
Nitrostyrenes can act as good Michael acceptors because of the high electron-withdrawing power of the nitro group. E. C. Taylor *et al.* have performed the reaction successfully between 2,6-diaminopyrimidine and nitroalkenes under biphasic solvent system (EtOAc/H_2O, 1:1) without base.[116, 120, 121] In 6-aminopyrimidine derivatives, the electron releasing 6-amino group can activate C5 toward electrophilic α,β-unsaturated carbonyl compounds.[122]

Actually, we can provide some explanations for the reason that 6-amino-4-oxo pyrimidine derivatives can act as effective Michael donors. First, the 6-amino group can provide electron density at C5 through the pyrimidine ring (Scheme 2.3, a-b). Second, if the N3 anion is formed, it can also increase the electron density at C5, allowing this carbon to act as a Michael donor through electron delocalisation (Scheme 2.3, c-d). These can be the reasonable explanations why the 6-amino-4-pyrimidinone scaffold has a high propensity to act as a good Michael donor.



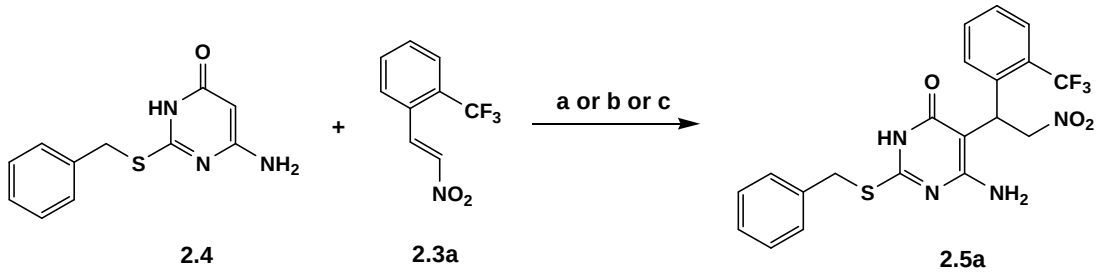
Scheme 2.3. C5 activation by 6-NH_2 and N3.

Scheme 2.4 depicts the two possible mechanisms of Michael addition between a 6-amino-4-pyrimidinone and a nitrostyrene based on the explanation in Scheme 2.3. The pathway-A shows the activation of C5 by 6-amino group and the pathway-B describes the activation of C5 by N3.



Scheme 2.4. Two plausible mechanisms of Michael addition. (A: activation by 6-NH_2, B: activation by N3).

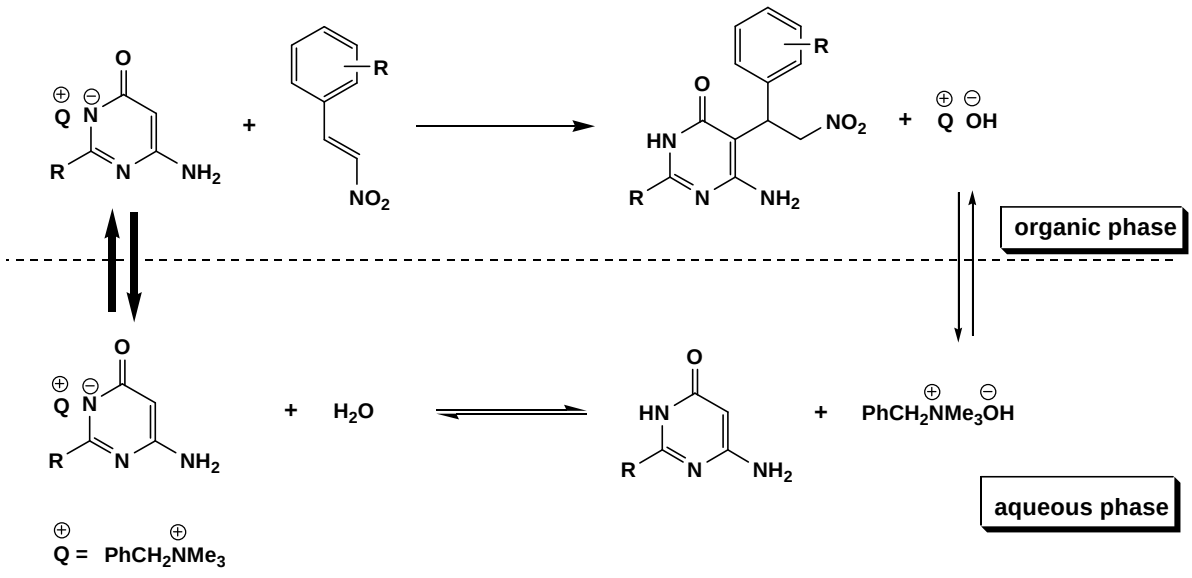
Because of Taylor's satisfactory work using the Michael addition under neutral biphasic solvent system (50% water in ethyl acetate, v/v),[116, 120, 121] we adopted these reaction conditions in our reaction between 2-thiobenzyl-6-aminopyrimidine **2.4** and a nitrostyrene **2.3a** (Scheme 2.5).



Scheme 2.5. Reagents, conditions and yields: (a) EtOAc/H_2O (1:1), reflux, 3 d, 35%. (b) EtOAc/H_2O (1:1), NaOH, reflux, 5 d, 38%. (c) EtOAc/H_2O (1:1), $NaHCO_3$, reflux, 5 d, 33%.

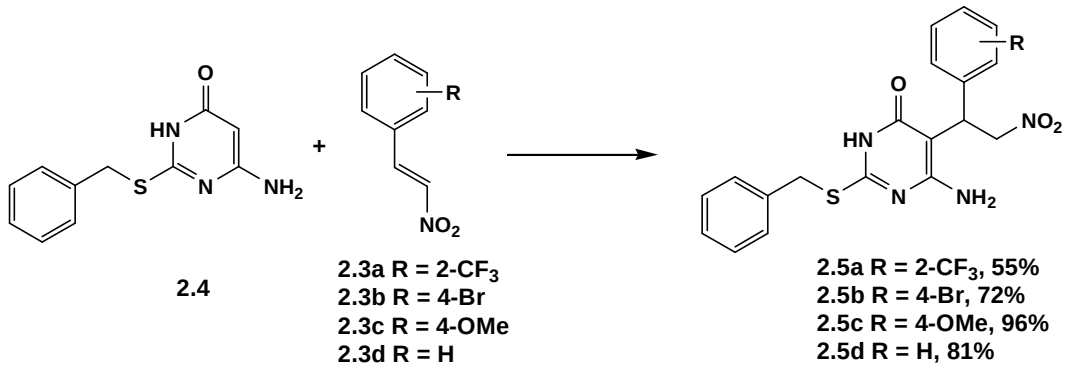
However, the reaction did not occur at room temperature. This may be due to the poor solubility and low permeability of the pyrimidine **2.4** from the aqueous phase into the organic phase to react with a Michael acceptor. We could obtain the product **2.5a** in a relatively low yield (35%) under reflux for several days. Subsequently, we added sodium hydroxide or sodium bicarbonate using these biphasic conditions to investigate any change in yield and reaction rate. These changes did not produce any remarkable differences in either yield or reaction rate. The products were only obtained in yields of 33 ~ 38%, by heating at reflux for five days (Scheme 2.5).

From the above observations on the Michael addition, it was concluded that the pH itself does not affect the reactivity of Michael donors. Thus, we decided to use a phase transfer catalyst (PTC) to activate the pyrimidine **2.4** to act as an effective Michael donor using biphasic conditions. Quaternary ammonium salt ($PhCH_2N^+Me_3HO^-$) was employed as a PTC in our reaction. Scheme 2.6 accounts for the role of the PTC which acts as a shuttling agent by extracting the anion from the aqueous phase into the organic reaction phase where the anionic compound can react with the organic reactant (Michael acceptor) already located in the organic phase.



Scheme 2.6. Action of a phase transfer catalyst in a biphasic solvent system.

Accordingly, we could imagine that the PTC's action may reduce the reaction time and allow lowering the temperature in the two-phase solvent system. Results that are summarised in Table 2.1 and Scheme 2.7 also substantiate the effectiveness of the PTC.



Scheme 2.7. Reagents and conditions: EtOAc/H_2O (1:1), $PhCH_2N^+Me_3HO^-$, reflux or mild heating in an oil bath (50 °C), 4 ~ 72 h.

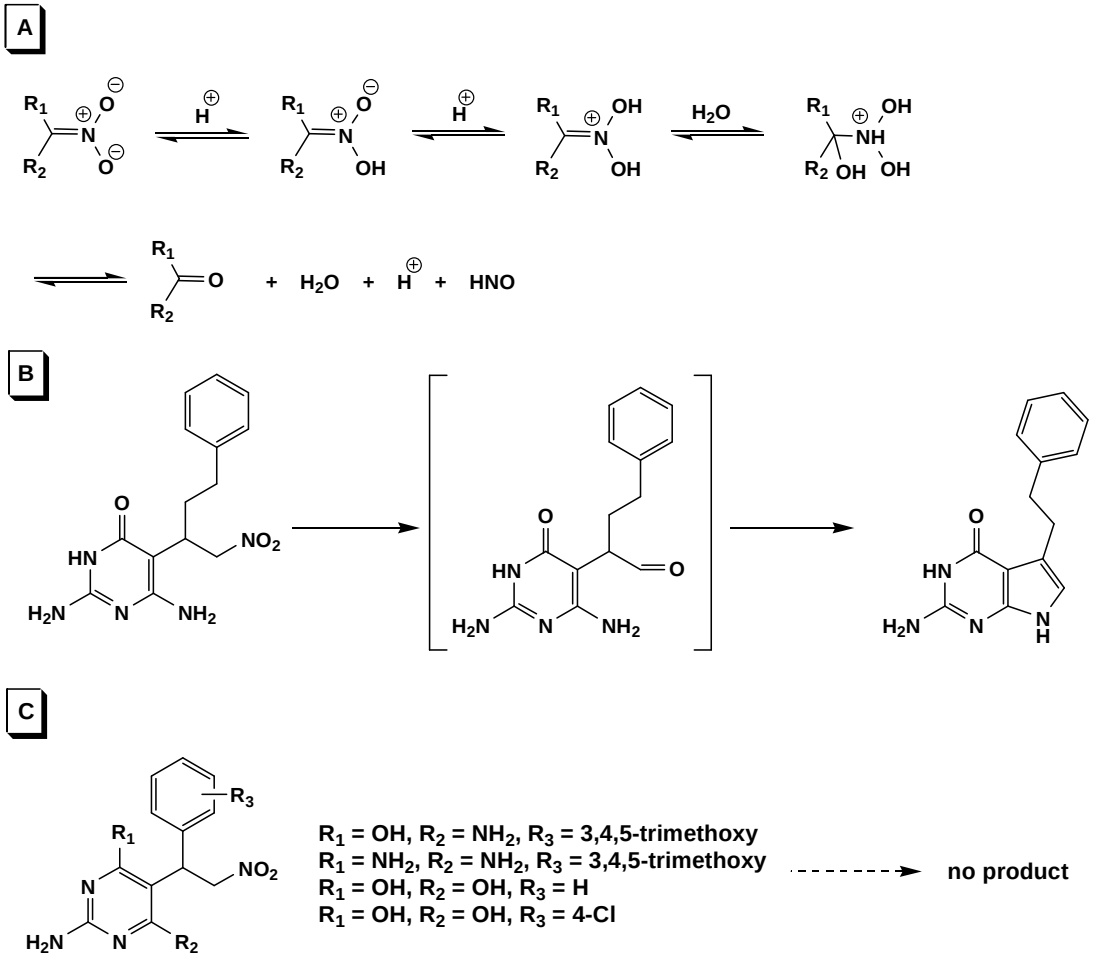
Table 2.1. Michael addition results using phase transfer catalyst in the biphasic solvent system.

Compounds	Reaction period (h)	Temperature (°C)	Yields (%)
2.5a	72	80	55
2.5b	4	70	72
2.5c	48	60	96
2.5d	4	60	81

Ring closure by Nef reaction

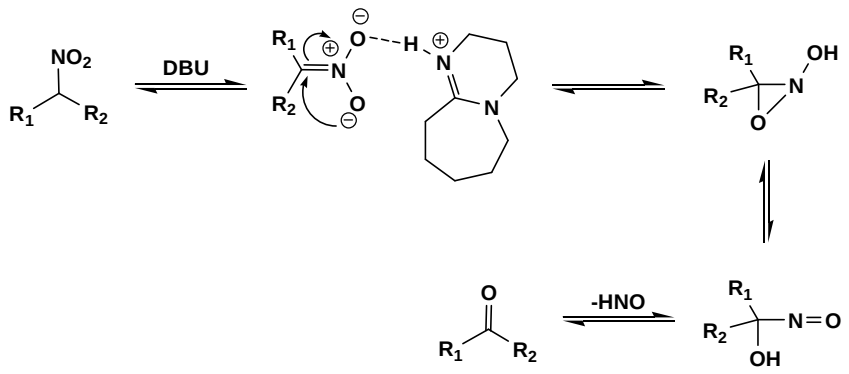
The Nef reaction, the conversion of a nitro group into a carbonyl functionality is one of the most important functional group transformations.[123] It is also a crucial step in our overall synthetic plan because the Nef reaction is the penultimate step to form pyrrolo[2,3-*d*]pyrimidine framework. E. C. Taylor *et al.* used the classical Nef reaction, this involved hydrolysis of strongly acidic conditions of a nitronate salt

produced by basic treatment of a nitroalkene (Scheme 2.8, A).[123, 124] The produced intermediate carbonyl compound then generated the pyrrole ring *via* intramolecular condensation (Scheme 2.8, B).[123, 125] E. C. Taylor *et al*. reported 40~57% yield of cyclised products in most cases among the 17 different compounds through the conventional Nef reaction. However, they also reported some limitations:[123] (i) This transformation failed to afford the expected products from methoxy-substituted precursors at their extended phenyl ring; (ii) if the precursor has a hydroxy group at C6 instead of an amino group, the reaction also failed to generate the corresponding pyrrolo[2,3-*d*]pyrimidines (Scheme 2.8, C).



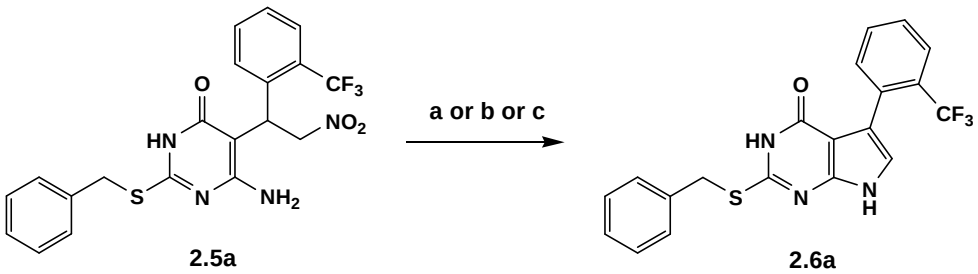
Scheme 2.8. A: conventional Nef reaction, B: ring formation *via* Nef reaction, C: samples that do not undergo ring formation.

Therefore, to overcome this limitation and to improve the yield of this important step, we decided to investigate other reaction conditions. Our first attempt to improve the cyclisation procedure was encouraged by R. Ballini *et al.* who reported the one-step conversion of secondary nitro compounds into the corresponding carbonyl derivatives using 1,8-diazabicyclo[5.4.0]undec-7-ene (DBU) in acetonitrile (Scheme 2.9).[126a]



Scheme 2.9

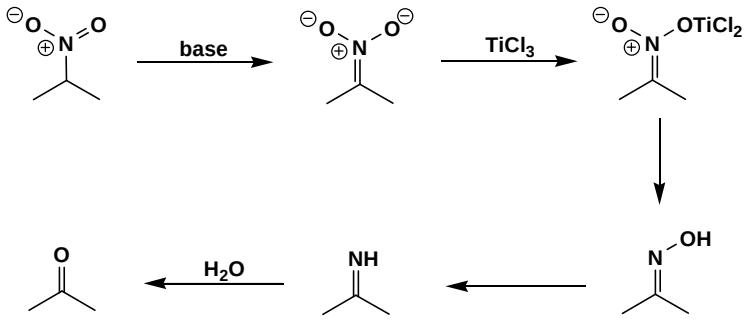
In our cases, the cyclised product **2.6a** was obtained in relatively low yields (16-26%) using DBU under microwave or normal thermal reactions (Scheme 2.10). Although nitroalkanes are remarkably acidic in water (pKa ≈ 10), acidity decreases significantly in nonhydroxylic solvents such as acetonitrile (pKa ≈ 30), mainly due to the decreased stabilisation of nitronate anions by loss of hydrogen bondings from aprotic solvents. In addition, P. H. Boyle *et al.* have demonstrated that DBU in acetonitrile is not able to efficiently convert nitroethane into the corresponding nitronate.[126b] In the same manner, the primary nitroalkane moiety in our substrate **2.5a** may not readily form the nitronate anion for conversion into the corresponding aldehyde. Indeed, R. Ballini commented that the DBU system is chemoselective toward secondary nitro groups in the presence of primary ones.[126a] Hence, the cyclisation using DBU was not very satisfactory in our case.



2.5a 2.6a

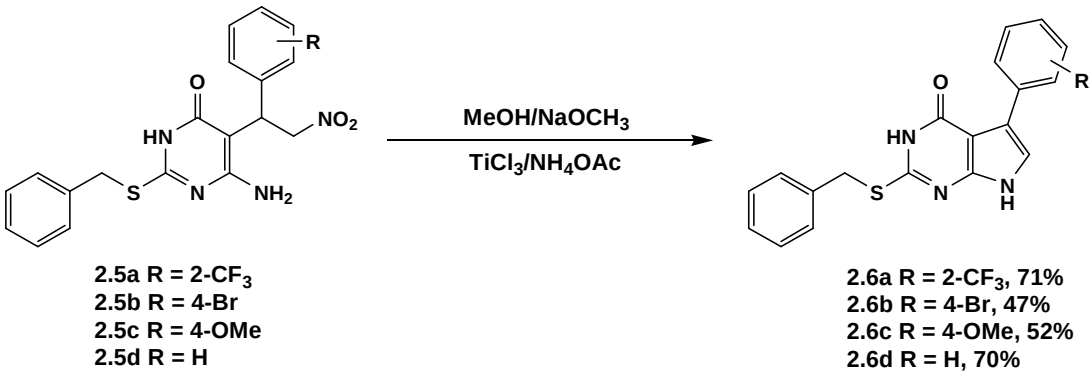
Scheme 2.10. Reagents, conditions and yields: (a) DBU, MeCN, microwave, 85 °C, 1h, 16%. (b) DBU, DMF, microwave, 85 °C, 1 h, 24%. (c) DBU, MeCN, 60 °C, 48 h, 26%.

The second approach involved a reductive method known as the McMurry method.[127] This employs titanium trichloride to reduce nitronate salts into aldehydes or ketones. A likely intermediate in this process is the oxime that can be obtained by reduction of the titanium nitronate. The oxime is further reduced to the imino derivative and then hydrolysed to the parent carbonyl compound (Scheme 2.11). The details of the N-O bond displacement step are not clear although radicals are probably involved since Ti(III) is a one-electron reducing agent.[127]



Scheme 2.11[127]

We could obtain the cyclised products in good yields (47 ~ 71%) smoothly using the McMurry method[127] with overnight stirring under mild conditions (room temperature ~ 60 °C, Scheme 2.12). The reactant **2.5c** which contained a 4-methoxy group also produced the anticipated cyclised product **2.6c** in this reaction.

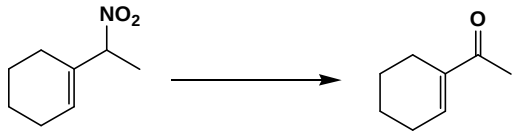


2.5a R = 2-CF_3
2.5b R = 4-Br
2.5c R = 4-OMe
2.5d R = H

2.6a R = 2-CF_3, 71%
2.6b R = 4-Br, 47%
2.6c R = 4-OMe, 52%
2.6d R = H, 70%

Scheme 2.12

As an alternative metal-mediated Nef reaction, tin(II) chloride dihydrate was introduced in our reaction. Although a tin containing reaction is undesirable for the medicinal synthesis due to the toxicity of tin, with regard to the synthetic feasibility, a tin-mediated reaction can be more convenient without the need for any additive reagents and may be a cheaper way to synthesise the targeting compounds. N. B. Das *et al.* reported an example of tin(II) chloride dihydrate reduction of β,γ-unsaturated nitroalkenes (Scheme 2.13).[128]

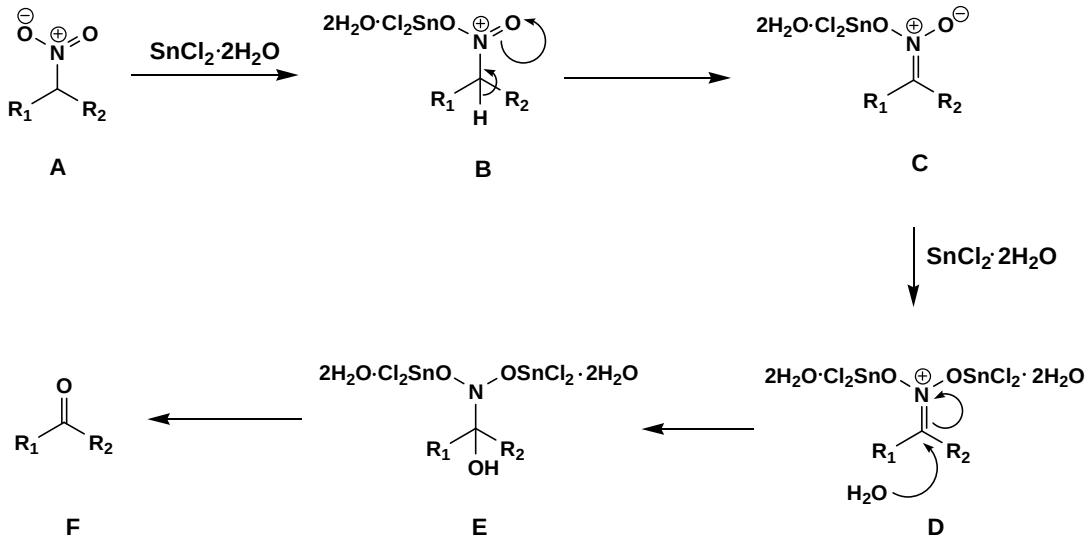


2.7 **2.8**

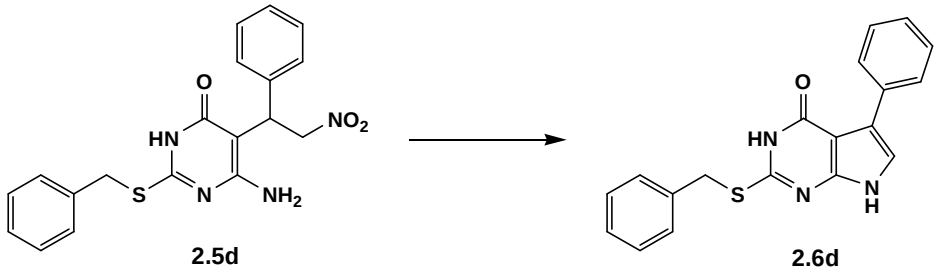
Scheme 2.13. Reagents, conditions and yield: $SnCl_2 \cdot 2H_2O$ (3 eq), THF, rt, 6 h, 70%.

The possible mechanism for this transformation was proposed to be taking place through the hydrolysis of the intermediate stannylnitronate (Scheme 2.14, **D**) Formation of intermediate **D** is possible due to the strong co-ordination of tin(II) chloride with oxygen of the nitro group which facilitates the removal of the proton from the α-carbon atom. In our reaction, the cyclised compound **2.6d** was also successfully synthesised under tin(II) chloride dihydrate-mediated system (Scheme

2.15).



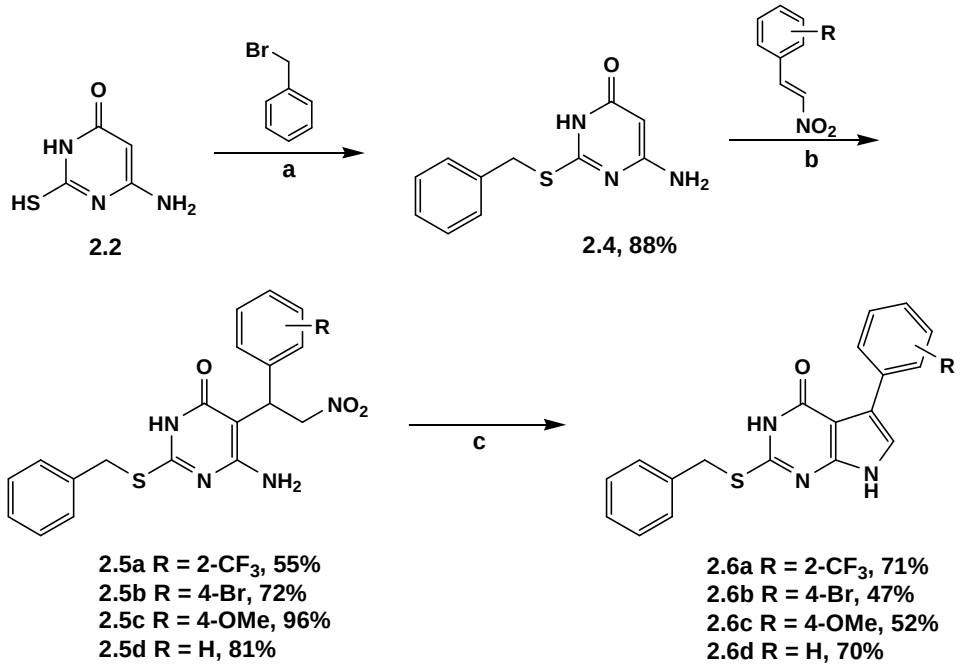
Scheme 2.14[128]



Scheme 2.15. Reagents, conditions and yield: $SnCl_2 \cdot 2H_2O$ (3 eq), EtOAc, 90 °C, 24 h, 72%.

Overall reaction pathway

The overall successful reaction pathway to form 2-thiobenzyl-6-substituted pyrrolo[2,3-*d*]pyrimidines is shown in Scheme 2.16.

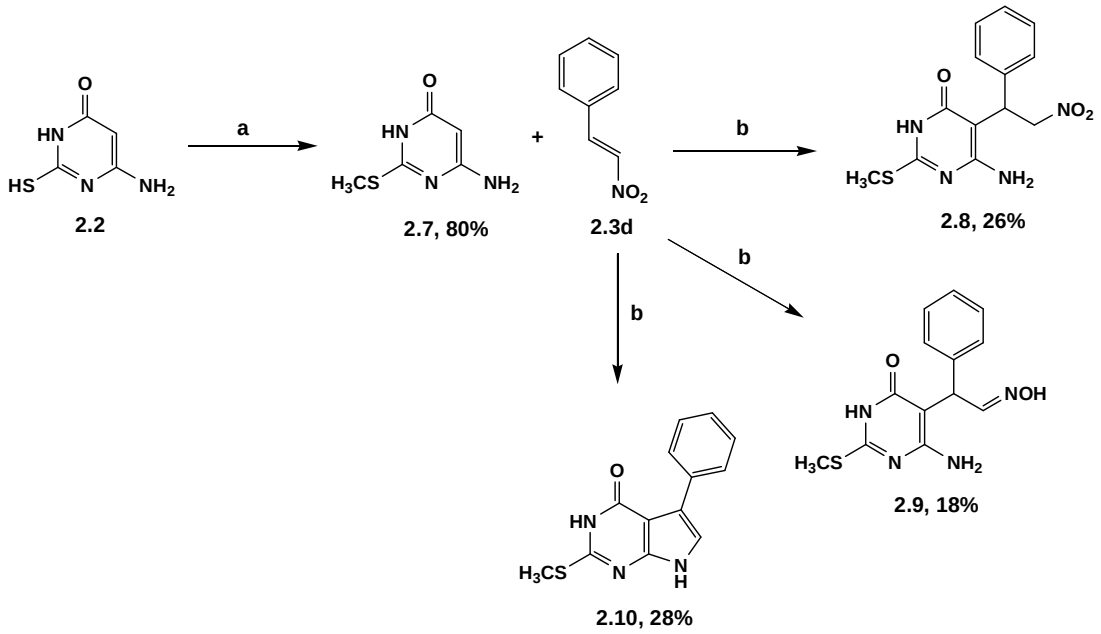


Scheme 2.16. Reagents and conditions: (a) H_2O/EtOH (3:5), Et_3N. (b) EtOAc/H_2O (1:1), $PhCH_2N^+Me_3HO^-$, 60~90 °C, 4 ~ 72 h. (c) MeOH, NaOMe, $TiCl_3$ (4 ~ 6 eq), NH_4OAc, rt ~ 60 °C, overnight.

2.2.1.2. 2-Thiomethyl pyrrolo[2,3-*d*]pyrimidine

In a similar sequence of reactions, we synthesised a 2-thiomethyl pyrrolo[2,3-*d*]pyrimidine scaffold **2.10** (Scheme 2.17). Methylation was successfully carried out using the same reaction conditions as benzylation (Section 2.1.1.1, Scheme 2.1). However, in the following step, using a biphasic system with phase transfer catalyst produced not only uncyclised product **2.8** but also the oxime intermediate **2.9** and cyclised product **2.10** at the same time. We presume that the thiomethyl moiety might give less hydrophobicity to the pyrimidine scaffold rather than the thiobenzyl one. Thereby, uncyclised product **2.8** may have more opportunities to form a nitro salt through deprotonation by hydroxyl anion of phase transfer catalyst in water layer. Then the quaternary ammonium cation may result in the formation of imminium ion which can be attacked by water to produce corresponding aldehyde and oxime intermediate **2.9**. Finally, these intermediates can generate the cyclised product **2.10**

via intramolecular condensation.



Scheme 2.17. Reagents and conditions: (a) EtOH/H_2O (2:1), Et_3N, MeI, rt, 5 h. (b) EtOAc/H_2O (1:1), $PhCH_2N^+Me_3HO^-$, 60 °C, 3 h.

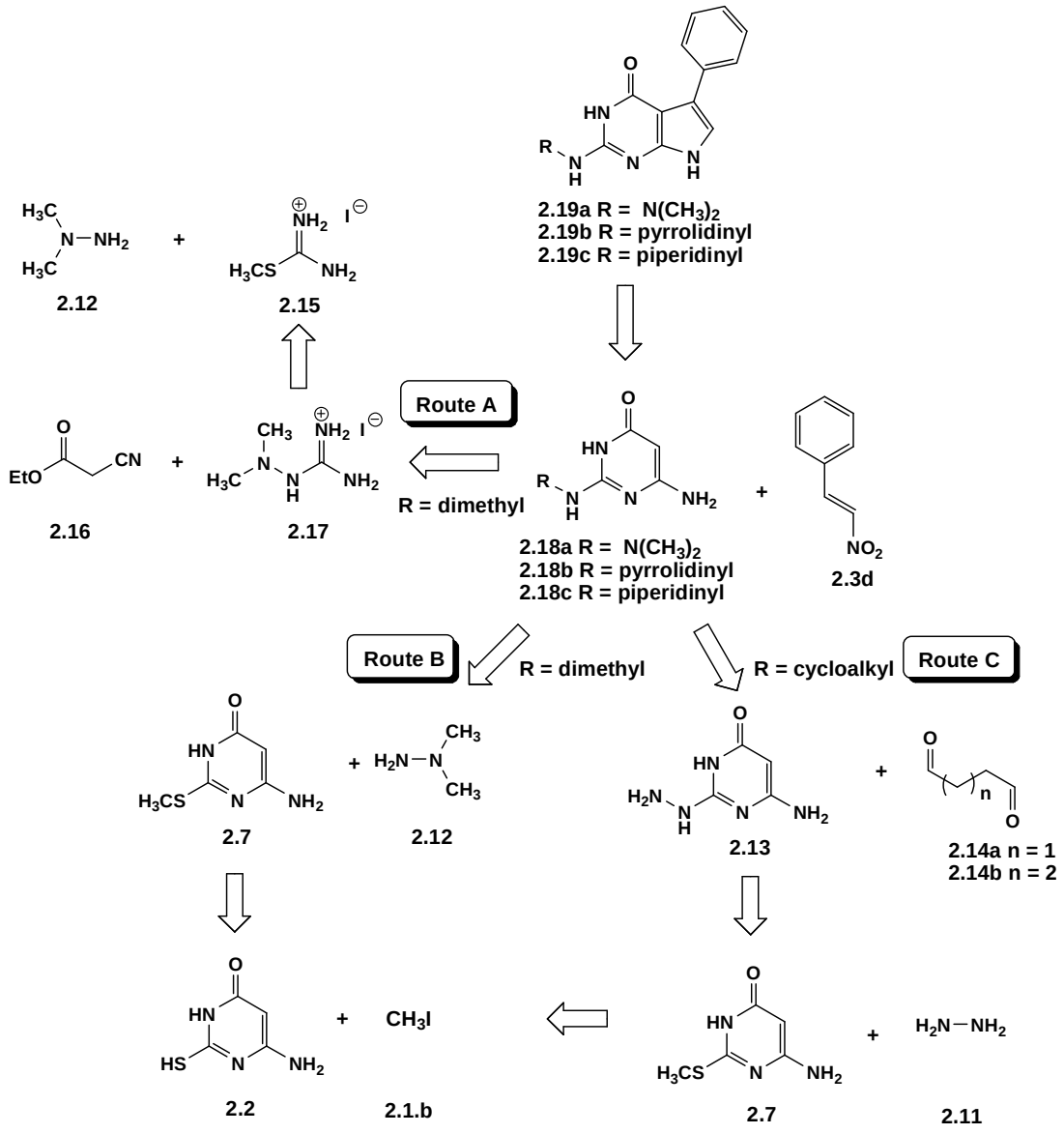
2.2.1.3. 2-Alkylhydrazino pyrrolo[2,3-*d*]pyrimidine

To exploit a different approach of C2 displacement, 2-alkylhydrazino-5-substituted pyrrolo[2,3-*d*]pyrimidines **2.19a** ~ **2.19c** were designed, the hydrazine group can be displaced by amine nucleophiles after protonation at N1' of the alkylhdrazine. In this section, formation of 2-alkylhydrazino-5-substituted pyrrolo[2,3-*d*]pyrimidine is reported and the results of C2 displacement will be discussed including the case of 2-thioalkyl-5-substituted pyrrolo[2,3-*d*]pyrimidine.

Retrosynthetic analysis (Scheme 2.18)

To synthesise 2,2-dialkylhydrazino substituents at C2, we explored three different

pathways. The main scaffold **2.19** can be prepared from Michael addition-Nef reaction route (Section 2.1.1.1). 2-(2,2-Dimethylhydrazino) pyrimidine **2.18a** can be formed from amino(2,2-dimethylhydrazino)methaniminium iodide **2.17** and ethyl 2-cyanoacetate **2.16** (Route A). Amino(2,2-dimethylhydrazino)methaniminium iodide **2.17** can be obtained from amino(methylsulfanyl)methaniminium iodide **2.15** and 1,1-dimethylhydrazine **2.12**.



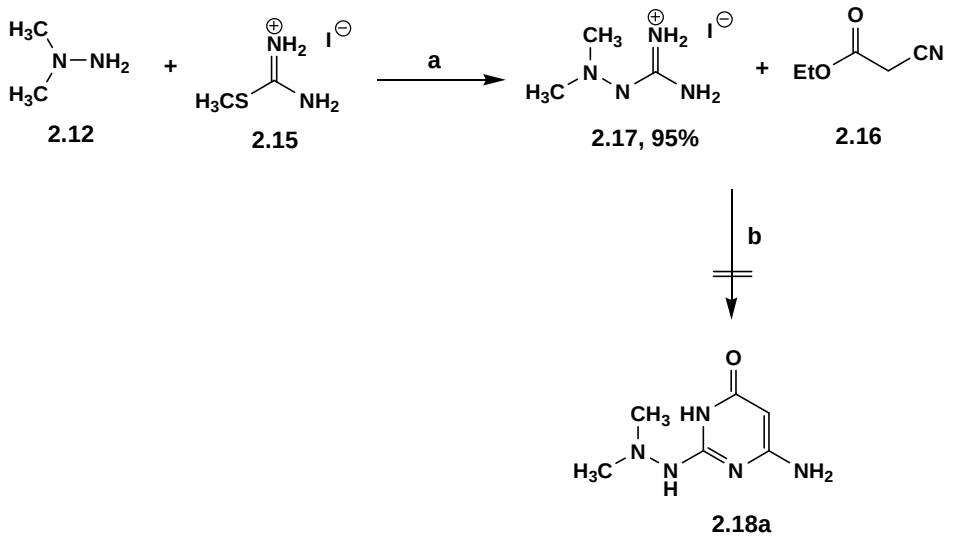
Scheme 2.18. Retrosynthetic analysis of 2-alkylhydrazino-5-substituted pyrrolo[2,3-*d*]pyrimidines.

Alternatively, compound **2.18a** can be obtained from 2,2-dimethylhydrazine **2.12** and 2-thiomethyl pyrimidine **2.7** through C2 displacement (Route B). 2-Thiomethyl pyrimidine **2.7** can be easily obtained from 2-thio pyrimidine **2.2** and iodomethane (Section 2.1.1.2).

Alternatively, 2-cycloalkylhydrazino pyrimidine **2.18b** and **2.18c** may be synthesized from 2-hydrazino pyrimidine **2.13** and a dialdehyde **2.14** *via* reductive amination. 2-Hydrazino pyrimidine **2.13** can be prepared from 2-thiomethyl pyrimidine **2.7** and hydrazine **2.11** (Route C).

Failure of Route A

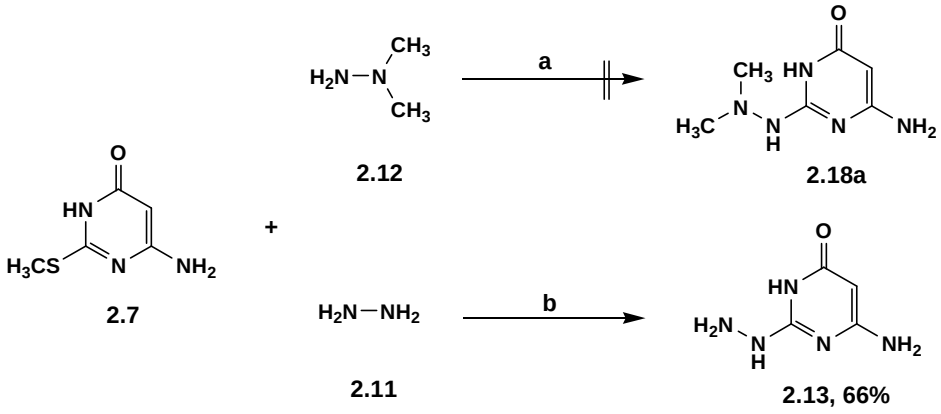
Route A is a ring formation strategy to give a key intermediate **2.18a** (Scheme 2.18). As reported,[129] amino(methylsulfanyl)methaniminium iodide **2.15** was successfully displaced by 1,1-dimethylhydrazine **2.12** to produce **2.17**. However, the ring formation step was unsuccessful using several conditions (Scheme 2.19, b). Therefore, we decided to try the C2 substitution of 2-thiomethyl pyrimidine **2.7** with 1,1-dimethylhydrazine **2.12** (Scheme 2.18, Route B)



Scheme 2.19. Reagents, conditions and yields: (a) 1,1-dimethylhydrazine, reflux, 20 h. (b) EtOH, NaOEt, reflux, 15 h or Et_3N, EtOH, reflux, 15 h or DBU, EtOH, reflux, 15 h.

Displacement at C2 by hydrazine and 1,1-dimethylhydrazine

As previously reported,[130] the 2-thiomethyl moiety of compound **2.7** was successfully displaced by hydrazine **2.11** to give **2.13**. However, reaction of compound **2.7** with 1,1-dimethylhydrazine **2.12** under various conditions (Scheme 2.20, a) did not produced C2 displaced product **2.18a**. The obtained compound after recrystallisation still clearly showed the *S*-methyl group in the ^{1}H (2.41 ppm in DMSO-d_6, compound **2.7** = 2.40 ppm) and ^{13}C NMR (12.39 ppm in DMSO-d_6, compound **2.7** = 12.52), and no evidence was found for C2 substituted product **2.18a**. These results led us to focus on route C (Scheme 2.18) to synthesise 2-cycloalkylhydrazino-6-substituted pyrrolo[2,3-*d*]pyrimidine **2.19b** and **2.19c** (Scheme 2.18).

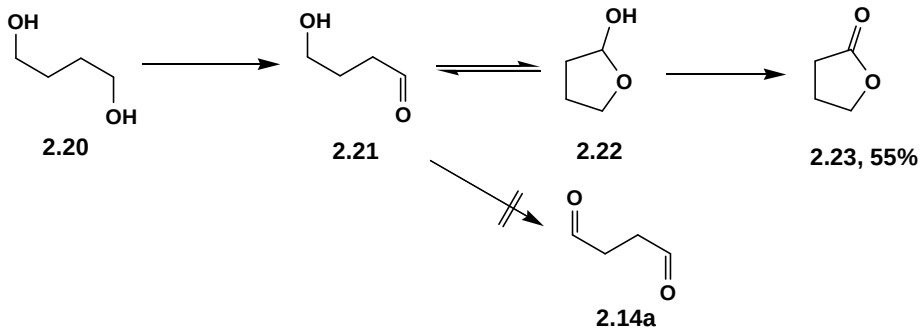


Scheme 2.20. Reagents and conditions: (a) *n*-BuOH, 1,1-dimethylhydrazine (5 eq), 125 °C, 2 d or *i*-PrOH, 1,1-dimethylhydrazine (5 eq), 130 °C, 2 d or 1,1,1-trifluoroethanol, TFA (1 eq), 1,1-dimethylhydrazine (5 eq), 80 °C, 1 d. (b) *n*-BuOH, hydrazine hydrate (2 eq), 125 °C, 4 h.

Failure of synthesis of succinaldehyde

In our first attempt, we wanted to synthesise 2-pyrrolidinylaminopyrimidine **2.18b** but partner substrate **2.14a** was not commercially available (Scheme 2.18). We simply thought that butane-1,4-diol **2.20** can be oxidized by relatively mild oxidant such as PCC (pyridinium chlorochromate) to produce dialdehyde **2.14a** as PCC will not fully oxidize the alcohol to the carboxylic acid. However, intramolecular ring

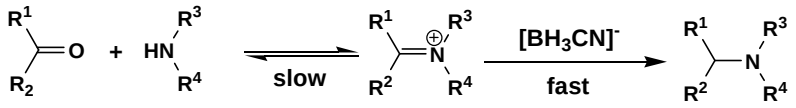
closure was predominant thereby the lactone **2.23** was solely produced (Scheme 2.21).



Scheme 2.21. Reagents and conditions: PCC, DCM, rt, 20 h.

Synthesis of 2-piperidinylaminopyrimidine by reductive amination

Fortunately, glutaraldehyde **2.14b** is commercially available, so reductive amination to obtain 2-piperidinylaminopyrimidine **2.18c** should be feasible (Scheme 2.18). Reaction of an aldehyde or ketone with ammonia, primary amine, or secondary amine at pH ~ 7 in the presence of $[BH_3CN]^-$ leads to primary, secondary, or tertiary amines, respectively, *via* reductive amination of the carbonyl group (Scheme 2.22).[131]

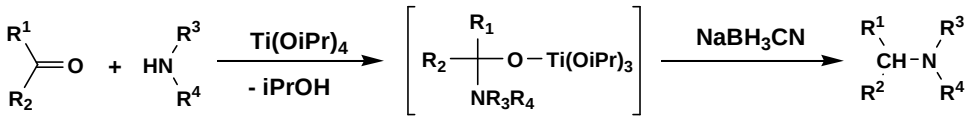


Scheme 2.22. Pathway of reductive amination.

In conventional reductive amination, a pH 6 ~ 8 is optimum for reductive amination, however, the key requirement appears to be the presence of enough proton source to generate positively charged iminium moiety. Under standard Borch reaction

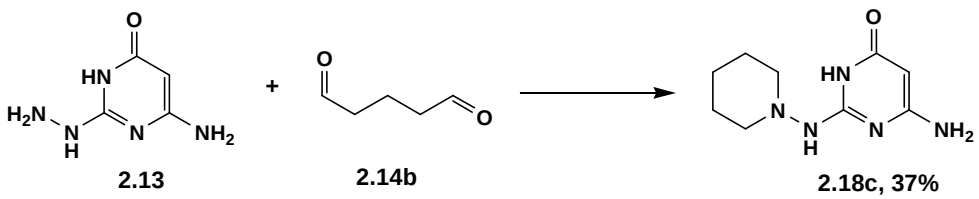
conditions,[131] reductive aminations are usually run using a five fold excess of the amine; although this improves the initial formation of the iminium intermediates, the main purpose is to prevent the product amine (when primary or secondary) from undergoing further reductive aminations.[131]

To overcome the need for a large excess of amine, modification of the conventional conditions have been reported.[132] For instance, R. J. Mattson *et al.* reported the use of titanium(IV) isopropoxide as a mild and effective Lewis acid catalyst for the reductive alkylation of amines with ketones and aldehyde (Scheme 2.23). They suggested a possible mechanism; once the stable complex is formed, which then is reduced either directly or *via* a transient iminium species.[132]



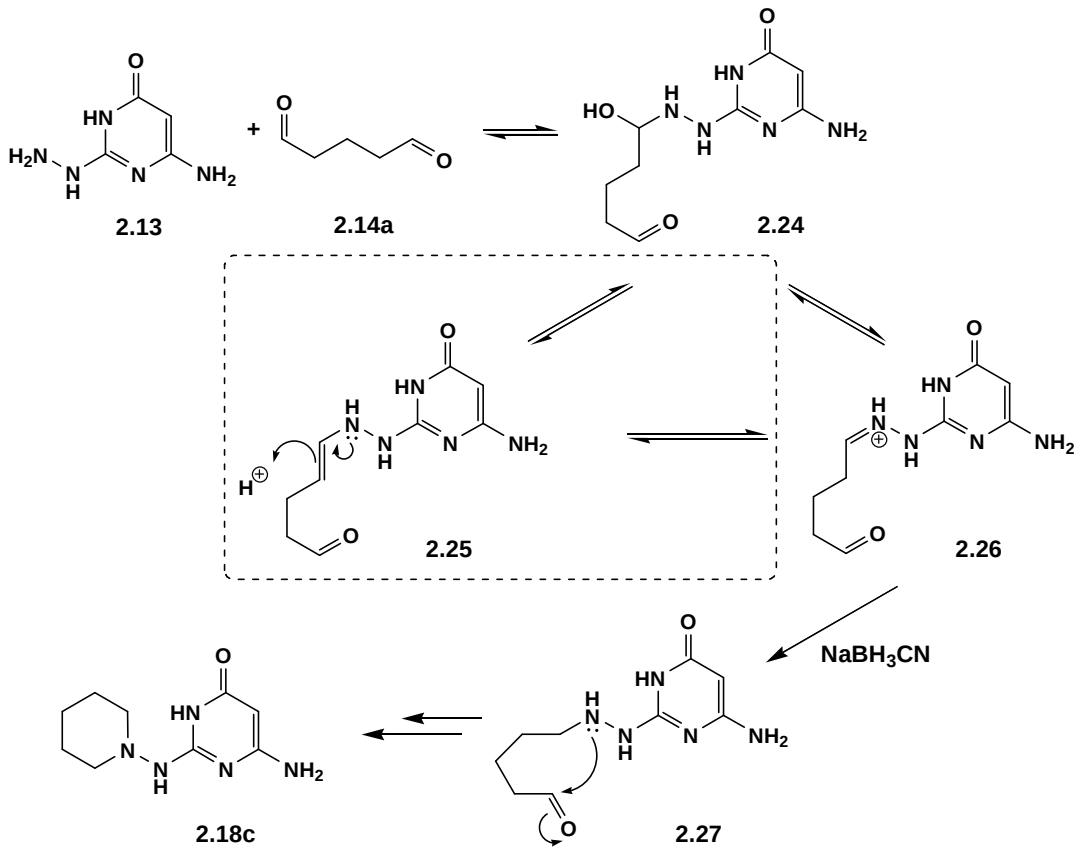
Scheme 2.23. Pathway of reductive amination in the presence of titanium(IV) isopropoxide.

In our case, in order to obtain the **2.18c**, the mono-alkylated intermediate must proceed by further intramolecular alkylation, thereby excess of the substrate **2.13** was unnecessary. In the same manner, high excess of partner dialdehyde **2.14b** was not required either. Actually, we could obtain the expected product **2.18c** in a moderate yield *via* conventional reductive amination conditions with 1.2 molar excess of glutaraldehyde **2.14b** (Scheme 2.24).



Scheme 2.24. Reagents and conditions: MeOH, HOAc, $NaBH_3CN$, rt, 19 h.

The possible reaction mechanism can be considered as shown in Scheme 2.25, where a slow and rate-determining preequilibrium step generates the iminium intermediate **2.26** which is then rapidly reduced to **2.27**. The carbinol amine (hemiaminal) **2.24** can also be converted into the iminium salt **2.26** *via* formation of the enamine **2.25** which can converted into the key intermediate **2.26** by rapid reversible protonation. Due to the short reaction time for enamine reduction (~ 15 min) compared to that of the reductive amination (several hours), it is possible that the enamine reduction pathway (Scheme 2.25, dotted line box) may be involved.[131]

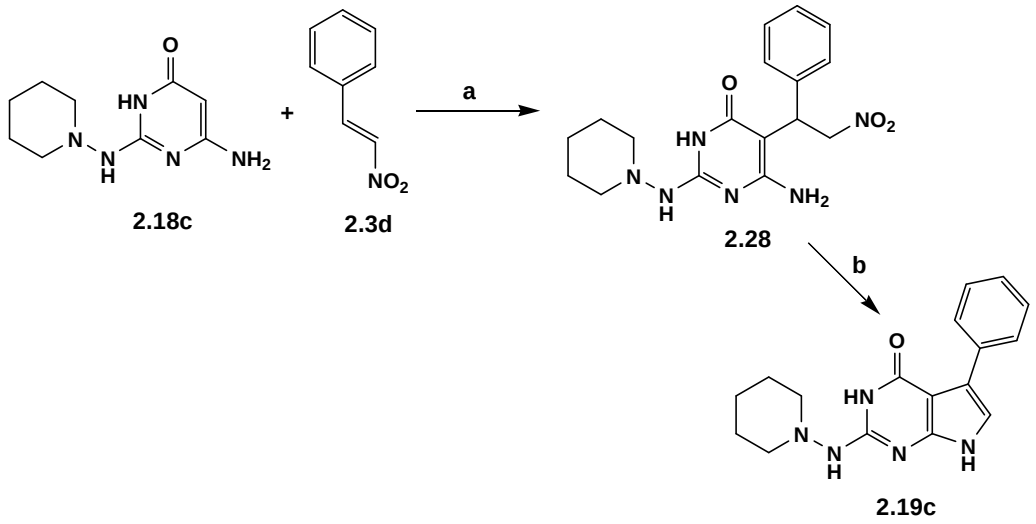


Scheme 2.25. The possible reaction mechanism of reductive amination.

Subsequent formation of a pyrrolo[2,3-*d*]pyrimidine scaffold

The obtained key intermediate **2.18c** was used to synthesise 2-piperidinylamino

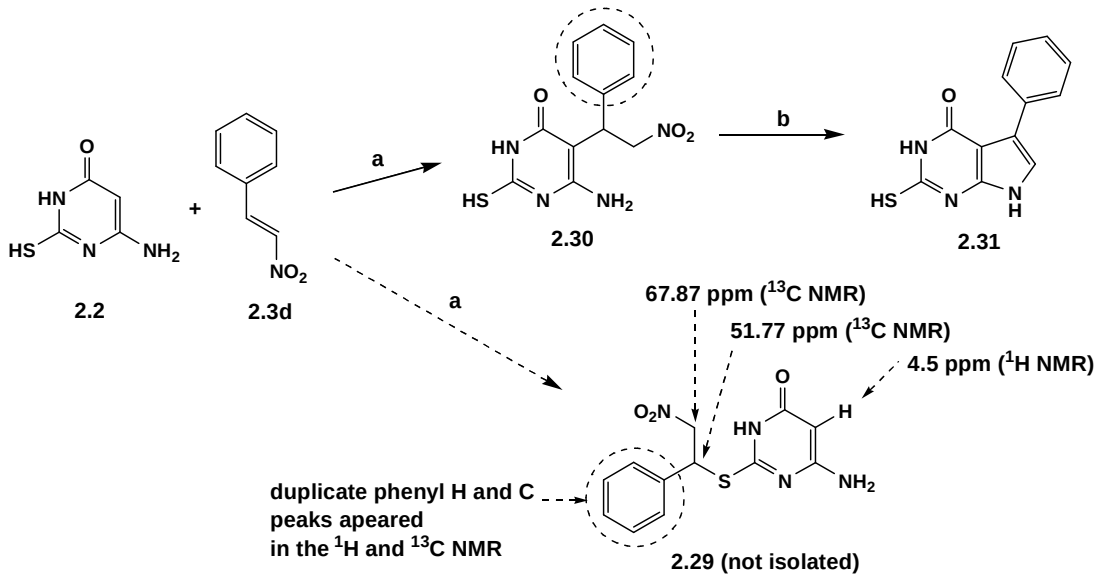
pyrrolo[2,3-*d*]pyrimidine **2.19c** through Michael addition followed by Nef reaction (Scheme 2.26). In this case, the both steps were also successful using conditions developed in Section 2.1.1.1.



Scheme 2.26. Reagents, conditions and yields: (a) EtOAc/H_2O (1:1), $PhCH_2N^+Me_3HO^-$, rt, 19 h, 58%. (b) $SnCl_2 \cdot 2H_2O$ (3 eq), EtOAc, 90 °C, 24 h, 48%.

2.2.1.4. 2-Mercapto pyrrolo[2,3-*d*]pyrimidine

The Michael-Nef reaction route was attempted in the case of 2-mercaptopyrimidine also. This was just to compare the reactivity and effectiveness of the route with 2-thioalkyl pyrimidines **2.4** and **2.7** (Section 2.2.1.1 and 2.2.1.2). Using the same reaction conditions as thiolalkyl pyrimidines, we could obtain the intermediate **2.30** and the cyclised product **2.31** in moderate yields, 42% and 45% respectively (Scheme 2.27). But the yields were relatively lower than those of 2-thiobenzyl pyrimidine in both steps (**2.5d** = 81%, Scheme 2.16 and **2.6d** = 72%, Scheme 2.15).



Scheme 2.27. Reagents, conditions, and yields: (a) EtOAc/H_2O (1:1), $PhCH_2N^+Me_3HO^-$, rt, 15 h, 42%. (b) $SnCl_2{\cdot}2H_2O$ (3 eq), EtOAc, 85 °C, 24 h, 45%. NMR spectra for **2.29** were obtained in DMSO-d_6.

In the Michael reaction step, we postulated that sulfur addition to **2.3d** would give compound **2.29**. However, although, the crude solid appeared as a mixture by ^{1}H and ^{13}C NMR before purification, only the C5 addition product **2.30** was isolated *via* column purification (Appendix I). We presume that the formation of unwanted by-product **2.29** might lead to lower yield in the Michael addition step.

The subsequent cyclisation of **2.30** proceeded well but typical organic-aqueous extraction was unsuccessful due to the lack of further hydrophobic group on the sulfur. Instead, careful filtration to remove unreacted tin(II) chloride followed by column purification gave cyclised product **2.31**.

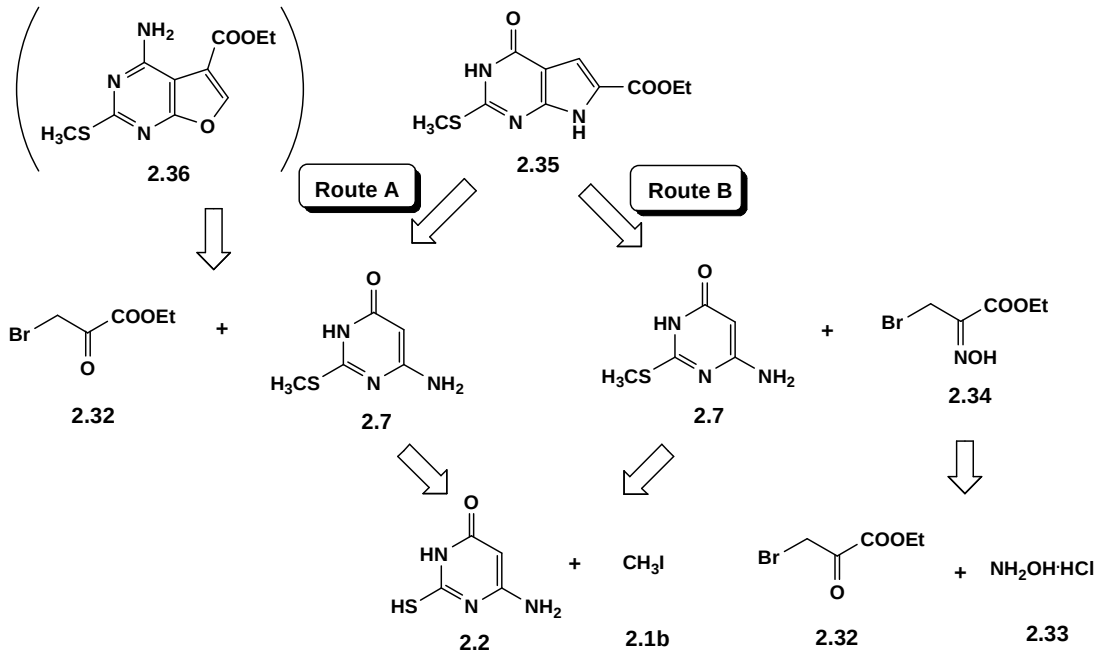
From these drawbacks including the formation of by-product and the lower yields, we conclude that alkylation of 2-mercaptopyrimidine is preferred before going to the Michael-Nef reaction route.

2.2.1.5. 2-Thiomethyl-6-carboxyl ethyl pyrrolo[2,3-*d*]pyrimidine

2-Thiomethyl-6-carboxyl ethyl pyrrolo[2,3-*d*]pyrimidine **2.35** was designed to study the C2 displacement reactivity as the 6-carboxyl group can reduce electron density of the fused ring due to its electron withdrawing character. Details for displacement reaction are discussed in the following section. Herein, we simply present synthetic method towards this compound.

Retrosynthetic analysis (Scheme 2.28)

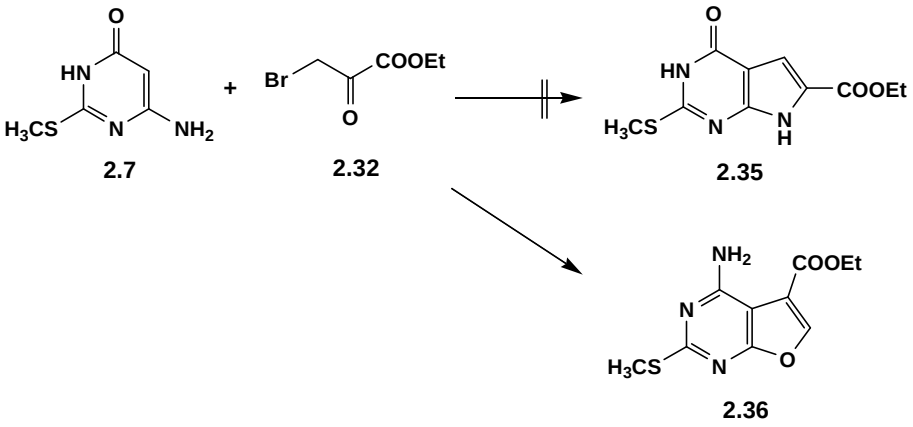
Route A is the cyclocondensation pathway with α-halo ketones described in Section 1.3.2.3. Although, there is a possibility of formation of furopyrimidine **2.36**, it is worthwhile to attempt as the reaction procedures are very simple. Route B was previously exploited by J. K Huggan.[117] Ethyl bromopyruvate oxime **2.34** can be obtained from the reaction of ethyl bromopyruvate **2.32** with hydroxylamine hydrochloride **2.33**.[133]



Scheme 2.28. Retrosynthetic analysis of 2-thiomethyl-6-carboxyl ethyl pyrrolo[2,3-*d*]pyrimidine **2.35**.

Route A

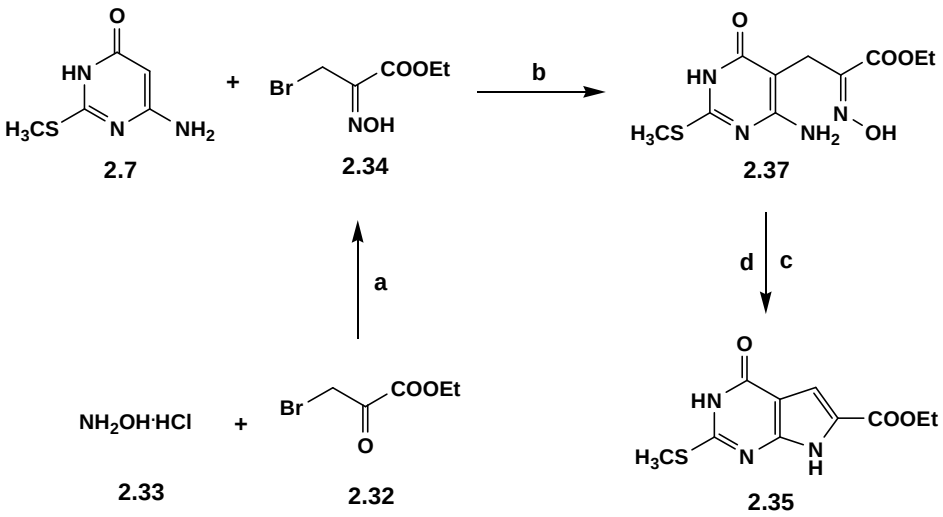
Initially, we expected that a mixture of the two products from the route A (Scheme 2.29), however, the furopyrimidine **2.36** was obtained as a sole product. Although, this would be a good point to synthesise C5-funtionalised furopyrimidines, it is not appropriate to obtain the required C6 extended 2-thioalkylpyrimidine.



Scheme 2.29. Reagent, conditions, and yield: DMF, rt, 3 d, 62%.

Route B

Route B was reported by J. K. Huggan (Scheme 2.30).[117] The partner substrate **2.34** was synthesised from hydroxylamine hydrochloride **2.33** and ethyl bromopyruvate **2.32** through the reported procedure by H. C. J. Ottenheijm *et al.*[133] The 2-thiomethylpyrimidine was reacted with oxime **2.34** in DMF to give the desired product **2.37**. However, in this work, Dowex-50 (H^+ form) was used as the acid catalyst instead of hydrochloric acid for the subsequent cyclisation. The Dowex-50 (H^+) catalysed reaction was more effective with regard to the yield (85% *vs.* 54%[117]).



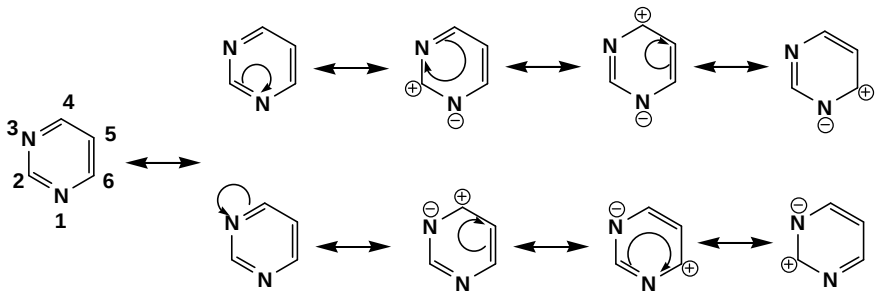
Scheme 2.30. Reagents, conditions, and yields: (a) $CHCl_3$, MeOH, rt, 24 h, 84%. (b) DMF, Et_3N, rt, 2 d, 21%. (c) H_2O/EtOH, HCl, benzaldehyde, reflux, 54%.[117] (d) Dowex-50 (H^+), H_2O, reflux, 15 h, 85%.

2.2.1.6. Displacement at C2 of pyrrolo[2,3-*d*]pyrimidines

Substitution at C2 in deazapurines/purines is a very useful synthetic route for the library synthesis for drug discovery.[134-137] However, the C2 substituion of purines[135, 136] and pyrrolo[3,2-*d*]pyrimidines[137] has proved extremely difficult and remained a challenging task.

Basic concept

Nitrogen has a higher electronegativity than carbon, and when present as –N= in an aromatic ring, nitrogen distorts the π electron distribution by its inductive ability. In the pyrimidine ring the annular nitrogen atoms are situated in the 1,3 positions, which results in a combination of their effects causing marked π electron deficiency at the 2, 4, and 6 positions (Scheme 2.31). Therefore, these active positions are readily attacked by nucleophiles, for example, resulting in ready substitution of leaving groups (e.g. halogens) at such positions by water, amines, etc.[138]

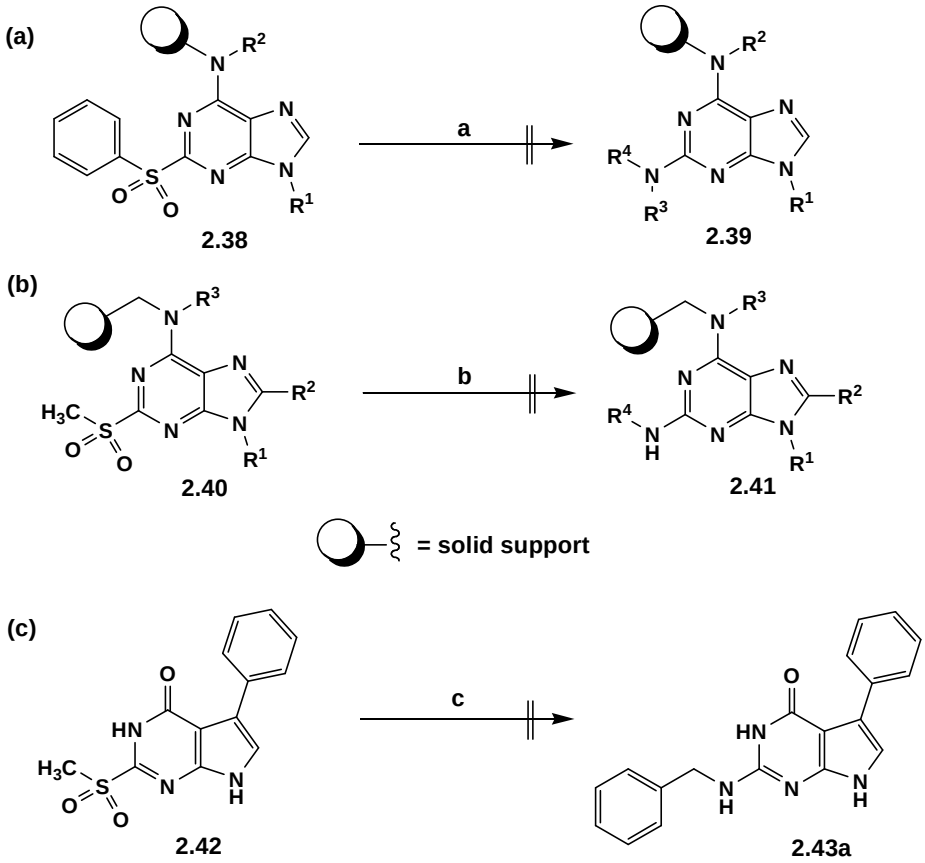


Scheme 2.31. Mesomeric forms of pyrimidine.

Difficulty of C2 displacement

Although the electron deficient character of C2, there are some reports of unsuccessful C2 substitution in purine scaffolds (Scheme 2.32, a and b)[135, 136] and pyrrolo[2,3-*d*]pyrimidine, as in our case (Scheme 2.32, c).

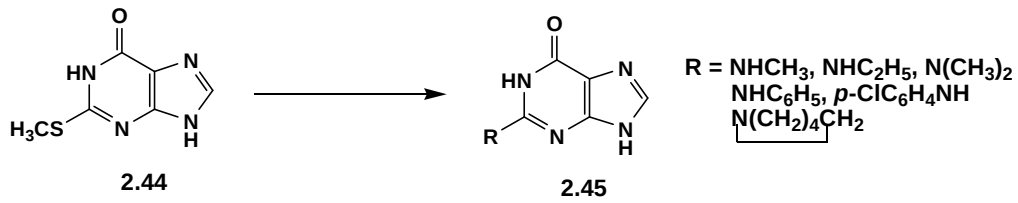
We can suggest reasons for the difficulties of C2 displacement in these motifs: (i) the introduction of electron-releasing substituents – hydroxy, amino, mercapto, etc. – counteracts the π electron deficiency of the pyrimidine rings[139] (amino group at C4 for the case a and b, Scheme 2.32); (ii) lone pair electrons of N1 and N3 can electronically hinder the approach of nucleophiles to the C2 position (Scheme 2.32, a and b); (iii) in the case c, the annular nitrogen (–NH– type), N7 gives the electron densities at the carbon sites of the fused ring by the fact that the nitrogen provides two electrons for the cyclic, conjugated, π bond system (Scheme 2.32, c); (iv) nucleophilic attack at C2 may be significantly aborogated as N3 is ionisable with basic nucleophiles (Scheme 2.32, c)



Scheme 2.32. Reagents and conditions: (a) R_3R_4NH (5 eq), 1,4-dioxane, 80 °C.[135] (b) R_4NH_2, DMF, 130 °C, 7 d.[136] (c) benzylamine (3 eq), DMF, 100 °C, 20 h.

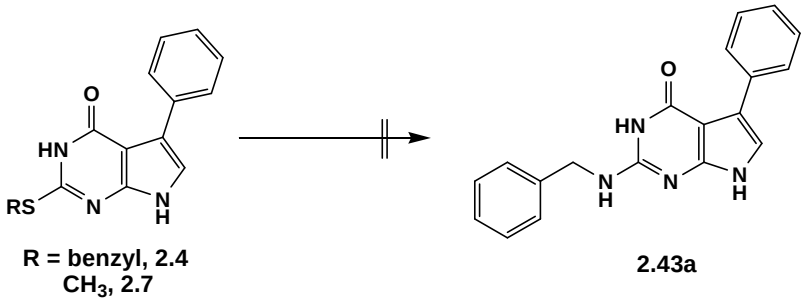
Direct displacement

From a review of the literature, it was found that successful C2 displacement had been reported by G. B. Elion *et al.* for 2-thiomethyl guanine **2.44** *via* direct thermal reaction (Scheme 2.33).[140] All the reactions were attempted at a relatively high temperature (140 or 160 °C) and under high concentration of amines (12 ~ 50%) in aqueous or alcoholic media or using no solvent. Through this direct method, G. B. Elion *et al.* obtained a series of amino-substituted guanines **2.45**.



Scheme 2.33. Reagents, conditions and yields: water or MeOH or EtOH or no solvent, corresponding amines (12 ~ 50% conc. or neat amines), 140 or 160 °C, 10 ~ 65%.

In our study, benzylamine was selected as a test nucleophilic amine in C2 substitution in pyrrolo[2,3-*d*]pyrimidines *via* a direct displacement method (Scheme 2.34). By varing the temperature, we observed that the formation of an inseparable mixture at over 130 °C (confirmed by ^{1}H NMR). Although, the ratio of by-product was reduced at 115 °C, the isolation of pure product was very difficult and the starting substrate consistently remained. At the lower temperature (103 °C), the by-product was not formed, however, the reaction did not go very well and the majority of starting substrate remained.

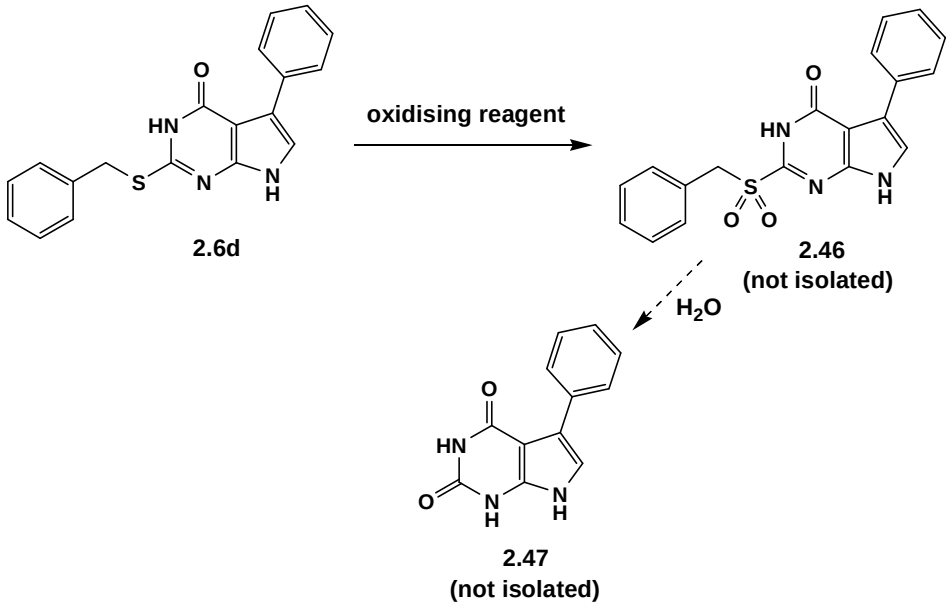


Scheme 2.34. Reagent and conditions: neat benzylamine, 103 ~ 150 °C, several days, no pure product was isolated.

Normal oxidation-substitution pathway

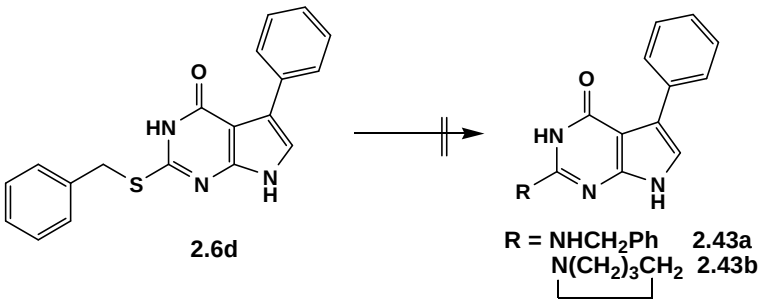
The oxidation of sulfides to sulfoxides or sulfones and substitution is a typical two-step method to replace a sulfide moiety on an annular carbon of fused pyrimidines.[141]

In our case, isolation of the sulfone **2.46** was not successful using several oxidizing agents such as *m*-chloroperbenzoic acid, Oxone®, $MoCl_2O_2$ with H_2O_2, SeO_2 with H_2O_2, Na_2WO_4 with H_2O_2 using various reaction conditions. In some cases, oxidation by Oxone® gave a mixture of sulfone and sulfoxide in low yields (> 30%). We presume that a possible cause of the ineffectiveness of isolation of sulfone **2.46** can be derived from nucleophilic attack by water during the aqueous reaction work-up, which can lead the water soluble compound **2.47** (Scheme 2.35).



Scheme 2.35. Ineffectiveness of isolation of sulfone **2.46**.

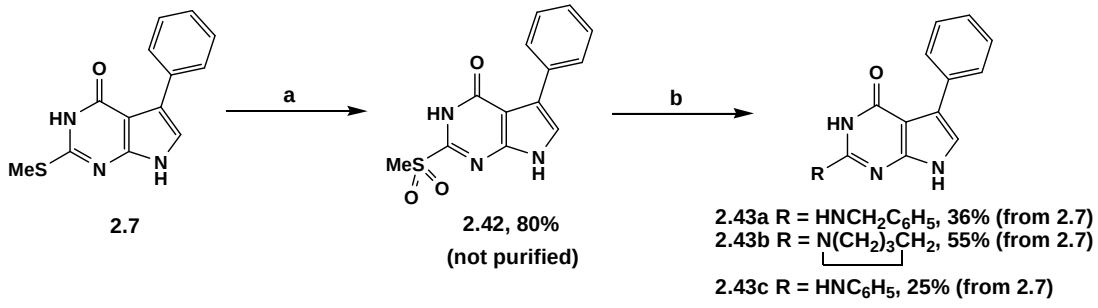
Alternatively, a one-pot consecutive oxidation-substitution method was attempted (Scheme 2.36) as isolation of sulfone **2.46** was difficult. However, the C2-substituted product was not obtained probably because, after oxidation, the remaining acid protonated the amine nucleophile.



Scheme 2.36. Reagent and conditions: *m*-CPBA (3 ~ 4 eq), DMF (or $CHCl_3$, THF), rt, 4 h then amines (5 ~ 10 eq), rt ~ 100 °C, 15 ~ 48 h.

Modified oxidation-substitution pathway

After failures in the direct thermal reaction and one-pot oxidation-substitution reaction, we developed an effective C2 substitution method (Scheme 2.37). Oxidation with *m*-chloroperbenzoic acid in DMF at room temperature for four hours was successful. After a simple work-up, involving the removal of DMF under vacuum, then washing with diethyl ether, the crude sulfone **2.42** was obtained in a good yield (80%). The identity was confirmed by a low-resolution mass spectrometry and indicated a predominant molecular ion peak (MH^+ = 290.1). The crude sulfone was then treated with selected neat amines without any solvents, and after stirring overnight at 100 °C, the C2 substituted products were obtained in acceptable yields (25 ~ 55%).



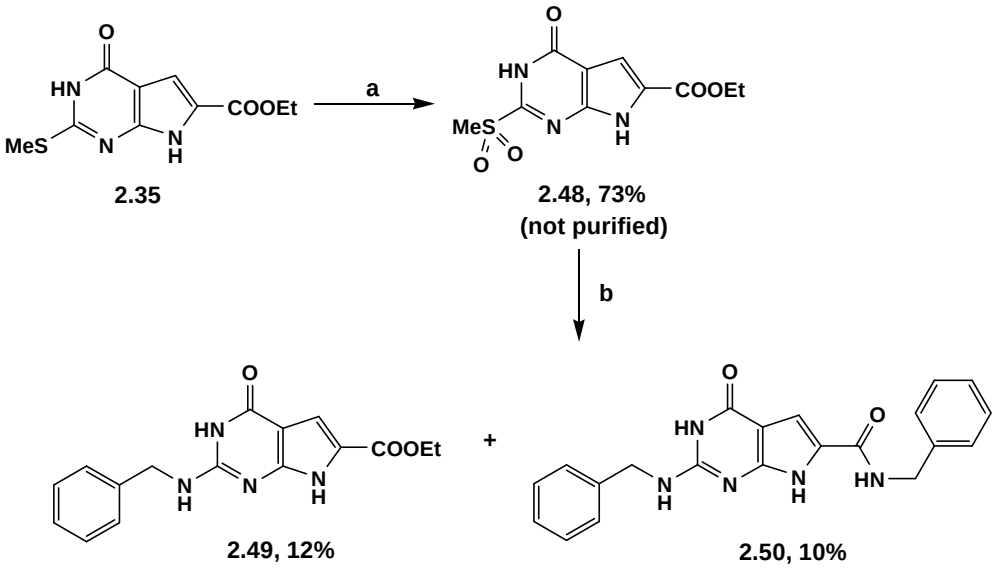
Scheme 2.37. Reagents and conditions: (a) *m*-CPBA (3 eq), DMF, rt, 4 h. (b) corresponding amines, 100 °C (in a sealed tube), 15 h.

Although oxidation followed by treating with amines are standard methods for this type of chemistry, two points are important in our case: (i) simple non aqueous work-up gave crude sulfone, (ii) high-concentration of amines without solvent. Interestingly, there are no reported cases for neat amine nucleophiles to cleave a sulfone moiety at C2 in the purine/pyrrolopyrimidine scaffolds. Therefore, we presume that our successful C2 displacement method can be applied to the similar type of frameworks for the library synthesis in the medicinal chemistry fields.

C2 Displacement reactivity of 6-ester containing pyrrolo[2,3-*d*]pyrimidine

To investigate C2 displacement reactivity, we synthesised a 6-ester containing model compound **2.35** (Scheme 2.30). The crude sulfone **2.48** was obtained by the same method described above, and then treated with neat benzylamine at lower temperature to compare any reactivity difference to 6-unsubstituted pyrrolo[2,3-*d*]pyrimidines (Scheme 2.38). The reaction progress was monitored by thin layer chromatography (TLC) and low resolution mass spectrometry (LRMS). After stirring for 14 hours at 40 °C, sulfone **2.48** was found predominantly. The temperature was increased to 60 °C and stirred for a further 24 hours. However, sulfone **2.48** was still the major product and compounds **2.49** and **2.50** formed concurrently together; the molecular ion peaks were at m/z 313.2 (MH^+) for **2.49** and 396.1 (MNa^+) for **2.50**. The reaction was completed at 100 °C after further 24 hours, however, a mixture of mono **2.49** and di-substituted compounds **2.50** was obtained after column purification. Isolation of the two compounds was accomplished *via* further separation using high-performance liquid chromatography (HPLC) and fully characterised.

Through this test, we conclude that there is no significant activating effect in the case of 6-ester connected pyrrolo[2,3-*d*]pyrimidines with regard to the reactivity of C2 displacement; the ester group is as reactive as C2 displacement.

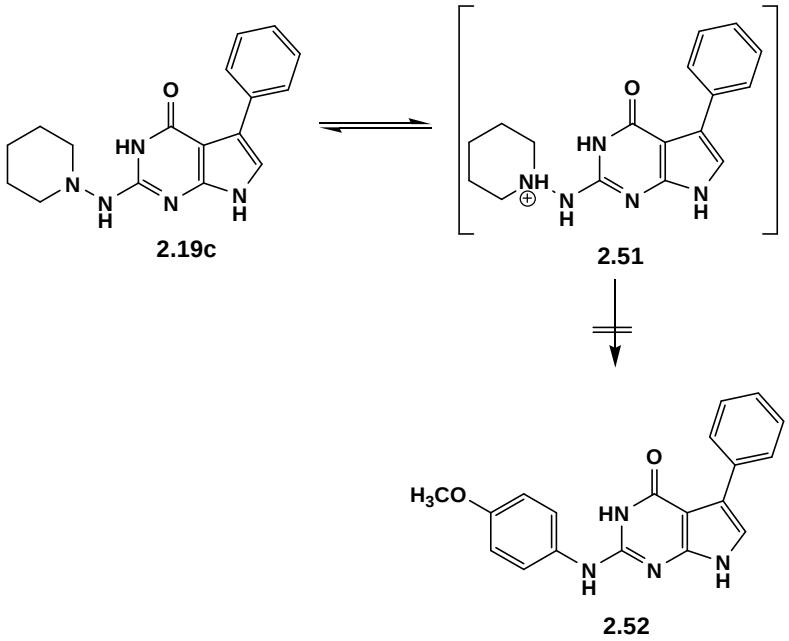


Scheme 2.38. Reagents and conditions: (a) *m*-CPBA (3 eq), DMF, rt, 4 h. (b) neat benzylamine, 100 °C, 24 h.

C2 Displacement of 2-piperidylamino pyrrolo[2,3-*d*]pyrimidine

To exploit a different approach for C2 displacement, compound **2.19c** was synthesised (Scheme 2.26). 4-Methoxyaniline was randomly selected as a partner amine and the corresponding hydrochloride salt was added as an acid catalyst to protonate at the piperidyl nitrogen. The ethanolic reaction mixture was heated at 85 °C for two and a half days in a sealed tube (Scheme 2.39). However, only starting substrate **2.19c** was recollected and any other by-product or decomposition were not found.

This displacement strategy was not fully investigated and further experiments are continuing in our research group. Finding an effective concentration of amines and the ratio of acid catalyst might be key points to accomplish this task.

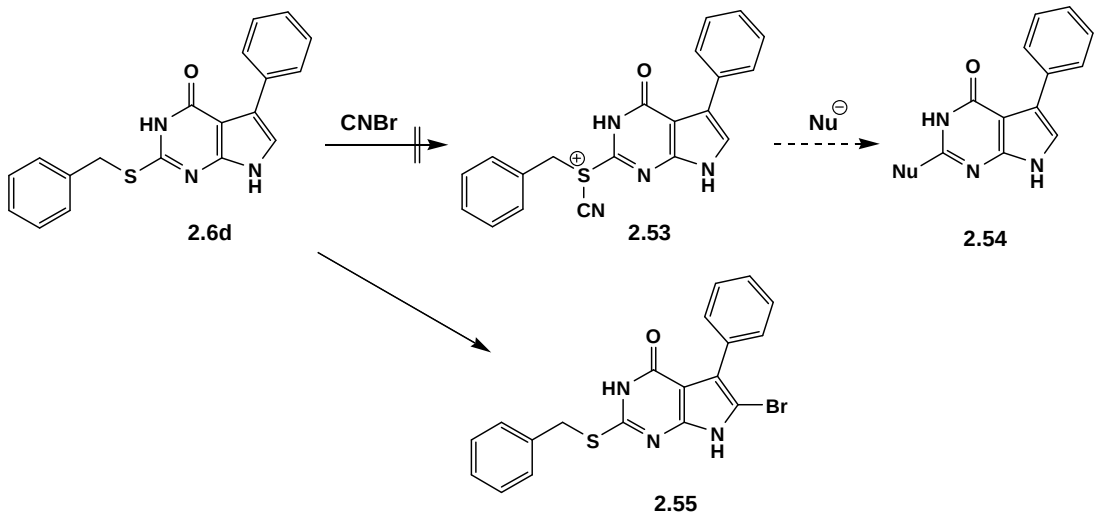


Scheme 2.39. Reagents and conditions: 4-methoxyaniline (1.2 eq), 4-methoxyaniline hydrochloride (1.2 eq), EtOH, 85 °C, 2.5 d.

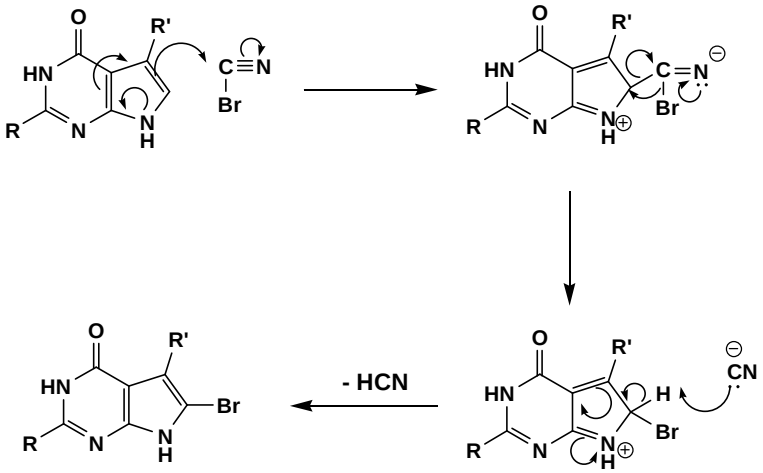
Two unexpected results

Whilst developing procedures for the C2 displacement reaction, we found two unexpected results. The first example is the reaction between cyanogen bromide and the 2-thiobenzylpyrrolo[2,3-*d*]pyrimidine **2.6d**. Originally, we thought that cyanation at sulfur would form more labile leaving group in **2.6d** to give **2.53** (Scheme 2.40). However, the intermediate **2.53** was not formed, instead, brominated product **2.55** was obtained in 93% yield.

The plausible mechanism is presented in Scheme 2.41.[142] The initial step is nucleophilic attack by the pyrrole on the carbon atom of cyanogen bromide followed by bromination at C6. Then the rearomatisation can occur by the loss of HCN to give 6-bromo product **2.55**. The obtained product **2.55** was fully characterised by HRMS, ^{1}H, ^{13}C-NMR, and IR spectrophotometry.



Scheme 2.40. Reagents, conditions and yield: CNBr, THF, 40 °C, 15 h, 93%.

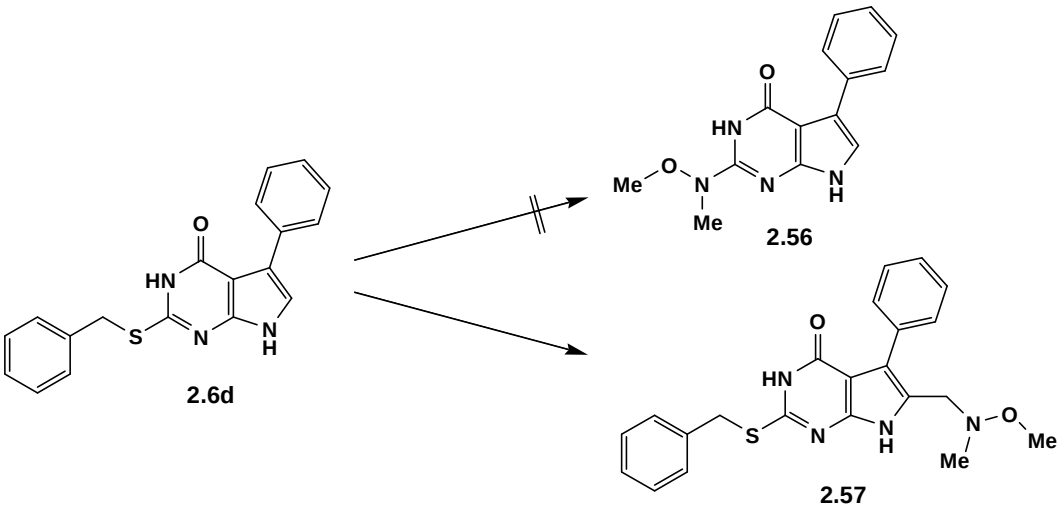


Scheme 2.41. The plausible mechanism of 6-brominated pyrrolo[2,3-*d*]pyrimidine.

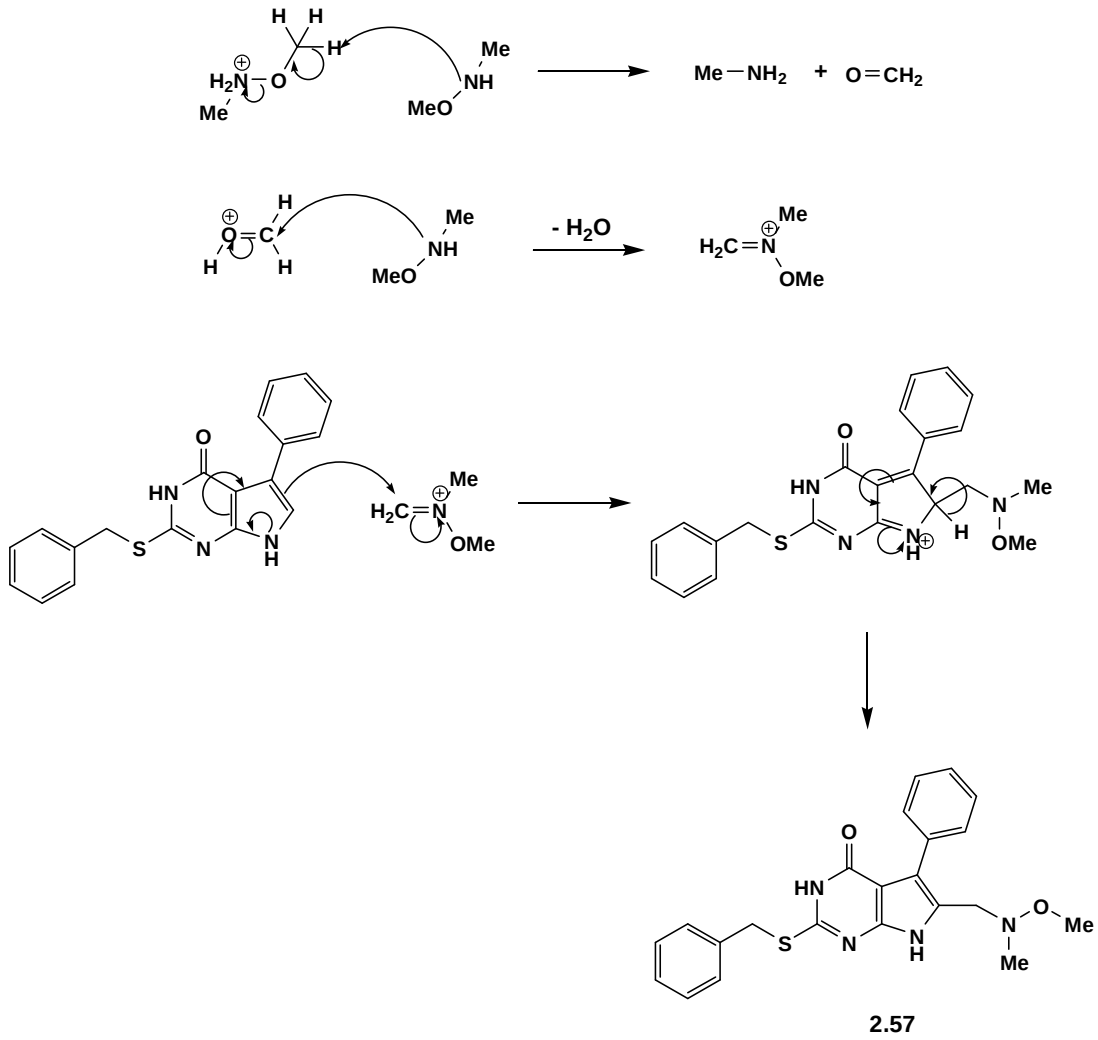
The second case is the reaction between *N*,*O*-dimethylhydroxylamine and the 2-thiobenzylpyrrolo[2,3-*d*]pyrimidine **2.6d** (Scheme 2.42). In this reaction, the C6 substituted product **2.57** was obtained instead of C2 substituted compound **2.56**.

Scheme 2.43 gives a possible mechanism for this reaction.[143] The lone pair of electrons of the free amine nitrogen remove one of the *O*-methyl protons of the amine salt to produce methylamine and formaldehyde. Again, the lone pair of

electrons of the free amine nitrogen can attack the carbonyl carbon of the aldehyde to give an iminium cation. Then the final product **2.57** can be obtained by the nucleophilic attack of the pyrrole on the carbon atom of iminium cation. The C6 substituted product **2.57** was fully characterised and the connection pattern was also confirmed by HMBC (heteronuclear multiple bond correlation) NMR spectrum (the H-C long range correlation, Appendix II).



Scheme 2.42. Reagents, conditions and yield: *N,O*-dimethylhydroxylamine, *N,O*-dimethylhydroxylamine hydrochloride, EtOH, 100 °C, 48 h, 15%.

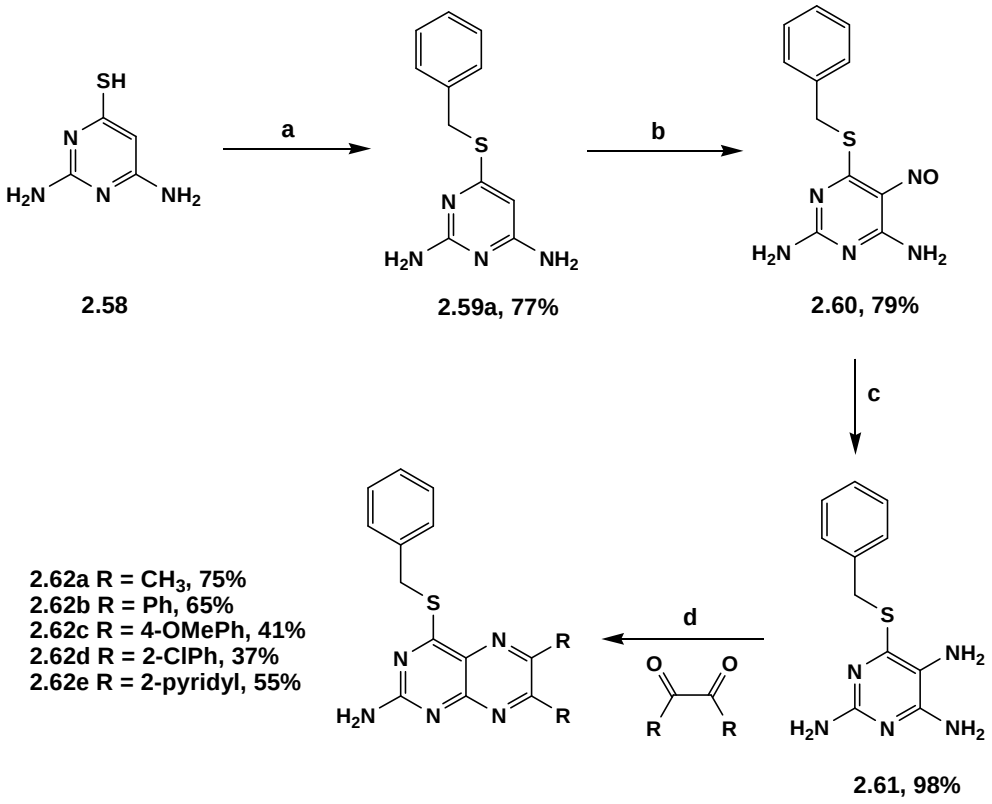


Scheme 2.43. The plausible mechanism of formation of compound **2.57**.

2.1.2. Pyrazino[2,3-*d*]pyrimidine framework

2.1.2.1. 6,7-Functionalised pteridines

6,7-Functionalised pteridines were synthesised successfully (Scheme 2.44). The reaction route was adopted from S. La Rosa,[144] a previous PhD student in our research group.



2.58 — 2.59a, 77% — 2.60, 79% — 2.61, 98%

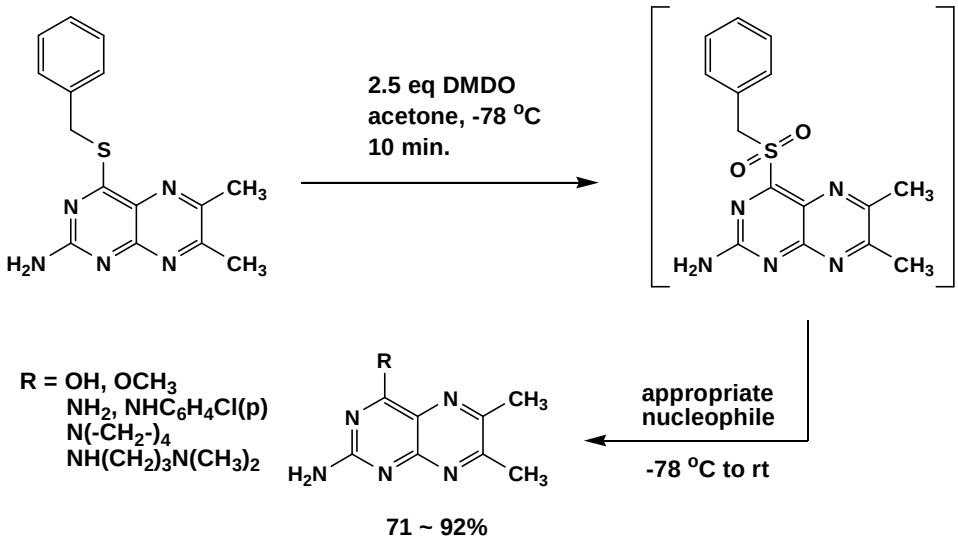
Scheme 2.44. Reagents and conditions: EtOH/H_2O, NaOH, benzyl bromide rt, 20 h. (b) $NaNO_2$, DMF/H_2O, AcOH, rt, 2 d. (c) NaS_2O_4, MeOH/H_2O, rt, 23 h. (d) DMF/100 °C or EtOH/reflux, , 4 h ~ 2 d.

From the starting material **2.58**, benzylation with benzyl bromide (Scheme 2.44, step a), nitrosation with sodium nitrite (Scheme 2.44, step b) and then the reduction with sodium dithionite (Scheme 2.44, step c) went well to produce compound **2.61** as

reported.[144] The cyclocondensation of **2.61** with five different biacetyls was also successful to give 6,7-functionalised compounds **2.62a ~ 2.62e** (Scheme 2.44, step d). DMF was used as a solvent for the cyclocondensation step by S. La Rosa[144] but we found that ethanol is a good alternative which is easier to evaporate during the reaction work-up and gives similar or better yields.

2.1.2.2. C4 Displacement of pteridines

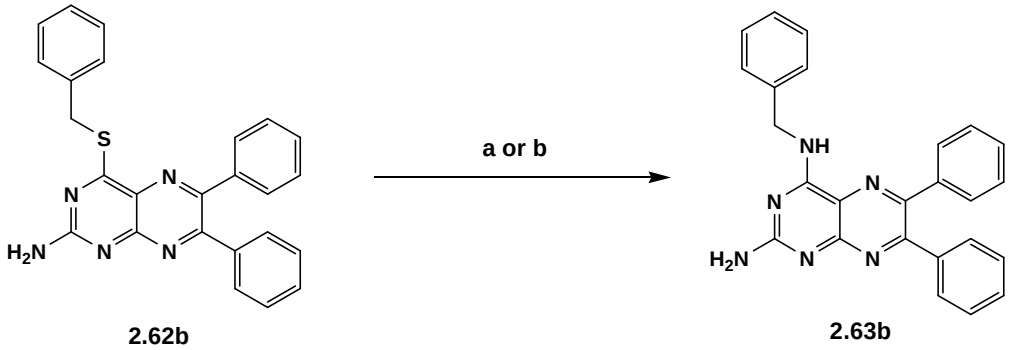
In the previous research, S. La Rosa reported an oxidation-subsitution strategy using DMDO (dimethyldioxirane) to replace the C4 thiobenzyl moiety (Scheme 2.45). Although, this pathway was successful in terms of yields, synthesizing DMDO is relatively difficult and ineffective. In addition, low temperatures (-78 °C) are required at the outset of the oxidation step. Therefore, we wanted to find convenient method to produce library compounds effectively.



Scheme 2.45. C4 displacement pathway by S. La Rosa.[144]

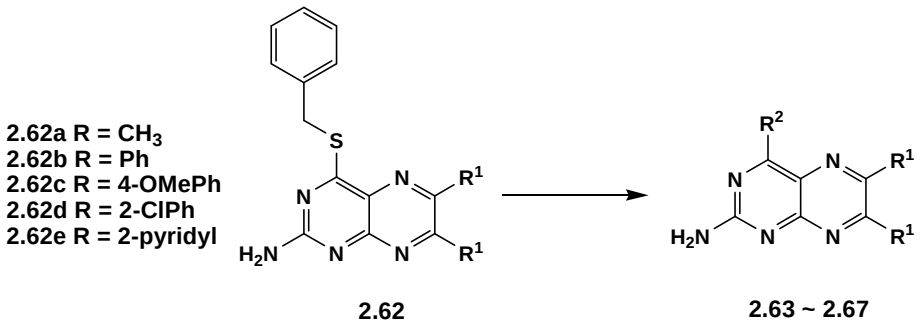
At first, we attempted direct thermal substitution using neat benzylamine without

prior oxidation. After stirring two and a half days at 100 °C, the expected product was obtained in 38% yield. Then, we decided to use microwave assisted reactions. Again, after one hour at 100 °C under microwave, the substituted product was obtained in 46% yield (Scheme 2.46). Because of the short reaction period (1 h), we were encouraged to synthesise a library of compounds *via* microwave reactions using five different amines, which were chosen to represent different structural types and reactivities.



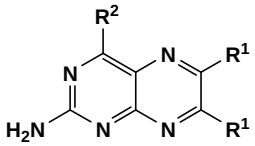
Scheme 2.46. Reagent, conditions and yields: (a) neat benzylamine, 100 °C, 2.5 d, 38%. (b) neat benzylamine, 100 °C, microwave (average 38 ~ 40 W), 1 h, 46%.

Finally, we obtained a library of twenty five 4,6,7-functionalised petridine compounds in moderate to good yields (Scheme 2.47 and Table 2.2). Biological activities of these compounds will be discussed in Section 2.2.2.



Scheme 2.47. Reagent and conditions: neat benzylamines, 100 °C, microwave (average 38 ~ 40 W), 1 or 2 h.

Table 2.2. Yields of 4,6,7-functionalised pteridine library compounds. (unit: %)



	R^2				
R^1	$NHCH_2Ph$	NHPh	$N(CH_2)_4$	$N(CH_2)_3N(CH_3)_2$	$N(CH_2)_4O$
CH_3	**2.63a**, 60	**2.64a**, 55	**2.65a**, 66	**2.66a**, 29	**2.67a**, 45
Ph	**2.63b**, 49	**2.64b**, 46	**2.65b**, 54	**2.66b**, 76	**2.67b**, 78
4-OCH_3Ph	**2.63c**, 53	**2.64c**, 25	**2.65c**, 53	**2.66c**, 55	**2.67c**, 44
2-ClPh	**2.63d**, 62	**2.64d**, 67	**2.65d**, 70	**2.66d**, 44	**2.67d**, 63
2-pyridyl	**2.63e**, 66	**2.64e**, 65	**2.65e**, 80	**2.66e**, 79	**2.67e**, 76

2.1.2.3. Reactivity comparison of C2 *vs.* C4 displacement

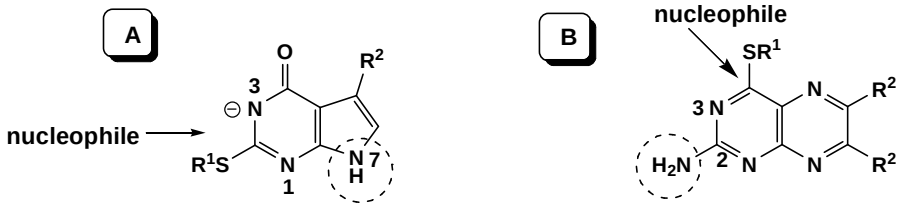
From the above success of one-step displacement of 4-thioalkyl pteridines without oxidation, we can conclude that there are different chemical environments between the C2 of pyrrolo[2,3-*d*]pyrimidines and C4 of pyrazino[2,3-*d*]pyrimidines. Explanations of factors which involve in displacement reactivities of the two ring systems can be given as follows.

(i) In general, pteridines are electron deficient compared to pyrrolo[2,3-*d*]pyrimidines as pteridines have two annular nitrogens in the fused ring.

(ii) In both cases, there are electron relasing sources (N7 for pyrrolo[2,3-*d*]pyrimidines, 2-NH_2 for pteridines) which can contribute electron richness to the ring carbons (Scheme 2.48).

(iii) However, a more significant effect is expected at C2 substitution of pyrrolo[2,3-*d*]pyrimidines due to the possibility of anion formation of N3 by basic nucleophiles (Scheme 2.48, A). In contrast, there is no anion formation and distinctive chemical moiety to electronically hinder

approaching of nucleophiles at the C4 of pteridines. Therefore, we suggest that this factor could be involved in the remarkable difference with regard to the displacement reactivity of the two different ring systems.



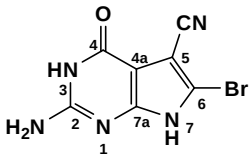
Scheme 2.48. Different chemical environments at C2 of pyrrolo[2,3-*d*]pyrimidine (A) and C4 of pyrazino[2,3-*d*]pyrimidine (B). Dotted circles: electron donating sources to the rings.

2.1.3. Design and synthesis of inhibitors for PTR1

2.1.3.1 Modeling study

A previous study

In collaboration with W. N. Hunter's group (University of Dundee), we found that compound **2.68** is a moderate inhibitor (80% inhibition at 100 uM) for *L. major* PTR1. Therefore, we have access to the crystal structure of the active site of *L. major* PTR1, which was co-crystallised with 5-cyano-6-bromo deazaguanine **2.68** (Fig. 2.1).[117] This study was performed in order to get an insight into the binding orientation of deazaguanine compounds and highlights the possible interactions with the residues in the active site. This also provides an idea of how much space is left for the accommodation of additional substituents in the active site.



2.68

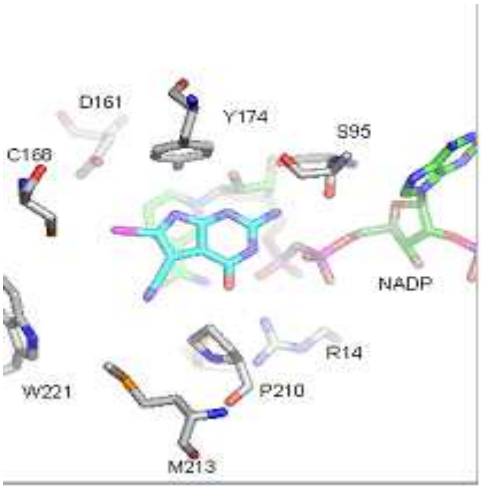


Figure 2.1. Crystal structure of **2.68** in the active site of *L. major* PTR1.[117] The nitrogens are coloured in dark blue and the bromine at the 6-position is in magenta. Image was provided by W. N. Hunter's group (University of Dundee).

An extended result with a model molecule

Through molecular modeling, the active site was located and molecule **2.69** was docked into the active site (Fig. 2.2). From this study, we could obtain several important clues to design inhibitors: (i) The deazaguanine framework fits well into

the active site; (ii) tyrosine (Tyr 194) allows a hydrogen bond to the 7-NH; (iii) the end of pocket A contains a hydrophobic tryptophan (Trp 221); (iv) space B also has a hydrophobic terminus but at the same time it contains an aspartate (Asp 161) which can be used to form hydrogen bonds; (v) although pocket C is not very big, it can accommodate small groups, so diversification at C2 seems to be possible.

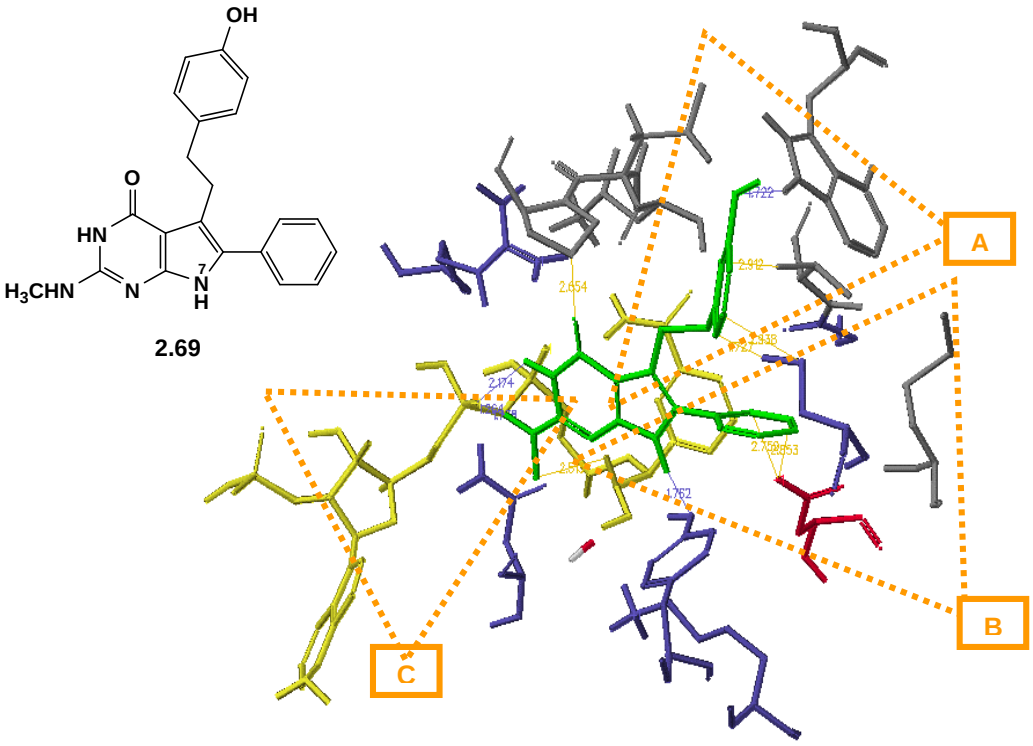


Figure 2.2. Structure of the active site of *L. major* PTR1 and its three pockets (two major A and B, one minor C). Green: model molecule **2.69**. Yellow: NADPH. Red: aspartate 161. Blue: polar residues including tyrosine 194 (bottom). Grey: non polar residues including tryptophan 221 (top right). Interatomic distances are shown for key potential interactions. The molecular model was obtained using the CAChe® molecular modeling system (Fujitsu, Poland) by superimposing a hypothetical inhibitor **2.69** in a crystal structure. Energy minimization was not carried out. Image was provided by C. J. Suckling, the supervisor of this thesis.

A specialized compound for pocket B

From the modeling study, a hydrogen bonding possibility was evident between the NH of C6 extended pyridone group of compound **2.70a** and the carboxylate anion of Asp 161 of the active site (Fig. 2.3). This clear possibility of hydrogen bonding meant that compound **2.70a** was a key target. The synthesis of **2.70a** would allow us to compare the inhibitory activity of C6-pyridone connected compound **2.70a** with the activity of C6-phenyl connected compound **2.72** (Fig. 2.4).

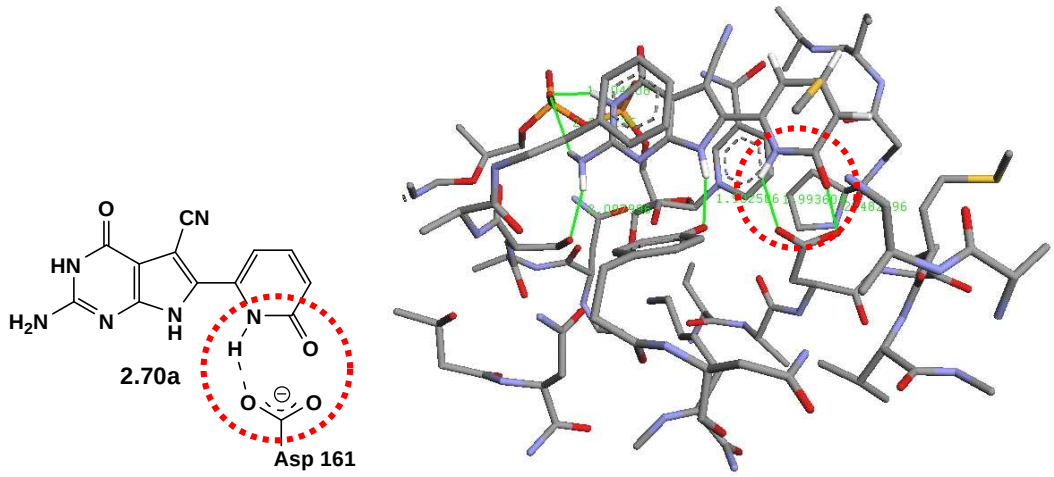


Figure 2.3. Structure of the active site of *L. major* PTR1 and its hydrogen bonding possibility with compound **2.70a** (red dotted circle). The molecular model was obtained using the CAChe® molecular modeling system (Fujitsu, Poland) by superimposing a hypothetical inhibitor **2.70a** in a crystal structure. Image was provided by C. J. Suckling, the supervisor of this thesis.

2.1.3.2. Design of inhibitors for PTR1

From the modeling study (Section 2.1.3.1), we have designed potential inhibitors of PTR1 to give diversity on the deazaguanine framework to fit with two major pockets (Fig. 2.2, A and B). Figure 2.4 shows our selected inhibitors and their target pockets, for the first stage of this project. The pyrimidine framework was selected from our previous biological results (Section 1.5.2.3, Fig. 1.39).[117] The synthetic results of

these compounds and the biological assays are given in Section 2.2.1.

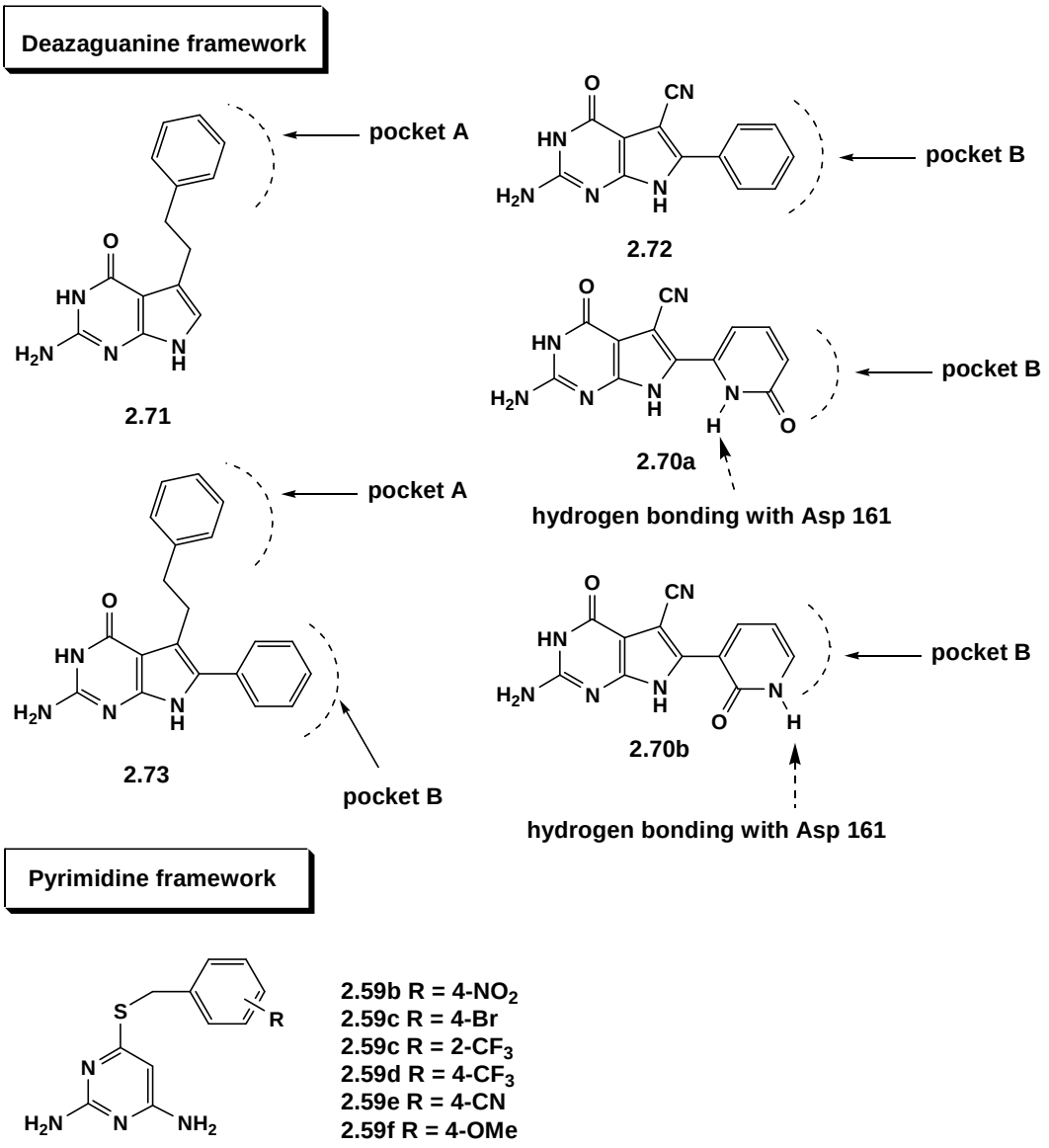
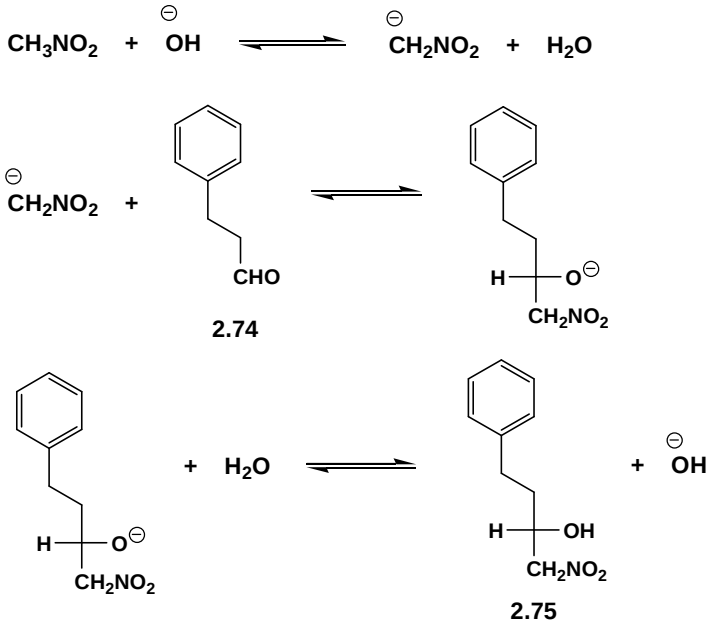


Figure 2.4. Potential inhibitors of PTR1 designed from a molecular modeling study of the active site of *L. major* (deazaguanine framework) and from previous biological results[117] (pyrimidine framework).

2.1.3.3. Synthesis of compound 2.71

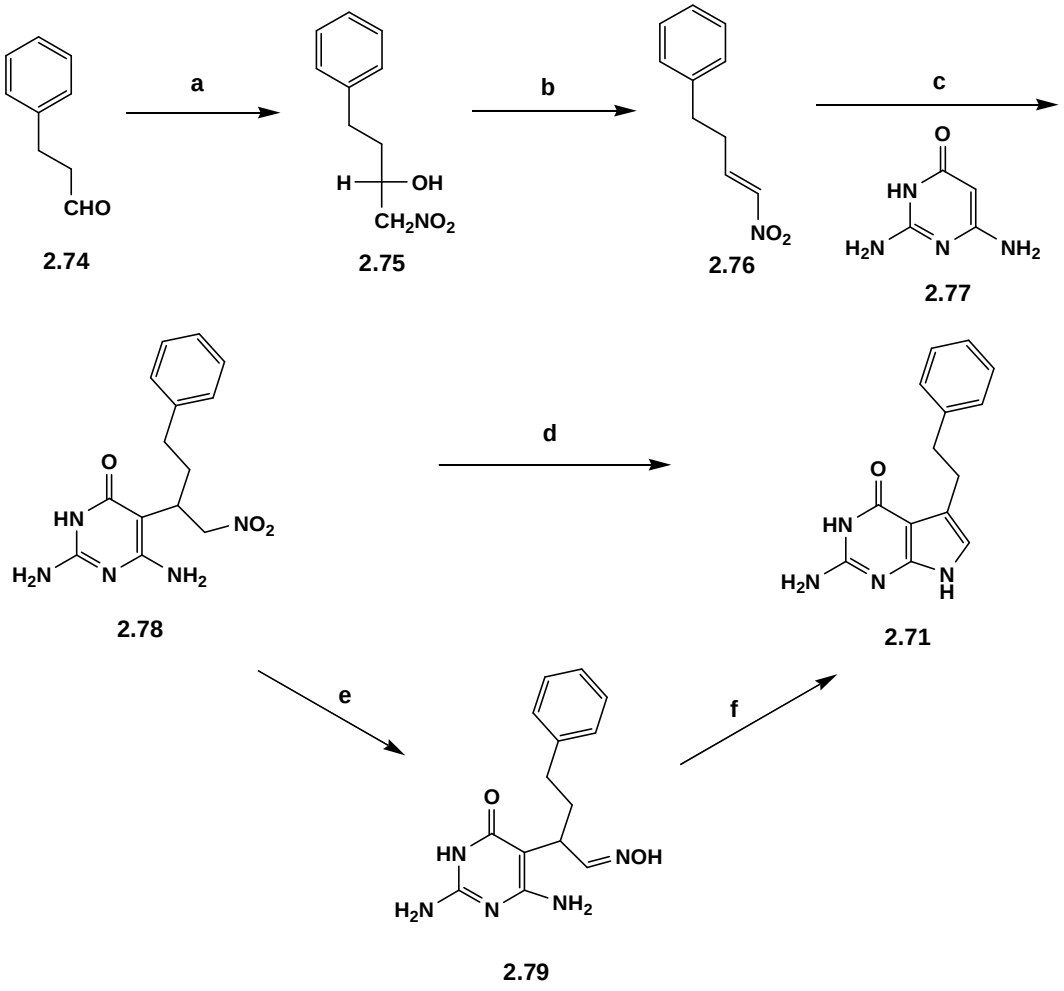
The successful synthesis of compound **2.71** was reported by E. C. Taylor and B. Liu.[116] Therefore, we prepared this compound in the same way as these workers (Scheme 2.50, route a, b, c and d). The first step involved a Henry reaction[116, 123] with aldehyde **2.74** and nitromethane which gave nitroaldol adduct **2.75** in excellent yield (94%, Scheme 2.49). Compound **2.75** was then dehydrated by mesylation followed by treatment with triethylamine to yield nitroalkene **2.76** as a yellow oil (Scheme 2.50).



Scheme 2.49. Henry reaction to form compound **2.75**.

Compound **2.78** was synthesised by Michael addition between 2,6-diaminopyrimidine (Michael donor) **2.77** and the nitroalkene (Michael acceptor) **2.76**. Then, a classical Nef reaction followed by intramolecular condensation gave the deazaguanine **2.71**. However, in our repeated attempts, we have found that the one-step cyclisation is not facile and purification is also difficult. Therefore, we decided to adopt a two-step reaction developed by D. Edmont *et al.*,[145] and the oxime

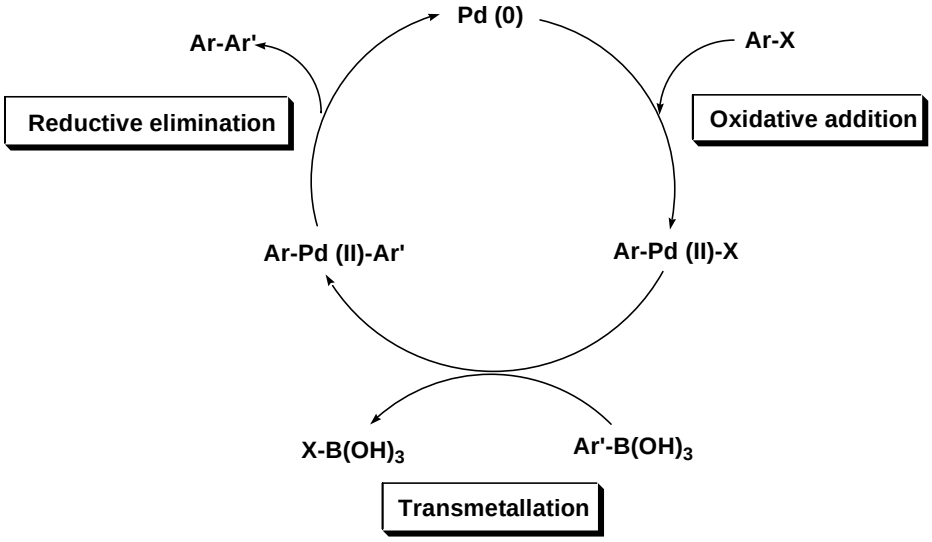
intermediate **2.79** and the cyclised product **2.71** were synthesised smoothly (Scheme 2.50, route e and f). Although this alternative is two-step, each step is relatively simple with regard to the reaction work-up, and do not require long reaction period and gives each product in good yields (58% for the oxime intermediate **2.79**, 90% for the cyclised product **2.71**).



Scheme 2.50. Reagents conditions and yields: (a) CH_3NO_2, NaOH, EtOH, 94%. (b) DCM, CH_3SO_2Cl, Et_3N, 55%. (c) EtOAc/H_2O 1:1, 90 °C, 24 h, 94%. (d) (i) aq NaOH, rt, 2 h; (ii) aq H_2SO_4 at 0 °C, 1 h; (iii) conc. NH_4OH to pH 7, rt, 1 h, 43%. (e) $SnCl_2$, benzenethiol, Et_3N, THF, rt, 1 h, 58 %. (f) Dowex-50 (H^+), H_2O, reflux, 15 h, 90%.

2.1.3.4. Synthesis of compound 2.72

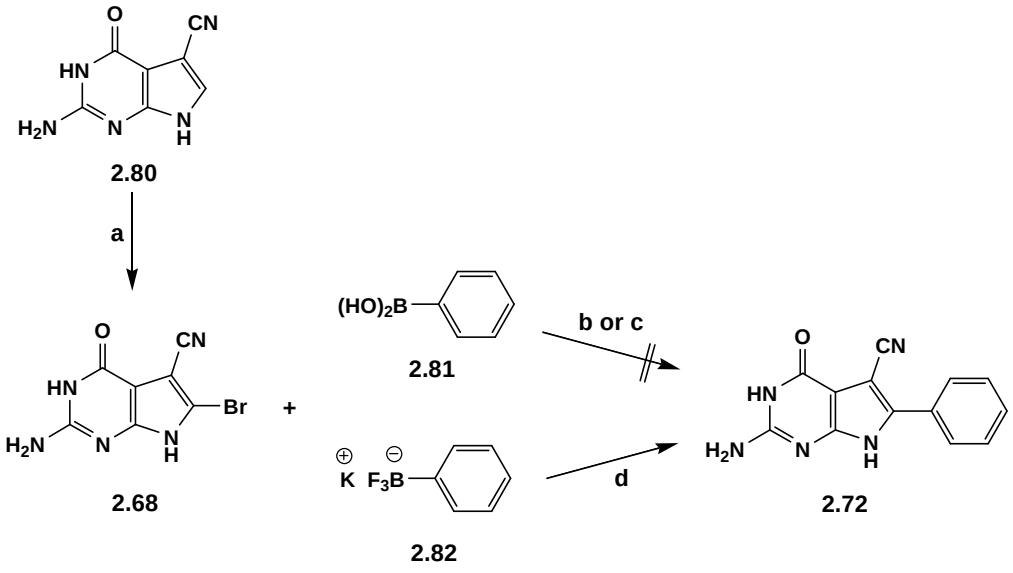
One of the major pockets of the active site of *L. major* PTR1 is potentially close to the C6 position of the deazaguanine framework (Section 2.1.3.1). Thus, introducing a phenyl ring at C6 to access the hydrophobic pocket has been a crucial step in our studies. The Suzuki reaction, involving palladium-catalysed cross-coupling reaction between organoboron compounds and organic halides can be a solution to this synthetic challenge (Scheme 2.51).[146] The innocuous nature of boronic acids, which are generally non-toxic and thermally, air, and moisture-stable, was a practical advantage of the Suzuki reaction.[147] However, as boronic acids are often subject to dimerisation and cyclic trimerisation through dehydration to form boronic acid anhydrides and boroxines, potassium organotrifluoroborate salts are widely employed as an alternative.[148, 149]



Scheme 2.51. Mechanism of Suzuki-cross coupling.[146]

Substrate **2.68** was prepared in the same way as J. K. Huggan[117] of these laboratories. Then, we attempted the conventional Suzuki coupling method between a halogenated substrate **2.68** and phenylboronic acid **2.81** as a coupling partner (Scheme 2.52, b and c). However, none of the anticipated products from either system, THF-

$PhCH_2N^+Me_3HO^-$-$PdCl_2(PPh_3)_2$ or THF-KF-$PdCl_2(PPh_3)_2$ were formed. Because substrate **2.68** is electron rich, the transmetallation seems not to be facile using conventional methods. There are several parameters in a Suzuki reaction – palladium source, ligand, additive, solvent, temperature, etc. – and there are, correspondingly, a large number of protocols for accomplishing the desired transformations. The choice of protocols depends on the structure of the reactants.[146] Therefore, we decided to change three parameters: (i) The solvent was changed from aprotic THF to protic IPA-H_2O (2:1); (ii) *t*-$BuNH_2$ was employed as the base; (iii) the coupling partner, potassium trifluorophenylborate was used in place of phenylboronic acid. Using these conditions, the anticipated product **2.72** was produced in excellent yield (92%). This modification was strongly encouraged by the observations of G. A. Molander, a pioneer of cross-coupling reactions using organotrifluoroborates.[150]



Scheme 2.52. Reagents, conditions and yield: (a) AcOH, Br_2, rt for 20 h then 60 °C for 1 d, 58%. (b) THF, $PhCH_2N^+Me_3HO^-$ (aq), $PdCl_2(PPh_3)_2$, 60 °C, 5 d. (c) THF, KF (aq), $PdCl_2(PPh_3)_2$, 60 °C, 5 d. (d) IPA/H_2O (2:1), *t*-$BuNH_2$, $PdCl_2(PPh_3)_2$, 85 °C, 4 d, 92%

Among the three parameters changed, which synergically can lead to significant difference in this reaction, the protic solvent system may be a crucial variable. Indeed, previous reports have indicated that water was required as a co-solvent for the

trifluoroborate coupling reactions.[150] Also, one or more hydroxyl groups displace fluorides on the tetracoordinate boron species involved in the transmetallation step of the catalytic cycle.[148, 151, 152] These conditions were more effective than our previous method – EtOH, Na_2CO_3 (aq), $PdCl_2(PPh_3)_2$, 26 ~ 74% yields - which was exploited by L. Keifer from these laboratories.[153] We now expect that we can smoothly and effectively obtain diverse C6 substituted potential inhibitors which have a deazaguanine scaffold through these improved cross-coupling reaction conditions.

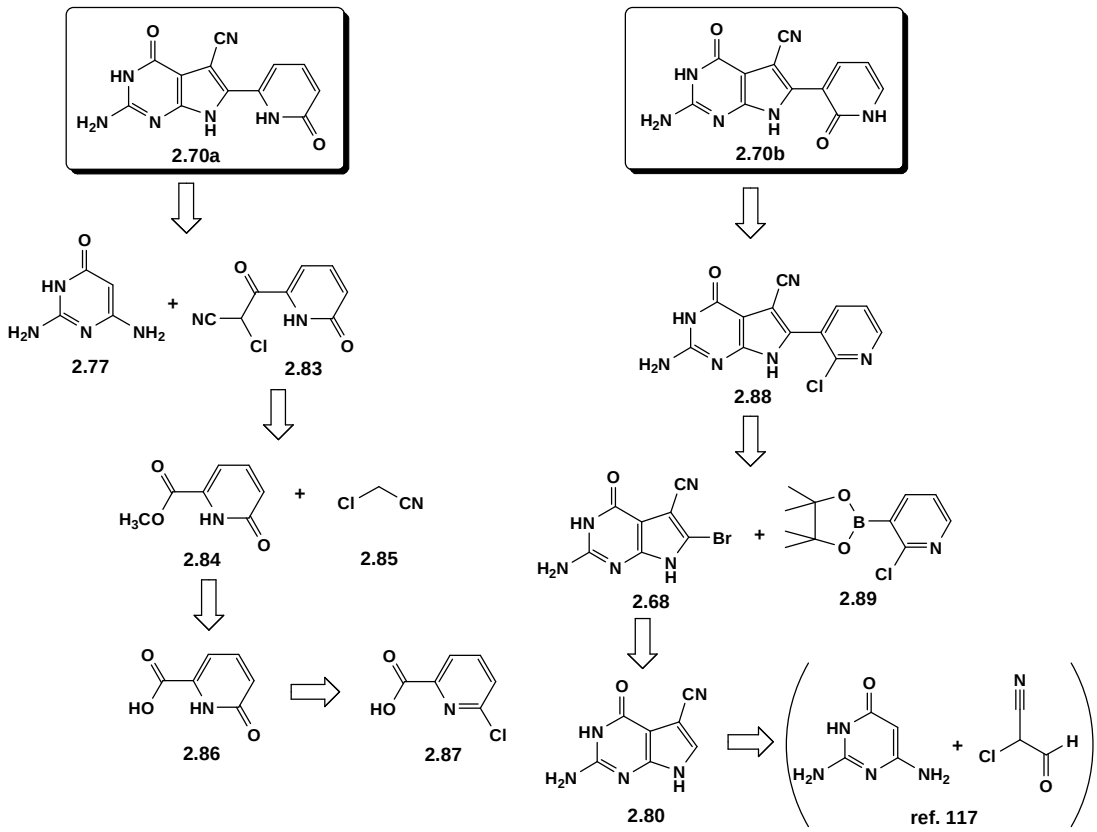
2.1.3.5. Synthesis of compound 2.70

Retrosynthetic analysis of 2.70 (Scheme 2.53)

Compounds were designed to occupy pocket B of the active site of PTR1 of *L. major* as well as hydrogen bonding to aspartic acid (Asp 161) of the active site (Fig 2.4).

Compound **2.70a** can be obtained *via* a cyclocondensation method with the key substrate **2.83**. Substrate **2.83** can be synthesised from the reaction of ester **2.84** and chloroacetonitrile **2.85** with a base. The ester **2.84** can be prepared by conventional esterification using methanol with acid catalyst. Carboxylic acid **2.86** can be obtained from commercially available compound **2.87** with aqueous acid.

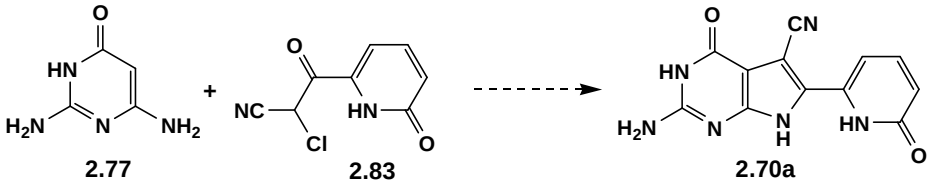
As boronic ester **2.89** is commercially available, a Suzuki coupling route was selected for compound **2.70b**, which can be prepared from compound **2.88** through hydrolysis. Compound **2.80** has been prepared previously,[117] which can be brominated at C6 to give substrate **2.68** (Section 2.1.3.4, Scheme 2.52).



Scheme 2.53. Retrosynthetic analysis of compound **2.70**.

Attempted synthesis of compound 2.70a

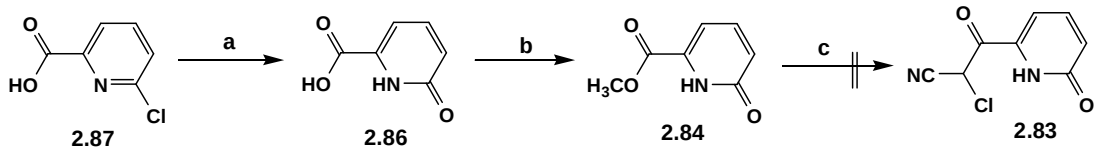
As discussed before, synthesis of compound **2.70a** depends on successful preparation of the key substrate **2.83** as this is not commercially available (Scheme 2.54).



Scheme 2.54. Synthesis of compound **2.70a**.

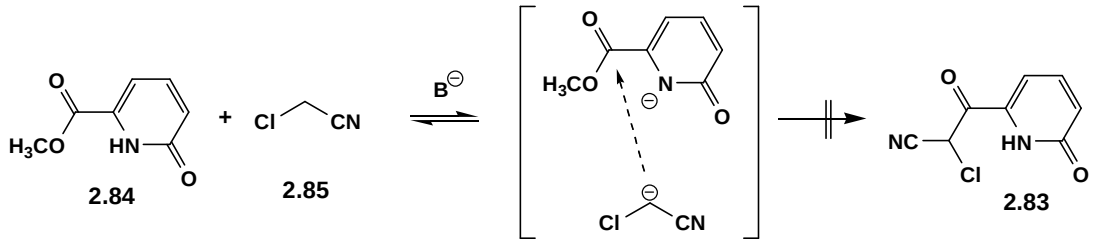
Ester substrate **2.84** was successfully synthesised using the procedure of T. Kametani

et al.[154] However, the key substrate **2.83** was not obtained from the reaction with chloroacetonitrile using two equivalent of sodium methoxide (Scheme 2.55).



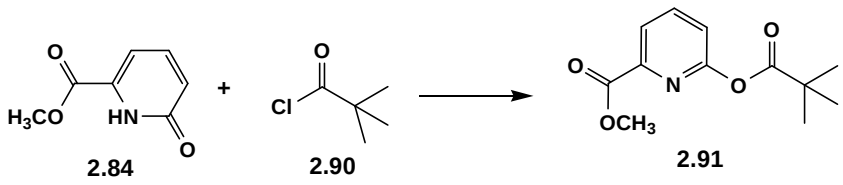
Scheme 2.55. Reagents, conditions and yields: (a) conc. HCl, 140 °C for 1 d then 120 °C 1 d, 84%. (b) MeOH, H_2SO_4, reflux, overnight, 62%. (c) NaOMe (2 eq), MeOH, 2-chloroacetonitril, reflux, overnight.

One plausible reason of this failure is the possibility of formation of pyridone anion with the base. This anion would electronically hinder the approaching of partner anion to attack the carbonyl carbon (Scheme 2.56).



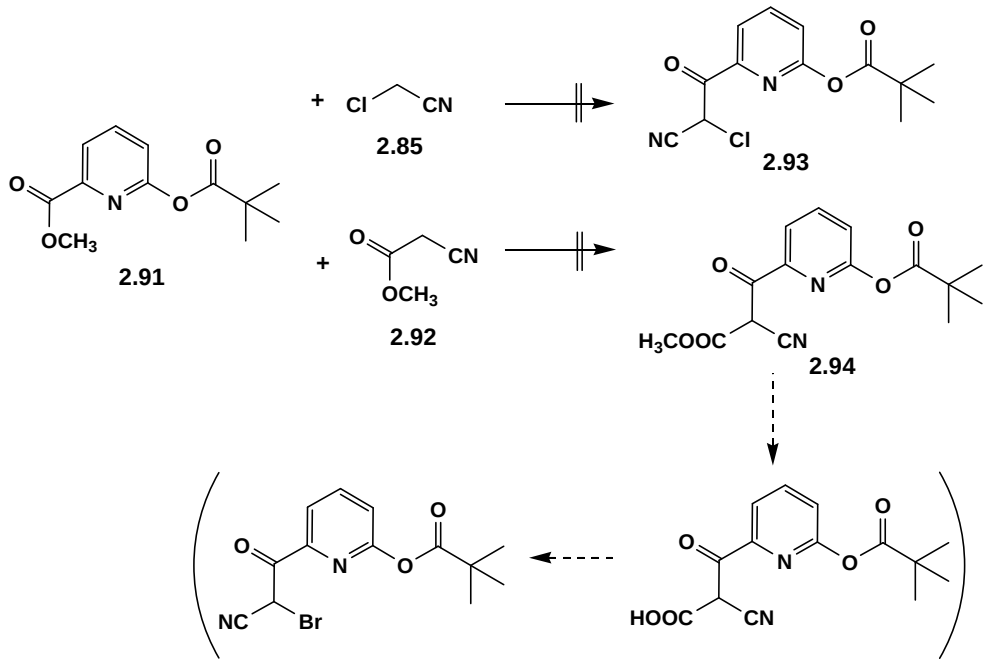
Scheme 2.56. Failure of formation of compound **2.83**.

In order to overcome the above problem, the pyridone **2.84** was protected using pivaloyl chloride. The protection reaction was performed in DMF with potassium carbonate and this gave the *O*-protected compound **2.91** solely (Scheme 2.57). This was confirmed by distinctive IR bands at 1722 and 1753 cm^{-1} (ester C=O stretching vibration: 1725 ~ 1750 cm^{-1}, Appendix III)[155] and any bands belong to amide C=O vibration (1650 ~ 1670 cm^{-1})[155] were not found.



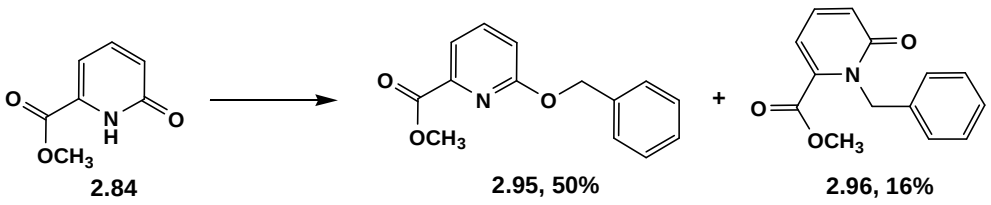
Scheme 2.57. Reagents, conditions and yields: DMF, K_2CO_3 (2 eq), 60 °C, 24 h, 53%.

The prepared *O*-pivaloyl protected compound **2.91** was reacted with chloroacetonitrile **2.85** or methyl 2-cyanoacetate **2.92** as an alternative (Scheme 2.58). There was no change in TLC after stirring for five hours at room temperature. The reaction was continued overnight under reflux. However, the substituted product was not obtained in either case, instead, only deprotected substrate **2.84** was found. This is probably because the electron withdrawing pyridine increases the electrophilicity of the pivaloyl carbonyl carbon.



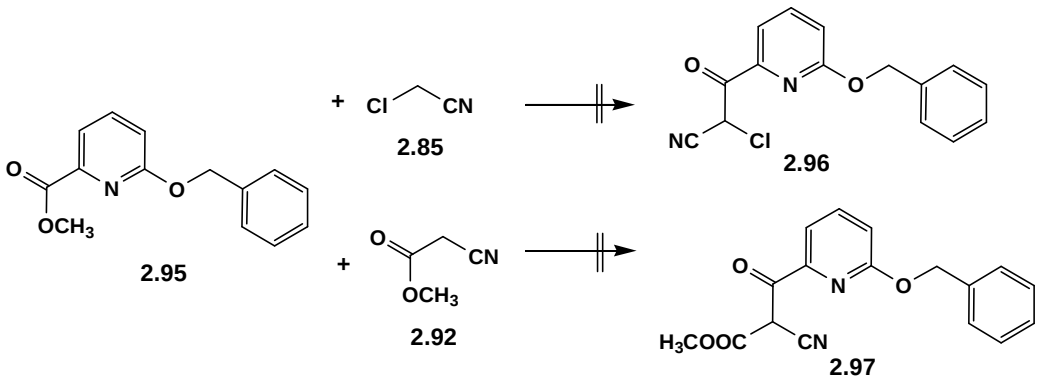
Scheme 2.58. Reagents, conditions: THF, NaOMe, rt for 5 h then reflux for overnight.

To clarify the feasibility of this chemistry, we decided to introduce a robust ether protecting group, benzyl group, instead of the pivalate ester. The reaction of substrate **2.84** with benzyl bromide gave two products, *O*-benzyl **2.95** and *N*-benzyl compound **2.96** (Scheme 2.59).



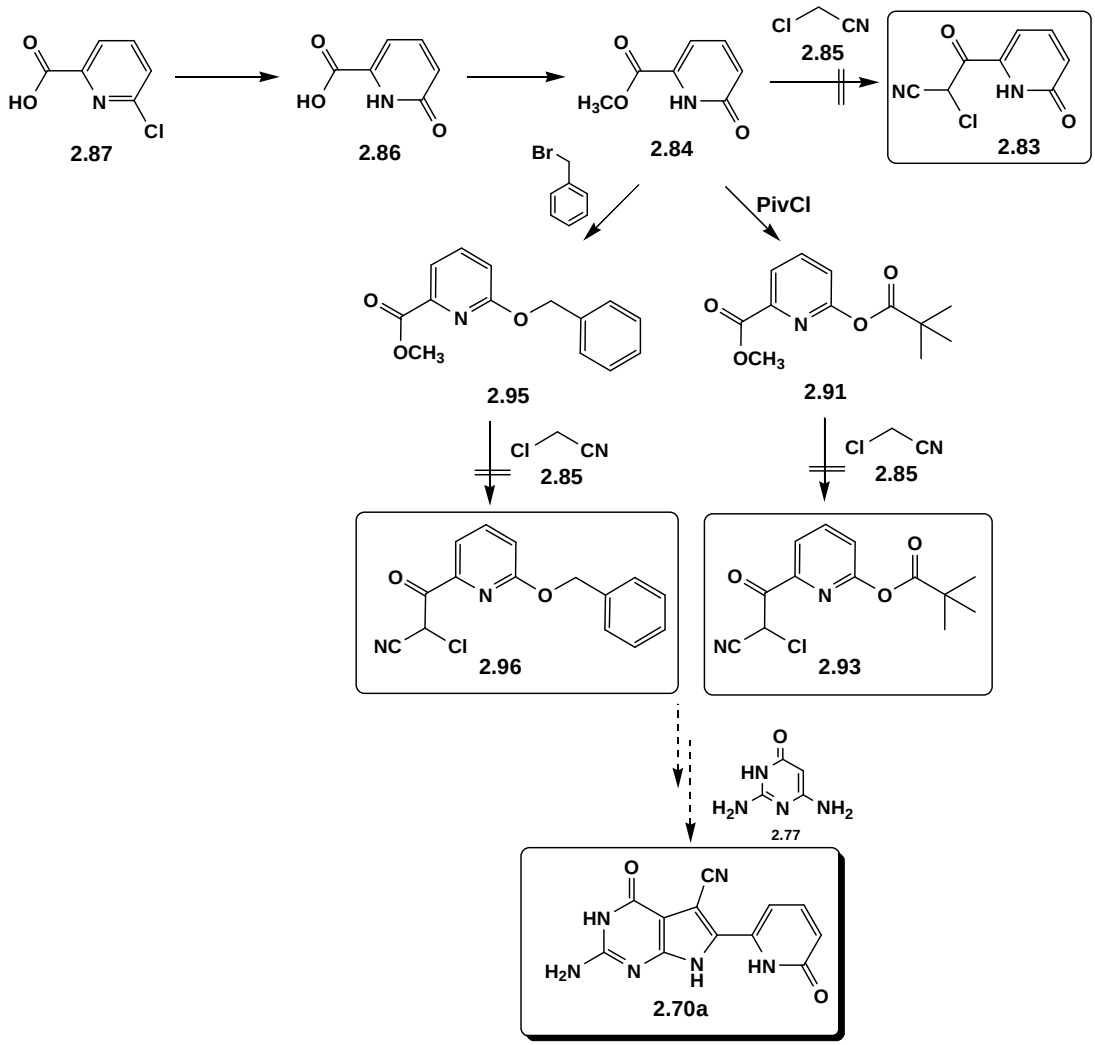
Scheme 2.59. Reagents, conditions and yields: DMF, NaH, benzyl bromide in THF, rt for 15 h then 100 °C for 4 h.

The two compounds were identified by significant difference of chemical shifts of benzyl carbons derived from the difference of electronegativity of nitrogen and oxygen. The *O*-benzyl carbon (less shielded carbon) appeared at 68.24 ppm, in contrast, 47.22 ppm was recorded for the *N*-benzyl carbon (more shielded carbon) in the ^{13}C NMR (APPENDIX IV). As the *O*-benzyl product **2.95** is less hindered than the *N*-benzyl product **2.96** toward the carbonyl cabon of the methyl ester moiety, partner reagents **2.85** or **2.92** were then reacted with compound **2.95** using sodium hydride as a base (Scheme 2.60).



Scheme 2.60. Reagents and conditions: DMF, NaH, 90 °C for overnight.

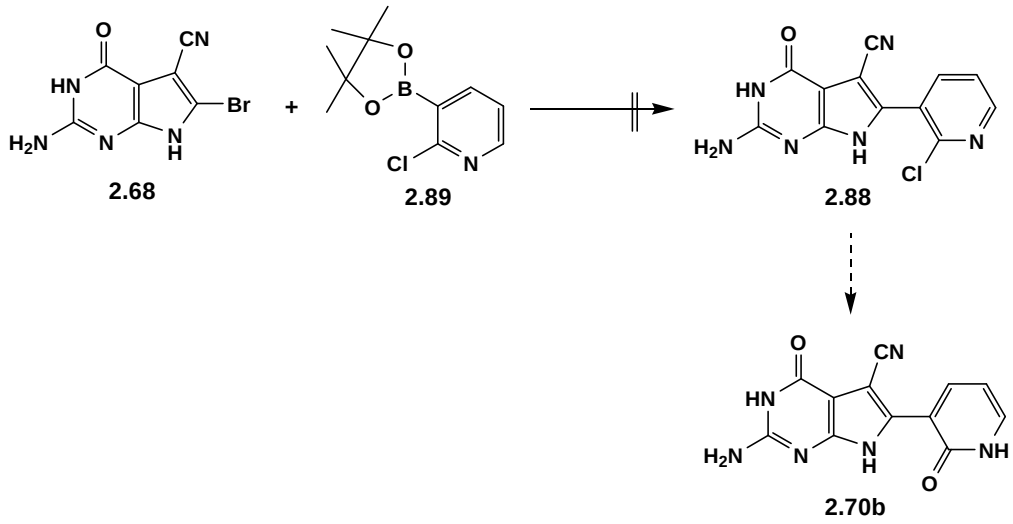
However, the reaction did not proceed and substrate **2.95** firmly remained. Because, we failed to synthesise the key substrate **2.83** (or **2.93** or **2.96)**, our target molecule **270a** could not be obtained (Scheme 2.61).



Scheme 2.61. Failure of synthesis of **2.70a**.

Attempted synthesis of compound 2.70b *via* Suzuki coupling

As boronic ester **2.89** is commercially available, we simply attempted a Suzuki coupling to obtain compound **2.88**. The latter can be hydrolysed to give the final target compound **2.70b** (Scheme 2.62).



Scheme 2.62. Reagents and conditions: IPA/H_2O (2:1) or DMF/H_2O (2:1), Et_3N or *t*-$BuNH_2$, $PdCl_2(PPh_3)_2$, 80 ~ 100 °C, 3 d.

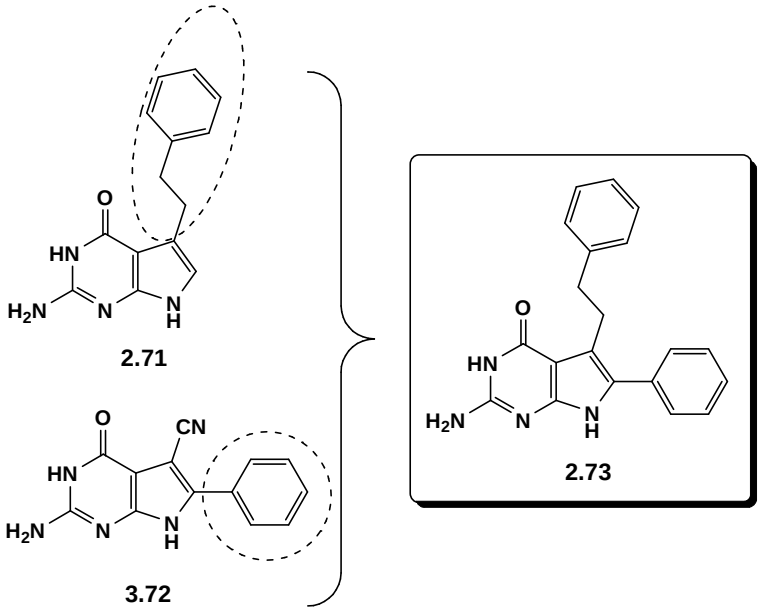
To avoid a co-coupling of chloro pyridine boronic ester **2.89**, in the first place, substrate **2.68** was activated with palladium catalyst and a base under the selected solvent systems, then boronic ester **2.89** was carefully added. However, we found that halopyridine boronic ester **2.89** was not smoothly coupled with the substrate. Although, in some cases, ^{1}H NMR spectra showed corresponding peaks of expected product **2.88**, even after column purification, the pure expected product **2.88** was not isolated but coeluted with starting substrate **2.68**.

Due to the failure of synthesis of compound **2.70a** and **2.70b**, other workers in our laboratory are now endeavouring to obtain similar compounds to **2.70a** and **2.70b** using different synthetic routes.

2.1.3.6. Attempted synthesis of compound 2.73

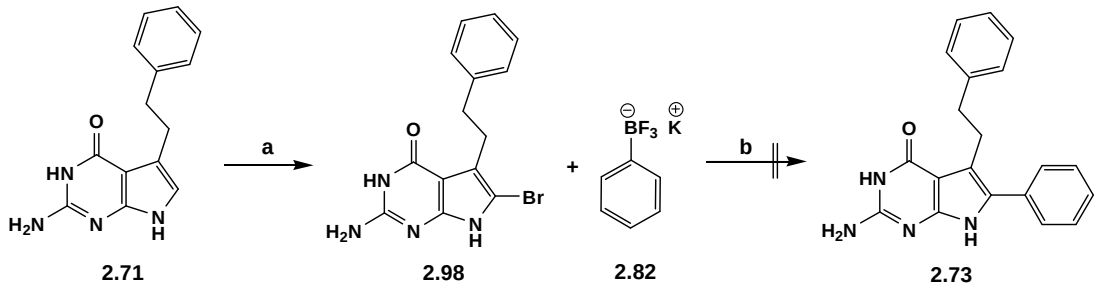
To occupy two major hydrophobic pockets of the active site of *L. major* PTR1 (Section 2.1.3.1, Fig. 2.22), we designed a hybrid compound **2.73** of the compound

2.71 and **2.72** (Scheme 2.63). We expect that this type of 5,6-substituted deazapurines may show better inhibitory activity/selectivity rather than C5 or C6 mono-substituted compounds.



Scheme 2.63. A model hybrid compound **2.73**.

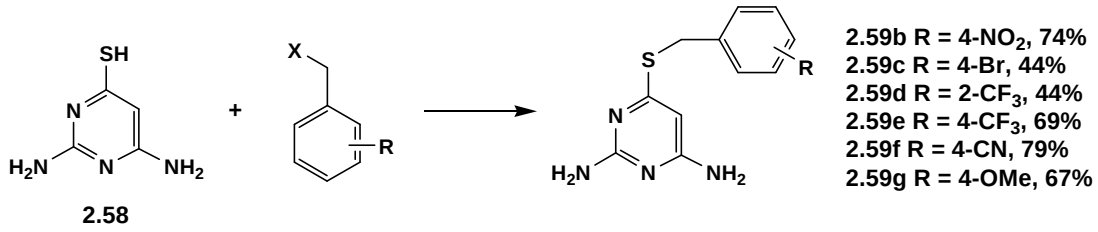
To synthesise the hybrid compound **2.73**, compound **2.71** was treated with bromine in acetic acid to give brominated compound **2.98** (Scheme 2.64). However, in the following step, incorporation of a phenyl ring at C6 was not successful by the improved Suzuki coupling conditions (Section 2.1.3.4). This is possibly because the phenethyl moiety at C5 can significantly hinder the approaching of the coupling partner **2.82**.



Scheme 2.64. Reagents, conditions and yield: (a) Br_2, AcOH, 50 °C ~ rt, 20 h, 70%. (b) IPA/H_2O (2:1), *t*-$BuNH_2$, $PdCl_2(PPh_3)_2$, 85 °C, 4 d.

2.1.3.7. Pyrimidine framework

6-(Benzylsulfanyl)-2,4-pyrimidinediamines **2.59b ~ 2.59g** were synthesised by simple benzylation of 2,6-diamino-4-pyrimidinethiol with six different benzyl halides (Scheme 2.65). The pyrimidinediamines **2.59b ~ 2.59g** were identified as potential synthetic targets based on our previous biological results with trypanosomatids PTR1 (Section 1.5.2.3, Fig. 1.39).[117] Biological results for these compounds will be discussed in the following section.



Scheme 2.65. Reagents and conditions: EtOH/H_2O, NaOH, rt ~ 80 °C, 19 ~ 46 h.

2.2. Biological results

Here, we describe biological data, PTR1 inhibitory activities for designed compounds (Section 2.2.1) and random screening results (cytotoxic, antibacterial and antiparasitic activities) for compounds synthesised by diversity oriented strategy (Section 2.2.2).

2.2.1. Inhibitory activity for PTR1

Among the synthesised compounds, eight (Figure 2.5) were investigated for their ability to inhibit PTR1 (*L. major* and *T. brucei*) by Lindsay Tulloch (W. N. Hunter's group, University of Dundee). The author of this thesis acknowledges their cooperation.

Assay method

Pteridine reductase was purified as described.[156] The enzyme (15 mg/ml, 50 mM Tris-HCl, 250 mM NaCl, 20% (v/v) glycerol, pH 7.5) was flash frozen in liquid nitrogen and stored at –20 °C until required. Potential inhibitors were dissolved in DMSO, while the substrate dihydrobiopterin was dissolved in 0.1 M NaOH. As initial assays (n=1), inhibition was performed at fixed concentrations, 20 μM for dihydrobiopterin and 10 μM for inhibitors. NADPH oxidation was followed, at 340 nm, in assays executed in a Beckman DU640 spectrophotometer.

Figure 2.6 shows the percentage inhibition results of the compounds compared with methotrexate (MTX), the archetypal antifolate drug.

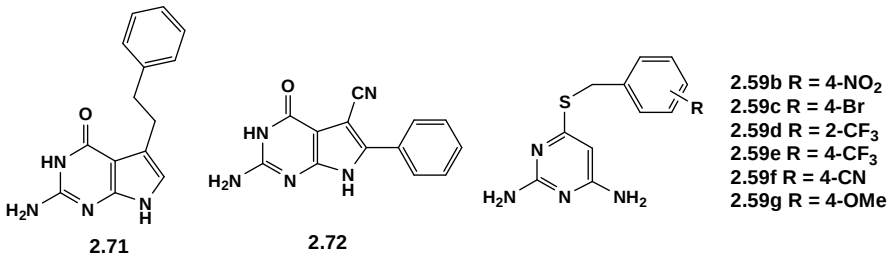


Figure 2.5. Biologically assayed compounds.

Compounds **2.71** and **2.72** were designed to fit into pockets A and B of the PTR1 active site, respectively (Section 2.1.3.1 and 2.1.3.2, Fig. 2.3 and 2.4). Interestingly, the percentage inhibitions of PTR1 activity by the two compounds were almost the same (~ 70%) for *T. brucei* though compound **2.71** inhibited the PTR1 activity more strongly (~ 25%) than compound **2.72** (~ 10%) in the case of *L. major*. This result indicates a C5 and C6 disubstituted deazaguanine may have a stronger inhibitory character due to the simultaneous interaction with the two major pockets of the active site. The series of compounds **2.59b ~ 2.59g** were shown to have a higher inhibitory action against *L. major* PTR1 activity (45 ~ 61%) than **2.71** or **2.72**. However, for *T. brucei*, the percentage inhibitions by **2.59b ~ 2.59g** were significantly lower (2 ~ 38%) than **2.71** and **2.72**. These data indicate that we need to study the crystal structure of PTR1 binding with one of the derivatives of **2.59** to allow the further design and synthesis of improved inhibitors.

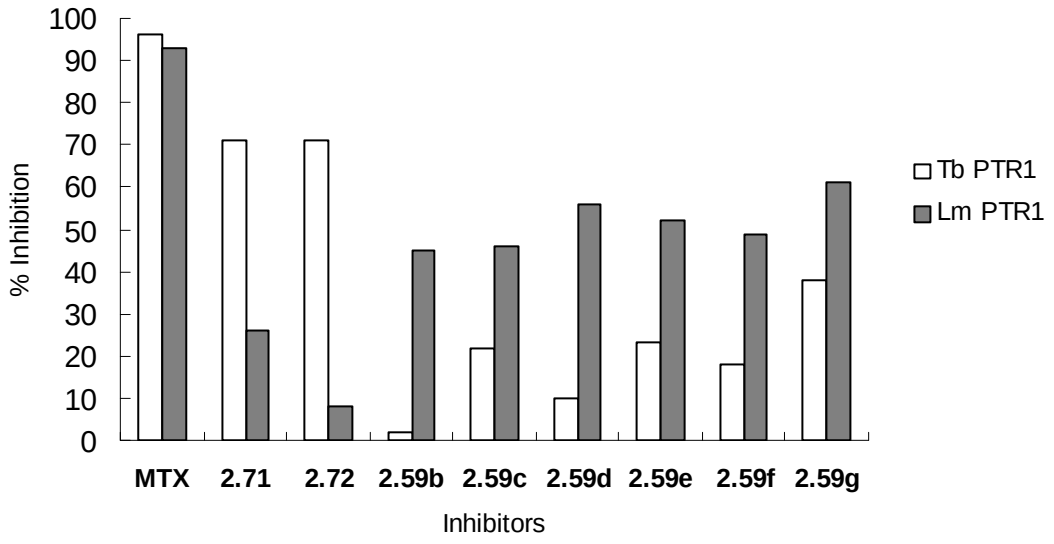


Figure 2.6. Percentage inhibition of PTR1 activity with dihydrobiopterin (fixed at 20 μM) by inhibitors (10 μM), n=1. Lm = *L. major* and Tb = *T. brucei*. MTX = methotrexate. Data was provided by W. N. Hunter's group, University of Dundee.

2.2.2. Screening assays

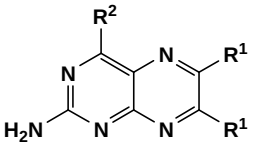
In this project, pyrazino[2,3-*d*]pyrimidines (Table 2.3) and most of pyrrolo[2,3-*d*]pyrimidines (Fig. 2.7) were synthesised to be screened for biological activities without regard for any particular protein targets. Therefore, we present partial biological screening results to show the potential of synthesised compounds to develop leads. The tested compounds are listed as follows (Table 2.3 and Fig. 2.7)

Cell based assays were performed by Carol Clements (Professor Alan Harvey's group) of SIDR (Strathclyde Institute for Drug Research). The authour of this thesis acknowledges their cooperation.

Assay method

Mammalian cell toxicity was evaluated for three normal cell lines H9C2 (rat, heart myoblast), HS27 (human foreskin fibroblast) and L929 (murine fibroblast), and three cancer cell lines HepG2 (hepatocellular carcinoma epithelial), ZR75 (epithelial breast cancer cell) and SHSY5Y (human neuroblastoma). Antibacterial activities were assayed for *Staphylococcus aureus* and *Escherichia coli*. Antiparasitic activity was investigated for *T. b. brucei* using the Alamar blue microplate method.[157a-c] The assay were conducted in 96 well microplates with incubation at 37 °C under a humidified 5% CO_2 atmosphere for 24 h for mammalian cells, 20 h for bacteria and 48 h for trypanosomes. Fluorescence was detected using a Wallac Victor 2 multilabel plate reader (exitation 560 nm, emmision 590 nm) giving % of control values. The initial screenings were done at a single concentration (100 μM for cytotoxicity and antiparasitic activity, 500 μM for antibacterial activity, n=1). IC_{50}s were obtained for some active compounds against *T. b. brucei* (n=2).

Table 2.3. Structures of assayed pyrazino[2,3-*d*]pyrimidines. (30 compounds)



	R^2					
R^1	SCH_2Ph	$NHCH_2Ph$	NHPh	$N(CH_2)_4$	$N(CH_2)_3N(CH_3)_2$	$N(CH_2)_4O$
CH_3	**2.62a**	**2.63a**	**2.64a**	**2.65a**	**2.66a**	**2.67a**
Ph	**2.62b**	**2.63b**	**2.64b**	**2.65b**	**2.66b**	**2.67b**
4-OCH_3Ph	**2.62c**	**2.63c**	**2.64c**	**2.65c**	**2.66c**	**2.67c**
2-ClPh	**2.62d**	**2.63d**	**2.64d**	**2.65d**	**2.66d**	**2.67d**
2-pyridyl	**2.62e**	**2.63e**	**2.64e**	**2.65e**	**2.66e**	**2.67e**

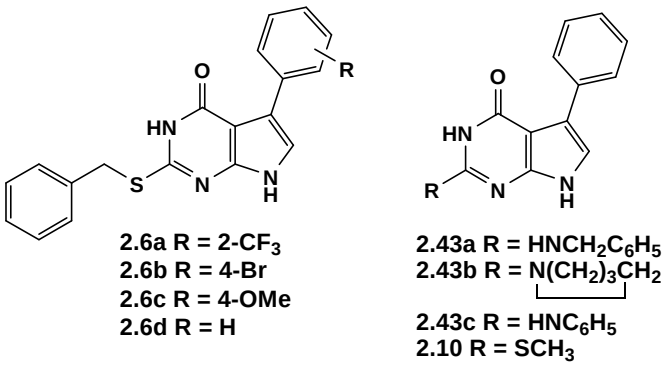


Figure 2.7. Structures of assayed pyrrolo[2,3-*d*]pyrimidines. (8 compounds)

2.2.2.1 Cytotoxicity

Cytotoxicity was examined for three normal cell lines and three cancer cell lines namely:

Normal cell lines

H9C2: rat, heart myoblast

HS27: human foreskin fibroblast

L929: murine fibroblast

Cancer cell lines

HepG2: hepatocellular carcinoma epithelial

ZR75: epithelial breast cancer cell

SHSY5Y: human neuroblastoma

Among the assayed 38 compounds, 14 compounds exhibited toxic effects for normal or cancer cells or both. The most active compounds are shown in Figure 2.8.

The data indicate the following.

(i) There were no compounds with significant toxicity for HepG2.

(ii) Two pteridine compounds with the dimethylaminopropyl chain, **2.66c** and **2.66d**, showed remarkable toxicity to ZR75 and SHSY5Y but again these two compounds were also significantly toxic to all three normal cells.

(iii) Although, toxicity to L929, a normal cell line, was found, compound **2.43c** exhibited good toxicity for ZR75, a breast cancer cell line. For compound **2.65d**, relatively less toxicity to ZR75 was found but no significant effects were indicated to the three normal cells.

(iv) Compound **2.62b** had toxicity to SHSY5Y (cancer cell) but it was slightly toxic to the L929 (normal cell) also.

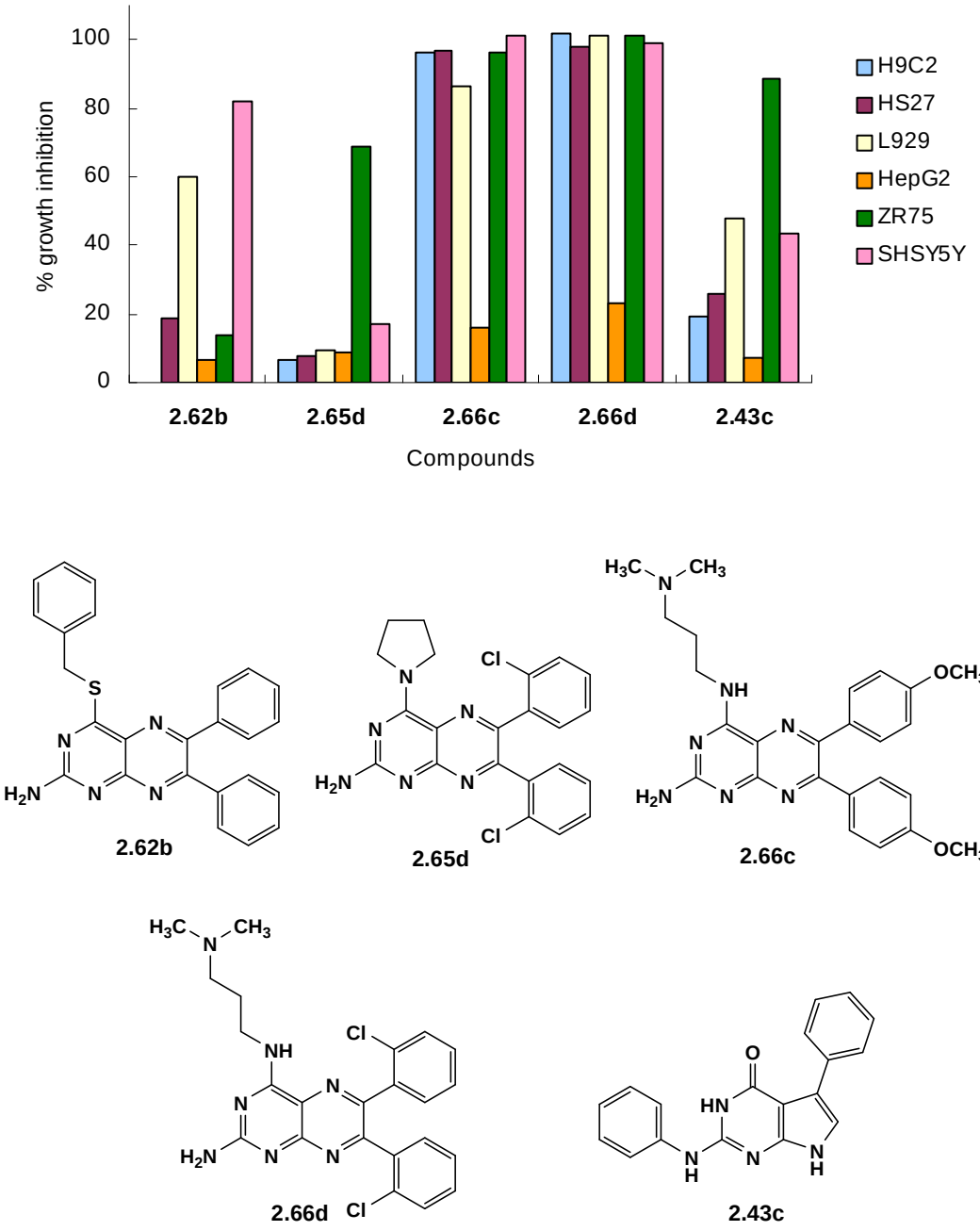


Figure 2.8. Percentage growth inhibition of normal cells (H9C2, HS27, L929) and cancer cells (HepG2, ZR75, SHSY5Y) with each compound (single concentration screen at 100 μM, n=1). Data provided by Professor A. L. Harvey (SIDR, University of Strathclyde).

Due to the relatively selective toxicity of compound **2.65d** to the ZR75 (epithelial breast cancer cell) and strong but nonselective toxicity of compound **2.66d**, structural modification at C4 to increase selectivity/potency for ZR75 might be worthwhile for

further research (Fig. 2.9).

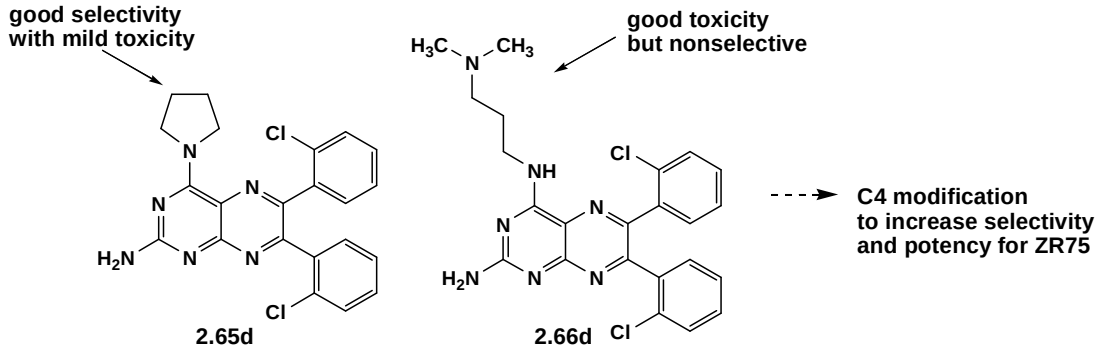


Figure 2.9. Possibility of new compounds with potential selectivity and potency for ZR75.

2.2.2.2. Antibacterial activity

For two species of bacteria, *S. aureus* and *E. coli*, antibacterial activities were not significant. The majority of tested compounds were not active against *S. aureus* and only **2.63d** showed moderate growth inhibition (48% at 200 μM). For *E. coli*, 10 compounds, eight pyrazino[2,3-*d*]pyrimidines and two pyrrolo[2,3-*d*]pyrimidines, exhibited weak growth inhibition (40~75% at 500 μM, a very high concentration). The data for six compounds are presented in Figure 2.10.

Although, this data does not provide any detailed information of structure-activity relationships, interestingly, we have found that four compounds have the same functional group (2-ClPh) at C5 and C6 among the five active pteridine compounds. Also, the most active compound **2.63d** has a benzylamino group at C4 that reflects the activity of **2.63c**. Compound **2.63c** does not have a 2-chlorophenyl group at C5 and C6. We presume that this basic but distinctive observation may provide a guide to produce new lead compounds *via* structural study of a specific target protein.

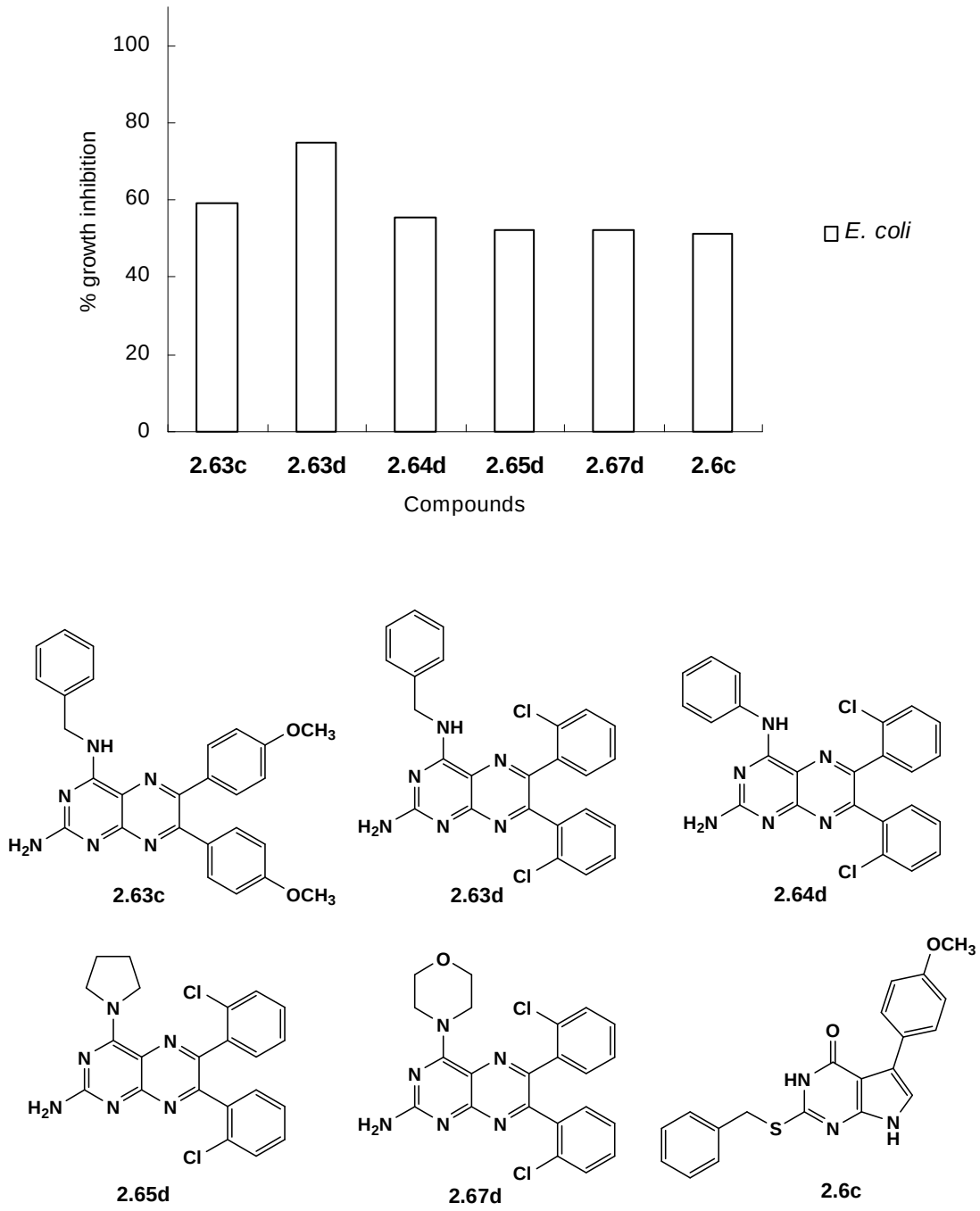


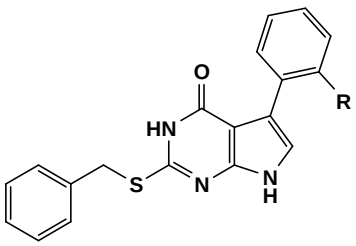
Figure 2.10. Percentage growth inhibition of *E. coli* with each compound (single concentration screen at 500 μM, n=1). Data provided by Professor A. L. Harvey (SIDR, University of Strathclyde).

2.2.2.3. Antiparasitic activity

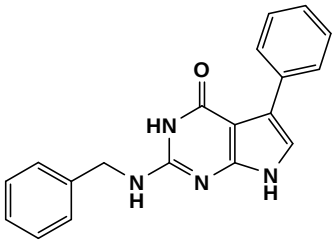
Antiparasitic activity was evaluated for *T. b. brucei*. Most encouragingly, among the 30 compounds, 24 compounds exhibited antiparasitic activity. Most of the pteridine compounds showed good cell growth inhibition (> 85% at 100 μM). Among eight pyrrolo[2,3-*d*]pyrimidines, four compounds showed antiparasitic activity. The percentage growth inhibition (88.2 ~ 98.5% at 100 μM) and IC_{50} values (12.5 ~ 50 μM) are presented in Table 2.4

Despite the fact that the screening results do not provide data about specific enzymes, we have found that diversification at C2 can also affect the antiparasitic activity in the case of pyrrolo[2,3-*d*]pyrimidines. The results imply that the variation at C2 of pyrrolo[2,3-*d*]pyrimidines may need to be considered to produce high-affinity (< 0.1 μM) leads for trypanosomatid parasites' enzymes, DHFR and PTR1. In addition, pteridine scaffolds are worthwhile to design potential inhibitors *via* molecular modeling study.

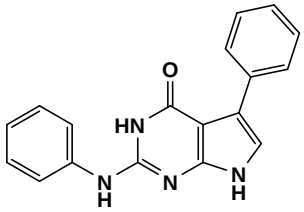
Table 2.4. The percentage growth inhibition and IC_{50} values (μM) for pyrrolo[2,3-*d*]pyrimidines for *T. b. brucei*. Data provided by Professor A. L. Harvey (SIDR, University of Strathclyde).



R = CF_3 2.6a
R = H 2.6d



2.43a



2.43c

Compounds	Growth inhibition (%) at 100 μM (n=1)	IC_{50}, μM (n=2)
2.6a	98.0	25
2.6d	97.9	25
2.43a	88.2	50
2.43c	98.5	12.5

3. CONCLUSION

3.1. Synthesis of pyrrolo[2,3-*d*]pyrimidines

2-Thioalkyl-6-amino-4-oxo pyrimidines were successfully reacted with functionalised nitroalkenes *via* a Michael addition reaction. Biphasic reactions (H_2O/EtOAc, 1:1) with a phase transfer catalyst ($PhCH_3N^+Me_3HO^-$) proved very effective in these Michael additions. Subsequent cyclisations were accomplished by the Nef reaction. Titanium(III) chloride or tin(II)chloride dihydrate mediated system provided cyclised products in better yields than those by the reaction using DBU.

Displacement of the thioalkyl moiety at C2 in pyrrolo[2,3-*d*]pyrimidines were also successful using a modified oxidation-substitution methodology; obtaining the crude sulfone by simple work-up followed by reaction in a neat amine. This achivement will be useful to diversify at C2 of pyrrolo[2,3-*d*]pyrimidines for library synthesis.

3.2. Synthesis of pyrazino[2,3-*d*]pyrimidines

6,7-Functionalised pteridines were synthesised by the Isay cyclocondensation. The thiobenzyl group at C4 was effectively displaced by five different amines without oxidative activation of the 4-thioether. Microwave assisted substitution reduced the reaction times and produced a 25 compound library in moderate to good yields.

3.3. Synthesis of inhibitors of PTR1

Through molecular modeling, a deazaguanine substructure was selected as an ideal framework to design potential inhibitors for trypanosomatid parasites' PTR1. Two

major hydrophobic pockets were found at C5 and C6 positions of a deazaguanine scaffold.

To occupy hydrophobic pockets at the active sites, phenethyl and phenyl groups were introduced at C5 and C6, respectively. The phenethyl group was successfully introduced using the method of E. C. Taylor *et al*.[116] Incorporation of a phenyl group at C6 was accomplished by Suzuki coupling in a mixed aqueous solvent system (H_2O/IPA, 1:2). However, a pyridone group, that may have provided additional hydrogen bonding interactions, could not be introduced by Suzuki coupling under various conditions. In addition, a hybrid compound, which was substituted with a phenethyl at C5 and a phenyl at C6, could not be obtained *via* Suzuki coupling. This was postulated to be due to steric hinderance from the C5 phenethyl moiety.

Six 4-thiobenzyl pyrimidines were also synthesised to investigate inhibitory abilities to PTR1.

3.4. Biological activities

Two deazaguanines and six pyrimidines were examined for their inhibitory activity of *L. major* and *T. brucei* PTR1. The two deazaguanines exhibited 70% inhibition at 10 μM for *T. brucei* but less than 30% inhibition for *L. major* PTR1 was found. Six pyrimidines showed better inhibitory activities (45 ~ 61%) for *L. major* than the two deazaguanines but lower activities (2 ~ 38%) were found for *T. brucei*.

Cytotoxic, antibacterial and antiparasitic activities were screened for thirty pyrazino[2,3-*d*]pyrimidines and eight pyrrolo[2,3-*d*]pyrimidines. In many cases, pteridines with 2-chlorophenyl groups at C5 and C6 exhibited cell growth inhibition for cancer/normal cells and bacterial cells. The majority of compounds had antiparasitic activity for *T. b. brucei* and four pyrrolo[2,3-*d*]pyrimidines showed different IC_{50}s (12.5~50 μM) depending on the functional group at C2 position.

3.5. Further study

In this section, a basic sketch for further study is suggested based on the obtained biological data (Section 2.2).

Some points for further structural modification of deazaguanine framework for PTR1 are presented in Section 3.5.1.

From the screening results, we obtained 28 hits (24 pteridines and 4 pyrrolo[2,3-*d*]pyrimidines) for *T. b. brucei*. This achievement provides a strong potential to produce high affinity leads. Related comments are introduced in Section 3.5.2.

3.5.1. Inhibitior for PTR1

3.5.1.1. Deazaguanine framework

The two synthesised compounds **2.71** and **2.72** exibited the same inhibitory activity for *T. b.* PTR1 (~ 70% at 10 μM, Section 2.2.1). This result supports the view that a hybrid molecule may have stronger inhibitory activity by synergy as this molecule occupies the two main hydrophobic pockets of PTR1 simultaneously. However, some detailed points may need to be considered to improve the inhibitory activity. These can be summarised as follows (Fig. 3.1)

(i) an optimal length of the alkyl chain between the main framework and the phenyl ring – Fig. 3.1, A.
(ii) a necessity of any heteroatom linker or branched linker (e.g. S, NH, N-CH_3) – Fig. 3.1, B.
(iii) an optimal functional group at C5 extended phenyl group with regard to variety and position – Fig. 3.1, C.
(iv) an optimal orientation of C5 extended phenyl ring which can be

affected by the factors (ii) and (iii) - Fig. 3.1, D.

(v) the incorporation of a pyridone group at C6 to obtain hydrogen bonding with Asp 161 (Section 2.1.3.1) – Fig. 3.1, E.

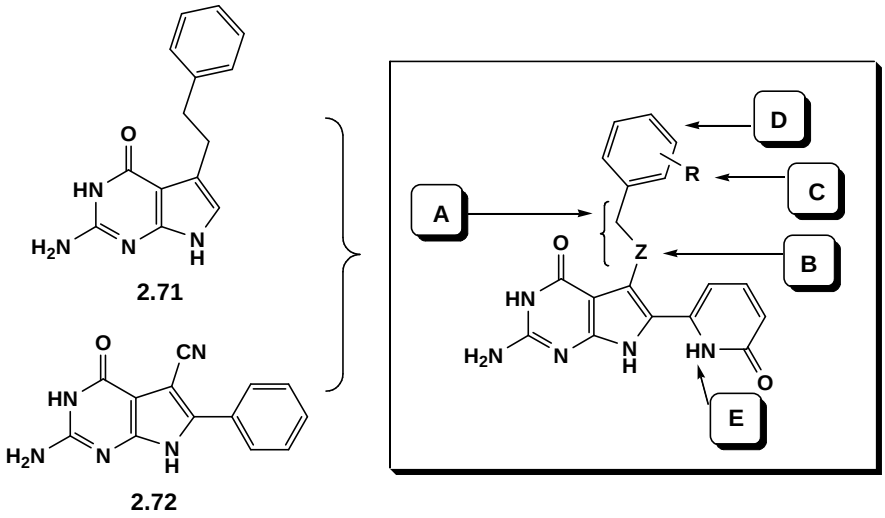


Figure 3.1. A postulated hybrid structure and considerable factors.

3.5.1.2. Pyrimidine framework

In the pyrimidine compounds **2.59b ~ 2.59g** (Fig. 3.2), no significant differences were found in the inhibitory activity for PTR1 from the variation of thiobenzyl group at C4 (Section 2.2.1).

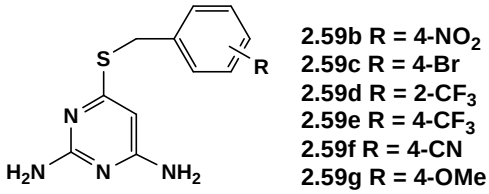


Figure 3.2. Six pyrimidine compounds.

However, these compounds exhibited better inhibitory activities to the *L. major* PTR1 (45 ~ 61%) than the deazaguanine compounds **2.71** and **2.72** (below 30%). Therefore, further structural design would be desirable through molecular modeling study.

3.5.2. Anti-*T. b. brucei* compounds

The screening results (Section 2.2.2.3) proved 24 hits of pteridines and 4 hits of pyrrolo[2,3-*d*]pyrimidines for *T. b. brucei*. For pteridine compounds, elaborate modeling studies with important protein targets, such as DHFR and PTR1, may provide more idea to develop a lead. The possibility of a dual inhibitor for DHFR and PTR1 may need to be considered also.

In the case of pyrrolo[2,3-*d*]pyrimidines, IC_{50} data (Section 2.2.2.3) demonstrate that C2 variation strategy may be an important issue for inhibitory activity for *T. b. brucei*. Thereby, more synthesis of pyrrolo[2,3-*d*]pyrimidines focused on C2 diversification seems also worthwhile to produce a lead by SAR (structure-activity relationship) study.

4. EXPERIMENTAL

4.1. Instrumentation and general materials

NMR spectra were determined on a Bruker Spectrospin spectrometer operating at 400 MHz for ^{1}H spectra and 100 MHz for ^{13}C spectra. Chemical shifts are reported as ppm relative to the residual ^{1}H in the solvents, DMSO-d_6 (2.50 ppm for ^{1}H, 39.5 ppm for ^{13}C) or $CDCl_3$ (7.27 ppm for ^{1}H, 77.0 ppm for ^{13}C) or TFA-*d* (164.2 ppm for ^{13}C). Multiplicities are indicated by s (singlet), brs (broad singlet), d (doublet), dd (double-doublet), dt (double-triplet), dq (double-quartet), t (triplet), q (quartet), qt (quintet), or m (multiplet). Coupling constant (*J* values) are given in Hz. IR spectra were recorded on a Nicolet Impact 400D FT spectrometer as KBr discs or neat samples on sodium chloride plates for oils. Melting points, when measurable, were determined on a Reichert hot stage apparatus and are uncorrected. The newly synthesised compounds were further characterised by HRMS. Data for electron impact (EI), chemical ionisation (CI) and fast atom bombardment (FAB) modes were obtained on a JEOL JMS-700 High Resolution Mass Spectrometer system. Data for electron spray ionisation (ESI) mode were obtained on a ThermoFinnigan LCQ mass spectrometer. Elemental analysis data, when obtainable, were also determined on a Perkin Elmer series II CHN analyser 2400. However, elemental analysis data are extremely difficult to obtain from polyazaheterocycles even using tungstate as a combustion catalyst. Results within the normally acceptable limits (± 0.4%) of anlaysis were not obtainable for many compounds prepared.

Microwave reactions were conducted using an Initiator Unit (Biotage, Uppsala, Sweden) using the stirring option. HPLC separation was carried out when necessary. Waters 1525 binary pump, Waters 2487 dual absorbance detector, and Breeze software (Column: Phenomenex Luna 5 micron C18, 60 × 21 mm, wavelength = 254 nm) were used on a gradient eluting system according to the following table.

Table 4.1. Gradient condition of mobile phases for HPLC separation.

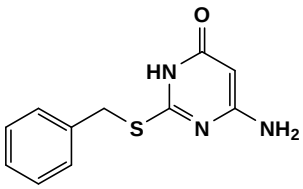
Time (min)	Flow rate (ml/min)	% A*	% B*
0	6.00	90	10
28.00	6.00	40	60
33.00	6.00	0	100
38.00	6.00	90	10
39.00	6.00	90	10
40.00	0.00	90	10
40.10	0.00	90	10

*A: water containing 0.1% TFA, B: acetonitrile containing 0.1% TFA.

TLC was carried out on silica (Merck 0.25 mm 60 F_{254}). Column chromatography was carried out using silica gel (230 ~ 400 mesh; 40 ~ 60 μm) using the method of W. C. Still *et al.*[158] Reagents were obtained from commercial suppliers and used without further purification. According to standard procedure,[159] solvents were dried over reasonable drying agents ($Mg(OMe)_2$ for MeOH and $Mg(OEt)_2$ for EtOH, CaH for CH_2Cl_2 and CH_3CN) prior to use or bought as anhydrous one (DMF from Aldrich Co., Gillingham, Dorset, UK). THF, Et_2O and DCM were dried using standard operating procedure for Innovative Technology Solvent Purification System (Pure-Solv® 400 Solvent Purification System).

4.2. Synthetic procedures

6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone

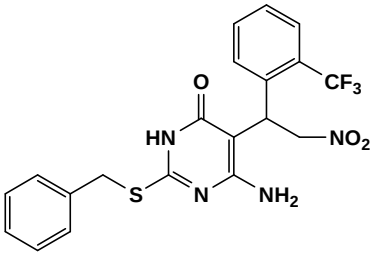


2.4

6-Amino-2-sulfanyl-4(3*H*)-pyrimidinone monohydrate **2.2** (8 g, 50 mmol) was suspended in a mixture of water (30 ml) and ethanol (50 ml). Triethylamine (10 g, 0.1 mol) was added and the solution became clear. Benzyl chloride (7 g, 55 mmol) was added to the stirred solution. Within a few minutes an exothermic reaction started with formation of a colourless precipitate. Stirring was continued for 30 min, the mixture was cooled to 4 °C and the precipitate was filtered and washed with water and diethyl ether to afford the product **2.4** as a white solid (10.3 g, 44 mmol, 88%; mp 241-243 °C, lit.[72] 248-253 °C).

^{1}H NMR (DMSO-d_6) δ 4.32 (2H, s, SCH_2), 4.95 (1H, s, C*H*=CN_2), 6.52 (2H, s, NH_2), 7.21-7.26 (1H, m, *P*h), 7.28-7.32 (2H, m, Ar*H*), 7.41-7.44 (2H, m, Ar*H*), 11.43 (1H, brs, *H*NCO). ^{13}C NMR (DMSO-d_6) δ 33.22 (S*C*H_2), 81.28 (*C*H=CN_2), 127.06, 128.34, 129.06 (5C, Ar*C*), 137.92 (1C, Ar*C*CH_2), 162.27 (CH=*C*N_2), 163.58 (S*C*=N), 164.57 (*C*=O). IR (KBr) 3448, 3294, 3151, 2931, 1847, 1598, 1443, 1304, 1219, 980, 818, 714 cm^{-1}.

6-Amino-2-(benzylsulfanyl)-5-{2'-nitro-1'-[2''-(trifluoromethyl)phenyl]ethyl}-4(3*H*)-pyrimidinone

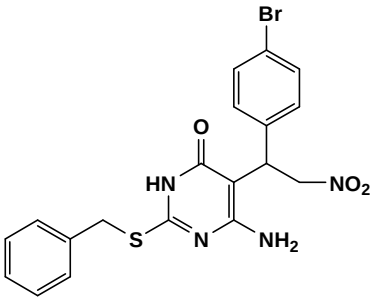


2.5a

To 6-amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **2.4 (**0.2 g, 0.86 mmol) in water (5 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 0.51 ml, 1.3 mmol). The mixture was stirred for 5 minutes and then 1-[(*E*)-2-nitroethenyl]-2'-(trifluoromethyl)benzene (0.19 g, 0.86 mmol) and ethyl acetate (5 ml) were added. The resulting mixture was stirred in an oil bath at 80 °C for three days, whereafter, ethyl acetate (30 ml) was added, and the organic layer was separated, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to 1:2) to give the product **2.5a** as a yellow solid (0.21 g, 0.47 mmol, 55%; mp 81-83 °C).

^{1}H NMR (DMSO-d_6) δ 4.34-4.80 (2H, s, SCH_2) 4.80 (1H, t, *J* 7.5, CHCH_2NO_2), 4.98-5.62 (2H, m, CHCH_2NO_2), 6.16 (2H, brs, NH_2), 7.22-7.25 (1H, m, *P*hSCH_2), 7.28-7.31 (2H, m, *P*hSCH_2), 7.41-7.43 (1H, m, *P*hSCH_2), 7.45 (1H, t, *J* 7.6, *P*hCF_3), 7.59 (1H, t, *J* 7.6, *P*hCF_3), 7.71 (1H, d, *J* 7.8, *P*hCF_3), 7.90 (1H, d, *J* 7.9, *P*hCF_3), 11.90 (1H, brs, OCNH). ^{13}C NMR (DMSO-d_6) δ 33.16 (SCH$_2$), 36.24 (CHCH$_2$NO_2), 75.33 (CHCH$_2$NO_2), 90.49 (C=CN_2), 120.49 (q, $J^1_{C\text{-}F}$ 272, CF$_3$), 126.23 (ArCCF_3), 126.29 (q, $J^2_{C\text{-}F}$ 29, ArCCF_3), 126.32, 127.28 (ArCCF_3), 127.79, 128.42, 129.18 (5C, ArCCH$_2$), 130.62, 132.59 (ArCCF_3), 137.49 (1C, ArCCH$_2$), 158.63 (C=CN$_2$), 160.17 (SC=N), 162.73 (C=O). IR (KBr) 3525, 3405, 2826, 1613, 1548 (CNO_2), 1423, 1375, 1311 (CF_3), 1163, 1110, 769 cm^{-1}. HRMS (EI) found 450.0976, $C_{20}H_{17}F_3N_4O_3S$ requires 450.0973 (M^+). Anal. Calcd for $C_{20}H_{17}F_3N_4O_3S$: C, 53.3; H, 3.8; N, 12.4; S, 7.1. Found: C, 53.3; H, 3.1; N, 11.7; S, 6.8.

6-Amino-2-(benzylsulfanyl)-5-[1'-(4''-bromophenyl)-2'-nitroethyl]-4(3*H*)-pyrimidinone

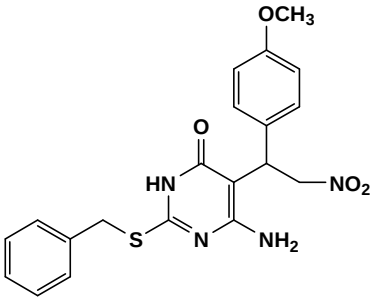


2.5b

To of 6-amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **2.4 (**0.4 g, 1.72 mmol) in water (10 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 1 ml, 2.6 mmol). The mixture was stirred for 5 minutes and then 1-bromo-4-[(*E*)-2-nitroethenyl]benzene (0.39 g, 1.72 mmol) and ethyl acetate (10 ml) were added. The resulting mixture was stirred in an oil bath at 70 °C for 4 hours, whereafter ethyl acetate (30 ml) was added, and the organic layer was separated, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to 1:1) to give the product **2.5b** as a brown solid (0.57 g, 1.23 mmol, 72%; mp 93-95 °C).

^{1}H NMR (DMSO-d_6) δ 4.33 (2H, s, SCH_2) 4.60 (1H, t, *J* 7.4, CHCH_2NO_2), 5.29-5.46 (2H, m, CHCH_2NO_2), 6.79 (2H, brs, NH_2), 7.22-7.45 (9H, m, ArH), 11.81 (1H, brs, HNC=O). ^{13}C NMR (DMSO-d_6) δ 34.60 (SCH$_2$), 38.47 (CHCH$_2$NO_2), 76.84 (CHCH$_2$NO_2), 90.88 (C=CN_2), 119.83 (1C, ArCBr), 127.20, 128.37, 129.12, 129.96, 130.94 (9C, ArC), 137.56 (1C, ArCCH$_2$), 139.65 (1C, ArCCHCH$_2$), 158.19 (C=CN$_2$), 159.99 (SC=N), 162.15 (C=O). IR (KBr) 3489, 3389, 1610, 1545 (CNO_2), 1488, 1420, 1374, 1222, 1073 (PhBr), 975, 698 cm^{-1}. HRMS (FAB) found 463.0271, $C_{19}H_{17}{}^{81}BrN_4O_3S$ requires 463.0264 (MH^+). Anal. Calcd for $C_{19}H_{14}BrN_3OS$: C, 55.3; H, 3.4; N, 10.1; S, 7.7. Found: C, 55.8; H, 3.1; N, 9.5; S, 7.3.

6-Amino-2-(benzylsulfanyl)-5-[1'-(4''-methoxyphenyl)-2'-nitroethyl]-4(3*H*)-pyrimidinone

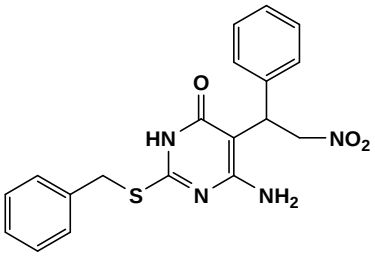


2.5c

To 6-amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **2.4 (**0.4 g, 1.72 mmol) in water (10 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 1 ml, 2.6 mmol). The mixture was stirred for 5 minutes and then 1-methoxy-4-[(*E*)-2-nitroethenyl]benzene (0.38 g, 2.1 mmol) and ethyl acetate (10 ml) were added. The resulting mixture was stirred in an oil bath at 65 °C for 48 hours, whereafter, ethyl acetate (30 ml) was added, and the organic layer was separated, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to ethyl acetate 100%) to give the product **2.5c** as a brown solid (0.68 g, 1.65 mmol, 96%; mp 88-90 °C).

^{1}H NMR (DMSO-d_6) δ 3.70 (3H, s, OCH_3), 4.33 (2H, s, SCH_2) 4.54 (1H, t, *J* 7.7, CHCH_2NO$_2$), 5.22-5.47 (2H, m, CHCH_2NO$_2$), 6.68 (2H, s, NH_2), 6.80 (2H, m, ArHOCH$_3$), 7.21-7.25 (1H, m, ArHCH$_2$), 7.27-7.31 (2H, m, ArHCH$_2$), 7.41-7.43 (4H, m, ArHCH$_2$ and ArHOCH$_3$), 11.77 (1H, s, HNC=O). ^{13}C NMR (DMSO-d_6) δ 33.06 (SCH$_2$), 38.47 (CHCH$_2$NO$_2$), 54.99 (OCH$_3$), 77.49 (CHCH$_2$NO$_2$), 91.73 (C=CN$_2$), 113.53 (2C, ArCOCH$_3$), 127.19, 128.39, 128.86, 129.12, 132.16 (3C+5C, ArCOCH$_3$ and ArCCH$_2$), 137.64 (1C, ArCCH$_2$), 157.88 (C=CN$_2$), 158.07 (1C, ArCOCH$_3$), 159.89 (SC=N), 162.33 (C=O). IR (KBr) 3486, 3385, 2835, 1610, 1546 (CNO$_2$), 1510, 1420, 1249, 1030, 971 cm^{-1}. HRMS (FAB) found 413.1286, $C_{20}H_{20}N_4O_4S$ requires 413.1284 (MH$^+$). Anal. Calcd for $C_{20}H_{20}N_4O_4S$: C, 58.2; H, 4.8; N, 13.5. Found: C, 58.8; H, 4.3; N, 13.2.

6-Amino-2-(benzylsulfanyl)-5-(2'-nitro-1'-phenylethyl)-4(3*H*)-pyrimidinone



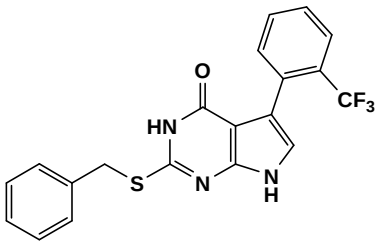
2.5d

To a mixture of 6-amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **2.4 (**0.4 g, 1.72 mmol) in water (10 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 1 ml, 2.6 mmol). The mixture was stirred for 5 minutes and then [(*E*)-2-nitroethenyl]benzene (0.26 g, 1.72 mmol) and ethyl acetate (10 ml) were added. The resulting mixture was stirred in an oil bath at 60 °C for 4 hours, ethyl acetate (30 ml) was added, and the organic layer was separated, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to ethyl acetate 100%) to give the product **2.5d** as a brown solid (0.53 g, 1.39 mmol, 81%; mp 83-85 °C).

^{1}H NMR (DMSO-d_6) δ 4.33 (2H, s, SCH_2) 4.62 (1H, t, *J* 7.5, CHCH_2NO_2), 5.28-5.51 (2H, m, CHCH_2NO_2), 6.72 (2H, s, NH_2), 7.17-7.49 (10H, m, ArH), 11.78 (1H, brs, HNCO). ^{*13}C NMR (DMSO-d_6) δ 33.11 (SCH$_2$), 77.18 (CHCH$_2$NO_2), 91.41 (C=CN_2), 126.68, 127.23, 127.75, 128.14, 128.41, 129.17 (5C + 5C, ArCCH and ArCCH_2), 137.60 (1C, ArCCH_2), 140.20 (1C, ArCCH), 158.07 (C=CN$_2$), 160.06 (SC=N), 162.34 (C=O). IR (KBr) 3484, 3389, 1610, 1545 (CNO_2), 1419, 1375, 1222, 973, 766, 698 cm^{-1}. HRMS (FAB) found 383.1183, $C_{19}H_{18}N_4O_3S$ requires 383.1178 (MH^+). Anal. Calcd for $C_{19}H_{18}N_4O_3S$: C, 59.6; H, 4.7; N, 14.6; S, 8.3. Found: C, 60.2; H, 4.8; N, 14.1; S, 8.6.

*The peak for CHCH_2NO_2 overlapped with the peaks of DMSO (38.88-40.13).

2-(Benzylsulfanyl)-5-[2'-(trifluoromethyl)phenyl]-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



2.6a

(a) Microwave reaction using DBU

6-Amino-2-(benzylsulfanyl)-5-{2'-nitro-1'-[2''-(trifluoromethyl)phenyl]ethyl}-4(3*H*)-pyrimidinone **2.5a** (0.1 g, 0.22 mmol) was suspended in solvent (3 ml, acetonitrile or dimethyl formamide) in a sealed vessel and then DBU (49 μl, 0.33 mmol) was added. The mixture was irradiated (average 27 W) at 85 °C for 1 hr and then ethyl acetate (30 ml) was added and evaporated to dryness. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:5 to 1:2) to give the product **2.6a** (green solid, 14 mg, 0.035 mmol, 16% in acetonitrile; brown solid, 21 mg, 0.052 mmol, 24% in dimethyl formamide). Spectroscopic data are detailed below.

(b) Thermal reaction using DBU

To a solution of 6-amino-2-(benzylsulfanyl)-5-{2'-nitro-1'-[2''-(trifluoromethyl) phenyl]ethyl}-4(3*H*)-pyrimidinone **2.5a (**0.09 g, 0.2 mmol) in acetonitrile (5 ml) was added DBU (45μl, 0.3 mmol). The reaction mixture was stirred 2 days in an oil bath (60 ˚C) under nitrogen. Through the same work-up and purification procedure described above for the microwave reaction, the desired product **2.6a** was obtained as a green solid (21 mg, 0.052 mmol, 26%). Spectroscopic data are detailed below.

(c) Tin(II) chloride reaction

To a mixture of 6-amino-2-(benzylsulfanyl)-5-{2'-nitro-1'-[2''-(trifluoromethyl) phenyl]ethyl}-4(3*H*)-pyrimidinone **2.5a** (0.1 g, 0.22 mmol) in ethyl acetate (15 ml) was added tin(II) chloride dihydrate (0.15 g, 0.66 mmol). The resulting mixture was stirred in an oil bath at 85 °C for 24 hours then poured into ethyl acetate (20 ml) and

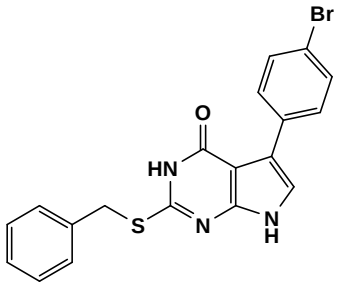
washed with saturated aqueous sodium bicarbonate (20 ml), 1% hydrochloric acid (10 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and concentrated under reduced pressure. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:2) to give the product **2.6a** as a yellow solid (45 mg, 0.11 mmol, 50%). Spectroscopic data are detailed below.

(d) Titanium(III) chloride reaction

6-Amino-2-(benzylsulfanyl)-5-{2'-nitro-1'-[2''-(trifluoromethyl)phenyl]ethyl}-4(3*H*)-pyrimidinone **2.5a** (0.16 g, 0.35 mmol) was dissolved in methanol (5 ml) and treated with one equivalent of sodium methoxide (18.9 mg, 0.35 mmol). A titanium(III) chloride solution was prepared separately by adding an aqueous solution of ammonium acetate (0.65 g, 8.4 mmol) in water (2 ml) to titanium(III) chloride (1.8 ml, 1.4 mmol, 10% solution in hydrochloric acid) under nitrogen. The prepared titanium(III) chloride solution was then added carefully to the anionic solution under nitrogen with vigorous stirring. The colour changed slowly from brown to yellow. The mixture was stirred overnight at room temperature then poured into ethyl acetate (20 ml) and separated into two phases. The aqueous phase was extracted with ethyl acetate (30 ml × 3). The organic extracts were combined, washed with 5% sodium bicarbonate (20 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 2:1) to afford the product **2.6a** as a yellow solid (0.1 g, 0.25 mmol, 71%; mp 188-190 °C).

^{1}H NMR (DMSO-d_6) δ 4.44 (1H, s, SCH_2), 6.89 (1H, d, *J* 1.9, C=C*H*NH), 7.25-7.75 (9H, m, Ar*H*), 11.99 (1H, s, C=CHN*H*), 12.04 (1H, s, *H*NCO). ^{13}C NMR (DMSO-d_6) δ 33.52 (S*C*H$_2$), 103.05 (*C*=CN$_2$), 115.45 (*C*=CHNH), 118.55 (C=*C*HNH), 122.98 (q, $J^1_{C\text{-}F}$ 272, *C*F$_3$), 125.43 (Ar*C*CF$_3$), 127.18 (m, $J^2_{C\text{-}F}$ 28, Ar*C*CF$_3$), 128.42, 129.11 (Ar*C*CH$_2$), 131.19 (Ar*C*CF$_3$), 133.49 (Ar*C*CH$_2$), 134.11 (Ar*C*CF$_3$), 137.32 (1C, Ar*C*CH$_2$), 147.91 (C=CN$_2$), 153, 69 (SC=N), 158.41 (C=O). IR (KBr) 3064, 1657, 1313 (CF$_3$), 1169, 1122, 1059, 1034, 972, 765, 697 cm^{-1}. HRMS (FAB) found 402.0890, $C_{20}H_{14}F_3N_3OS$ requires 402.0888 (MH^+). Anal. Calcd for $C_{20}H_{14}F_3N_3OS$: C, 59.8; H, 3.5; N, 10.4; S, 7.9. Found: C, 60.5; H, 3.1; N, 10.8; S, 8.4.

2-(Benzylsulfanyl)-5-(4'-bromophenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



2.6b

(a) Tin(II) chloride reaction

To a mixture of 6-amino-2-(benzylsulfanyl)-5-[1'-(4''-bromophenyl)-2'-nitroethyl]-4(3*H*)-pyrimidinone **2.5b** (0.1 g, 0.22 mmol) in ethyl acetate (15 ml) was added tin(II) chloride dihydrate (0.15 g, 0.65 mmol). The resulting mixture was stirred in an oil bath at 85 °C for 24 hours then poured into ethyl acetate (20 ml) and washed with saturated aqueous sodium bicarbonate (20 ml), 1% hydrochloric acid (10 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:1 to ethyl acetate 100%) to give the product **2.6b** as a yellow solid (23 mg, 0.056 mmol, 25%). Spectroscopic data are detailed below.

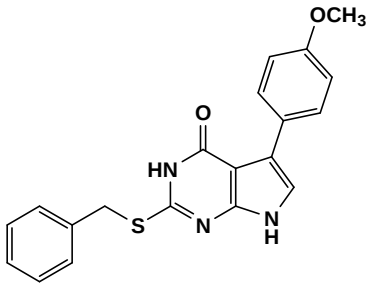
(b) Titanium(III) chloride reaction

6-Amino-2-(benzylsulfanyl)-5-[1'-(4''-bromophenyl)-2'-nitroethyl]-4(3*H*)-pyrimidinone **2.5b** (0.33 g, 0.72 mmol) was dissolved in methanol (10 ml) and treated with one equivalent of sodium methoxide (38.9 mg). A titanium(III) chloride solution was prepared separately by adding an aqueous solution of ammonium acetate (3.3 g, 43 mmol) in water (5 ml) to titanium(III) chloride (5.6 ml, 4.3 mmol, 10% solution in hydrochloric acid) under nitrogen. The prepared titanium(III) chloride solution was then added carefully to the anionic solution under nitrogen with vigorous stirring. The colour changed slowly from brown to yellow. The mixture was stirred overnight at room temperature then poured into ethyl acetate (20 ml) and separated into two phases. The aqueous phase was extracted with ethyl acetate (30 ml × 3). The organic extracts were combined, washed with 5% sodium bicarbonate (20 ml) and with brine

(20 ml), then dried with anhydrous magnesium sulfate, and concentrated. The residue was recrystallised from ethyl acetate and *n*-hexane to afford the product **2.6b** as a yellow solid (0.14 g, 0.34 mmol, 47%; mp 270-272 °C).

^{1}H NMR (DMSO-d_6) δ 4.44 (2H, s, SCH_2), 7.22-7.34 (3H, m, Ar*H*), 7.39 (1H, d, *J* 2.5, C=C*H*NH), 7.46-7.51 (4H, m, Ar*H*), 7.92-7.94 (2H, m, Ar*H*), 12.08 (1H, s, C=CHN*H*), 12.15 (1H, s, *H*NCO). ^{13}C NMR (DMSO-d_6) δ 33.53 (S*C*H$_2$), 101.16 (*C*=CN$_2$), 118.08 (1C, Ar*C*), 118.74 (*C*=CHNH), 118.77 (C=*C*HNH), 127.29, 128.45, 129.09, 129.68, 130.77 (9C, Ar*C*), 133.48 (1C, Ar*C*), 137.25 (1C, Ar*C*), 149.46 (C=*C*N$_2$), 153.79 (S*C*=N), 159.09 (*C*=O). IR (KBr) 3251, 2819, 1649, 1431, 1212, 1073 (PhBr), 1010, 970, 782, 729 cm^{-1}. HRMS (EI) found 411.0042, $C_{19}H_{14}{}^{79}BrN_3OS$ requires 411.0041 (M^+). Anal. Calcd for $C_{19}H_{14}BrN_3OS$: C, 55.4; H, 3.4; N, 10.2; S, 7.8. Found: C, 55.8; H, 3.2; N, 9.5; S, 7.3.

2-(Benzylsulfanyl)-5-(4'-methoxyphenyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*] pyrimidin-4-one



2.6c

(a) Tin(II) chloride reaction

To a mixture of 6-amino-2-(benzylsulfanyl)-5-[1'-(4''-methoxyphenyl)-2'-nitroethyl]-4(3*H*)-pyrimidinone **2.5c** (0.1 g, 0.24 mmol) in ethyl acetate (15 ml) was added tin(II) chloride dihydrate (0.18 g, 0.82 mmol). The resulting mixture was stirred in an oil bath at 85 °C for 24 hours then poured into ethyl acetate (20 ml) and washed with saturated aqueous sodium bicarbonate (20 ml), 1% hydrochloric acid (10 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and

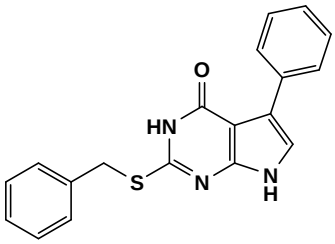
concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:1 to ethyl acetate 100%) to give the product **2.6c** as a grey solid (41 mg, 0.1 mmol, 42%). Spectroscopic data are detailed below.

(b) Titanium(III) chloride reaction

6-Amino-2-(benzylsulfanyl)-5-[1'-(4''-methoxyphenyl)-2'-nitroethyl]-4(3*H*)-pyrimi dinone **2.5c** (0.14 g, 0.34 mmol) was dissolved in methanol (10 ml) and treated with one equivalent of sodium methoxide (18.4 mg). Titanium(III) chloride solution was prepared separately by adding an aqueous solution of ammonium acetate (1.5 g, 20 mmol) in water (2.6 ml) to titanium(III) chloride (2.6 ml , 2.0 mmol 10% solution in hydrochloric acid) under nitrogen. The prepared titanium(III) chloride solution was then added carefully to the anionic solution under nitrogen with vigorous stirring. The colour changed slowly from brown to yellow. The mixture was stirred overnight at room temperature then poured into ethyl acetate (20 ml) and separated into two phases. The aqueous phase was extracted with ethyl acetate (30 ml × 3). The organic extracts were combined, washed with 5% sodium bicarbonate (20 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and concentrated The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:1) to give the product **2.6c** as a yellow solid (64 mg, 0.18 mmol, 52%; mp 233-235 °C).

^{1}H NMR (DMSO-d_6) δ 3.77 (3H, s, OCH_3), 4.43 (2H, s, SCH_2), 6.88 (2H, d, *J* 8.8, Ar*H*), 7.19 (1H, d, *J* 2.4, C=C*H*NH), 7.24-7.49 (5H, m, Ar*H*), 7.84 (2H, d, *J* 8.8, Ar*H*), 11.90 (1H, s, C=CHN*H*), 12.04 (1H, s, *H*NCO). ^{13}C NMR (DMSO-d_6) δ 33.49 (S*C*H_2), 54.99 (O*C*H_3), 101.15 (*C*=CN_2), 113.36 (2C, Ar*C*), 116.49 (*C*=CHNH), 119.84 (C=*C*HNH), 126.70, 127.24, 128.41, 128.88, 129.06 (5C + 3C, Ar*C*CH_2 and Ar*C*OCH_3), 137.30 (1C, Ar*C*CH_2), 149.07 (C=*C*N_2), 153.23 (S*C*=N), 157.60 (1C, Ar*C*OCH_3), 159.12 (*C*=O). IR (KBr) 3203, 2833, 1646, 1525, 1438, 1241, 1179, 1031, 970, 832 cm^{-1}. HRMS (FAB) found 364.1119, $C_{20}H_{17}N_3O_2S$ requires 364.1120 (MH^+). Anal. Calcd for $C_{20}H_{17}N_3O_2S$: C, 66.1; H, 4.7; N, 11.5; S, 8.8. Found: C, 65.6; H, 4.2; N, 10.8; S, 8.3.

2-(Benzylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



2.6d

(a) Tin(II) chloride reaction

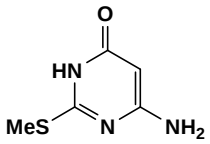
To a mixture of 6-amino-2-(benzylsulfanyl)-5-(2'-nitro-1'-phenylethyl)-4(3*H*)-pyrimidinone **2.5d** (0.4 g, 1.04 mmol) in ethyl acetate (30 ml) was added tin(II) chloride dihydrate (0.71 g, 3.1 mmol). The mixture was stirred in an oil bath at 90 °C for 24 hours. The organic layer was separated, washed with brine (40 ml), dried, and concentrated. The resulting solid was dried under vacuum to give the product **2.6d** as a yellow solid (0.25 g, 0.75 mmol, 72%). Spectroscopic data are detailed below.

(b) Titanium(III) chloride reaction

6-Amino-2-(benzylsulfanyl)-5-(2'-nitro-1'-phenylethyl)-4(3*H*)-pyrimidinone **2.5d** (0.53 g, 1.38 mmol) was dissolved in methanol (10 ml) and treated with one equivalent of sodium methoxide (74.5 mg). A titanium(III) chloride solution was prepared separately by adding an aqueous solution of ammonium acetate (6.3 g, 83 mmol) in water (10 ml) to titanium(III) chloride (10 ml, 8.3 mmol, 10% solution in hydrochloric acid) under nitrogen. The prepared titanium(III) chloride solution was then added carefully to the anionic solution under nitrogen with vigorous stirring. The colour changed slowly from brown to yellow. The mixture was stirred overnight at room temperature then poured into ethyl acetate (20 ml) and separated into two phases. The aqueous phase was extracted with ethyl acetate (30 ml × 3). The organic extracts were combined, washed with 5% sodium bicarbonate (20 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and concentrated. The residue was recrystallised from ethyl acetate and *n*-hexane to afford the product **2.6d** as a yellow solid (0.32 g, 0.96 mmol, 70%; mp 267-269 °C).

^{1}H NMR (DMSO-d_6) δ 4.44 (2H, s, SCH_2), 7.16-7.28 (2H, m, Ar*H*), 7.30 (1H, d, *J* 2.2, C=C*H*NH), 7.31-7.35 (4H, m, Ar*H*), 7.46-7.48 (2H, m, Ar*H*), 7.91-7.93 (2H, m, Ar*H*), 11.99 (1H, s, C=CHN*H*), 12.09 (1H, s, *H*NCO). ^{13}C NMR (DMSO-d_6) δ 33.53 (S*C*H_2), 101.26 (*C*=CN_2), 117.60 (*C*=CHNH), 120.06 (C=*C*HNH), 125.70, 127.28, 127.73, 127.89, 128.43, 129.09 (5C + 5C, ArC), 134.18 (1C, Ar*C*C=CH)), 137.29 (1C, Ar*C*CH_2), 149.33 (C=*C*N_2), 153.51 (S*C*=N), 159.08 (*C*=O). IR (KBr) 3203, 3061, 2829, 1643, 1433, 1212, 1095, 973, 753, 694 cm^{-1}. HRMS (FAB) found 334.1017, $C_{19}H_{15}N_3OS$ requires 334.1014 (MH^+). Anal.Calcd for $C_{19}H_{15}N_3OS$: C, 68.4; H, 4.5; N, 12.6. Found: C, 68.4; H, 4.6; N, 12.6.

6-Amino-2-(methylsulfanyl)-4(3*H*)-pyrimidinone

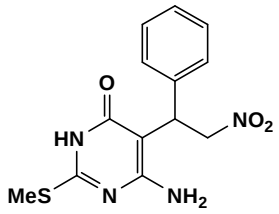


2.7

To a suspension of 6-amino-2-sulfanyl-4(3*H*)-pyrimidinone **2.2** (5 g, 31 mmol) in ethanol (50 ml) and water (25 ml) was added triethylamine (8.3 ml) and iodomethane (2.1 ml). The reaction mixture was stirred at room temperature for 5 hours. The precipitate was collected by filtration, washed with water (50 ml) and ether (50 ml), to afford the required product **2.7** as a white solid (4 g, 25 mmol, 80%; mp 270-273 °C).

^{1}H NMR (DMSO-d_6) δ 2.40 (3H, s, SCH_3), 4.88 (1H, s, C=C*H*), 6.43 (2H, s, NH_2), 11.46 (1H, brs, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 12.52 (S*C*H_3), 81.14 (*C*=CNH_2), 162.81 (*C*NH_2), 163.49 (*C*SCH_3), 164.20 (O*C*N). IR (KBr) 3499, 3472, 3320, 3203, 2923, 2741, 1640, 1616, 1588, 1448, 1303, 1247, 1227, 980, 905, 798, 534 cm^{-1}. HRMS (CI) found 158.0385, $C_5H_7N_3OS$ requires 153.0388 (MH^+).

6-Amino-2-(methylsulfanyl)-5-(2'-nitro-1'-phenylethyl)-4(3*H*)-pyrimidinone

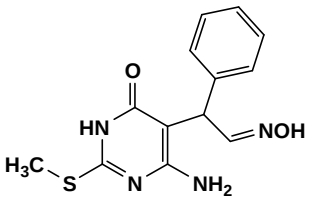


2.8

To a mixture of 6-amino-2-(methylsulfanyl)-4(3*H*)-pyrimidinone **2.7** (2 g, 12.7 mmol) in water (40 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 6 ml). The mixture was stirred for 10 minutes at 60 °C and then [(*E*)-2-nitroethenyl]benzene (2 g, 13.4 mmol) and ethyl acetate (40 ml) were added. The resulting mixture was stirred in an oil bath at 60 °C for 3 hours. The organic layer was separated, washed with brine (20 ml), dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 2:1 to 1:1) to give the product **2.8** as a purple solid (1.0 g, 3.3 mmol, 26%; mp 107-110 °C).

^{1}H NMR (DMSO-d_6) δ 2.40 (3H, s, SCH_3), 4.60 (1H, t, *J* 7.7, CHCH_2NO_2), 5.25-5.52 (2H, m, CHCH_2NO_2), 6.04 (2H, s, NH_2), 7.16-7.47 (5H, m, ArH), 11.78 (1H, s, CONH). ^{13}C NMR (DMSO-d_6) δ 12.35 (SCH$_3$), 38.85 (CHCH$_2$NO$_2$), 77.07 (CHCH$_2$NO$_2$), 91.13 (CCHPh), 126.60 (ArCH), 127.66, 128.07 (4C, ArCH), 140.18 (ArC), 159.02 (CCN$_2$), 160.06 (CSCH$_3$), 162.13 (OCN). IR (KBr) 3478, 3389, 3214, 1613, 1574, 1546 (C-NO$_2$), 1419, 1377, 1220, 971, 757, 699 cm^{-1}. HRMS (FAB) found 329.0693, $C_{13}H_{14}N_4O_3S$ requires 329.0684 (MNa$^+$).

(1'*E/Z*)-[4'-Amino-2'-(methylsulfanyl)-6'-oxo-1',6'-dihydro-5'-pyrimidinyl] (phenyl)ethanal oxime

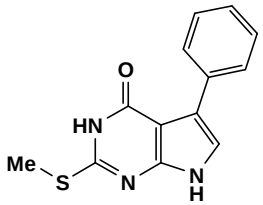


2.9

To a mixture of 6-amino-2-(methylsulfanyl)-4(3*H*)-pyrimidinone **2.7** (2 g, 12.7 mmol) in water (40 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 6 ml). The mixture was stirred for 10 minutes at 60 °C and then [(*E*)-2-nitroethenyl]benzene (2 g, 13.4 mmol) and ethyl acetate (40 ml) were added. The resulting mixture was stirred in an oil bath at 60 °C for 3 hours. The organic layer was separated, washed with brine (20 ml), dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 2:1 to ethyl acetate 100%) to afford a brown solid **2.9** (0.68 g, 2.3 mmol, 18%; mp 105-107 °C) as an *E*/*Z* mixture (3:2 by ^{1}H NMR).

^{1}H NMR (*E* isomer, DMSO-d_6) δ 2.43 (3H, s, SC*H*$_3$), 4.75 (1H, d, *J* 7.6, C*H*CHNOH), 6.43 (2H, brs, N*H*$_2$), 7.12-7.26 (5H, m, Ar*H*), 7.98 (1H, d, *J* 7.6, CHC*H*NOH), 10.42 (1H, s, O*H*), 11.78 (1H, brs, OCN*H*). ^{13}C NMR (*E* isomer, DMSO-d_6) δ 12.41 (S*C*H$_3$), 40.27 (*C*HCHNOH), 92.74 (OC*C*), 126.03 (Ar*C*H), 127.34, 128.02 (4C, Ar*C*H), 140.86 (Ar*C*), 150.68 (CH*C*HNOH), 159.02 (C=*C*N$_2$), 159.79 (S*C*N$_2$), 162.16 (O*C*N). IR (KBr) 3376, 3208, 1613, 1577, 1541, 1420, 1226, 967, 699, 543 cm^{-1}. HRMS (FAB) found 291.0924, $C_{13}H_{14}N_4O_2S$ requires 291.0916 (MH^+).

2-(Methylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



2.10

(a) Tin(II) chloride reaction

To a mixture of 6-amino-2-(methylsulfanyl)-5-(2'-nitro-1'-phenylethyl)-4(3*H*)-pyrimidinone **2.8** (0.2 g, 0.65 mmol) in ethyl acetate (10 ml) was added tin(II) chloride dihydrate (0.44 g, 1.95 mmol). The mixture was stirred in an oil bath at 90 °C for 24 hours. The organic layer was separated, washed with saturated aqueous sodium bicarbonate (20 ml) and with brine (20 ml), then dried with anhydrous magnesium sulfate, and concentrated. The residue was dried under vacuum to give the product **2.10** as a yellow solid (74 mg, 0.29 mmol, 45%). Spectroscopic data are detailed below.

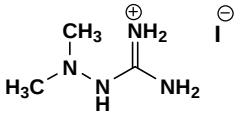
(b) one-step reaction

To a mixture of 6-amino-2-(methylsulfanyl)-4(3*H*)-pyrimidinone **2.7** (2 g, 12.7 mmol) in water (40 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 6 ml). The mixture was stirred for 10 minutes at 60 °C and then [(*E*)-2-nitroethenyl]benzene (2 g, 13.4 mmol) and ethyl acetate (40 ml) were added. The resulting mixture was stirred in an oil bath at 60 °C for 3 hours. The precipitate was collected by filtration, washed with water (20 ml) and *n*-hexane (20 ml), to afford the required product **2.10** as a light purple solid (0.92 g, 3.6 mmol, 28%; mp >260 °C).

^{1}H NMR (DMSO-d_6) δ 2.52 (3H, s, SCH_3), 7.15-7.19 (1H, m, Ar*H*), 7.27 (1H, d, *J* 2.2, CCN*H*), 7.29-7.32 (2H, m, Ar*H*), 7.91 (2H, m, Ar*H*), 11.97 (1H, s, CCN*H*), 12.10 (1H, s, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 12.75 (S*C*H$_3$), 101.02 (OC*C*), 117.45 (PhC=*C*H), 119.94 (Ph*C*=CH), 125.66 (Ar*C*H), 127.71, 127.88 (4C, Ar*C*H), 134.19 (Ar*C*), 149.55 (OCC=*C*NH), 154.73 (*C*SCH$_3$), 159.02 (O*C*N). IR (KBr) 3417, 3170,

3050, 2906, 2829, 1655, 1584, 1523, 1429, 1294, 1207, 1095, 970, 752, 692 cm^{-1}. HRMS (FAB) found 258.0699, $C_{13}H_{11}N_3OS$ requires 258.0701 (MH^+). Anal. Calcd for $C_{13}H_{11}N_3OS \cdot 0.1H_2O$: C, 60.2; H, 4.4; N, 16.2; S, 12.4. Found: C, 60.5; H, 3.9; N, 15.4; S, 12.1.

Amino(2,2-dimethylhydrazino)methaniminium iodide



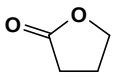
2.17

A mixture of amino(methylsulfanyl)methaniminium iodide **2.15** (0.9 g, 4.1 mmol) and 2,2-dimethylhydrazine (6 ml) was refluxed for twenty hours, cooled and diluted with ether (10 ml). The remaining amine and ether were evaporated under vacuum (510 mmHg) and ethanol (20 ml) was added, kept it at room temperature for one hour, and a small amount of white solid was precipitated. The ethanol layer was carefully decanted and evaporated under vacuum (510 mmHg) to give the product **2.17** as a light brown semisolid (0.9 g, 3.9 mmol, 95%).

*^{1}H NMR (DMSO-d_6) δ 2.92 (3H × 2, s, NCH_3), 7.10 (2H × 2, brs, NH_2). ^{13}C NMR (DMSO-d_6) δ 37.76 (N*C*H$_3$), 156.79 (*C*NH$_2$). IR (KBr) 3372, 3219, 1645, 1435, 1344, 1116, 1064, 780, 710, 628, 538 cm^{-1}. Anal. Calcd for $C_3H_{11}N_4I_1Cl_2 \cdot 0.3H_2O \cdot 0.5$ CH_3CH_2OH: C, 18.6; H, 5.7; N, 21.7. Found: C, 18.6; H, 5.3; N, 20.9.

*-NH- proton was not found.

Dihydro-2(3*H*)-furanone

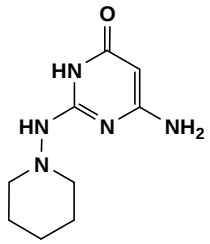


2.23

To a suspension of pyridinium chlorochromate (24 g, 0.11 mol) in dichloromethane (100 ml) was added a solution of 1,4-butanediol **2.20** (2 g, 22 mmol) in dichloromethane (10 ml) over a period of one hour at room temperature. After a total reaction time of 20 hours, ether (200 ml) was added. The resulting brown solution was passed through Florisil (30 g, 30-60 mesh) and the solution was evaporated under vacuum (510 mmHg) to give the product **2.23** as a yellow oil (1.1 g, 12.2 mmol, 55%).

^{1}H NMR ($CDCl_3$) δ 2.23-2.31 (2H, m, $OCH_2CH_2CH_2$), 2.48-2.52 (2H, m, $OCH_2CH_2CH_2$), 4.36 (2H, t, *J* 7.0, $OCH_2CH_2CH_2$). ^{13}C NMR ($CDCl_3$) δ 22.15 ($OCH_2CH_2CH_2$), 27.76 ($OCH_2CH_2CH_2$), 68.49 ($OCH_2CH_2CH_2$), 177.69 (OCO). IR (KBr) 3522, 2992, 2917, 1769 (OC=O), 1461, 1423, 1377, 1280, 1168, 1036, 991, 930, 870, 801, 676, 636, 535, 492 cm^{-1}.

6-Amino-2-(1'-piperidinylamino)-4(3*H*)-pyrimidinone



2.18c

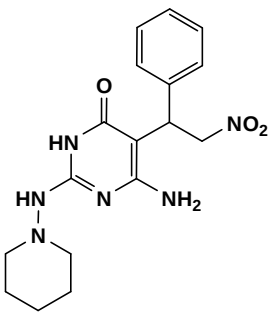
To a suspension of 6-amino-2-hydrazino-4(3*H*)-pyrimidinone **2.13** (0.19 g, 1.3 mmol) in methanol (10 ml) was added acetic acid (80 μl). After five minutes, a solution of pentanedial (50% solution in water, 0.28 ml) in methanol (2 ml) was

added and then a solution of sodium cyanoborohydride (0.2 g) in methanol (2 ml). The resulting mixture was stirred at room temperature for 19 hours then the methanol was evaporated under reduced pressure (450 mmHg) and water (20ml) was added. This mixture was poured into ethyl acetate (20 ml) and extracted (20 ml × 3). The organic layer was combined and washed with saturated aqueous sodium bicarbonate (30 ml), and with brine (30 ml), then dried with anhydrous magnesium sulfate, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate 100% to ethyl acetate:methanol = 1:1) to give the product **2.18c** as a white solid (100 mg, 0.48 mmol, 37%, mp 155-157 °C).

^{1}H NMR (DMSO-d_6) δ 1.22-1.46 (2H, m, $NCH_2CH_2CH_2$), 1.57-1.63 (4H, m, $NCH_2CH_2CH_2$), 2.65 (4H, brs, $NCH_2CH_2CH_2$), 4.47 (1H, s, OCC*H*), 5.95 (2H, s, NH_2), 8.36 (1H, s, CH_2NNH), 8.97 (1H, s, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 23.29 ($NCH_2CH_2CH_2$), 25.72 ($NCH_2CH_2CH_2$), 56.65 ($NCH_2CH_2CH_2$), 77.19 (OC*C*H), 154.91 (CNH_2), 162.56 (HN*C*NH), 165.86 (O*C*NH). IR (KBr) 3344, 2938, 2854, 1631, 596, 1493, 1467, 1284, 1100, 1036, 973, 876, 795 cm^{-1}. HRMS (ESI) found 210.1348, $C_9H_{15}N_5O_1$ requires 210.1349 (MH^+). Anal. Calcd for $C_9H_{15}N_5O_1 \cdot 1.0H_2O$: C, 47.6; H, 7.5; N, 30.8. Found: C, 47.6; H, 7.5; *N, 27.1.

*A satisfactory N analysis could not be obtained due to the difficulty of complete burning even when aided by WO_3.

6-Amino-5-(2'-nitro-1'-phenylethyl)-2-(1"-piperidinylamino)-4(3*H*)-pyrimidinone



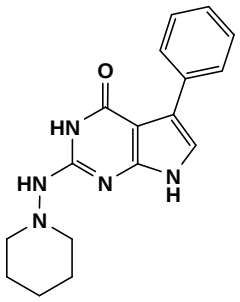
2.28

To a mixture of 6-amino-2-(1'-piperidinylamino)-4(3*H*)-pyrimidinone **2.18c (**0.1 g, 0.48 mmol) in water (10 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 0.28 ml, 0.72 mmol). The mixture was stirred for 5 minutes and then [(*E*)-2-nitroethenyl]benzene (71 mg, 0.48 mmol) and ethyl acetate (10 ml) were added. The resulting mixture was stirred at room temperature for 15 hours, ethyl acetate (30 ml) was added, and the organic layer was separated, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:1 to ethyl acetate 100%) to give the product **2.28** as a white solid (0.1 g, 0.28 mmol, 58%; mp 138-140 °C).

^{1}H NMR (DMSO-d_6) δ 1.23-1.45 (2H, m, $NCH_2CH_2CH_2$), 1.58-1.60 (4H, m, $NCH_2CH_2CH_2$), 2.63 (4H, brs, $NCH_2CH_2CH_2$), 4.56 (1H, t, J 7.7, $CHCH_2NO_2$), 5.25-5.30, 5.45-5.50 (2H, m, $CHCH_2NO_2$), 6.15 (2H, brs, NH_2), 7.13-7.17 (1H, m, ArH), 7.21-7.25, 7.49-7.51 (2H × 2, m, Ar*H*), 8.34 (1H, s, CH_2NNH), 9.12 (1H, s, OCN*H*). ^{*13}C NMR (DMSO-d_6) δ 22.75 ($NCH_2CH_2CH_2$), 25.18 ($NCH_2CH_2CH_2$), 56.16 ($NCH_2CH_2CH_2$), 77.56 ($CHCH_2NO_2$), 86.34 (OC*C*), 126.32 (Ar*C*), 127.72, 127.90 (2C × 2, Ar*C*), 141.21 (Ar*C*), 152.69 (CNH_2), 161.38 (HN*C*NH), 162.24 (O*C*NH). IR (KBr) 3402, 2941, 1610, 1547 (CNO_2), 1494, 1451, 1377, 1080, 1034, 877, 790, 770, 700 cm^{-1}. HRMS (ESI) found 359.1825, $C_{17}H_{22}N_6O_3$ requires 359.1826 (MH^+). Anal. Calcd for $C_{17}H_{22}N_6O_3 \cdot 0.3H_2O$: C, 56.1; H, 6.3; N, 23.1. Found: C, 56.5; H, 6.5; N, 22.4.

*The peak for $CHCH_2NO_2$ overlapped with the peaks of DMSO (38.88-40.13).

5-Phenyl-2-(1'-piperidinylamino)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



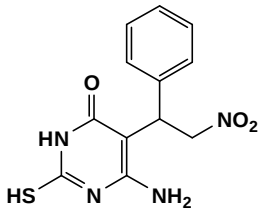
2.19c

To a mixture of 6-amino-5-(2'-nitro-1'-phenylethyl)-2-(1''-piperidinylamino)-4(3*H*)-pyrimidinone **2.28** (84 mg, 0.23 mmol) in ethyl acetate (10 ml) was added tin(II) chloride dihydrate (0.16 g, 0.7 mmol). The mixture was stirred in an oil bath at 90 °C for 24 hours. Ethyl acetate (20 ml) was added, then washed with saturated aqueous sodium bicarbonate (20 ml), brine (20 ml), 1% hydrochloric acid (10 ml), dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:1 to ethyl acetate 100%) to give the product **2.19c** as a white solid (35 mg, 0.11 mmol, 48%; mp 258-260 °C).

^{1}H NMR (DMSO-d_6) δ 1.36 (2H, brs, NCH$_2$CH$_2$C*H*$_2$), 1.63-1.66 (4H, m, NCH$_2$C*H*$_2$CH$_2$), 2.72 (4H, brs, NC*H*$_2$CH$_2$CH$_2$), 7.01 (1H, d, *J* 2.4, HNC*H*), 7.11-7.15 (1H, Ar*H*), 7.26-7.29, 7.90-7.92 (2H × 2, m, Ar*H*), 9.48 (1H, s, CHN*H*), 11.27 (OCN*H*). ^{13}C NMR (DMSO-d_6) δ 22.93 (NCH$_2$CH$_2$*C*H$_2$), 25.95 (NCH$_2$*C*H$_2$CH$_2$), 57.79 (N*C*H$_2$CH$_2$CH$_2$), 99.18 (OC*C*), 114.67 (HN*C*H), 122.15 (HNCH*C*), 126.34 (Ar*C*), 128.06, 128.21 (2C × 2, Ar*C*), 134.05 (Ar*C*), 151.60 (OCC*C*NH), 151.69 (HN*C*NH), 158.65 (O=*C*). IR (KBr) 3424, 3307, 3203, 2932, 1653, 1610, 1435, 1289, 1144, 1119, 1031, 824, 757, 693 cm^{-1}. HRMS (ESI) found 310.1662, $C_{17}H_{19}N_5O_1$ requires 310.1662 (MH^+). Anal. Calcd for $C_{17}H_{19}N_5O_3$: C, 66.0; H, 6.2; N, 22.6. Found: C, 65.5; H, 6.4; *N, 21.5.

*A satisfactory N analysis could not be obtained due to the difficulty of complete burning even when aided by WO_3.

6-Amino-5-(2'-nitro-1'-phenylethyl)-2-sulfanyl-4(3*H*)-pyrimidinone



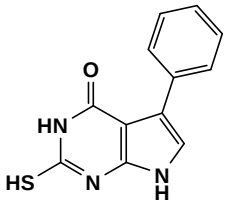
2.30

To a mixture of 6-amino-2-sulfanyl-4(3*H*)-pyrimidinone **2.2** (0.5 g, 3.1 mmol) in water (20 ml) was added *N*,*N*,*N*-trimethyl(phenyl)methanaminium hydroxide (40% solution in water, 1.8 ml). The mixture was stirred for 10 minutes at room temperature and then [(*E*)-2-nitroethenyl]benzene (0.46 g, 3.1 mmol) and ethyl acetate (20 ml) were added. The resulting mixture was stirred at room temperature for 15 hours. The precipitate was collected by filtration and purified by column chromatography (silica gel/ethyl acetate 100%) to give the product **28e** as a light yellow solid (0.34 g). The mother liquid was extracted by ethyl acetate (20 ml × 2). The organic extracts were combined, dried with anhydrous magnesium sulfate. The ethyl acetate was evaporated and the residual solid was dried under vacuum to give the product **2.30** as a yellow solid (0.05 g, total 0.39 g, 1.3 mmol, 42%; mp 185-187 °C).

^{1}H NMR (DMSO-d_6) δ 4.60 (1H, t, *J* 7.3, PhC*H*), 5.27-5.45 (2H, m, PhCHCH_2), 6.60 (2H, brs, NH_2), 7.18-7.42 (5H, m, Ar*H*), 11.50 (1H, s, S*H*), 11.81 (1H, s, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 37.97 (Ph*C*H) 76.67 (PhCH*C*H_2), 88.08 (OC*C*), 126.75 (Ar*C*H), 127.51, 128.17 (4C, Ar*C*H), 139.53 (Ar*C*), 151.73 (*C*NH_2), 161.03 (*C*SH), 173.15 (O*C*NH). IR (KBr) 3450, 3362, 3230, 2910, 2379, 1626, 1550, 1429, 1374, 1209, 1163, 765, 696, 537 cm^{-1}. HRMS (FAB) found 293.0713, $C_{12}H_{12}N_4O_3S$ requires 293.0708 (MH^+). Anal. Calcd for $C_{12}H_{12}N_4O_3S$: C, 49.3; H, 4.1; N, 19.2; S, 11.0. Found: C, 50.0; H, 4.0; *N, 18.0; S, 10.5.

*A satisfactory N analysis could not be obtained due to the difficulty of complete burning even when aided by WO_3.

5-Phenyl-2-sulfanyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one

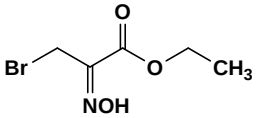


2.31

To a mixture of 6-Amino-5-(2'-nitro-1'-phenylethyl)-2-sulfanyl-4(3*H*)-pyrimidinone **2.30** (0.13 g, 0.44 mmol) in ethyl acetate (20 ml) was added tin(II) chloride dihydrate (0.3 g, 1.33 mmol). The resulting mixture was stirred in an oil bath at 85 °C for 24 hours. The reaction mixture was then diluted with ethyl acetate (20 ml), and the remaining tin(II) chloride was removed by filtration, and the liquid portion was concentrated in vacuo (270 mmHg). The residue was purified by column chromatography (silica gel/ethyl acetate 100%) to give the product **2.31** as a yellow solid (0.05 g, 0.20 mmol, 45%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 7.03 (1H, s, HNC*H*), 7.16-7.83 (5H, m, Ar*H*), 11.41 (1H, s, *H*NCH), 11.87 (1H, s, OCN*H*), 13.23 (1H, brs, S*H*). ^{13}C NMR (DMSO-d_6) δ 99.21 (HN*C*H), 120.81 (HNCH*C*Ph), 126.10 (Ar*C*H), 127.73, 127.92 (4C, Ar*C*H), 133.29 (Ar*C*), 140.11 (OCC*C*N), 157.86 (HS*C*), 171.17 (O*C*N). IR (KBr) 3445, 3208, 1659, 1610, 1410, 1292, 1174, 1116, 740, 691, 600, 513. cm^{-1}. HRMS (ESI) found 244.0533, $C_{12}H_9N_3OS$ requires 244.0539 (MH^+).

Ethyl (2*E*)-3-bromo-2-(hydroxyimino)propanoate[133]



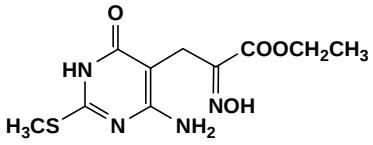
2.34

Hydroxylamine hydrochloride (1.8 g, 25.9 mmol) was added to a stirred solution of

ethyl bromopyruvate **2.32** (5.1 g, 26.2 mmol) in chloroform (70 ml) and methanol (50 ml) at room temperature under a nitrogen atmosphere. The mixture was stirred at room temperature for 24 hours prior to being concentrated to dryness on a rotary evaporator in vacuo (450 mmHg). The residue was dissolved in dichloromethane (50 ml) and washed with 0.1 N hydrochloric acid (50 ml). The organic layer was collected and washed with brine (50 ml) and dried with anhydrous magnesium sulfate. Evaporation of the solvent under vacuum (510 mmHg) yielded the title compound **2.34** as a white crystalline solid (4.6 g, 21.9 mmol, 84%, mp 71-73 °C, lit.[133] 78-79 °C).

^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, J 7.1, CH_2CH_3), 4.19 (2H, s, $BrCH_2$), 4.21 (2H, q, J 7.1, CH_2CH_3), 13.17 (1H, s, NO*H*). ^{13}C NMR (DMSO-d_6) δ 13.88 (CH_2CH_3), 30.88 ($BrCH_2$), 61.22 (CH_2CH_3), 146.74 (*C*NOH), 162.01 (*C*OO). IR (KBr) 3285 (OH), 3054, 2983, 2873, 1725, 1440, 1325, 1226, 1187, 1026, 856, 776, 721. cm^{-1}.

Ethyl-3-[4'-amino-2'-(methylsulfanyl)-6-oxo-1',6'-dihydro-5'-pyrimidinyl]-2-(hydroxyimino)propanoate[117]



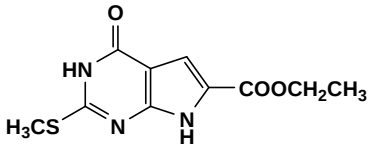
2.37

6-Amino-2-(benzylsulfanyl)-4(3*H*)-pyrimidinone **2.7** (0.63 g, 4.0 mmol) was dissolved in dry DMF (8 ml). Triethylamine (0.40 g, 4.0 mmol) was added and the mixture was stirred under nitrogen at room temperature for 1 h. A solution of ethyl bromopyruvate oxime (0.94 g, 4.4 mmol) in dry DMF (8 ml) was added over a period of 4 hours. Stirring was continued for a further 2 days. DMF was evaporated under vacuum and the residue was purified by column chromatography (silica gel/ethyl acetate 100%). The desired product **2.37** was crystallised from ethyl acetate and hexane, and dried to give a yellow solid (0.25 g, 0.87 mmol, 21%, mp 181-

183 °C).

^{1}H NMR (DMSO-d_6) δ 1.15 (3H, t, J 7.1, CH_2CH_3), 2.40 (3H, s, SCH_3), 3.41 (2H, s, CH_2C=NOH), 4.07 (2H, q, J 7.1, CH_2CH_3), 6.51 (2H, brs, NH_2), 11.73 (1H, brs, NO*H*), 12.14 (1H, s, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 12.40 (SCH_3), 13.86 (CH_2CH_3), 19.20 (CH_2CNOH), 60.47 (CH_2CH_3), 87.94 ($C$$CH_2$CNOH), 150.24 ($C$NOH), 159.72 ($C$$NH_2$), 162.41 ($C$$SCH_3$), 163.41 (O$C$NH), 163.67 ($C$OO). IR (KBr) 3467, 3373, 2934, 2851, 1717, 1621, 1580, 1552, 1423, 1316, 1248, 1031, 1001, 968, 773 cm^{-1}.

Ethyl 2-(methylsulfanyl)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-car boxylate

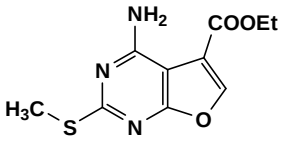


2.35

Ethyl-3-[4'-amino-2'-(methylsulfanyl)-6'-oxo-1',6'-dihydro-5'-pyrimidinyl]-2-(hydroxyimino)propanoate **2.37** (0.24 g, 0.83 mmol) was heated for 15 hours at reflux with Dowex-50 (H^+ form, 0.14 g) in water (20 ml). The reaction mixture was then diluted with methanol (150 ml), and the Dowex resin filtered. The methanol was evaporated in vacuo (450 mmHg) to give the product **2.35** as a brown solid (0.18 g, 0.71 mmol, 85%, mp > 270 °C, lit.[117] > 230 °C).

^{1}H NMR (DMSO-d_6) δ 1.27 (3H, t, J 7.1, CH_2CH_3), 2.53 (3H, s, SCH_3), 4.23 (2H, q, CH_2CH_3), 7.02 (1H, s, OCCC*H*), 12.13 (1H, brs, OCCN*H*), 12.59 (OCN*H*). ^{13}C NMR (DMSO-d_6) δ 12.87 (SCH_3), 14.18 (CH_2CH_3), 60.24 (CH_2CH_3), 105.39 (OC*C*CNH), 109.10 (*C*=CCOO), 122.35 (C=*C*COO), 149.61 (OCC*C*NH), 158.15 (*C*SCH3), 158.60 (O*C*NH), 160.21 (*C*OO). IR (KBr) 3478, 3241, 3126, 2934, 2840, 1651, 1569, 1492, 1401, 1273, 1242, 1212, 1166, 1020, 957, 762, 526 cm^{-1}.

Ethyl 4-amino-2-(methylsulfanyl)furo[2,3-*d*]pyrimidine-5-carboxylate

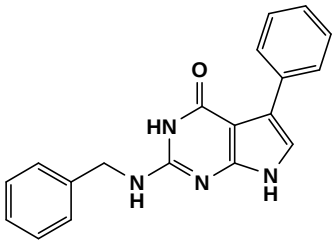


2.36

To a mixture of 6-amino-2-(methylsulfanyl)-4(3*H*)-pyrimidinone **2.7** (0.5 g, 3.2 mmol) in DMF (10ml) was added ethyl 3-bromo-2-oxopropanoate (0.44 ml). The mixture was at room temperature for 3 days. The DMF was evaporated in vacuo (17 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:2 to ethyl acetate 100%) to give the product **2.36** as a light yellow solid (0.52 g, 2.0 mmol, 62%; mp 156-158 °C).

^{1}H NMR (DMSO-d_6) δ 1.30 (3H, t, *J* 7.1, $COOCH_2CH_3$), 2.46 (3H, s, SCH_3), 4.31 (2H, q, *J* 7.1, $COOCH_2CH_3$), 7.51 (1H, s, NH_2), 7.93 (1H, s, NH_2), 8.46 (1H, s, C=C*H*). ^{13}C NMR (DMSO-d_6) δ 13.35 (SCH_3), 13.97 ($COOCH_2CH_3$), 61.44 ($COOCH_2CH_3$), 94.01 (NH_2CC=C), 113.09 (*C*COO), 146.81 (C=*C*HO), 158.04 (*C*OO), 163.49 (C=*C*NO), 167.42 ($CSCH_3$), 167.90 (C=NH_2). IR (KBr) 3373, 3131, 1703, 1640, 1593, 1550, 1465, 1412, 1369, 1355, 1253, 1100, 1064, 1037, 927, 754, 595, 565 cm^{-1}. HRMS (CI) found 254.0603, $C_{10}H_{11}N_3O_3S$ requires 254.0599 (MH^+). Anal. Calcd for $C_{10}H_{11}N_3O_3S$: C, 47.4; H, 4.4; N, 16.6; S, 12.7. Found: C, 47.7; H, 4.0; N, 16.1; S, 12.3.

2-(Benzylamino)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one

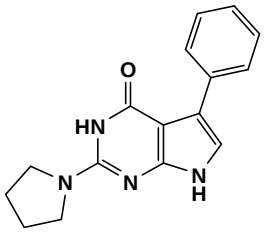


2.43a

To a mixture of 2-(methylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **2.7** (0.2 g, 0.78 mmol) in DMF (5 ml) was added *m*-CPBA (77%, 0.52 g, 2.3 mmol). The resulting mixture was stirred for 4 hours at 3 °C. The DMF was evaporated in vacuo (17 mmHg). The resulting solid was washed with ether (50 ml), to afford the crude sulfone as a light pink solid (0.18 g, 80%, confirmed by LRMS-ESI: M + 1 = 290.1). The crude sulfone intermediate was heated with benzylamine (1 ml) in a sealed tube for 17 hours at 100 °C. The excess benzylamine was removed under vacuum (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:2 to ethyl acetate 100%) to afford the required product **2.43a** as a yellow solid (88 mg, 0.28 mmol, 36%, mp 278-280 °C).

^{1}H NMR (DMSO-d_6) δ 4.49 (2H, d, *J* 5.8, PhCH_2), 6.51 (1H, t, *J* 5.8, *H*NCH_2Ph), 6.99 (1H, d, *J* 2.4, PhC=C*H*), 7.11-7.34 (8H, m, Ar*H*), 7.91-7.93 (2H, m, Ar*H*), 10.30 (1H, brs, PhC=CHN*H*), 11.34 (1H, brs, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 43.67 (Ph*C*H$_2$), 97.28 (O=C*C*), 115.19 (PhC=*C*H), 119.69 (Ph*C*=CH), 125.23, 126.86 (2C, Ar*C*H), 127.02, 127.38, 127.76, 128.31 (8C, Ar*C*H), 134.79, 139.34 (2C, Ar*C*), 151.50 (O=CC=*C*), 152.11 (*C*NHCH$_2$Ph), 158.96 (O*C*N). IR (KBr) 3412, 3197, 2785, 1654, 1477, 1292, 1100, 749, 694, 655 cm^{-1}. HRMS (EI) found 316.1326, $C_{19}H_{16}N_4O$ requires 316.1324 (M^+).

5-Phenyl-2-(1'-pyrrolidinyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one

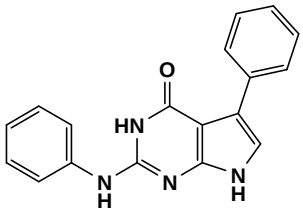


2.43b

To a mixture of 2-(methylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **2.7** (0.2 g, 0.78 mmol) in DMF (5 ml) was added *m*-CPBA (77%, 0.52 g, 2.3 mmol). The resulting mixture was stirred for 4 hours at room temperature. The DMF was evaporated in vacuo (17 mmHg). The resulting solid was washed with ether (50 ml), to afford the crude sulfone as a light pink solid (0.19 g, 84%, confirmed by LRMS-ESI: M + 1 = 290.1). The crude sulfone intermediate was heated with pyrrolidine (1 ml) in a sealed tube for 15 hours at 100 °C. The pyrrolidine was removed under vacuum (270 mmHg) and the residue was recrystallised from methanol and ether to afford the product **2.43b** as a brown solid (0.12 g, 0.43 mmol, 55%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.88 (4H, t, *J* 6.6, NCH$_2$C*H*$_2$), 3.43 (4H, t, *J* 6.6, NC*H*$_2$CH$_2$), 6.98 (1H, d, *J* 2.2, PhC=C*H*), 7.11-7.29 (3H, m, Ar*H*), 7.91-7.93 (2H, m, Ar*H*), 9.86 (1H, brs, PhC=CHN*H*), 11.30 (1H, brs, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 24.85 (NCH$_2$*C*H$_2$), 46.65 (N*C*H$_2$CH$_2$), 96.27 (O=C*C*), 115.28 (PhC=*C*H), 119.35 (Ph*C*=CH), 125.15 (Ar*C*H), 127.37, 127.78 (4C, Ar*C*H), 134.95 (Ar*C*), 150.20 (O=CC=*C*), 152.57 (*C*NCH$_2$), 159.68 (O*C*N). IR (KBr) 3439, 3126, 2956, 1646, 1519, 1344, 1127, 1078, 905, 746, 694 cm^{-1}. HRMS (FAB) found 303.1219, $C_{16}H_{16}N_4ONa$ requires 303.1222 (MNa$^+$). Anal. Calcd for $C_{16}H_{16}N_4O\cdot0.2H_2O$: C, 67.7; H, 5.8; N, 19.7. Found: C, 67.5; H, 5.8; N, 19.7.

2-Anilino-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one

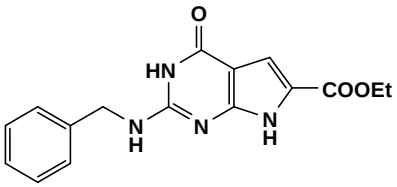


2.43c

To a mixture of 2-(methylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **2.7** (0.2 g, 0.78 mmol) in DMF (5 ml) was added *m*-CPBA (77%, 0.52 g, 2.3 mmol). The resulting mixture was stirred for 4 hours at room temperature. The DMF was evaporated in vacuo (17 mmHg). The resulting solid was washed with ether (50 ml), to afford the crude sulfone as a light pink solid (0.20 g, 89%, confirmed by LRMS-ESI: M + 1 = 290.1). The crude sulfone intermediate was heated with aniline (1 ml) in a sealed tube for 17 hours at 100 °C. The aniline was removed under vacuum (16 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to ethyl acetate 100%) to afford the required product **2.43c** as a yellow solid (58 mg, 0.19 mmol, 24%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 6.99-7.17 (3H, m, Ar*H*), 7.27-7.34 (3H, m, Ar*H*), 7.30 (1H, d, *J* 2.7, PhC=C*H*), 7.64-7.66 (2H, m, Ar*H*), 7.94-7.96 (2H, m, Ar*H*), 8.63 (1H, s, PhN*H*), 10.32 (1H, brs, PhC=CHN*H*), 11.62 (1H, brs, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 98.55 (O=C*C*), 116.15 (PhC=*C*H), 118.94 (2C, Ar*C*), 119.87 (Ph*C*=CH), 122.09, 125.42 (Ar*C*H), 127.46, 127.83, 128.79 (6C, Ar*C*H), 134.56, 139.11 (Ar*C*), 148.04 (O=CC=*C*), 150.88 (*C*NCH$_2$), 158.65 (O*C*N). IR (KBr) 3391, 2923, 2852, 1672, 1632, 1595, 1565, 1497, 1441, 1329, 1264, 1146, 855, 751, 689 cm^{-1}. HRMS (FAB) found 303.1255, $C_{18}H_{14}N_4O$ requires 303.1246 (MH^+).

Ethyl 2-(benzylamino)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylate

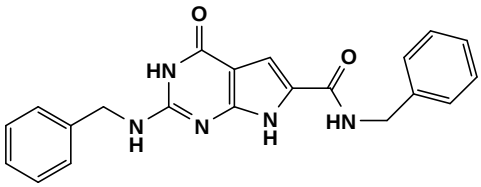


2.49

To a mixture of 2-methylsulfanyl-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid ethyl ester **2.35** (0.16 g, 0.63 mmol) in DMF (5 ml) was added *m*-CPBA (77%, 0.42 g, 1.9 mmol). The resulting mixture was stirred for 4 hours at room temperature. The DMF was evaporated under reduced pressure (17 mmHg). The resulting solid was washed with ether (50 ml), to afford the crude sulfone as a brown solid (0.13 g, 73%, confirmed by LRMS-ESI: MH^+ = 286.0). The crude sulfone intermediate was heated with benzylamine (1 ml) in a sealed tube for 15 hours at 100 °C. The excess benzylamine was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate 100%) and separate from the disubstituted product by HPLC to afford the required product **2.49** as a white solid (24 mg, 0.077 mmol, 12%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.23 (3H, t, *J* 7.1, CH_2CH_3), 4.17 (2H, q, *J* 7.1, CH_2CH_3), 4,49 (2H, d, *J* 5.8, $HNCH_2$), 6.79 (1H, t, *J* 5.8, $HNCH_2$), 6.91 and 6.92 (1H, OCCC*H*), 7.22-7.34 (5H, m, Ar*H*), 10.49 (1H, s, OCCCN*H*), 11.91 (1H, s, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 14.24 (CH_2CH_3), 43.73 ($HNCH_2$), 59.74 (CH_2CH_3), 101.87 (OC*C*CNH), 109.72 (H*C*CNH), 120.27 (HC*C*NH), 126.96 (Ar*C*H), 127.24, 128.36 (4C, Ar*C*H), 138.97 (Ar*C*), 152.36 (OCC*C*NH), 152.96 (HN*C*NH), 158.75 (*C*OO), 160.28 (O*C*NH). IR (KBr) 3422, 3120, 2978, 1662, 1615, 1552, 1522, 1410, 1303, 1240, 1196, 1026, 776, 696, 548 cm^{-1}. HRMS (FAB) found 313.1311, $C_{16}H_{16}N_4O_3$ requires 313.1301 (MH^+). Anal. Calcd for $C_{16}H_{16}N_4O_3$: C, 61.5; H, 5.2; N, 17.9. Found: C, 61.9; H, 5.2; N, 17.6.

***N*-Benzyl-2-(benzylamino)-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxamide**

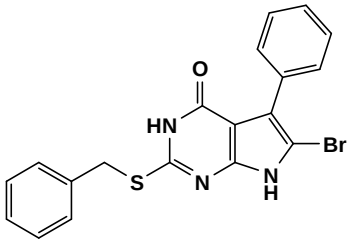


2.50

To a mixture of 2-methylsulfanyl-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-6-carboxylic acid ethyl ester **2.48** (0.16 g, 0.63 mmol) in DMF (5 ml) was added *m*-CPBA (77%, 0.42 g, 1.9 mmol). The resulting mixture was stirred for 4 hours at room temperature. The DMF was evaporated in vacuo (17 mmHg). The resulting solid was washed with ether (50 ml), to afford the crude sulfone as a brown solid (0.13 g, 73%, confirmed by LRMS-ESI: MH^+ = 286.0). The crude sulfone intermediate was heated with benzylamine (1 ml) in a sealed tube for 15 hours at 100 °C. The excess benzylamine was removed under reduced pressure and the residue was purified by column chromatography (silica gel/ethyl acetate 100%) and separated from the mono-substituted product by HPLC to afford the required product **2.50** as a white solid (24 mg, 0.064 mmol, 10%, mp > 250 °C)

^{1}H NMR (DMSO-d_6) δ 4.40 (2H, d, *J* 5.9, OCNHCH_2), 4.47 (2H, d, *J* 5.8, NCNHCH_2), 6.65 (1H, t, *J* 5.8, NCN*H*CH_2), 7.01 and 7.02 (1H, OCCC*H*), 7.21-7.34 (10H, m, Ar*H*), 8.53 (1H, t, *J* 5.9, OCN*H*CH_2), 10.37 (1H, s, OCCN*H*), 11.54 (1H, s, OCN*H*CN). ^{13}C NMR (DMSO-d_6) δ 41.98 (OCNHC*C*H_2), 43.75 (NCNHC*C*H_2), 101.19 (OC*C*CN), 104.95 (OCC*C*H), 124.41 (OC*C*CH), 126.68, 126.93 (Ar*C*H), 127.16, 127.29, 128.25, 128.33 (8C, Ar*C*H), 139.12, 139.77 (2C, Ar*C*), 151.46 (OCC*C*N), 152.43 (OCNH*C*N), 158.90 (O*C*NHCH_2), 160.27 (O*C*NHCN). IR (KBr) 3422, 1613, 1544, 1451, 1292, 1245, 1215, 784, 749, 696 cm^{-1}. HRMS (FAB) found 374.1618, $C_{21}H_{19}N_5O_2$ requires 374.1617 (MH^+).

2-(Benzylsulfanyl)-6-bromo-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one

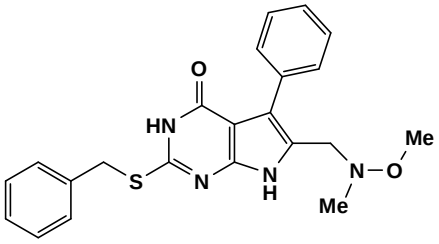


2.55

To a suspension of 2-(benzylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **2.6d** (0.05 g, 0.15 mmol) in tetrahydrofuran (2 ml) was added cyanogen bromide (0.5 ml, 3 M in dichloromethane). The reaction mixture was stirred at 40 °C for 15 hours. The solvent was evaporated in vacuo (400 mmHg) to afford the required product **2.55** as a dark green solid (58 mg, 0.14 mmol, 93%, mp >250 °C).

^{1}H NMR (DMSO-d_6) δ 4.43 (2H, s, SCH_2), 7.25-7.52 (10H, m, Ar*H*), 12.22 (1H, s, *H*NCO), 12.78 (1H, s, C=CHN*H*). ^{13}C NMR (DMSO-d_6) δ 33.52 (S*C*H$_2$), 101.55 (*C*=CN$_2$), 102.62 (C=*C*BrNH), 119.32 (*C*=CBrNH), 126.62, 127.34, 127.51, 128.48, 129.17, 130.18 (10C, Ar*C*H), 132.17 (1C, Ar*C*), 137.29 (1C, Ar*C*), 148.58 (C=*C*N$_2$), 154.45 (*C*N$_3$), 157.52 (*C*=O). IR (KBr) 3423, 3027, 2923, 1651, 1550, 1418, 1209, 1111, 973, 757, 691, 620 (CBr), 526 cm^{-1}. HRMS (FAB) found 412.0113, $C_{19}H_{14}{}^{79}BrN_3OS$ requires 412.0119 (MH^+).

2-(Benzylsulfanyl)-6-{[methoxy(methyl)amino]methyl}-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one

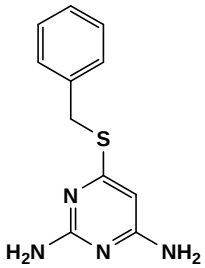


2.57

To a mixture of 2-(benzylsulfanyl)-5-phenyl-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **2.6d** (68 mg, 0.20 mmol) in ethanol (6 ml) in was added *N*,*O*-dimethylhydroxylamine (0.12 g, 2.0 mmol) and *N*,*O*-dimethylhydroxylamine hydrochloride (0.2 g, 2.0 mmol). The mixture in a sealed tube was stirred in an oil bath at 100 °C for 24 hours and then a further aliquot of *N*,*O*-dimethylhydroxylamine (0.12 g, 2.0 mmol) was added. The resulting mixture was stirred at 100 °C for 24 hours. The ethanol was evaporated in vacuo (210 mmHg) and the residue was extracted by ethyl acetate (20 ml × 3), washed with brine (20 ml), dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:2 to ethyl acetate 100%) to give the product **2.57** as a white solid (13 mg, 0.03 mmol, 15%; mp 185-187 °C).

^{1}H NMR ($CDCl_3$) δ 2.60 (3H, s, NCH_3), 3.50 (3H, s, OCH_3), 3.91 (2H, s, NCH_2), 4.46 (2H, s, SCH_2), 7.20-7.23 (2H, m, Ar*H*), 7.28-7.35 (4H, m, Ar*H*), 7.39-7.42 (2H, m, Ar*H*), 7.55-7.57 (2H, m, Ar*H*), 9.08 (1H, s, C=CN*H*), 10.99 (1H, s, *H*NCO). ^{13}C NMR ($CDCl_3$) δ 35.18 (S*C*H_2), 44.36 (N*C*H_3), 54.26 (N*C*H_2), 59.94 (O*C*H_3), 102.74 (O=C*C*), 119.66 (NCH$_2$C=*C*), 126.09 (NCH$_2$*C*=C), 126.66, 127.65, 127.71, 128.68, 129.10, 130.55 (10C, Ar*C*H), 132.75 (1C, Ar*C*), 136.25 (1C, Ar*C*), 148.50 (OC*C*NH), 153.99 (S*C*=N), 159.89 (O*C*NH). IR (KBr) 3423, 2929, 1657, 1551, 1524, 1195 (CH_2-N-CH_3), 1044, 976, 762, 697 cm^{-1}. HRMS (FAB) found 407.1536, $C_{22}H_{22}N_4O_2S$ requires 407.1542 (MH^+).

6-(Benzylsulfanyl)-2,4-pyrimidinediamine[144]

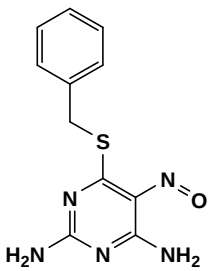


2.59a

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (6.3 g, 26.2 mmol) and sodium hydroxide (1.6 g, 41.6 mmol) in ethanol (150 ml) and water (100 ml) was added benzyl bromide (3.4 ml, 28.8 mmol). The reaction mixture was stirred at room temperature for 20 hours. The precipitate was collected by filtration, washed with water (50 ml) and *n*-hexane (50 ml), to afford the required product **2.59a** as a white solid (4.7 g, 20.2 mmol, 77%; mp 144-146 °C, lit.[144] 144-146 °C).

^{1}H NMR (DMSO-d_6) δ 4.31 (2H, s, SCH_2), 5.78 (1H, *H*C=CS), 6.74, 7.03 (2H × 2, brs, NH_2), 7.24-7.42 (5H, m, Ar*H*). ^{13}C NMR (DMSO-d_6) δ 32.85(S*C*H_2), 90.64 (H*C*=NH_2), 127.22 (1C, Ar*C*H), 128.47, 128.93 (4C, Ar*C*H), 137.05 (1C, Ar*C*), 158.41 (*C*N_3), 161.93 (HC=*C*N2), 163.35 (S*C*=N). IR (KBr) 3342, 3166, 1642, 1533, 1503, 1245, 1111, 991, 699, 661, 617, 537 cm^{-1}.

6-(Benzylsulfanyl)-5-nitroso-2,4-pyrimidinediamine[144]

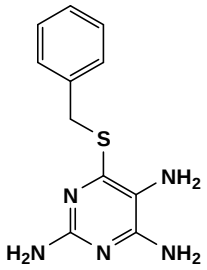


2.60

To a suspension of 6-(benzylsulfanyl)-2,4-pyrimidinediamine **2.59** (4.1 g, 17.5 mmol) and sodium nitrite (1.5g, 21.7 mmol) in DMF (40 ml) and water (40 ml) was added glacial acetic acid (7 ml). The reaction mixture was stirred at room temperature for 2 days. The precipitate was collected by filtration, washed with water (50 ml) and *n*-hexane (50 ml), to afford the required product **2.60** as a purple solid (3.6 g, 13.8 mmol, 79%; mp 253-255 °C).

^{1}H NMR (DMSO-d_6) δ 4.45(1H, s, SCH_2), 7.21-7.51 (5H, m, Ar*H*), 8.00-8.08 (2H, s, N_2CNH_2), 8.14 (1H, d, *J* 3.7, NH_2CCNO), 9.67 (1H, d, *J* 3.7, NH_2CCNO). ^{13}C NMR (DMSO-d_6) δ 31.81(S*C*H$_2$), 126.98(1C, Ar*C*H), 128.37, 129.29 (4C, Ar*C*H)138.10 (1C, Ar*C*) 145.29 (*C*NO), 149.19 (NH_2*C*CNO), 160.74 (*C*N$_3$), 180.51 (S*C*N). IR (KBr) 3488, 3303, 3174, 1619, 1542, 1492, 1361, 1325, 1256, 1142, 1008(C-NO), 921, 788, 694, 609, 525 cm^{-1}.

6-(Benzylsulfanyl)-2,4,5-pyrimidinetriamine[144]

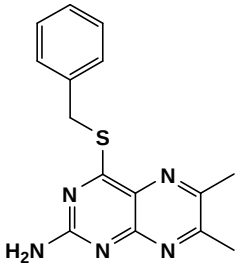


2.61

To a suspension of 6-(benzylsulfanyl)-5-nitroso-2,4-pyrimidinediamine **2.60** (2.6 g, 9.9 mmol) in methanol (30 ml) and water (30 ml) was added sodium dithionite (5.1 g, 29.3 mmol). The reaction mixture was stirred at room temperature for 3 hours and a further aliquot of sodium dithionite (5.1 g, 29.3 mmol) was added and stirred for 20 hours. The precipitate was collected by filtration, washed with water (50 ml) and *n*-hexane (50 ml), to afford the required product **2.61** as a light yellow solid (2.4 g, 9.7 mmol, 98%; mp 173-175 °C, lit.[144] 175-177 °C).

^{1}H NMR (DMSO-d_6) δ 4.26 (2H, s, SCH_2), 5.71, 6.29, 6.40 (2H × 3, brs, NH_2), 7.19-7.35 (5H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 34.15(SCH$_2$), 116.89 (SCCNH$_2$), 127.02 (ArC), 128.32 (2C, ArCH), 128.90 (2C, ArCH), 138.02 (ArC), 153.72 (CN3), 155.72 (NH$_2$CCN$_2$), 166.31 (SCN). IR (KBr) 3329, 3166, 1642, 1533, 1503, 1245, 1111, 991, 699, 661, 617, 537 cm^{-1}.

4-(Benzylsulfanyl)-6,7-dimethyl-2-pteridinamine[144]

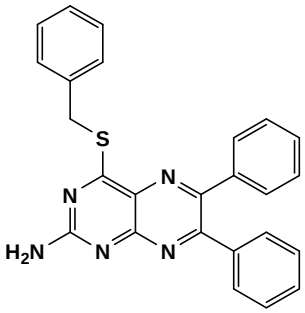


2.62a

To a suspension of 6-(benzylsulfanyl)-2,4,5-pyrimidinetriamine **2.61** (0.8 g, 3.2 mmol) in ethanol (45 ml) was added biacetyl (0.34 ml). The reaction mixture was refluxed for 4 hours. The precipitate was collected by filtration, washed with ether (25 ml) and *n*-hexane (25 ml), to afford the required product **2.62a** as a yellow solid (0.71 g, 2.4 mmol, 75%; mp 210-213 °C, lit.[144] 208-210 °C).

^{1}H NMR (DMSO-d_6) δ 2.48, 2.54 (3H × 2, s, CH_3), 4.55 (2H, s, SCH_2), 7.20 (2H, brs, NH_2), 7.22-7.25 (1H, m, ArH), 7.28-7.32 (2H, m, ArH), 7.48-7.50 (2H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 21.71, 23.24 (2C, CH$_3$), 32.04 (SCH$_2$), 125.73 (SCCN), 127.03 (ArCH), 128.34, 129.21 (4C, ArCH), 137.85 (ArC), 147.73, 152.93 (2C, NCCH$_3$), 160.75 (NCNCCH3), 161.33 (CN$_3$), 172.49 (SCN). IR (KBr) 3413, 3153, 1630, 1556, 1553, 1407, 1363, 1193, 932, 696, 642 cm^{-1}.

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine

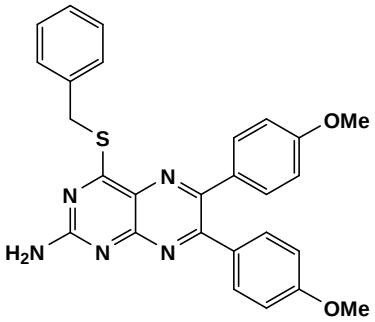


2.62b

To a suspension of 6-(benzylsulfanyl)-2,4,5-pyrimidinetriamine **2.61** (0.8 g, 3.2 mmol) in ethanol (60 ml) was added benzyl (0.75 g, 3.6 mmol). The reaction mixture refluxed for 20 hours. The precipitate was collected by filtration, washed with ether (50 ml), to afford the required product **2.62b** as a yellow solid (0.88 g, 9.7 mmol, 65%; mp 175-177 °C).

^{1}H NMR (DMSO-d_6) δ 4.52 (1H, s, SCH_2), 7.23-7.53 (17H, m, ArH + NH_2). ^{13}C NMR (DMSO-d_6) δ 32.22 (SCH$_2$), 126.48 (SCCN), 127.15, 128.01, 128.40, 129.28, 129.43, 129.56 (15C, ArCH), 137.63, 138.08, 138.35 (3C, ArC), 146.84 (SCCNC), 152.66 (NCNCPh), 159.09 (NCNCPh), 161.23 (CN$_3$), 173.87 (SCN). IR (KBr) 3347, 3065, 1658, 1448, 1412, 1369, 1113, 919, 768, 698, 618 cm^{-1}. HRMS (FAB) found 422.1444, $C_{25}H_{19}N_5S$ requires 422.1439 (MH^+).

4-(Benzylsulfanyl)-6,7-bis(4'-methoxyphenyl)-2-pteridinylamine

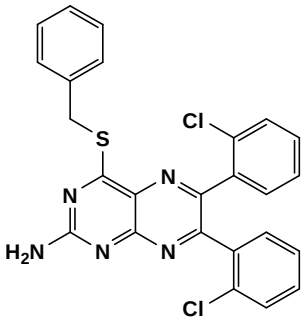


2.62c

To a suspension of 6-(benzylsulfanyl)-2,4,5-pyrimidinetriamine **2.61** (1.16 g, 4.7 mmol) in DMF (25 ml) was added 1,2-bis(4'-methoxyphenyl)-1,2-ethanedione (2.5 g, 9.4 mmol). The reaction mixture was stirred at 100 °C for 17 hours. The solvent was evaporated in vacuo (17 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:n-hexane = 1:2 to ethyl acetate:methanol = 1:1) to afford the required product **2.62c** as a yellow solid (0.94 g, 1.95 mmol, 41%, mp 199-202 °C).

^{1}H NMR (DMSO-d_6) δ 3.75 (3H, s, OCH_3), 3.77 (3H, s, OCH_3), 4.50 (2H, s, SCH_2), 6.87-6.92 (4H, m, Ar*H*), 7.23-7.32 (5H, m, Ar*H*), 7.38-7.41 (2H, m, Ar*H*), 7.44 (2H, brs, NH_2), 7.51-7.53 (2H, m, Ar*H*). ^{13}C NMR (DMSO-d_6) δ 32.18 (S*C*H$_2$), 55.12, 55.18 (2C, O*C*H$_3$), 113.53, 113.56 (2C, Ar*C*H), 126.04 (SC*C*N), 127.12 (Ar*C*H), 128.39, 129.26 (4C, Ar*C*H), 130.66, 130.69 (2C, Ar*C*), 130.71, 131.17 (4C, Ar*C*H), 137.71 (Ar*C*), 146.55 (SCCN*C*), 152.47 (NCN*C*Ph), 158.48 (Ar*C*OMe), 159.43 (N*C*NCPh), 160.18 (Ar*C*OMe), 161.13 (CN$_3$), 173.37 (S*C*N). IR (KBr) 3472, 3126, 1624, 1553, 1525, 1404, 1336, 1108, 1067, 1048, 1004, 990, 910, 768, 738, 705, 595, 471 cm^{-1}. HRMS (FAB) found 482.1656, $C_{27}H_{23}N_5O_2S$ requires 482.1651 (MH^+).

4-(Benzylsulfanyl)-6,7-bis(2'-chlorophenyl)-2-pteridinylamine

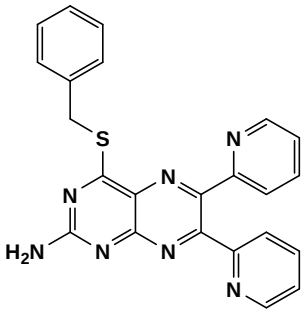


2.62d

To a suspension of 6-(benzylsulfanyl)-2,4,5-pyrimidinetriamine **2.61** (0.5 g, 2.0 mmol) in ethanol (30 ml) was added 1,2-bis(2'-chlorophenyl)-1,2-ethanedione (0.67 g, 2.4 mmol). The reaction mixture was refluxed for 2 days. The solvent was evaporated in vacuo (200 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:n-hexane = 1:1 to ethyl acetate 100%) to afford the required product **2.62d** as a yellow solid (0.36 g, 0.73 mmol, 37%, mp 251-253 °C).

^{1}H NMR (DMSO-d_6) δ 4.52 (1H, s, SCH_2), 7.23-7.42 (11H, m, Ar*H*), 7.51-7.53 (2H, m, Ar*H*), 7.68 (2H, brs, NH_2). ^{13}C NMR (DMSO-d_6) δ 28.94 (S*C*H$_2$), 126.79 (SC*C*N), 127.18 (Ar*C*H), 128.40, 129.29 (4C, Ar*C*H), 130.43, 130.67, 131.27 (6C, Ar*C*H), 131.60 (Ar*C*), 131.85 (2C, Ar*C*H), 132.29 (Ar*C*), 135.97, 136.34 (2C, Ar*C*Cl), 137.53 (Ar*C*), 145.55 (SCCN*C*), 152.92 (NCN*C*Ph), 158.38 (N*C*NCPh), 161.43 (*C*N$_3$), 174.36 (S*C*CN). IR (KBr) 3472, 3126, 1624, 1553, 1525, 1404, 1336, 1108, 1067, 1048, 1004, 990, 910, 768, 738, 705, 595, 471 cm^{-1}. HRMS (FAB) found 490.0660, $C_{25}H_{17}{}^{35}Cl_2N_5S$ requires 490.0664 (MH^+). Anal. Calcd for $C_{25}H_{17}Cl_2N_5S$: C, 61.2; H, 3.5; N, 14.3; Cl, 14.5; S, 6.5. Found: C, 61.2; H, 3.5; N, 14.0; Cl, 14.5; S, 6.8.

4-(Benzylsulfanyl)-6,7-di(2'-pyridinyl)-2-pteridinamine

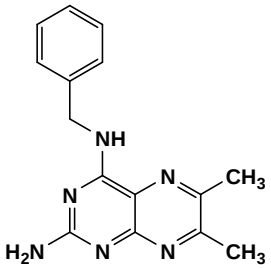


2.62e

To a suspension of 6-(benzylsulfanyl)-2,4,5-pyrimidinetriamine **2.61** (1 g, 4.0 mmol) in ethanol (60 ml) was added 1,2-di(2'-pyridinyl)-1,2-ethanedione (0.85 g, 4.0 mmol). The reaction mixture was refluxed for 4 hours. The precipitate was collected by filtration, washed with ether (50 ml), to afford the required product **2.62e** as a yellow solid (0.92 g, 2.2 mmol, 55%; mp 260-263 °C).

^{1}H NMR (DMSO-d_6) δ 4.53 (2H, s, SCH_2), 7.24-7.36 (5H, m, Ar*H*), 7.53-7.55 (2H, m, Py*H*), 7.65 (2H, brs, NH_2), 7.80-7.95 (4H, m, Py*H*), 8.19-8.24 (2H, m, Py*H*). ^{13}C NMR (DMSO-d_6) δ 32.32 (S*C*H_2), 12.92, 123.45, 123.64, 123.89 (4C, Py*C*H), 126.38 (SC*C*=C), 127.20, 128.43, 129.34 (5C, Ar*C*H), 136.73, 136.82 (2C, Py*C*H), 137.49 (Ar*C*), 146.15(N=*C*C=N), 147.91, 147.97 (2C, Py*C*H), 152.87 (N=C*C*=N), 156.27, 156.52 (2C, Py*C*), 158.31 (C=*C*N_2), 161.52 (*C*N_3), 174.07 (S*C*N). IR (KBr) 3475, 3054, 1624, 1576, 1536, 1484, 1399, 1355, 1242, 1091, 787, 743, 617, 546, 466 cm^{-1}. HRMS (FAB) found 424.1338, $C_{23}H_{17}N_7S$ requires 424.1344 (MH^+).

N^4-Benzyl-6,7-dimethyl-2,4-pteridinediamine



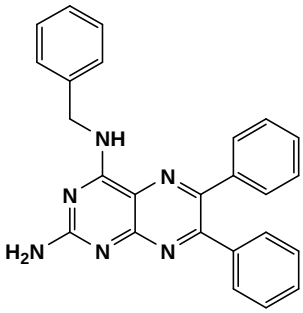
2.63a

4-(Benzylsulfanyl)-6,7-dimethyl-2-pteridinamine **2.62a** (0.15 g, 0.5 mmol) was suspended in benzylamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess benzylamine was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate 100% to ethyl acetate:methanol = 5:1) to afford the required product **2.63c** as an orange solid (85 mg, 0.3 mmol, 61%, mp 230-232 °C).

^{1}H NMR (DMSO-d_6) δ 2.51 (6H, s, *CH_3), 4.66 (2H, d, J 6.4, NHCH_2), 6.45 (2H, s, NH_2), 7.19-7.23 (1H, m, ArH), 7.27-7.31 (2H, m, ArH), 7.34-7.36 (2H, m, ArH), 8.40 (1H, t, J 6.4, NHCH$_2$). ^{13}C NMR (DMSO-d_6) δ 21.40, 22.85 (CH$_3$), 43.12 (NHCH$_2$), 119.56 (HNCCN), 126.67 (ArCH), 127.39, 128.17 (4C, ArCH), 139.51, 145.09 (CCH3), 154.27 (ArC), 158.68 (HNCCC), 160.21 (CNH$_2$), 162.24 (HNCN). IR (KBr) 3467, 3379, 3296, 3115, 1635, 1593, 1481, 1437, 1418, 1363, 1336, 1215, 976, 823, 754, 699, 606 cm^{-1}. HRMS (ESI) found 281.1507, $C_{15}H_{16}N_6$ requires 281.1509 (MH$^+$). Anal. Calcd for $C_{15}H_{16}N_6 \cdot 0.1H_2O$: C, 63.9; H, 5.8; N, 29.8. Found: C, 63.8; H, 5.2; N, 29.1.

*The peak of CH_3 partially overlapped with DMSO.

N^4-Benzyl-6,7-diphenyl-2,4-pteridinediamine



2.63b

(a) Thermal reaction

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine **2.62b** (0.1 g, 0.24 mmol) was heated with benzylamine (1 ml) in a sealed tube for 2 days at 100 °C. Excess benzylamine was removed under vacuum (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to ethyl acetate 100%) to afford the required product **2.63c** as a yellow solid (36 mg, 0.09 mmol, 38%).

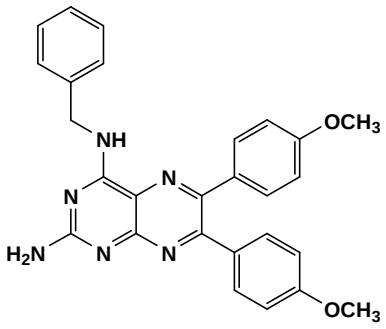
(b) Microwave reaction

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine **2.62b** (0.1 g, 0.24 mmol) was suspended in benzylamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess benzylamine was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to ethyl acetate 100%) to afford the required product **2.63c** as a yellow solid (46 mg, 0.11 mmol, 46%, mp 246-248 °C).

^{1}H NMR (DMSO-d_6) δ 4.74 (2H, d, *J* 6.4, PhC*H*$_2$NH), 6.77 (2H, brs, N*H*$_2$), 7.21-7.44 (15H, m, Ar*H*), 8.75 (1H, t, *J* 6.4, PhCH$_2$N*H*). ^{13}C NMR (DMSO-d_6) δ 43.26 (Ph*C*H$_2$), 120.94 (NHC*C*=C), 126.73, 127.37, 127.83, 127.91, 127.98, 128.22, 128.80, 129.50, 129.63 (15C, Ar*C*H), 138.47, 138.91, 139.35 (Ar*C*), 144.66, 154.23 (Ph*C*=N), 157.13 (NHCC=*C*), 160.42 (*C*NH$_2$), 163.15 (HN*C*). IR (KBr) 3467, 3412,

3379, 3269, 3060, 1733, 1629, 1593, 1572, 1445, 1421, 1349, 1242, 1176, 1105, 696 cm^{-1}. HRMS (EI) found 404.1746, $C_{25}H_{20}N_6$ requires 404.1749 (M^+). Anal. Calcd for $C_{25}H_{20}N_6 \cdot 0.5H_2O$: C, 72.6; H, 5.1; N, 20.3. Found: C, 72.8; H, 4.7; N, 19.9.

N^4-Benzyl-6,7-bis(4'-methoxyphenyl)-2,4-pteridinediamine

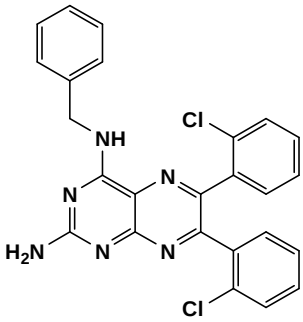


2.63c

4-(Benzylsulfanyl)-6,7-bis(4'-methoxyphenyl)-2-pteridinylamine **2.62c** (0.1 g, 0.21 mmol) was suspended in benzylamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess benzylamine was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:methanol = 1:5) to afford the required product **2.63c** as an yellow solid (55 mg, 0.11 mmol, 52%, mp 101-103 °C).

^{1}H NMR (DMSO-d_6) δ 3.74, 3.77 (3H × 2, s, OCH_3), 4.73 (2H, d, J 6.4, HNCH_2), 6.67 (2H, brs, NH_2), 6.85-7.4 (13H, m, ArH), 8.64 (1H, t, J 6.4, HNCH$_2$). ^{13}C NMR (DMSO-d_6) δ 32.27 (HNCH$_2$), 55.10, 55.13 (2C, OCH$_3$), 113.36, 113.46 (ArCH), 120.32 (HNCCN), 126.64, 126.72, 127.38, 128.21, 128.92 (ArCH), 130.85, 130.95 (4C, ArCH), 131.07, 131.26, 139.42 (3C, ArC), 144.41 (NC=CN), 153.91 (N=CC=N), 156.49 (ArCOMe), 159.09 (CC=CN), 159.77 (ArCOMe), 160.36 (H$_2$NC), 162.92 (HNCN). IR (KBr) 3379, 3170, 2934, 2840, 1591, 1569, 1437, 1347, 1245, 1174, 1026, 828, 699, 595, 540 cm^{-1}. HRMS (ESI) found 465.2039, $C_{27}H_{24}N_6O_2$ requires 465.2034 (MH^+).

N^4-Benzyl-6,7-bis(2'-chlorophenyl)-2,4-pteridinediamine

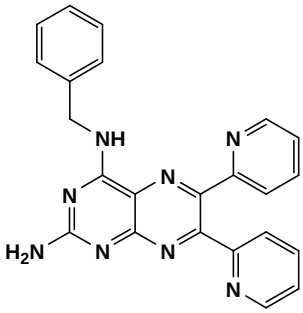


2.63d

4-(Benzylsulfanyl)-6,7-bis(2'-chlorophenyl)-2-pteridinylamine **2.62d** (0.15 g, 0.3 mmol) was suspended in benzylamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Benzylamine was removed under vacuum and the residue was purified by column chromatography (silica gel/ethyl acetate 100%) to afford the required product **2.63d** as a yellow solid (88 mg, 0.19 mmol, 63%, mp 139-141 °C).

^{1}H NMR (DMSO-d_6) δ 4.69 (2H, d, *J* 6.2, CH_2NH), 6.99 (2H, s, NH_2), 7.20-7.46 (13H, m, Ar*H*), 8.86 (1H, t, *J* 6.2, CH_2N*H*). ^{13}C NMR (DMSO-d_6) δ 43.38 (*C*H_2NH), 121.58 (HNC*C*), 126.47, 126.54, 126.78 (Ar*C*H), 127.61, 128.22 (2C × 2, Ar*C*H), 129.02, 129.22, 130.17, 130.36, 131.10 (ArCH), 131.72 (Ar*C*), 132.01 (Ar*C*H), 132.58 (Ar*C*), 136.57, 136.69 (2C, Ar*C*Cl), 139.11 (Ar*C*), 143.80 (N=C*C*=N), 154.13 (N=*C*C=N), 156.51 (HNCC*C*), 160.25 (H_2N*C*), 162.94 (CH_2NH*C*). IR (KBr) 3395, 1591, 1569, 1456, 1437, 1352, 1179, 1111, 1042, 740, 699 cm^{-1}. HRMS (ESI) found 473.1048, $C_{25}H_{18}{}^{35}Cl_2N_6$ requires 473.1043 (MH^+). Anal. Calcd for $C_{25}H_{18}Cl_2N_6$: C, 63.4; H, 3.8; N, 17.7; Cl, 15.0. Found: C, 63.3; H, 3.6; N, 17.4; Cl, 14.6.

***N*[4]-Benzyl-6,7-di(2'-pyridinyl)-2,4-pteridinediamine**



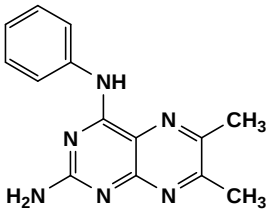
2.63e

4-(Benzylsulfanyl)-6,7-di(2'-pyridinyl)-2-pteridinamine **2.62e** (0.15 g, 0.35 mmol) was suspended in benzylamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. ExceBenzylamine was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate 100%) to afford the required product **2.63e** as an orange solid (95 mg, 0.23 mmol, 66%, mp 205-207 °C).

^{1}H NMR (DMSO-d_6) δ 4.76 (2H, d, *J* 6.3, CH_2NH), 6.30 (2H, brs, NH_2), 7.16-8.26 (13H, m, Ar*H*), 8.86 (1H, t, *J* 6.3, CH_2N*H*). ^{13}C NMR (DMSO-d_6) δ 43.30 (*C*H_2NH), 120.88 (HNC*C*), 122.51, 123.21, 123.67, 123.72 (Py*C*H), 126.76 (Ar*C*H), 127.39, 128.24 (2C × 2, Ar*C*H), 136.35, 136.59 (Ar*C*H), 139.22 (Ar*C*), 144.16 (N=*C*C=N), 147.71, 147.84 (Py*C*H), 154.41 (N=C*C*=N), 155.63, 156.29 (Py*C*), 157.29 (HNCC*C*), 160.36 (H_2N*C*), 163.44 (CH_2NH*C*). IR (KBr) 3445, 3390, 3126, 1559, 1569, 1445, 1421, 1352, 1111, 803, 749, 696 cm^{-1}. HRMS (ESI) found 407.1732, $C_{23}H_{18}N_8$ requires 407.1727 (MH^+). Anal. Calcd for $C_{23}H_{18}N_8$: C, 68.0; H, 4.5; N, 27.6. Found: *C, 67.4; *H, 3.9; *N, 25.8.

*A satisfactory C, H, and N analysis could not be obtained due to the difficulty of complete burning even when aided by WO_3.

6,7-Dimethyl-N^4-phenyl-2,4-pteridinediamine

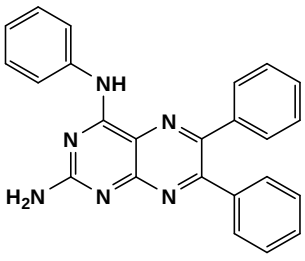


2.64a

4-(Benzylsulfanyl)-6,7-dimethyl-2-pteridinamine **2.62a** (0.15 g, 0.5 mmol) was suspended in aniline (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess aniline was removed in vacuo (15 mmHg) and the residue was recrystallised from methanol and diethyl ether to afford the product **2.64a** as a brown solid (78 mg, 0.27 mmol, 54%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 2.55, 2.59 (3H × 2, s. CH_3), 6.29 (2H, brs, NH_2), 7.07-8.05 (5H, m, ArH), 9.61 (1H, brs, PhNH). ^{13}C NMR (DMSO-d_6) δ 21.47, 22.79 (2C, CH$_3$), 119.47 (NCCC), 121.58 (2C, ArCH), 123.84 (ArCH), 128.52 (2C, ArCH), 138.48 (ArC), 147.21, 151.40 (CCH3), 158.03 (NCCC), 159.62 (H_2NC), 159.99 (NCCC). IR (KBr) 3346, 3181, 1618, 1596, 1555, 1454, 1388, 1363, 1111, 754, 688, 617 cm^{-1}. HRMS (ESI) found 267.1345, $C_{14}H_{14}N_6$ requires 267.1353 (MH^+).

N^4,6,7-Triphenyl-2,4-pteridinediamine



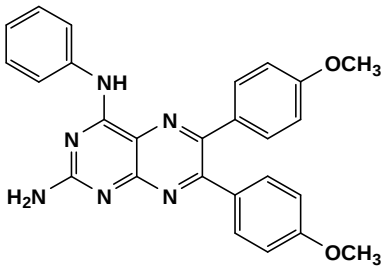
2.64b

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine **2.62b** (0.15 g, 0.36 mmol) was

suspended in aniline (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess aniline (15 mmHg) was removed under vacuum and the residue was purified by column chromatography (silica gel/*n*-hexane:ethyl acetate = 2:1 to ethyl acetate 100%) to afford the required product **2.64b** as a yellow solid (65 mg, 0.17 mmol, 47%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 7.22-7.26 (1H, m, Ar*H*), 7.28-7.49 (10H, m, *ArH*), 7.54-7.57 (2H, m, Ar*H*), 7.74 (1H, brs, NH_2), 7.94-7.98 (2H, m, Ar*H*), 8.26 (1H, brs, NH_2), 10.54 (2H, s, PhN*H*). ^{13}C NMR (DMSO-d_6) δ 121.46 (HNC*C*C), 123.98, 124.10, 126.25 (Ar*C*H), 128.17, 128.39, 128.73 (2C × 3, Ar*C*H), 129.07 (Ar*C*H), 129.65, 129.94 (2C × 2, Ar*C*H), 136.76, 136.99, 137.04 (Ar*C*), 144.73, 149.28 (Ph*C*N), 135.96 (HNCC*C*), 157.19 (H_2N*C*), 158.52 (HN*C*CC). IR (KBr) 3501, 3364, 3102, 1629, 1596, 1557, 1533, 1493, 1449, 1347, 1241, 1108, 701, 630 cm^{-1}. HRMS (ESI) found 391.1654, $C_{24}H_{18}N_6$ requires 391.1666 (MH^+). Anal. Calcd for $C_{24}H_{18}N_6$: C, 73.8; H, 4.6; N, 21.5. Found: C, 73.4; H, 4.7; N, 21.4.

6,7-Bis(4'-methoxyphenyl)-*N*^{4}-phenyl-2,4-pteridinediamine

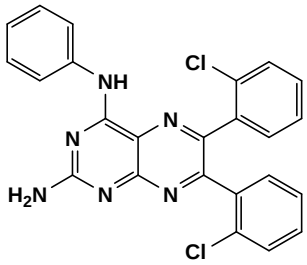


2.64c

4-(Benzylsulfanyl)-6,7-bis(4'-methoxyphenyl)-2-pteridinamine **2.62c** (0.15 g, 0.31 mmol) was suspended in aniline (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 2 hour. Ecess aniline was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/*n*-hexane:ethyl acetate = 2:1 to ethyl acetate 100%) to afford the required product **2.64c** as a yellow solid (35 mg, 0.08 mmol, 26%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 3.77, 3.78 (3H × 2, s, OCH_3), 6.89-6.93 (4H, m, ArH), 6.93 (2H, brs, NH_2), 7.10-8.05 (9H, m, ArH), 9.58 (1H, s, PhNH). ^{13}C NMR (DMSO-d_6) δ 55.14, 55.17 (OCH$_3$), 113.44, 113.52 (2C × 2, ArCH), 120.04 (HNCCC), 121.35 (2C, ArCH), 123.44 (ArCH), 128.49 (2C, ArCH), 130.86 (ArC), 131.06 (4C, ArCH), 131.12 (ArC), 138.85 (ArC), 144.96, 153.88 (OCH$_3$PhCN), 156.95 (ArC), 158.07 (HNCCC), 159.23 (ArC), 159.91 (H$_2$NC), 162.47 (HNCCC). IR (KBr) 3471, 3122, 2923, 1603, 1565, 5113, 1434, 1346, 1243, 1176, 1018, 836, 752, 604 cm^{-1}. HRMS (ESI) found 451.1865, $C_{26}H_{22}N_6O_2$ requires 451.1877 (MH$^+$). Anal. Calcd for $C_{26}H_{22}N_6O_2$: C, 69.3; H, 4.9; N, 18.7. Found: C, 69.2; H, 4.8; N, 18.4.

6,7-Bis(2'-chlorophenyl)-*N*^{4}-phenyl-2,4-pteridinediamine



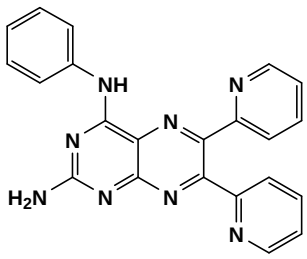
2.64d

4-(Benzylsulfanyl)-6,7-bis(2'-chlorophenyl)-2-pteridinamine **2.62d** (0.15 g, 0.32 mmol) was suspended in aniline (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess aniline was removed in vacuo (15 mmHg) and the residue was purified by column chromatography (silica gel/*n*-hexane:ethyl acetate = 3:1 to ethyl acetate 100%) to afford the required product **2.64d** as a yellow solid (62 mg, 0.13 mmol, 41%; mp 233-235 °C).

^{1}H NMR (DMSO-d_6) δ 7.09-7.14 (1H, m, ArH), 7.16 (2H, brs, NH_2), 7.24-7.41 (9H, m, ArH), 7.50-7.53 (1H, m, ArH), 8.01-8.04 (2H, m, ArH), 8.79 (1H, PhNH). ^{13}C NMR (DMSO-d_6) δ 121.39 (NCCC), 121.75 (2C, ArCH), 123.71, 126.47, 126.57 (ArCH), 128.42 (2C, ArCH), 129.05, 129.25, 130.24, 130.42, 131.17 (ArCH), 131.75 (ArC), 132.20 (ArCH), 132.63, 136.46, 136.65, 138.63 (ArC), 144.10, 154.50

(ClPh*C*N), 156.95 (HNCC*C*), 158.26 (H_2NC), 162.86 (HN*C*CC). IR (KBr) 3483, 3351, 3065, 1610, 1593, 1561, 1539, 1476, 1437, 1412, 1349, 1231, 1111, 1056, 751, 625, 469 cm^{-1}. HRMS (ESI) found 459.0875, $C_{24}H_{17}N_6{}^{35}Cl_2$ requires 459.0886 (MH^+). Anal. Calcd for $C_{24}H_{17}N_6Cl_2$: C, 60.4; H, 4.1; N, 19.2; Cl, 16.2. Found: C, 59.9; H, 3.8; N, 18.7; Cl, 16.5.

N^4-Phenyl-6,7-di(2'-pyridinyl)-2,4-pteridinediamine

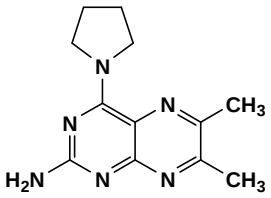


2.64e

4-(Benzylsulfanyl)-6,7-di(2'-pyridinyl)-2-pteridinamine **2.62e** (0.15 g, 0.35 mmol) was suspended in aniline (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess aniline was removed in vacuo (15 mmHg) and the residue was recrystallised from methanol and diethyl ether to afford the product **2.64e** as a yellow solid (90 mg, 0.23 mmol, 66%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 7.12 (2H, brs, NH_2), 7.14-7.42 (5H, m, Ar*H*), 7.86-8.26 (8H, m, Ar*H*), 9.78 (1H, s, PhN*H*). ^{13}C NMR (DMSO-d_6) δ 120.65 (NCCC), 121.64 (2C, Ar*C*H), 122.64, 123.28, 123.70, 123.75, 123.95 (Ar*C*H), 128.50 (2C, Ar*C*H), 136.46, 136.64 (Ar*C*H), 138.70 (Ar*C*), 144.67 (Py*C*N), 147.67, 147.86 (Ar*C*H), 154.29 (Py*C*N), 156.43 (NCC*C*), 157.15, 157.21 (Py*C*), 158.15 (H_2NC), 162.97 (HN*C*CC). IR (KBr) 3428, 3379, 3142, 1624, 1585, 1558, 1536, 1484, 1445, 1412, 1358, 1242, 1149, 1122, 995, 765, 617 cm^{-1}. HRMS (ESI) found 393.1562, $C_{22}H_{16}N_8$ requires 393.1571 (MH^+).

6,7-Dimethyl-4-(1'-pyrrolidinyl)-2-pteridinamine

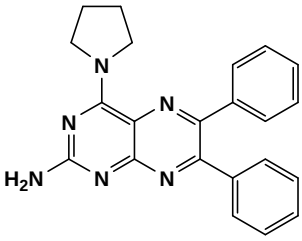


2.65a

4-(Benzylsulfanyl)-6,7-dimethyl-2-pteridinamine **2.62a** (0.15 g, 0.5 mmol) was suspended in pyrrolidine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess pyrrolidine was removed in vacuo (270 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:methanol = 1:1) to afford the required product **2.65a** as a yellow solid (81 mg, 0.33 mmol, 66%, mp > 250 °C, lit.[158] > 240 °C).

^{1}H NMR (DMSO-d_6) δ 1.87, 1.91 (2H × 2, brs, NCH_2CH_2), 2.46, 2.47 (3H × 2, s, CH_3), 3.63, 4,17 (2H × 2, brs, NCH_2CH_2), 6.30 (2H, s, NH_2). ^{13}C NMR (DMSO-d_6) δ 22.59 (2C, CH_3), 25.53, 28.14 (2C, NCH_2CH_2), 55.80, 57.59 (2C, NCH_2CH_2), 125.04 (NCCC), 146.44, 153.64 (2C, CCH3), 153.64 (NCCC), 156.13 (H_2NC), 162.40 (NCCC). IR (KBr) 3368, 3126, 2972, 2950, 2879, 1643, 1552, 1530, 1451, 1426, 1338, 1311, 1223, 973, 820, 642, 460 cm^{-1}.

6,7-Diphenyl-4-(1'-pyrrolidinyl)-2-pteridinamine



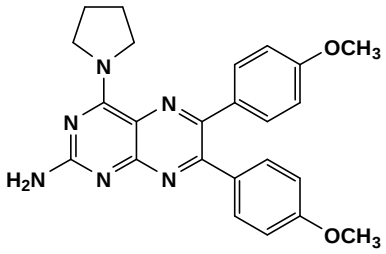
2.65b

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine **2.62b** (0.1 g, 0.24 mmol) was

suspended in pyrrolidine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess pyrrolidine was removed in vacuo (270 mmHg) and the residue was recrystallised from methanol and diethyl ether to afford the product **2.65b** as a yellow solid (47 mg, 0.13 mmol, 54%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.86-1.91, 2.00-2.03 (2H × 2, m, NCH_2CH_2), 3.69-3.72, 4.24-4.27 (2H × 2, m, NCH_2CH_2), 6.61 (2H, NH_2), 7.28-7.44 (10H, Ar*H*). ^{13}C NMR (DMSO-d_6) δ 23.23, 26.67 (2C, NCH_2CH_2), 49.45, 50.77 (2C, NCH_2CH_2), 114.23 (NC*C*C), 127.78, 128.04, 128.92, 129.16, 129.49 (5C × 2, Ar*C*H), 138.76, 138.90 (Ar*C*), 155.35, 158.36 (Ph*C*N), 160.84 (NCC*C*), 162.52 (HN_2C), 166.34 (N*C*CC). IR (KBr) 3434, 2983, 2763, 1629, 1566, 1525, 1451, 1426, 1322, 1240, 1116, 691, 614, 458 cm^{-1}. HRMS (ESI) found 369.1828, $C_{22}H_{20}N_6$ requires 369.1822 (MH^+).

6,7-Bis(4'-methoxyphenyl)-4-(1-pyrrolidinyl)-2-pteridinylamine



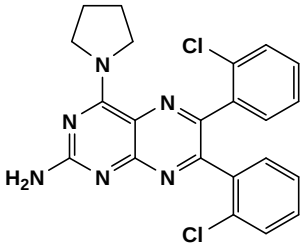
2.65c

4-(Benzylsulfanyl)-6,7-bis(4'-methoxyphenyl)-2-pteridinamine **2.62c** (0.15 g, 0.31 mmol) was suspended in pyrrolidine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess pyrrolidine was removed in vacuo (270 mmHg) and the residue was recrystallised from methanol and diethyl ether to afford the product **2.65c** as a yellow solid (70 mg, 0.16 mmol, 52%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.88, 2.00 (2H × 2, brs, NCH_2CH_2), 3.69 (2H, brs, NCH_2CH_2), 3.75, 3.77 (3H × 2, s, OCH_3), 4.24 (2H, brs, NCH_2CH_2), 6.51 (2H, brs, NH_2), 6.87-7.42 (8H, m, Ar*H*). ^{13}C NMR (DMSO-d_6) δ 23.23, 26.62 (2C, NCH_2CH_2),

49.34, 50.69 (2C, N*C*H_2CH_2), 55.06, 55.14 (2C, O*C*H_3), 113.49, 113.57 (2C × 2, Ar*C*H), 122.32 (NC*C*C), 130.35, 130.97 (2C × 2, Ar*C*H), 131.02, 131.49 (Ar*C*), 142.95, 154.58 (OCH_3Ph*C*N), 155.07 (Ar*C*), 158.29 (NCC*C*), 158.90 (Ar*C*), 159.84 (H_2N*C*), 162.33 (N*C*CC). IR (KBr) 3439, 2961, 1610, 1563, 1528, 1508, 1434, 1349, 1322, 1245, 1174, 1028, 836, 598, 546 cm^{-1}. HRMS (ESI) found 429.2039, $C_{24}H_{24}N_6O_2$ requires 429.2033 (MH^+).

6,7-Bis(2'-chlorophenyl)-4-(1''-pyrrolidinyl)-2-pteridinylamine

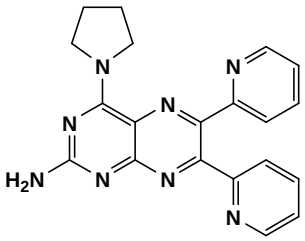


2.65d

4-(Benzylsulfanyl)-6,7-bis(2'-chlorophenyl)-2-pteridinamine **2.62d** (0.1 g, 0.2 mmol) was suspended in pyrrolidine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess pyrrolidine was removed in vacuo (270 mmHg) and the residue was recrystallised from methanol and diethyl ether to afford the product **2.65d** as a yellow solid (62 mg, 0.14 mmol, 70%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.86-1.87, 1.94-1.96 (2H × 2, m, NCH_2CH_2), 3.7, 4.1 (2H × 2, brs, NCH_2CH_2), 6.73 (2H, brs, NH_2), 7.18-7.42 (8H, m, Ar*H*). ^{13}C NMR (DMSO-d_6) δ 23.15, 26.48 (2C, NCH_2*C*H_2), 49.59, 50.74 (2C, N*C*H_2CH_2), 123.45, (NC*C*C), 126.43, 126.67, 129.17, 129.46, 129.86, 130.31 (8C, Ar*C*H), 131.68, 132.59 (Ar*C*), 136.70, 136.99 (2C, Ar*C*Cl), 142.12, 155.08 (ClPh*C*N), 155.79 (NCC*C*), 158.25 (H_2N*C*), 162.74 (N*C*CC). IR (KBr) 3478, 3291, 3060, 2978, 1637, 1566, 1530, 1437, 1421, 1322, 1231, 754, 738, 466 cm^{-1}. HRMS (ESI) found 437.1051, $C_{22}H_{18}N_6{}^{35}Cl_2$ requires 437.1043 (MH^+). Anal. Calcd for $C_{22}H_{18}N_6Cl_2$: C, 60.4; H, 4.1; N, 19.2; Cl, 16.2. Found: C, 59.9; H, 3.8; N, 18.7; Cl, 16.5.

6,7-Di(2'-pyridinyl)-4-(1''-pyrrolidinyl)-2-pteridinamine

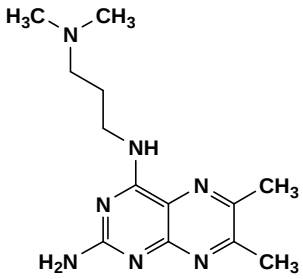


2.65e

4-(Benzylsulfanyl)-6,7-di(2'-pyridinyl)-2-pteridinamine **2.62e** (0.14 g, 0.33 mmol) was suspended in pyrrolidine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess pyrrolidine was removed in vacuo (270 mmHg) and the residue was recrystallised from methanol and diethyl ether to afford the product **2.65e** as a brown solid (98 mg, 0.26 mmol, 79%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.88-1.91, 2.02-2.05 (2H × 2, m, NCH_2CH_2), 3.71-3.74, 4.26-4.29 (2H × 2, m, NCH_2CH_2), 6.71 (2H, s, NH_2), 7.21-8.24 (8H, m, Ar*H*). ^{13}C NMR (DMSO-d_6) δ 23.23, 26.65 (NCH_2CH_2), 49.54, 50.83 (2C, NCH_2CH_2), 122.32 (Ar*C*H), 122.76 (NC*C*C), 122.90, 123.23, 123.65, 136.61, 136.66 (Ar*C*H), 143.02(Py*C*N), 147.72, 147.83 (Ar*C*H), 155.29 (Py*C*N), 155.55 (NCC*C*), 157.03, 157.24 (Ar*C*), 158.23 (H_2NC), 162.75 (N*C*CC). IR (KBr) 3450, 3126, 2972, 2862, 1632, 1561, 1528, 1440, 1418, 1325, 1144, 1097, 803, 620, 480 cm^{-1}. HRMS (ESI) found 371.1733, $C_{20}H_{18}N_8$ requires 371.1727 (MH^+).

N^4-[3'-(dimethylamino)propyl]-6,7-dimethyl-2,4-pteridinediamine

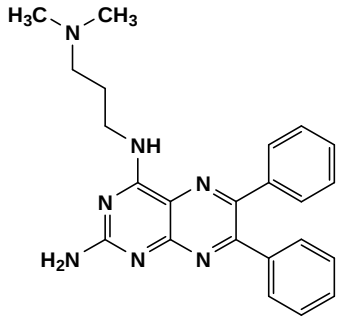


2.66a

4-(Benzylsulfanyl)-6,7-dimethyl-2-pteridinamine **2.62a** (0.15 g, 0.5 mmol) was suspended in N^1,N^1-dimethyl-1,3-propanediamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess N^1,N^1-dimethyl-1,3-propanediamine was removed in vacuo (120 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.66a** as a light yellow solid (40 mg, 0.14 mmol, 28%; mp 223-225 °C).

^{1}H NMR (DMSO-d_6) δ 1.73 (2H, q, J 6.9, HNCH$_2$CH_2CH$_2$), 2.15 (6H, s, CH_3NCH_3), 2.30 (2H, t, J 6.8, HNCH$_2$CH$_2$CH_2), 3.46 (2H, q, J 6.8, HNCH_2CH$_2$CH$_2$), 6.38 (2H, s, NH_2), 8.14 (1H, t, J 5.6, HNCH$_2$CH$_2$CH$_2$). ^{13}C NMR (DMSO-d_6) δ 21.42, 22.81 (2C, CH$_3$), 26.47 (HNCH$_2$$CH_2CH_2$), 39.10 (HN$CH_2CH_2CH_2$), 45.14 ($CH_3NCH_3$), 57.26 (HNCH$_2CH_2$$CH_2$), 119.69 (HNC$C$C), 144.81, 154.18 (N$CCH_3$), 158.36 (HNCC$C$), 160.16 (H$_2NC$), 162.38 (HN$C$CC). IR (KBr) 3496, 3121, 2857, 2819 (NCH$_3$), 2772 (NCH$_3$), 1630, 1595, 1568, 1465, 1418, 1349, 1238, 1145, 1112, 700, 615 cm^{-1}. HRMS (ESI) found 276.1929, $C_{13}H_{21}N_7$ requires 276.1931 (MH$^+$).

N^4-[3'-(dimethylamino)propyl]-6,7-diphenyl-2,4-pteridinediamine



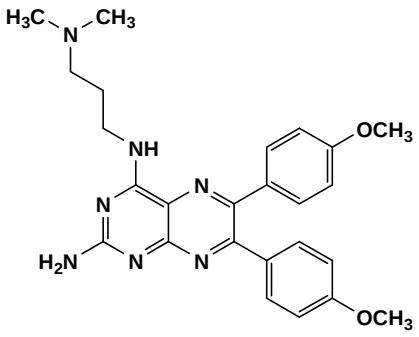
2.66b

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine **2.62b** (0.1 g, 0.24 mmol) was suspended in N^1,N^1-dimethyl-1,3-propanediamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess N^1,N^1-dimethyl-1,3-propanediamine was removed in vacuo (120 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.66b** as a yellow solid (71 mg, 0.18 mmol, 75%; mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.77 (2H, q, J 6.7, HNCH$_2$CH_2CH$_2$), 2.15 (6H, s, CH_3NCH_3), 2.34 (2H, t, J 6.6, HNCH$_2$CH$_2$CH_2), 3.54 (2H, q, J 6.7, HNCH_2CH$_2$CH$_2$), 6.71 (2H, s, NH_2), 7.28-7.41 (10H, m, ArH), 8.58 (1H, t, J 5.6, HNCH$_2$CH$_2$CH$_2$). ^{*13}C NMR (DMSO-d_6) δ 25.94 (HNCH$_2$$CH_2CH_2$), 45.15 ($CH_3NCH_3$), 57.60 (HNCH$_2CH_2$$CH_2$), 121.06 (HNC$C$C), 127.82 (2C, Ar$C$H), 127.89 (Ar$C$H), 127.96 (2C, Ar$C$H), 128.76 (Ar$C$H), 129.48 (4C, Ar$C$H), 138.55, 138.91 (Ar$C$), 144.41, 154.09 (Ph$C$N), 156.89 (HNCC$C$), 160.25 (H$_2NC$), 163.71 (HN$C$CC). IR (KBr) 3379, 3162, 2942, 2813 (NCH$_3$), 2789, 2763 (NCH$_3$), 1639, 1582, 1438, 1337, 1219, 1037, 977, 822 cm^{-1} HRMS (ESI) found 400.2234, $C_{23}H_{25}N_7$ requires 400.2244 (MH$^+$).

*The peak for HNCH$_2$CH$_2$CH$_2$ overlapped with the peaks of DMSO (38.88-40.13).

N^4-[3'-(dimethylamino)propyl]-6,7-bis(4''-methoxyphenyl)-2,4-pteridinediamine



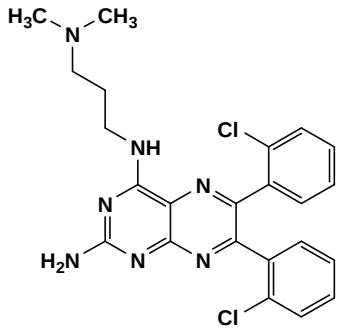
2.66c

4-(Benzylsulfanyl)-6,7-bis(4'-methoxyphenyl)-2-pteridinamine **2.62c** (0.1 g, 0.21 mmol) was suspended in N^1,N^1-dimethyl-1,3-propanediamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess N^1,N^1-dimethyl-1,3-propanediamine was removed in vacuo (120 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:n-hexane = 2:1 to methanol:triethylamine = 99:1) to afford the required product **2.66c** as an orange solid (52 mg, 0.11 mmol, 52%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 1.76 (2H, q, J 6.6, HNCH$_2$CH_2CH$_2$), 2.15 (6H, s, CH_3NCH_3), 2.33 (2H, t, J 6.6, HNCH$_2$CH$_2$CH_2), 3.53 (2H, q, J 6.6, HNCH_2CH$_2$CH$_2$), 3.75, 3.76 (3H × 2, s, OCH_3), 6.63 (2H, s, NH_2), 6.86-6.90 , 7.35-7.38 (4H × 2, m, ArH), 8.48 (1H, t, J 5.6, HNCH$_2$CH$_2$CH$_2$). ^{*13}C NMR (DMSO-d_6) δ 26.02 (HNCH$_2$$CH_2CH_2$), 45.20 ($CH_3NCH_3$), 55.11, 55.12 (2C, OC$H_3$), 57.64 (HNCH$_2CH_2$$CH_2$), 113.36, 113.45 (2C × 2, ArCH), 120.45 (HNCCC), 130.71, 130.95 (2C × 2, ArCH), 131.16, 131.28 (ArC), 144.17, 153.78 (OPhCN), 156.25 (HNCCC), 159.04, 159.74 (ArCOMe), 160.19 (H$_2$NC), 163.04 (HNCCC). IR (KBr) 3424, 2934, 2824 (NCH$_3$), 2774 (NCH$_3$), 1592, 1513, 1436, 1346, 1294, 150, 1175, 1033, 831 cm^{-1}. HRMS (ESI) found 460.2446, $C_{25}H_{29}N_7O_2$ requires 460.2456 (MH$^+$). Anal. Calcd for $C_{25}H_{29}N_7O_2$·1.2H$_2$O: C, 62.4; H, 6.6; N, 20.4. Found: C, 62.0; H, 6.0; N, 20.3.

*The peak for HNCH_2CH$_2$CH$_2$ overlapped with the peaks of DMSO (38.88-40.13).

6,7-Bis(2'-chlorophenyl)-N^4-[3''-(dimethylamino)propyl]-2,4-pteridinediamine



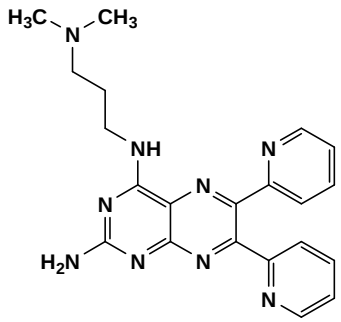
2.66d

4-(Benzylsulfanyl)-6,7-bis(2'-chlorophenyl)-2-pteridinamine **2.62d** (0.13 g, 0.26 mmol) was suspended in N^1,N^1-dimethyl-1,3-propanediamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess N^1,N^1-dimethyl-1,3-propanediamine was removed in vacuo (120 mmHg) and the residual solid was washed with diethyl ether to afford the required product **2.66d** as a yellow solid (55 mg, 0.12 mmol, 46%; mp 235-237 °C).

^{1}H NMR (DMSO-d_6) δ 1.74 (2H, q, *J* 6.8, HNCH$_2$C*H*$_2$CH$_2$), 2.09 (6H, s, C*H*$_3$NC*H*$_3$), 2.29 (2H, t, *J* 6.8, HNCH$_2$CH$_2$C*H*$_2$), 3.50 (2H, q, *J* 6.8, HNC*H*$_2$CH$_2$CH$_2$), 6.83 (2H, s, N*H*$_2$), 7.25-7.43 (8H, m, Ar*H*), 8.57 (1H, t, *J* 5.6, *H*NCH$_2$CH$_2$CH$_2$). ^{13}C NMR (DMSO-d_6) δ 25.97 (HNCH$_2$*C*H$_2$CH$_2$), 39.40 (HN*C*H$_2$CH$_2$CH$_2$), 45.02 (*C*H$_3$N*C*H$_3$), 57.25 (HNCH$_2$CH$_2$*C*H$_2$), 121.65 (HNC*C*C), 126.48 (2C, Ar*C*H), 129.02, 129.18, 130.08, 130.28, 131.12 (Ar*C*H), 131.73 (Ar*C*), 131.94 (Ar*C*H), 132.56 (Ar*C*), 136.73, 136.87 (Ar*C*Cl), 143.35, 154.54 (ClPh*C*N), 156.27 (HNCC*C*), 160.21 (H$_2$N*C*), 163.52 (HN*C*CC). IR (KBr) 3374, 3192, 2942, 2813 (NCH$_3$), 2716 (NCH$_3$), 1646, 1595, 1573, 1464, 1435, 1345, 1187, 1110, 1048, 768, 742 cm^{-1}. HRMS (ESI) found 468.1453, $C_{23}H_{23}N_7{}^{35}Cl_2$ requires 468.1465 (MH^+). Anal. Calcd for $C_{23}H_{23}N_7Cl_2\cdot 0.5H_2O$: C, 57.9; H, 5.1; N, 20.5; Cl, 14.9. Found: C, 58.2; H, 5.2; [*]N, 21.6; Cl, 14.1.

[*]A satisfactory N and Cl analysis could not be obtained due to the difficulty of complete burning even when aided by WO_3.

N^4-[3'-(Dimethylamino)propyl]-6,7-di(2''-pyridinyl)-2,4-pteridinediamine

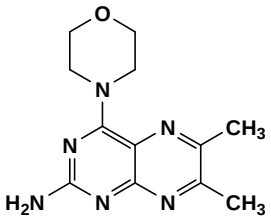


2.66e

4-(Benzylsulfanyl)-6,7-di(2'-pyridinyl)-2-pteridinamine **2.62e** (0.15 g, 0.35 mmol) was suspended in N^1,N^1-dimethyl-1,3-propanediamine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess N^1,N^1-dimethyl-1,3-propanediamine was removed in vacuo (120 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.66e** as a yellow solid (112 mg, 0.28 mmol, 80%, mp 251-253 °C).

^{1}H NMR (DMSO-d_6) δ 1.79 (2H, q, J 6.6, HNCH$_2$CH_2CH$_2$), 2.17 (6H, s, CH_3NCH_3), 2.36 (2H, t, J 6.6, HNCH$_2$CH$_2$CH_2), 3.55 (2H, q, J 6.6, HNCH_2CH$_2$CH$_2$), 6.82 (2H, s, NH_2), 7.23-7.33 (2H, m, ArH), 7.81-7.99 (4H, m, ArH), 8.16-8.24 (2H, m, ArH), 8.65 (1H, t, J 5.6, HNCH$_2$CH$_2$CH$_2$). ^{13}C NMR (DMSO-d_6) δ 25.96 (HNCH$_2$$CH_2CH_2$), 39.64 (HN$CH_2CH_2CH_2$), 44.89 ($CH_3NCH_3$), 57.51 (HNCH$_2CH_2$$CH_2$), 122.47, 123.18, 123.44, 123.69, 136.33, 136.58 (ArCH), 144.00 (PyCN), 147.74, 147.82 (ArCH), 154.28 (PyCN), 156.58, 156.76 (ArC), 157.29 (HNCCC), 160.17 (H$_2$NC), 163.54 (HNCCC). IR (KBr) 3486, 3123, 2853, 2824 (NCH$_3$), 2774 (NCH$_3$), 1599, 1562, 1466, 1446, 1414, 1350, 1144, 1116, 809, 738, 616, 463 cm^{-1}. HRMS (ESI) found 402.2143, $C_{21}H_{23}N_9$ requires 402.2149 (MH$^+$).

6,7-Dimethyl-4-(4'-morpholinyl)-2-pteridinamine



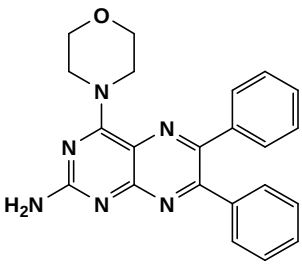
2.67a

4-(Benzylsulfanyl)-6,7-dimethyl-2-pteridinamine **2.62a** (0.1 g, 0.34 mmol) was suspended in morpholine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess morpholine was removed in vacuo (75 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.67a** as a yellow solid (40 mg, 0.15 mmol, 44%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 2.46 (3H, s, *CH_3), 3.71 (2H × 2, t, J 3.9, NCH_2CH$_2$O), 4.25 (2H × 2, brs, NCH$_2$CH_2O), 6.52 (2H, s, NH_2). ^{13}C NMR (DMSO-d_6) δ 21.67, 22.71 (*C*H$_3$), 42.97 (N*C*H$_2$CH$_2$O), 66.24 (NCH$_2$*C*H$_2$O), 121.35 (NC*C*C), 143.72, 155.75 (N*C*CH$_3$), 155.75 (NCC*C*), 160.83 (H$_2$N*C*), 166.35 (N*C*CC). IR (KBr) 3422, 1642, 1556, 1388, 1327, 1441, 1141(O-CH$_2$), 1113 (O-CH$_2$), 1022, 876, 636, 616 cm^{-1}. HRMS (ESI) found 261.1452, $C_{12}H_{16}N_6O_1$ requires 261.1458 (MH$^+$).

*The other CH_3 peak overlapped with the peaks of DMSO.

4-(4'-Morpholinyl)-6,7-diphenyl-2-pteridinamine



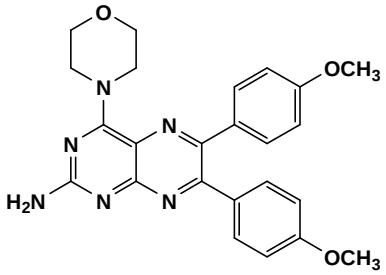
2.67b

4-(Benzylsulfanyl)-6,7-diphenyl-2-pteridinamine **2.62b** (0.15 g, 0.36 mmol) was suspended in morpholine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess morpholine was removed in vacuo (75 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.67b** as a yellow solid (107 mg, 0.28 mmol, 78%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 3.75 (2H × 2, t, *J* 3.9, NC*H*$_2$CH$_2$O), 4.31 (2H × 2, brs, NCH$_2$C*H*$_2$O), 6.78 (2H, brs, N*H*$_2$). 7.26-7.45 (10H, m, Ar*H*). ^{*13}C NMR (DMSO-d_6) δ 66.27 (NCH$_2$*C*H$_2$O), 122.25 (NC*C*C), 128.00 (Ar*C*H), 128. 05, 128.13 (2C × 2, Ar*C*H), 129.05 (Ar*C*H), 129.16, 129.50 (2C × 2, Ar*C*H), 138.43, 13857 (Ar*C*), 143.04, 155.55 (Ph*C*N), 155.78 (NCC*C*), 159.06 (H$_2$N*C*), 161.99 (N*C*CC). IR (KBr) 3495, 3360, 2951, 1622, 1567, 1526, 1453, 1427, 1340, 1269, 1240, 1144 (O-CH$_2$), 1104 (O-CH$_2$), 870, 694, 636, 619 cm^{-1}. HRMS (ESI) found 385.1763, $C_{22}H_{20}N_6O_1$ requires 385.1771 (MH^+).

*The peak of NC*H*$_2$CH$_2$O overlapped with the peaks of DMSO (38.88-40.13).

6,7-Bis(4'-methoxyphenyl)-4-(4''-morpholinyl)-2-pteridinylamine



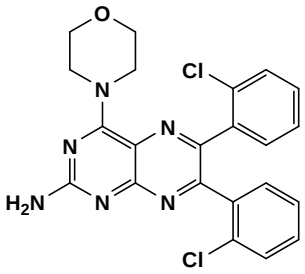
2.67c

4-(Benzylsulfanyl)-6,7-bis(4'-methoxyphenyl)-2-pteridinamine **2.62c** (0.15 g, 0.31 mmol) was suspended in morpholine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess morpholine was removed in vacuo (75 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.67c** as a yellow solid (60 mg, 0.13 mmol, 42%, mp 217-219 °C).

^{1}H NMR (DMSO-d_6) δ 3.73 (2H × 2, m, NCH_2CH$_2$O), 3.75, 3.76 (3H, s, OCH3), 4.29 (2H × 2, brs, NCH$_2$CH_2O), 6.69 (2H, brs, NH_2). 6.85-6.91 (4H, m, Ar*H*), 7.28-7.31, 7.40-7.43 (2H × 2, m, Ar*H*). ^{*13}C NMR (DMSO-d_6) δ 55.09, 55.15 (2C, O*C*H$_3$), 66.27 (NCH$_2$*C*H$_2$O), 113.54, 113.67 (2C × 2, Ar*C*H), 121.59 (NC*C*C), 130.38 (2C, Ar*C*H), 130.72 (Ar*C*), 131.00 (2C, Ar*C*H), 131.17 (Ar*C*), 142.78, 155.05 (OPh*C*N), 155.25 (NCC*C*), 159.10 (H$_2$N*C*), 159.57, 160.00 (Ar*C*OMe), 161.79 (N*C*CC). IR (KBr) 3437, 1609, 1561, 1437, 1338, 1297, 1252, 1176, 1138 (O-CH$_2$), 1113 (O-CH$_2$), 1026, 620 cm^{-1}. HRMS (ESI) found 445.1975, $C_{24}H_{24}N_6O_3$ requires 445.1983 (MH$^+$). Anal. Calcd for $C_{24}H_{24}N_6O_3 \cdot 0.1H_2O$: C, 62.33; H, 5.67; N, 18.17. Found: C, 61.90; H, 5.06; N, 18.91.

*The peak of NCH_2CH$_2$O overlapped with the peaks of DMSO (38.88-40.13).

6,7-Bis(2'-chlorophenyl)-4-(4''-morpholinyl)-2-pteridinylamine



2.67d

4-(Benzylsulfanyl)-6,7-bis(2'-chlorophenyl)-2-pteridinamine **2.62d** (0.15 g, 0.2 mmol) was suspended in morpholine (1 ml) in a microwave tube. The mixture was irradiated at 100 °C for 1 hour. Excess morpholine was removed in vacuo (75 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.67d** as a yellow solid (57 mg, 0.12 mmol, 60%, mp 253-255 °C).

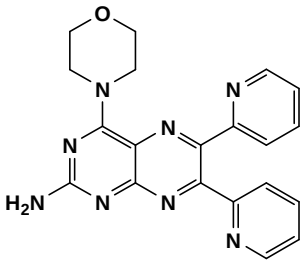
^{1}H NMR (DMSO-d_6) δ 3.71 (2H × 2, m, NCH_2CH$_2$O), 4.31 (2H × 2, brs, NCH$_2$CH_2O), 6.89 (2H, brs, NH_2). 7.17-7.45 (8H, m, Ar*H*). ^{*13}C NMR (DMSO-d_6) δ 66.19 (NCH$_2$*C*H$_2$O), 122.94 (NC*C*C), 126.50, 126.73, 129.19, 129.32, 130.03, 130.44, 131.51(Ar*C*H), 131.61 (Ar*C*), 131.81 (Ar*C*H), 132.43 (Ar*C*), 136.23, 136.74

(Ar*C*Cl), 141.62, 155.52 (ClPh*C*N), 156.06 (NCC*C*), 159.43 (H_2NC), 162.23 (N*C*CC). IR (KBr) 3511, 3025, 1631, 1560, 1522, 1434, 1419, 1346, 1192, 1111 (O-CH_2), 1025, 761, 741 cm^{-1}. HRMS (ESI) found 453.0985, $C_{22}H_{18}N_6O_1{}^{35}Cl_2$ requires 453.0992 (MH^+). Anal. Calcd for $C_{22}H_{18}N_6O_1Cl_2 \cdot 0.5H_2O$: C, 57.2; H, 4.1; N, 18.2. Found: C, 57.5; H, 3.8; **N, 16.7.

*The peak of NCH_2CH_2O overlapped with the peaks of DMSO (38.88-40.13).

**A satisfactory N analysis could not be obtained due to the difficulty of complete burning even when aided by WO_3.

4-(4'-Morpholinyl)-6,7-di(2''-pyridinyl)-2-pteridinamine



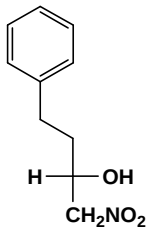
2.67e

4-(Benzylsulfanyl)-6,7-di(2'-pyridinyl)-2-pteridinamine **2.62e** (0.15 g, 0.35 mmol) was suspended in morpholine (1 ml) in a microwave tube. The mixture was irradiated at 100 ˚C for 1 hr. Excess morpholine was removed in vacuo (75 mmHg) and the residual solid was washed by diethyl ether to afford the required product **2.67e** as a yellow solid (103 mg, 0.27 mmol, 77%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 3.77 (2H × 2, m, NCH_2CH$_2$O), 4.33 (2H × 2, brs, NCH$_2$CH_2O), 6.87 (2H, brs, NH_2). 7.22-7.35 (2H, m, Ar*H*), 7.82-7.94 (4H, m, Ar*H*), 8.17-8.24 (2H, m, Ar*H*). *^{13}C NMR (DMSO-d_6) δ 66.25 (NCH$_2$*C*H$_2$O), 122.10 (NC*C*C), 122.50, 122.92, 123.40, 123.69 (Ar*C*H), 136.71 (2C, Ar*C*H), 142.76 (Py*C*N), 147.90 (2C, Ar*C*H), 155.58 (Py*C*N), 155.77 (NCC*C*), 156.73, 156.86 (Ar*C*), 159.55 (H_2NC), 162.24 (NCC*C*). IR (KBr) 3469, 1632, 1562, 1523, 1420, 1342, 1144 (O-CH_2), 1109 (O-CH_2), 1033, 872, 793, 619 cm^{-1}. HRMS (ESI) found 387.1670, $C_{20}H_{18}N_8O_1$ requires 387.1676 (MH^+).

*The peak of NCH_2CH_2O overlapped with the peaks of DMSO (38.88-40.13).

1-Nitro-4-phenyl-2-butanol[116]

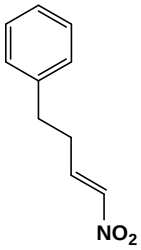


2.75

To a stirred mixture of nitromethane (3.7 g, 3.3 ml, 60 mmol), ethanol (2 ml), and 10 N aqueous NaOH (0.1 ml) was added 3-phenylpropanal (6.7 g, 6.6 ml, 50 mmol) at room temperature. After two-thirds of the propanal was added, 10 N aqueous NaOH (0.1 ml) and water (0.4 ml) were added, then the remainder of the propanal was added. The resulting mixture was stirred at 38 °C for 30 h and then neutralised with 2 N aqueous HCl. A yellow precipitate was collected, which was washed with *n*-hexane, and dried in vacuo (390 mmHg) to give the product **2.75** as a yellow solid (9.1 g, 47 mmol, 94%; mp 75-77 °C)

^{1}H NMR ($CDCl_3$) δ 1.80-1.98 (2H, m, C*H*$_2$$CH_2$Ph), 2.77-2.84 (2H, m, CH_2C*H*$_2$Ph), 2.88-2.95 (1H, m, CH_2C*H*OH), 4.33-4.39 (1H, m, CH_2CHO*H*), 4.44-4.46 (2H, m, C*H*$_2$$NO_2$), 7.25-7.38 (5H, m, *P*h). ^{13}C NMR ($CDCl_3$) δ 31.28 (PhCH_2*C*H_2), 35.09 (Ph*C*H_2CH_2) 67.76 (CH_2*C*HOH), 80.53 (*C*H_2NO_2), 126.29 (1C, Ar*C*H), 128.37, 128.61 (4C, Ar*C*H), 140.58 (1C, Ar*C*). IR (KBr) 3360, 3028, 2947, 1549 (CNO_2), 1454, 1384, 1206, 1102, 881, 758 cm^{-1}.

[(3*E*)-4-Nitro-3-butenyl]benzene[116]

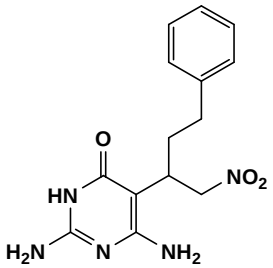


2.76

To a solution of 1-nitro-4-phenyl-2-butanol **2.75** (2.94 g, 15 mmol) in methylene chloride (20 ml) at 0 °C was added methanesulfonyl chloride (1.7 g, 15 mmol) followed by addition of triethyl amine (3.02 g, 30 mmol). The mixture was warmed to room temperature, and stirred for 20 min, then poured into water (15 ml) and extracted with methylene chloride (20 ml). The organic extract was washed with aqueous saturated sodium bicarbonate (20 ml × 3) then dried with anhydrous magnesium sulfate and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:9) to afford the product **2.76** as a yellow oil which was stored at –20 °C (1.45 g, 8.2 mmol, 55%).

^{1}H NMR ($CDCl_3$) δ 2.65 (2H, dq, *J* 7.7, 1.4, $PhCH_2CH_2$), 2.90 (2H, t, *J* 7.7, $PhCH_2CH_2$), 7.02 (1H, dt, *J* 13.4, 1.4, $CH{=}CHNO_2$), 7.25-7.42 (6H, m, $CH{=}CHNO_2$ and *P*h). ^{13}C ($CDCl_3$) δ 30.23 (CH_2CH_2Ph), 34.08 (CH_2CH_2Ph), 126.77 (1C, ArCH), 128.44, 128.87 (4C, ArCH), 139.75 ($CH{=}CHNO_2$), 140.16 (1C, ArC), 141.55 ($CH{=}CHNO_2$). IR (neat) 3028, 2929, 1953, 1734, 1648, 1523 (CNO_2), 1454, 1351, 1089, 953 (CH=CH), 840, 751, 700 cm^{-1}.

2,6-Diamino-5-[1'-(nitromethyl)-3'-phenylpropyl]-4(3*H*)-pyrimidinone[116]

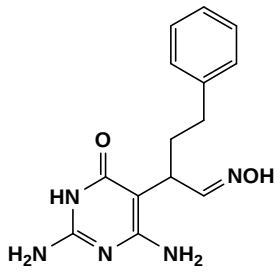


2.78

To a mixture of [(3*E*)-4-Nitro-3-butenyl]benzene **2.76** (1.27 g, 7.2 mmol) in a mixture of water (15 ml) and ethyl acetate (15 ml) at room temperature was added 2,6-diamino-4(3*H*)-pyrimidinone **2.77** (0.9 g, 7.2 mmol). The resulting mixture was stirred in an oil bath at 90 °C for 24 hours. The organic layer was separated, washed with brine (30 ml), dried, and concentrated. The resulting solid was dried in vacuo (120 mmHg) to give the product **2.78** as a yellow solid (2.06 g, 6.8 mmol, 94%: mp 143-145 °C).

^{1}H NMR (DMSO-d_6) δ 1.67-1.76 (1H, m, CH_2CH$_2$Ph), 2.08-2.18 (1H, m, CH_2CH$_2$Ph), 2.43-2.64 (2H, m, CH$_2$CH_2Ph), 3.42 (1H, brs, CHCH$_2$CH$_2$Ph), 4.77-5.07 (2H, m, CH_2NO$_2$), 5.95 (2H, brs, C=CNH_2), 6.08 (2H, brs, H_2NCN$_2$), 7.13-7.41 (5H, m, ArH), 9.85 (1H, brs, OCNH). ^{13}C NMR (DMSO-d_6) δ 31.83 (*C*HCH$_2$CH$_2$), 32.98 (CH*C*H$_2$CH$_2$), 35.09 (CHCH$_2$*C*H$_2$), 77.65 (*C*H$_2$NO$_2$), 84.08 (*C*=CN$_2$), 125.55 (1C, Ar*C*), 127.99, 128.22 (4C, Ar*C*H), 142.34 (1C, Ar*C*), 153.55 (C=*C*N$_2$), 161.93 (*C*N$_3$), 162.81 (*C*=O). IR (KBr) 3471, 3401, 3183, 2859, 1625, 1595, 1536 (CNO$_2$), 1493, 1450, 1378, 697 cm^{-1}.

(1*E*/*Z*)-2-(2',4'-Diamino-6'-oxo-1',6'-dihydro-5'-pyrimidinyl)-4-phenylbutanal oxime

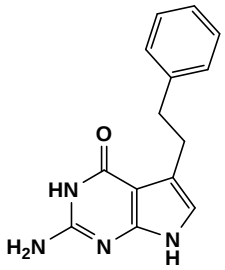


2.79

To a suspension of 2,6-diamino-5-[1'-(nitromethyl)-3'-phenylpropyl]-4(3*H*)-pyrimidinone **2.78** (0.82 g, 2.7 mmol) and tin(II) chloride (0.77 g, 4.0 mmol) in tetrahydrofuran (70 ml) was added benzenthiol (1.2 ml) and triethylamine (1.8 ml). The reaction mixture was stirred at room temperature for 1 hour. The remaining tin(II) chloride was removed by filtration, and the liquid portion was concentrated in vacuo (400 mmHg). The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:1 to ethyl acetate:methanol = 1:1) to afford a white solid **2.79** (0.45 g, 1.56 mmol, 58%, mp > 250 °C) as an *E*/*Z* mixture (3:1 by ^{1}H NMR).

^{1}H NMR (*E* isomer, DMSO-d_6) δ 1.94-2.01 (2H, m, CH_2CH$_2$Ph), 2.42-2.48 (2H, m, CH$_2$CH_2Ph), 3.45 (1H, q, *J* 7.4, C*H*CH$_2$CH$_2$Ph), 5.77 (2H, brs, C=CNH_2), 6.03 (2H, brs, H_2NCN$_2$), 7.13-7.27 (5H, m, Ar*H*), 7.58 (1H, d, *J* 6.9, C*H*=NOH), 9.83 (1H, brs, OCN*H*), 10.60 (1H, s, CH=NO*H*). ^{13}C NMR (*E* isomer, DMSO-d_6) δ 35.47 (*C*HCH$_2$CH$_2$), 32.45 (CH*C*H$_2$CH$_2$), 33.37 (CHCH$_2$*C*H$_2$), 152.10 (*C*HNO), 86.45 (*C*=CN$_2$), 125.45 (1C, Ar*C*), 128.07, 128.17 (4C, Ar*C*), 153.51 (C=*C*N$_2$), 161.81 (*C*N$_3$), 161.99 (C=O). IR (KBr) 3371, 3104, 1621, 1596, 1503, 1430, 1374, 1020, 987, 787, 694, 559 cm^{-1}. HRMS (FAB) found 288.1462, $C_{14}H_{17}N_4O_2$ requires 288.1460 (MH$^+$).

2-Amino-5-(2'-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



2.71

(a) Cyclisation with Dowex-50 H^+

(1*E*/*Z*)-2-(2',4'-Diamino-6'-oxo-1',6'-dihydro-5'-pyrimidinyl)-4-phenylbutanal oxime **2.79** (0.2 g, 0.7 mmol) was heated for 15 hours at reflux with Dowex-50 (H^+ form, 0.33 g) in water (25 ml). The reaction mixture was then diluted with methanol (50 ml), and the Dowex resin filtered. The methanol was evaporated under vacuum and the precipitated was filtered and washed with water (15 ml) to give the product **2.71** as a brown solid (0.16 g, 0.63 mmol, 90%). Spectroscopic data are detailed below.

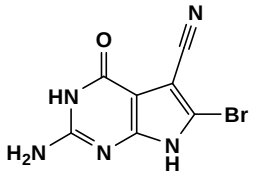
(b) Classical Nef reaction method[116]

To an aqueous solution of sodium hydroxide (0.2 g, 5.0 mmol) in water (5 ml) was added 2,6-diamino-5-[1'-(nitromethyl)-3'-phenylpropyl]-4(3*H*)-pyrimidinone **2.78** (0.25 g, 0.82 mmol) at room temperature. The mixture was stirred for 2 hours, and then it was slowly added to an aqueous solution of sulfuric acid (97%, 0.69 g, 7.0 mmol) in water (5 ml) at 0 °C. The resulting mixture was stirred at 0 °C for one hour and at room temperature for one hour. The colour of the mixture changed to grey. Concentrated ammonium acetate was added at 0 °C to adjust the pH to 7. The precipitated solid was collected and purified by column chromatography (silica gel/ethyl acetate:methanol = 9:1) to give the product **2.71** as a dark brown solid (0.09 g, 0.35 mmol, 43%; mp 155-157 °C).

^{1}H NMR (DMSO-d_6) δ 2.81-2.85 (2H, m, CH_2CH$_2$Ph), 2.88-2.92 (2H, m, CH$_2$CH_2Ph), 5.99 (2H, s, NH_2), 6.31 (1H, d, *J* 1.5, C=C*H*NH), 7.13-7.28 (5H, m, Ar*H*), 10.16 (1H, brs, C=CHN*H*), 10.59 (1H, s, OCN*H*). ^{13}C NMR (DMSO-d_6) δ

28.34 (ArCH$_2$*C*H$_2$), 36.37 (Ar*C*H$_2$CH$_2$), 98.92 (*C*=CN$_2$), 113.50(*C*=CHNH), 118.07 (C=*C*HNH), 125.60 (1C, Ar*C*H), 128.20, 128.37 (4C, ArCH), 142.51 (1C, Ar*C*), 151.37 (C=*C*N$_2$), 152.27 (*C*N$_3$), 159.48 (*C*=O). IR (KBr) 3340, 3925, 1631, 1433, 1343, 1137, 786, 698, 620 cm^{-1}.

2-Amino-6-bromo-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile

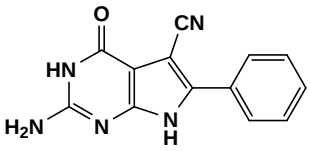


2.68

To a suspension of 2-amino-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **2.80** (1.0 g, 5.7 mmol) in glacial acetic acid (50 ml) was added bromine (0.43 ml, 8.4 mmol). The mixture was stirred at room temperature for 20 hours then heated to 60 °C for one day. The resulting solid was filtered and washed with water (100 ml), *n*-hexane (100 ml), and dried in vacuo (120 mmHg) to give the product **2.68** as green solid (0.84 g, 3.3 mmol, 58%, mp > 250, lit.[117] > 300 °C).

^{1}H NMR (DMSO-d_6) δ 6.53 (2H, brs, NH_2), 10.78 (1H, s, *H*NCBr), 12.82 (1H, brs, OCN*H*). ^{13}C NMR (DMSO-d_6) δ 88.30 (*C*C≡N), 99.91 (OC*C*), 110.81(*C*Br), 114.53 (C*C*≡N), 151.92 (*C*NHCBr), 153.97 (H$_2$N*C*), 156.35 (O*C*NH). IR (KBr) 3104, 2235 (C≡N), 1684, 1535, 1505, 1415, 1359, 1233, 876, 773, 630, 586 cm^{-1}.

2-Amino-4-oxo-6-phenyl-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile

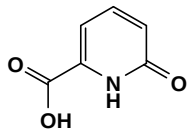


2.72

2-Propanol (5 ml) was mixed with water (2.5 ml) under nitrogen. Into this stirred solution were added 2-Amino-6-bromo-4-oxo-4,7-dihydro-3*H*-pyrrolo[2,3-*d*]pyrimidine-5-carbonitrile **2.68** (0.1 g, 0.39 mmol) and $PdCl_2(PPh_3)_2$ (14 mg, 0.02 mmol). After five minutes, potassium trifluoro(phenyl)borate (86 mg, 0.47 mmol) and *t*-$BuNH_2$ 86 mg (1.17 mmol) were added. The resulting mixture was stirred in an oil bath at 85 °C for four days, methanol (20 ml) was added, and concentrated. The residue was purified by column chromatography (silica gel/methanol:ethyl acetate = 1:1) to give the product **2.72** as a yellow solid (0.9 g, 0.36 mmol, 92%; mp > 250 °C, lit.[117] > 230 °C).

^{1}H NMR (DMSO-d_6) δ 6.50 (2H, brs, NH_2), 7.42 (1H, t, *J* 7.3, Ar*H*), 7.49 (2H, t, *J* 7.3, Ar*H*), 7.82 (2H, d, *J* 7.3, ArH), 10.73 (1H, brs, C=CN*H*), 12.38 (1H, brs, O=CN*H*). ^{13}C NMR (DMSO-d_6) δ 82.25 (C=*C*Ph), 100.24 (*C*=CN_2 and *C*C≡N), 116.51 (*C*≡N), 126.22, 128.95, 129.29 (5C, ArC), 138.39 (1C, ArC), 152.15 (C=*C*N_2), 154.03 (*C*N_3), 157.32 (*C*=O). IR (KBr) 3421, 2978, 2891, 2694, 2216 (C≡N), 1670, 1595, 1401, 778, 687 cm^{-1}.

6-Oxo-1,6-dihydro-2-pyridinecarboxylic acid



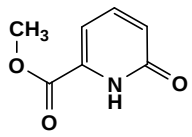
2.86

A solution of 6-chloro-2-pyridinecarboxylic acid **2.87** (1g, 6.3 mmol) in 36% hydrochloric acid (20 ml) was heated at 120-135 °C for 2 days. After cooling, the solvent was removed in vacuo (120 mmHg) and the residue was recrystallised from methanol to afford the required product **2.86** as a white solid. (0.74 g mg, 5.3 mmol, 84%, mp 280-282 °C, decomposition 260-262 °C, lit.[154] 265 °C).

[*1]H NMR (DMSO-d_6) δ 6.61-6.64 (1H, m, HN(CO)C*H*), 6.95-6.97 (1H, m, HN(COOH)C*H*), 7.53-7.57 (1H, m, HN(CO)CHC*H*), 12.42 (1H, brs, COO*H*). ^{13}C NMR (DMSO-d_6) δ 109.93, 123.34 (Ar*C*H), 137.58 (Ar*C*COOH), 139.91 (Ar*C*H), 162.11 (Ar*C*=O), 162.85 (*C*OOH). IR (KBr) 3430, 3091, 1638 (O=CNH), 1541, 1464, 1309, 1273, 1006, 938, 822, 768, 588, 543, 473 cm^{-1}. HRMS (ESI) found 387.1670, $C_{20}H_{18}N_8O_1$ requires 387.1676 (MH^+).

[*]The peak of OCN*H* was not observable.

Methyl 6-oxo-1,6-dihydro-2-pyridinecarboxylate



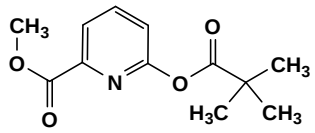
2.84

A solution of 6-oxo-1,6-dihydro-2-pyridinecarboxylic acid **2.86** (0.74g, 5.3 mmol) in methanol (20 ml) with concentrated sulfuric acid (2 ml) was refluxed for one day. Removal of the solvent under pressure gave a yellowish oil, which was neutralised with aqueous sodium bicarbonate, extracted with chloroform (30 ml × 3), and dried over anhydrous magnesium sulfate. Evaporation of solvent gave the required product **2.84** as a white solid. (0.5 g mg, 3.3 mmol, 62%, mp > 108-110 °C, lit.[154] 105 °C).

^{1}H NMR (DMSO-d_6) δ 3.83 (3H, s, CH_3), 6.71-6.73 (1H, m, HN(CO)C*H*), 7.14-7.16 (1H, m, HN(COOMe)C*H*), 7.61-7.65 (1H, m, HN(CO)CHC*H*), 11.52 (1H, s, N*H*). ^{13}C NMR (DMSO-d_6) δ 52.56 (CH3), 112.20, 121.11 (Ar*C*H), 138.89 (Ar*C*COOMe), 139.94 (Ar*C*H), 162.54 (Ar*C*=O), 162.73 (COO). IR (KBr) 3451, 3059, 2939, 1735,

1657 (O=CNH), 1602, 1438, 1291, 1264, 1137, 1008, 895, 829, 763, 577, 556 cm^{-1}.

Methyl 6-[(2',2'-dimethylpropanoyl)oxy]-2-pyridinecarboxylate

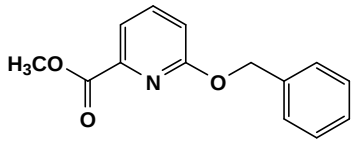


2.91

To a solution of methyl 6-oxo-1,6-dihydro-2-pyridinecarboxylate **2.84** (0.29 g, 1.9 mmol) in dry DMF (7 ml) was added potassium carbonate (0.51 g, 3.7 mmol) under nitrogen at room temperature. The resulting mixture was stirred for 15 minutes and then 2,2-dimethylpropanoyl chloride (0.87 g, 7.2 mmol) was slowly added. The reaction mixture was stirred at 60 °C for one day. The DMF was evaporated in vacuo (12 mmHg), whereafter ethyl acetate (30 ml) was added, and the organic layer was separated, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:2) to give the product **2.91** as a colorless oil (0.24 g, 1.0 mmol, 53%).

^{1}H NMR ($CDCl_3$) δ 1.40 (3H × 3, s, CC*H*$_3$), 4.00 (3H, s, OC*H*$_3$), 7.23 (1H, dd, *J* 8.0, 0.8, Ar*H*), 7.93 (1H, t, *J* 8.0, 7.5, Ar*H*), 8.05 (1H, dd, *J* 7.5, 0.8, Ar*H*). ^{13}C NMR ($CDCl_3$) δ 27.04 (3C, C*C*H$_3$), 39.25 (*C*CH$_3$), 53.03 (O*C*H$_3$), 120.57, 123.31, 140.12, 147.06, 158.10 (Ar*C*), 164.89 (*C*OCH$_3$), 176.61 (O*C*CCH$_3$). IR (neat) 3087, 2972, 2873, 1753 (OC=O), 1722 (OC=O), 1588, 1451, 1434, 1396, 1366, 1319, 1264, 1226, 1102, 1028, 995, 886, 760, 672 cm^{-1}. HRMS (CI) found 238.1080, $C_{12}H_{15}N_1O_4$ requires 238.1079 (MH^+).

Methyl 6-(benzyloxy)-2-pyridinecarboxylate

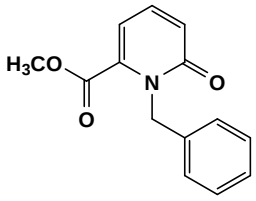


2.95

To a mixture of methyl 6-oxo-1,6-dihydro-2-pyridinecarboxylate **2.84** (3.0 g, 2.0 mmol) in dry DMF (3 ml) was added sodium hydride (96 mg, 4.0 mmol) under nitrogen at room temperature. The resulting mixture was stirred for about ten minutes, and then benzyl bromide (0.23 ml, 2.0 mmol) in tetrahydrofuran (3 ml) was added. The reaction mixture was stirred at room temperature for 15 hours and further four hours at 100 °C then water (20 ml) was added to quench the reaction. The sample was extracted with ethyl acetate (30 ml × 3) and the organic phases were combined, dried, and concentrated. The residue was purified by column chromatography (silica gel/ethyl acetate:*n*-hexane = 1:3 to ethyl acetate 100%) to give the product **2.95** as colorless oil (0.25 g, 1.0 mmol, 50%).

^{1}H NMR ($CDCl_3$) δ 3.99 (3H, s, OC*H*$_3$), 5.48 (2H, s, OC*H*$_2$), 6.97-7.00 (1H, m, Ar*H*), 7.31-7.41 (3H, m, Ar*H*), 7.51-7.53 (2H, m, Ar*H*), 7.68-7.75 (2H, m, Ar*H*). ^{13}C NMR ($CDCl_3$) δ 52.83 (O*C*H$_3$), 68.24 (O*C*H$_2$), 115.77 (Ar*C*H), 119.05 (Ar*C*H), 128.20, 128.65, 128.69 (5C, Ar*C*H), 137.10 (Ar*C*), 139.35 (Ar*C*H), 145.55 (*C*COOMe), 163.58 (CH$_2$O*C*), 165.90 (O=*C*). IR (KBr) 3626, 3534, 3032, 3951, 2597, 1739, 1724, 1595, 1573, 1497, 1452, 1372, 1328, 1270, 1194, 1161, 1139, 1074, 1016, 987, 913, 824, 793, 769, 752, 699, 635, 529 cm^{-1}. HRMS (ESI) found 244.0963, $C_{14}H_{13}N_1O_3$ requires 244.0968 (MH^+).

Methyl 1-benzyl-6-oxo-1,6-dihydro-2-pyridinecarboxylate

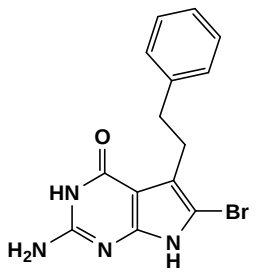


2.96

Through the same reaction procedure for compound **2.84**, the product **2.96** was obtained as colorless oil (80 mg, 0.33 mmol, 16%).

^{1}H NMR ($CDCl_3$) δ 3.74 (3H, s, OCH_3), 5.47 (2H, s, NCH_2), 6.70-6.72, 6.80-6.83 (1H × 2, m, Ar*H*), 7.14-7.36 (6H, m, Ar*H*). ^{13}C NMR ($CDCl_3$) δ 47.22 (N*C*H$_2$), 53.15 (O*C*H$_3$), 111.03 (Ar*C*H), 125.19 (Ar*C*H), 127.61 (Ar*C*H), 127.68, 128.74 (2C × 2, Ar*C*H), 137.46 (Ar*C*), 137.73 (Ar*C*H), 138.38 (*C*COOMe), 162.86 (N*C*=O), 162.97 (O=*C*). IR (KBr) 3453, 3032, 2593, 1732, 1662, 1591, 1544, 1496, 1439, 1270, 1194, 1136, 1079, 1050, 1028, 949, 911, 880, 819, 788, 758, 735, 698, 563, 512 cm^{-1}. HRMS (ESI) found 244.0965, $C_{14}H_{13}N_1O_3$ requires 244.0968 (MH^+).

2-Amino-6-bromo-5-(2'-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one



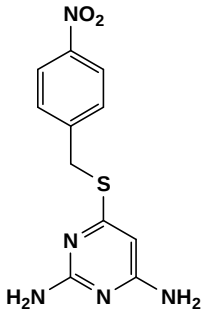
2.98

2-Amino-5-(2'-phenylethyl)-3,7-dihydro-4*H*-pyrrolo[2,3-*d*]pyrimidin-4-one **2.71** (0.05 g, 0.2 mmol) was heated at 50 °C for 1 hour in glacial acetic acid (1 ml).

Bromine (10 µl) was added and the reaction mixture was stirred at room temperature for 20 hours. The acetic acid was evaporated in vacuo (50 mmHg) and the residue was purified by column chromatography (silica gel/ethyl acetate:methanol = 9:1) to afford the required product **2.98** as a dark brown solid (46 mg, 0.14 mmol, 70%, mp > 250 °C).

^{1}H NMR (DMSO-d_6) δ 2.74-2.78 (2H, m, CH_2CH_2Ph), 2.83-2.87 (2H, m, CH_2CH_2Ph), 6.12 (2H, s, NH_2), 7.13-7.27 (5H, m, Ar*H*), 10.27 (1H, s, OCN*H*), 11.42 (1H, C=CBrN*H*). ^{13}C NMR (DMSO-d_6) δ 27.34 (*C*H_2CH_2Ph), 35.94 (CH_2*C*H_2Ph), 97.27 (*C*=CN_2), 99.31 (*C*=CBrNH), 116.53 (C=*C*BrNH), 125.62 (1C, Ar*C*H), 128.11, 128.23 (4C, Ar*C*H), 141.80 (1C, Ar*C*), 151.32 (C=*C*N_2), 152.56 (*C*N_3), 158.05 (*C*=O). IR (KBr) 3493, 3396, 2923, 2857, 1670, 1641, 1604, 1580, 1492, 1432, 1382, 1075, 866, 776, 713, 691, 620 (CBr) cm^{-1}. HRMS (FAB) found 333.0349, $C_{12}H_9{}^{79}BrN_4O$ requires 333.0351 (MH^+).

6-[(4'-Nitrobenzyl)sulfanyl]-2,4-pyrimidinediamine

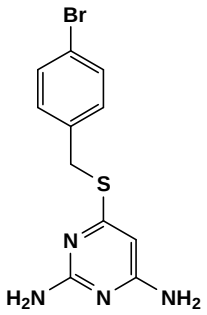


2.59b

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (0.6 g, 2.5 mmol) and sodium hydroxide (0.16 g, 4.0 mmol) in ethanol (15 ml) and water (10 ml) was added 1-(bromomethyl)-4-nitrobenzene (0.6 g, 2.8 mmol). The reaction mixture was stirred in an oil bath at 80°C overnight. The precipitate was collected by filtration, washed with water (10 ml) and *n*-hexane (10 ml), to afford the required product **2.59b** as a yellow solid (0.51 g, 1.84 mmol, 74%; mp 200-202 °C).

^{1}H NMR (DMSO-d_6) δ 4.06 (2H, s, SCH_2), 5.62 (1H, s, CH=CN_2), 6.14 (2H, brs, CH=CNH_2), 6.31 (2H, brs, N_2CNH_2), 7.67 (2H, d, J 8.7, Ph), 8.13 (2H, d, J 8.7, Ph). ^{13}C NMR (DMSO-d_6) δ 31.08 (SCH$_2$), 90.17 (CHCN_2), 123.36, 130.15 (4C, ArC), 146.30, 147.27 (2C, ArC), 162.08 (CN$_3$), 163.50 (CCN$_2$), 164.23 (CSCH$_2$). IR (KBr) 3431, 3308, 3158, 1628, 1559, 1516 (PhNO_2), 1430, 1342, 1157, 904, 789 cm^{-1}. HRMS (EI) found 277.0635, $C_{11}H_{11}N_5O_2S$ requires 277.0633 (M^+).

6-[(4'-Bromobenzyl)sulfanyl]-2,4-pyrimidinediamine

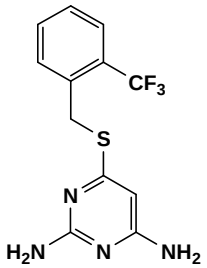


2.59c

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (0.6 g, 2.5 mmol) and sodium hydroxide (0.16 g, 4.0 mmol) in ethanol (15 ml) and water (10 ml) was added 1-bromo-4-(bromomethyl)benzene (0.69 g, 2.75 mmol). The reaction mixture was stirred in an oil bath at 80 °C for 24 hours. The precipitate was collected by filtration, washed with water (10 ml) and *n*-hexane (10 ml), to afford the required product **2.59c** as a white solid (0.34 g, 1.09 mmol, 44%; mp 198-200 °C).

^{1}H NMR (DMSO-d_6) δ 4.27 (2H, s, SCH_2), 5.67 (1H, s, CH=CN_2), 6.39 (2H, brs, CH=CNH_2), 6.61 (2H, brs, N_2CNH_2), 7.36 (2H, d, J 8.4, ArH), 7.48 (2H, d, J 8.4, ArH). ^{13}C NMR (DMSO-d_6) δ 31.59 (SCH$_2$), 90.39 (CCN_2), 120.07 (1C, ArC), 131.11, 131.21 (4C, ArCH), 137.61 (1C, ArC), 160.38 (CN$_3$), 162.69 (CHCN$_2$), 164.19 (CSCH$_2$). IR (KBr) 3312, 3139, 1651, 1554, 1486, 1436, 1158, 1069 (PhBr), 1012, 905, 791 cm^{-1}. HRMS (EI) found 309.9890, $C_{11}H_{11}{}^{79}BrN_4S$ requires 309.9888 (M^+).

6-{[2'-(Trifluoromethyl)benzyl]sulfanyl}-2,4-pyrimidinediamine

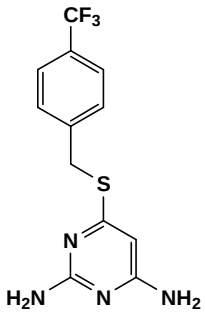


2.59d

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (0.55 g, 2.3 mmol) and sodium hydroxide (0.16 g, 4.0 mmol) in ethanol (15 ml) and water (10 ml) was added 1-(bromomethyl)-2-(trifluoromethyl)benzene (0.6 g, 2.55 mmol). The reaction mixture was stirred at room temperature for 28 hours. The precipitate was collected by filtration, washed with water (10 ml) and *n*-hexane (10 ml), to afford the required product **2.59d** as a light yellow solid (0.30 g, 1.0 mmol, 44%; mp 162-165 °C).

^{1}H NMR (DMSO-d_6) δ 4.47 (2H, s, SCH_2), 5.60 (1H, s, CH=CN_2), 6.11 (2H, brs, CH=CNH_2), 6.27 (2H, brs, N_2CNH_2), 7.45 (1H, t, J 7.6, ArH), 7.61 (1H, t, J 7.5, ArH), 7.69 (1H, d, J 7.8, ArH), 7.74 (1H, d, J 7.7, ArH). ^{13}C NMR (DMSO-d_6) δ 28.20 (SCH$_2$), 89.94 (CCN$_2$), 123.01 (q, $J^1_{C\text{-}F}$ 272, CF_3), 125.73 (ArCH), 126.51 (q, $J^2_{C\text{-}F}$ 29, ArC), 127.69, 131.97, 132.78 (ArCH), 136.84 (ArC), 162.22 (CN$_3$), 163.61 (C=CN$_2$), 164.43 (CSCH$_2$). IR (KBr) 3474, 3300, 3157, 1640, 1558, 1438, 1317 (CF_3), 1179, 1114, 1035, 903, 772 cm^{-1}. HRMS (EI) found 300.0658, $C_{12}H_{11}F_3N_4S$ requires 300.0657 (M^+). Anal. Calcd for $C_{12}H_{11}F_3N_4S\cdot0.2H_2O$: C, 47.4; H, 3.8; N, 18.4; S, 10.6. Found: C, 47.0; H, 2.9; N, 18.7; S, 11.0.

6-{[4'-(Trifluoromethyl)benzyl]sulfanyl}-2,4-pyrimidinediamine

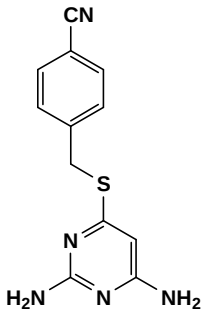


2.59e

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (0.55 g, 2.3 mmol) and sodium hydroxide (0.16 g, 4.0 mmol) in ethanol (15 ml) and water (10 ml) was added 1-(bromomethyl)-4-(trifluoromethyl)benzene (0.6 g, 2.5 mmol). The reaction mixture was stirred at room temperature for 28 hours. The precipitate was collected by filtration, washed with water (10 ml) and *n*-hexane (10 ml), to afford the required product **2.59e** as a white solid (0.48 g, 1.59 mmol, 69%; mp 184-186 °C).

^{1}H NMR (DMSO-d_6) δ 4.36 (2H, s, SCH_2), 5.60 (1H, s, CH=CN_2), 6.02 (2H, brs, CH=CNH_2), 6.19 (2H, brs, N_2CNH_2), 7.61-7.66 (4H, m, ArH). ^{13}C NMR (DMSO-d_6) δ 31.19 (SCH$_2$), 90.06 (CCN_2), 120.20 (q, $J^1_{C\text{-}F}$ 270, CF_3), 125.07 (2C, ArCH), 126.92 (1C, $J^2_{C\text{-}F}$ 32, ArC), 129.65 (2C, ArCH), 143.91 (1C, ArC), 162.43 (CN$_3$), 163.71 (C=CN$_2$), 164.70 (CSCH$_2$). IR (KBr) 3461, 3302, 3175, 1618, 1557, 1374, 1323 (CF_3), 1174, 1129, 1066, 852, 786 cm^{-1}. HRMS (EI) found 300.0656, $C_{12}H_{11}F_3N_4S$ requires 300.0657 (M^+).

4-{[(2',6'-Diamino-4'-pyrimidinyl)sulfanyl]methyl}benzonitrile

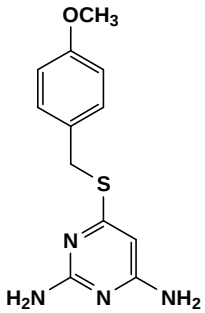


2.59f

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (0.6 g, 2.5 mmol) and sodium hydroxide (0.16 g, 4.0 mmol) in ethanol (15 ml) and water (10 ml) was added 4-(bromomethyl)benzonitrile (0.54 g, 2.75 mmol). The reaction mixture was stirred at room temperature for 19 hours. The precipitate was collected by filtration, washed with water (10 ml) and *n*-hexane (10 ml), to afford the required product **2.59f** as a white solid (0.51 g, 1.98 mmol, 79%; mp 194-196 °C).

^{1}H NMR (DMSO-d_6) δ 4.29 (2H, s, SCH_2), 5.62 (1H, s, CH=CN_2), 6.13 (2H, brs, CH=CNH_2), 6.31 (2H, brs, N_2CNH_2), 7.59 (2H, d, *J* 8.1, *P*h), 7.44 (2H, d, *J* 8.1, *P*h). ^{13}C NMR (DMSO-d_6) δ 31.44 (S*C*H$_2$), 90.20 (*C*CN_2), 109.55 (2C, Ar*C*H), 118.80 (*C*≡N), 129.90 (2C, Ar*C*H), 132.17 (Ar*C*), 144.93 (Ar*C*), 161.93 (*C*N_3), 163.43 (C=*C*N_2), 164.38 (*C*SCH$_2$). IR (KBr) 3433, 3321, 3132, 2224 (C≡N), 1633, 1546, 1429, 1382, 1167, 900, 801 cm^{-1}. HRMS (EI) found 257.0733, $C_{12}H_{11}N_5S$ requires 257.0735 (M^+).

6-[(4'-Methoxybenzyl)sulfanyl]-2,4-pyrimidinediamine



2.59g

To a suspension of 2,6-diamino-4-pyrimidinethiol **2.58** (0.6 g, 2.5 mmol) and sodium hydroxide (0.16 g, 4.0 mmol) in ethanol (15 ml) and water (10 ml) was added 1-(bromomethyl)-4-methoxybenzene (0.6 g, 2.55 mmol). The reaction mixture was stirred at room temperature for 46 hours. The precipitate was collected by filtration, washed with water (10 ml) and *n*-hexane (10 ml), to afford the required product **2.59g** as a white solid (0.44 g, 1.68 mmol, 67%; mp 189-191 °C).

^{1}H NMR (DMSO-d_6) δ 3.72 (3H, s OCH_3), 4.25 (2H, s, SCH_2), 5.81 (1H, s, $CH{=}CN_2$), 6.86 (4H, d, *J* 8.6, *P*h and $CH{=}CNH_2$), 7.11 (2H, brs, N_2CNH_2), 7.32 (2H, d, *J* 8.6, *P*h). ^{13}C NMR (DMSO-d_6) δ 32.63 (SCH_2), 55.06 (OCH_3), 90.89 ($CH{=}CN_2$), 113.9 (2C, Ar*C*H), 128.54 (Ar*C*), 130.16 (2C, Ar*C*H), 158.16 (CN_3), 158.48 (Ar*C*), 161.94 ($C{=}CN_2$), 163.16 ($CSCH_2$). IR (KBr) 3335, 3146, 1656, 1512, 1303, 1249, 1102, 972, 754, 614 cm^{-1}. HRMS found (EI) 262.0889, $C_{12}H_{14}N_4OS$ requires 262.0888 (M^+).

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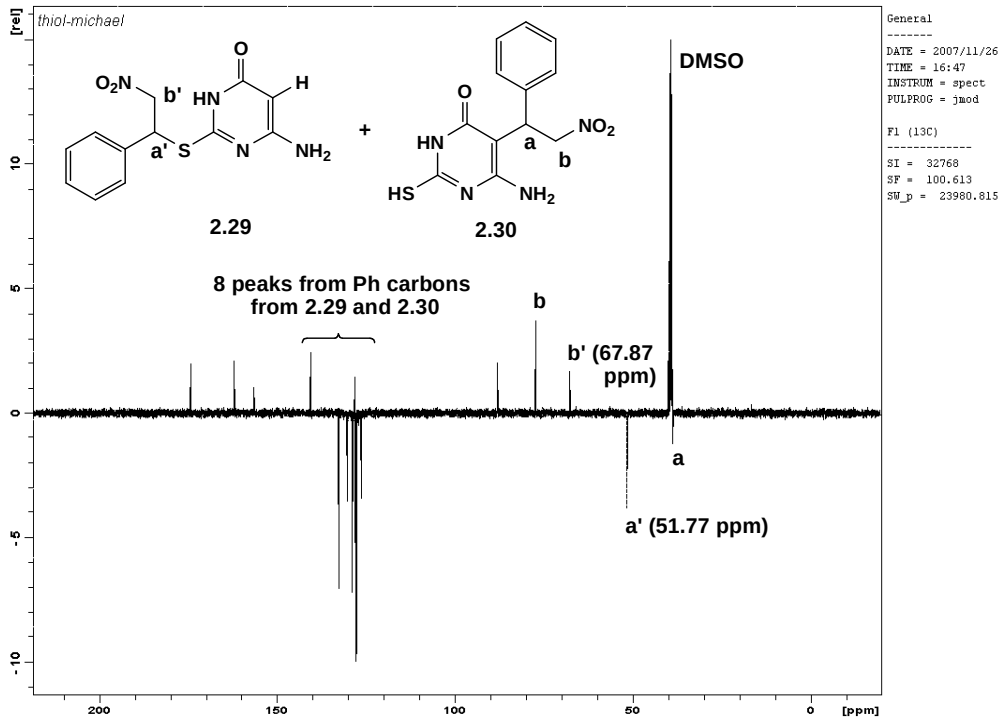
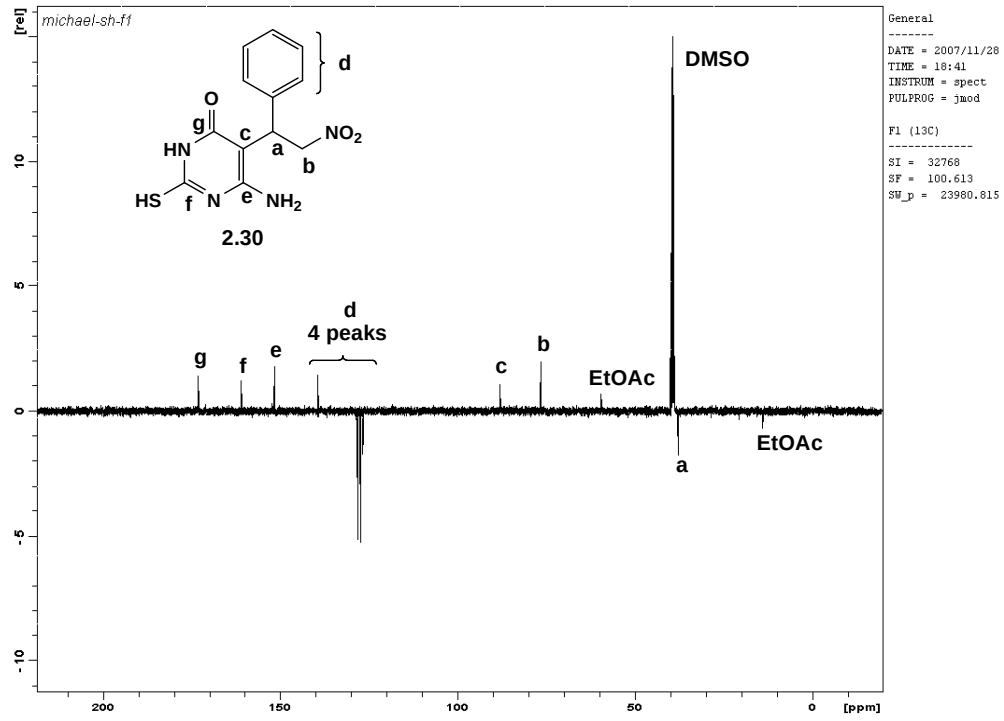
APPENDIX I

^{13}C NMR spectra of compound 2.29 and 2.30

Before purification

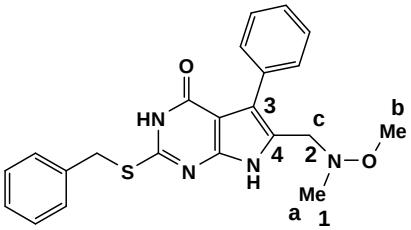


After column purification

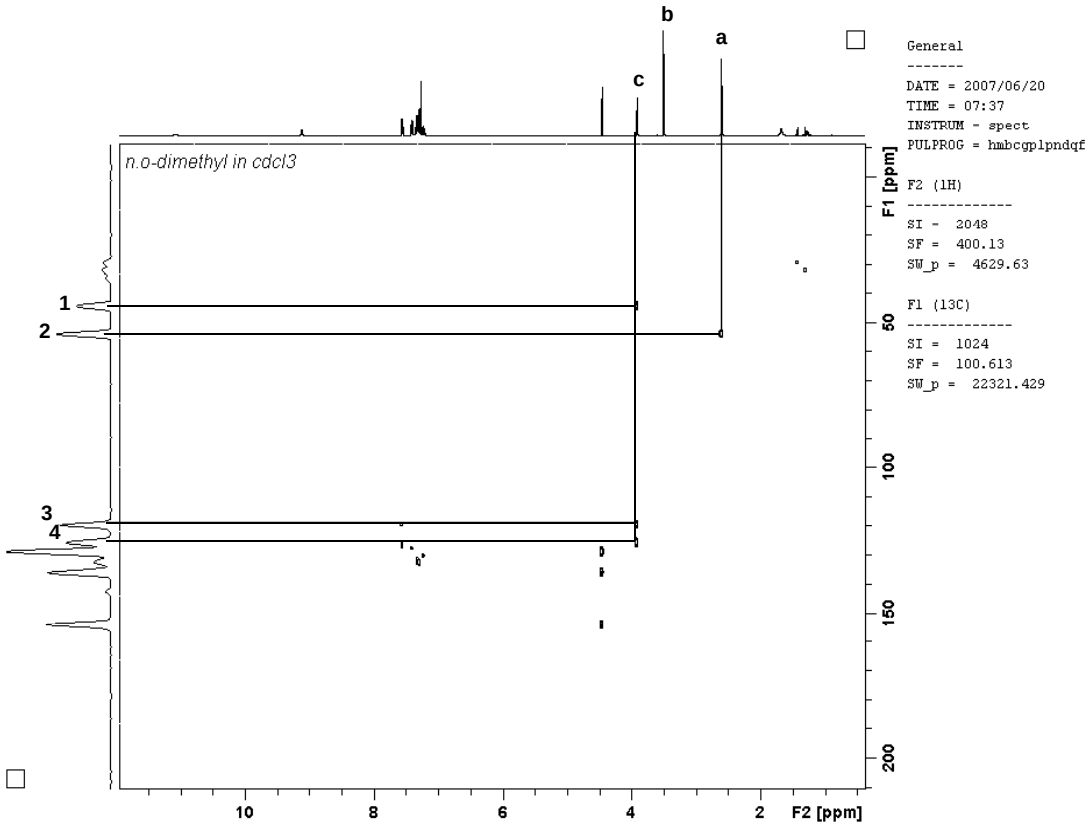


APPENDIX II

HMBC (the H-C long range correlation) data for compound 2.57

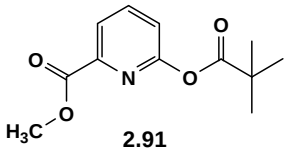


H(a): correlation with C(2)
H(b): no correlation
H(c): correlation with C(1), C(3), C(4)



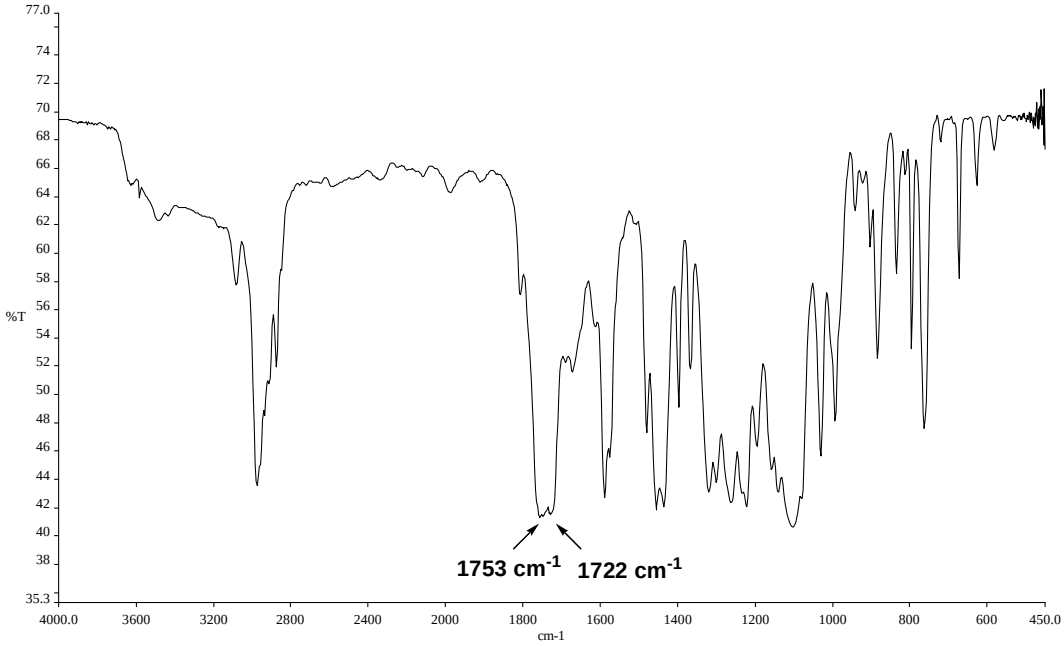
APPENDIX III

IR spectrum of compound 2.91



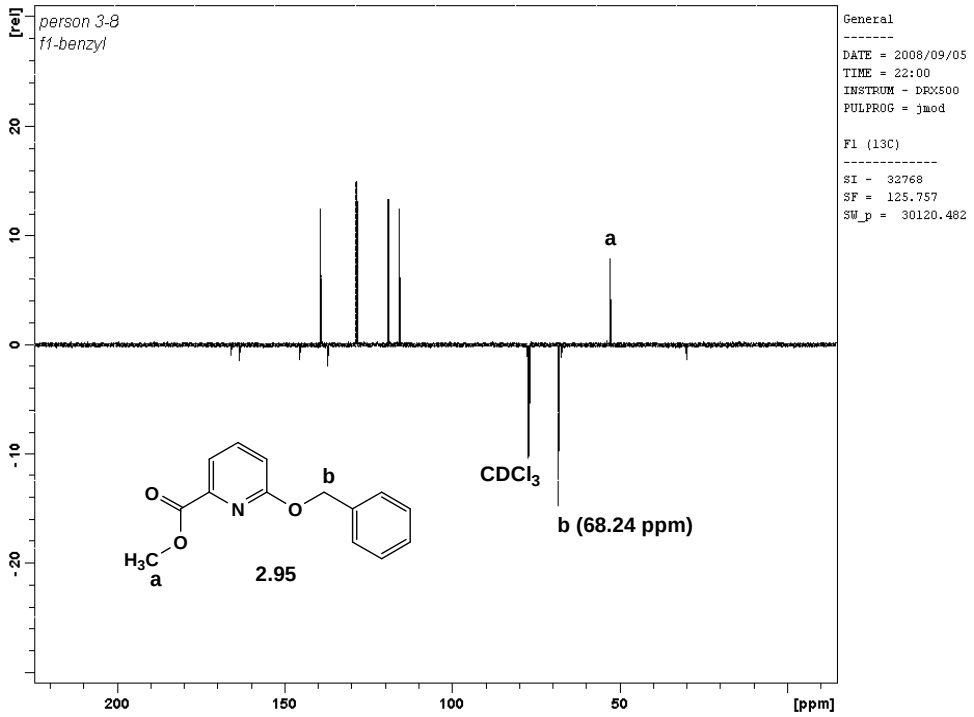
2.91

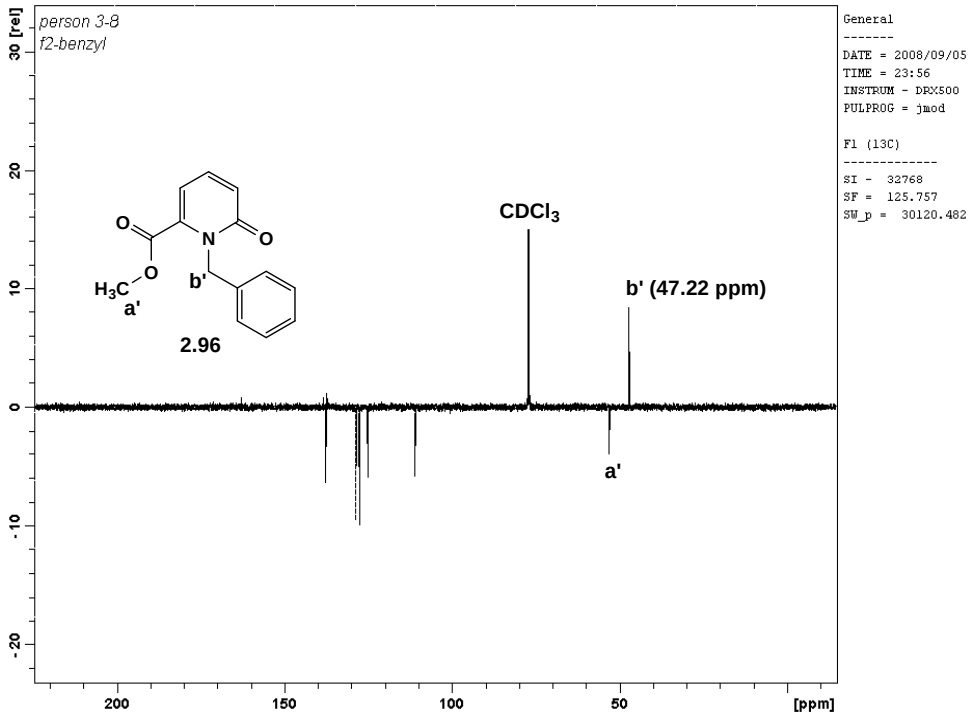
: ester C=O stretching at 1722 and 1753 cm^{-1}



APPENDIX IV

^{13}C NMR spectra of compound 2.95 and 2.96





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