Synthesis and Structure-Activity Relationship Studies of Novel Anti-infectives for Cross Screening in Tuberculosis and Malaria Disease Models

A thesis presented in fulfilment of the requirement for the degree of

DOCTOR OF PHILOSOPHY

Department of Chemistry

Faculty of Science

UNIVERSITY OF CAPE TOWN

August 2008

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Supervisor: Prof. KELLY CHIBALE

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ABSTRACT

Some 12-16 hours after the invasion of the human erythrocyte by the malaria parasite *Plasmodium falciparum*, there appear in the erythrocyte membrane 'new permeability pathways' which mediate an increased permeability of the infected cell to a range of low molecular weight solutes, including anions, cations, amino acids, polyols and nucleosides. There is evidence that the pathways have an important bi-functional role: firstly, that the new permeability pathways are required for the uptake of essential nutrients, and secondly, for the removal of metabolic wastes from the infected cells. Reported screening of 165 analogues of the new permeability pathways inhibitor furosemide, and the related compound bumetanide, for their effect on the malaria parasite-induced choline influx resulted in the identification of 13 effective compounds. Of these, 5 showed inhibitory activity *in vitro* against the parasite at a concentration of 10 µM. Analysis of the data on all the 165 compounds revealed some preliminary structure-activity relationships. Based on this preliminary structure-activity relationship data, compounds with specific diversity sites were designed for synthesis.

Acetolactate synthase (also known as acetohydroxyacid synthase) is the enzyme which catalyzes the first step in the biosynthesis of branched chain amino acids, including valine, leucine and isoleucine. It is a target for several classes of herbicides including sulfonyl ureas and imidazolinones. The complete crystal structure of yeast acetolactate synthase has been shown to share 26% homology with the *Mycobacterium tuberculosis* enzyme. On this basis, docking studies were initiated, which resulted in the generation of a virtual library of biaryl-based sulfonyl ureas. Exploratory libraries of sulfonyl ureas, imidazolinones, sulfonylcyanoguanidines, acylthioureas and related compounds (phthalimides) with the potential of having antituberculosis activity, presumably targeting acetolactate synthase, were synthesized.

Studying the general approaches to the synthesis of sulfonyl ureas, the general procedure is to either react a sulfonamide with an isocyanate in the presence of a weak base, or to react a sulfonyl

isocyanate with a primary or secondary amine. Both approaches work well chemically. However, the lack of diverse commercially available (sulfonyl) isocyanates, as well as the instability of isocyanates in general are drawbacks. A method that generates a vast selection of (sulfonyl) isocyanates from a range of commercially available starting materials would, hence, be very useful. A new approach to the synthesis of sulfonyl ureas was envisaged. This strategy involves the use of 1,2,4-dithiazolidine-3,5-dione, which should provide an alternative route to the arylsulfonyl ureas.

(i) Compound **H157** (one the five compounds that showed inhibitory activity *in vitro* against the parasite at a concentration of 10 μM) was chosen as a basis for structure-activity relationship studies. **H157** and its derivatives bearing an altered "side chain" length, numerous carboxylic acid and ester derivatives bearing hetero atoms, heterocyclic as well as aromatic moieties (in the place of the naphthyl functionality of **H157**) and the hydroxamic acid bioisostere of **H157**, were synthesized. All potential new permeability pathways inhibitors and intermediates, as well as, all available potential acetolactate synthase inhibitors and their respective intermediates, were tested in the sorbitol uptake assay for their potential of inhibiting the new permeability pathways. Four compounds were identified to demonstrate significant inhibition of the sorbitol uptake, namely **RKG256** (synthesized **H157**), **RKG340** (an extended 'side-chain' derivative of **H157**), **RKG404P2** (hydroxamic bioisostere of **H157**) and **RKG359** (sulfonyl urea, and analogue of classical Cl^- anion inhibitor, glibenclamide). **RKG359** and **RKG340** showed almost complete inhibition down to the concentration of 9 μM. **RKG404P2** proved to be the most active derivative [(IC_{50} = 1.2 μM)]. The compounds were further tested for *in vitro* activity against the chloroquine-sensitive D10 strain and chloroquine-resistant W2 strains of *Plasmodium falciparum*. The most active compound was found to be a 7-chloroquinoline bearing **H157** derivative having equipotent activity against both strains [D10 (IC_{50} = 1.28 μM) and W2 (IC_{50} = 0.89 μM)]. From the four compounds that showed good new permeability inhibition in the sorbitol uptake assay, only **RKG256** (**H157**) [D10 (IC_{50} = 17.9 μM)] and **RKG404P2** [D10 (IC_{50} = 16.9 μM) and W2 (IC_{50} = 17.7 μM)] showed notable *in vitro* activity. This activity is not as potent when compared to the control chloroquine, but presents the possibility that the activity demonstrated may be a result of inhibition of the new permeability pathways.

(ii) Synthesized sulfonyl ureas, imidazolinones, sulfonylcyanoguanidines, acylthioureas, phthalimides, intermediate compounds, and a collection of 13 commercially available herbicides were tested against the *Mycobacterium tuberculosis* H_{37}Rv strain using the Alamar blue assay, as well as in the *Mycobacterium tuberculosis* Resazurin assay. Only commercially available ethoxysulfuron (**RKGC5Et**) showed inhibition of greater than 90% in the primary screening against the H_{37}Rv strain at the concentration of 6.25 μg/ml. Other compounds that showed low to moderate inhibition (21-41%) at the concentration 6.25 μg/ml were acylthioureas: **RKG162A**, **RKG1541**, **RKG1542** and **RKG1543**; and commercially available sulfonyl ureas: triasulfuron (**RKGC11Tria**) and thifensulfuron-methyl (**RKGC12Th**). From the primary screening in the resazurin assay, only acylthioureas **RKG162A** and **RKG1541** showed good inhibition of the reduction of resazurin [approximate (IC_{50} = 16.9 μM) and (IC_{50} = 10.7 μM)].

(iii) The new synthesis of sulfonyl ureas envisaged using 1,2,4-dithiazolidine-3,5-dione as the starting scaffold, which was proposed to work via a 4-arenesulfonyl-1,2,4-dithiazolidine-3,5-dione intermediate, was unsuccessful. The coupling of 1,2,4-dithiazolidine-3,5-dione with a sulfonyl chloride was attempted under a variety of conditions, however, the 4-arenesulfonyl-1,2,4-dithiazolidine-3,5-dione intermediate could not be isolated or confirmed. Another new route to sulfonyl ureas was envisaged involving the reaction of *N*-alkyl-1,2,4-dithiazolidine-3,5-dione with sulfonamides in the presence of triphenylphosphine and a weak base. This alternate method was successful, and eleven sulfonyl ureas were synthesized to demonstrate the success of the method with yields ranging from 32 – 79%. Due to this new method being initially limited to *N*-alkyl-1,2,4-dithiazolidine-3,5-diones, broadening this new synthetic method to include *N*-aryl-1,2,4-dithiazolidine-3,5-diones was envisaged. Transition metal-catalyzed (Pd and Cu) amidation methodology was used to attempt the coupling of 1,2,4-dithiazolidine-3,5-dione with arylbromides. Unfortunately, the methodology using both the Pd- and Cu-catalyzed systems was unsuccessful.

DECLARATION

I declare that the work described in this thesis is my own unaided work submitted for the degree of Doctor of Philosophy (PhD), and it has not been submitted previously for a degree or examination at this or any other university. Relevant sources used and people that contributed are referenced and acknowledged.

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Richard Klaus Gessner

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LIST OF ABBREVIATIONS

AcOH	- acetic acid
AIDS	- acquired immunodeficiency syndrome
ALS	- acetolactate synthase
Ar	- aromatic
ATP	- adenosine triphosphate
CQ	- chloroquine
CQR	- chloroquine resistant
CQS	- chloroquine sensitive
DCM	- dichloromethane
DMAP	- 4-(dimethylamino)pyridine
DMF	- dimethylformamide
DMSO	- dimethyl sulfoxide
E1cB	- E1: unimolecluar elimination; cB: conjugate base; leaving group is lost from *conjugate base* of starting material
ED_{50}	- mean effective dose of drug needed to produce a therapeutic effect in 50% of test sample
EI(MS)	- electron impact mass spectroscopy
EPA	- United States Environmental Protection Agency
equiv.	- equivalents
Et_3N	- triethylamine
EtOAc	- ethyl acetate
EtOH	- ethanol
FGI	- functional group interchange
h or hrs	- hour/s
H-bond	- hydrogen bond
HEPES	- 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HIV	- human immunodeficiency virus

HRMS	- high resolution mass spectroscopy
IC_{50}	- inhibitor concentration reducing V_{max} by 50%
*i*Pr	- *iso*-propyl
IR	- infrared
K_i	- inhibition constant
LD_{50}	- lethal dose required to kill 50% of a group of animals
LRMS	- low resolution mass spectroscopy
M.	- genus *Mycobacterium*
MDR	- multi-drug resistant
MEM	- a general cell culture medium
MeOH	- methanol
MHz	- mega hertz
MIC	- minimum inhibitory concentration
min	- minutes
m.p.	- melting point
MS	- mass spectroscopy
m/z	- mass to charge ratio
NMR	- nuclear magnetic resonance
NMP	- 1-methyl-2-pyrrolidinone
NPP	- new permeability pathways
NPPB	- 5-nitro-2-(3-phenylpropylamino)-benzoic acid
P.	- genus *Plasmodium*
Ph	- phenyl
R_f	- ratio of movement of solute to solvent in TLC
Rpm	- rotations per minute
RPMI 1640	- a cell culture medium (Seromed, Munich)
rt	- ambient temperature
SAR	- structure-activity relationship
S_N	- nucleophilic substitution reaction

T.	- genus *Trypanosoma*
TB	- tuberculosis
THF	- tetrahydrofuran
TI	- therapeutic index = parasite ED_{50}/Mammalian cells ED_{50}
TLC	- thin layer chromatography
UV	- ultraviolet
WHO	- World Health Organization
Xantphos	- (9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis(diphenylphosphine
XDR	- extensively drug resistant

Nuclear Magnetic Resonance (NMR) abbreviations:

Ar	- aromatic
br	- broad
$CDCl_3$	- deuterated chloroform
CD_3CN	- deuterated acetonitrile
COSY	- correlated spectroscopy [(H, H)-correlated NMR spectroscopy]
d	- doublet
d^x-	- deuterated solvent (where x = number of hydrogen atoms replaced by deuterium atoms)
dd	- doublet of doublets
D_2O	- deuterated water (H_2O)
dquar	- doublet of quartets
dt	- doublet of triplets
hep	- heptet
HETCOR	- correlated spectroscopy [(H, C)-correlated NMR spectroscopy]
J	- coupling constant
m	- multiplet
MeOD	- deuterated methanol

oct - octet

ppm - parts per million

quar - quartet

quar-d - quartet of doublets

quin - quintet

s - singlet

δ - chemical shift in ppm

t - triplet

td - triplet of doublets

TMS - tetramethylsilane

Infrared (IR) abbreviations:

br - broad

m - medium

s - strong

ν_{max} - maximal velocity (cm^{-1})

w - weak

Chapter 1

Malaria

Malaria is a disease caused by protozoan parasites of the genus '*Plasmodium'*. These are four species which are pathogenic to humans. It is spread by the bite of an infected female *Anopheles* mosquito, and is transferred from the infected bloodfeeding female mosquitoes (as male mosquitoes do not bite) into the bloodstream of the human hosts. These four species include: *Plasmodium falciparum, P. vivax, P. ovale, P. malariae*. *Plasmodium falciparum* is the most lethal of the malaria infections, and results in the most severe clinical malaria cases and deaths. It is estimated that a child dies as a result of malaria infection every 30 seconds,[1] and that an estimated 350 – 500 million cases of malaria, resulting in approximately 700,000 – 2.7 million deaths, occur annually worldwide.[2]

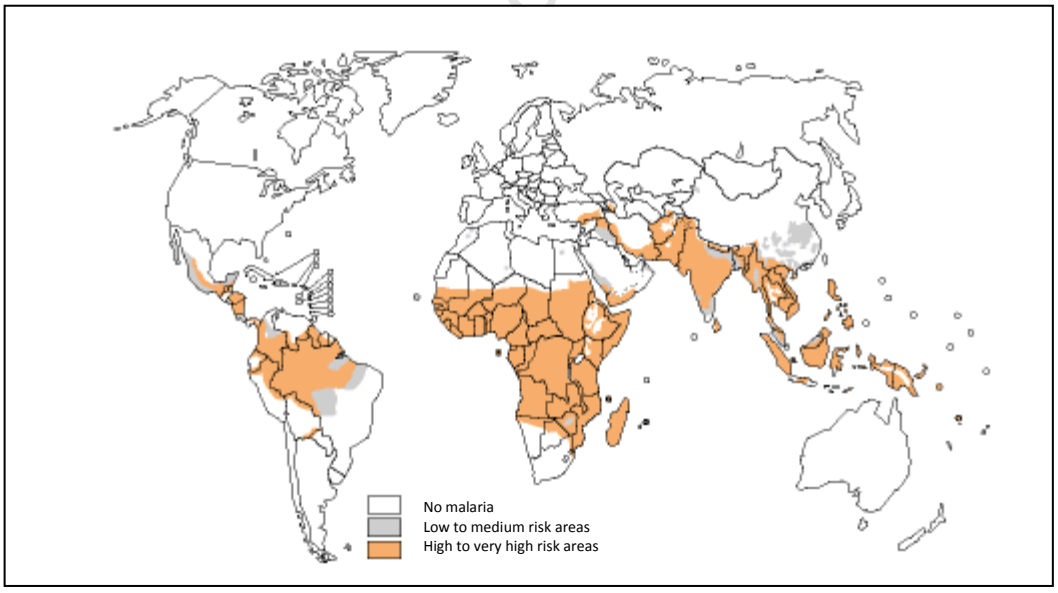


Figure 1.1: Geographical distribution of malaria endemicity[3]

Most infections and fatalities occur in sub-Saharan Africa, however, parts of Asia, Europe, Latin America and the Middle East are also at risk. It is further approximated that 40% of the world's population is at risk[1] (risk areas are displayed in Figure 1.1).

1.1 Life Cycle of the Parasite *Plasmodium falciparum*

The spread of the malaria parasite, in nature, is reliant on the successive infection of two hosts: the female *Anopheles* mosquito and humans. Starting at the infected mosquito host (Figure 1.2), threadlike structures (known as sporozoites) in the salivary glands are injected into the human host, along with the mosquito's saliva, when the mosquito takes a blood meal. The

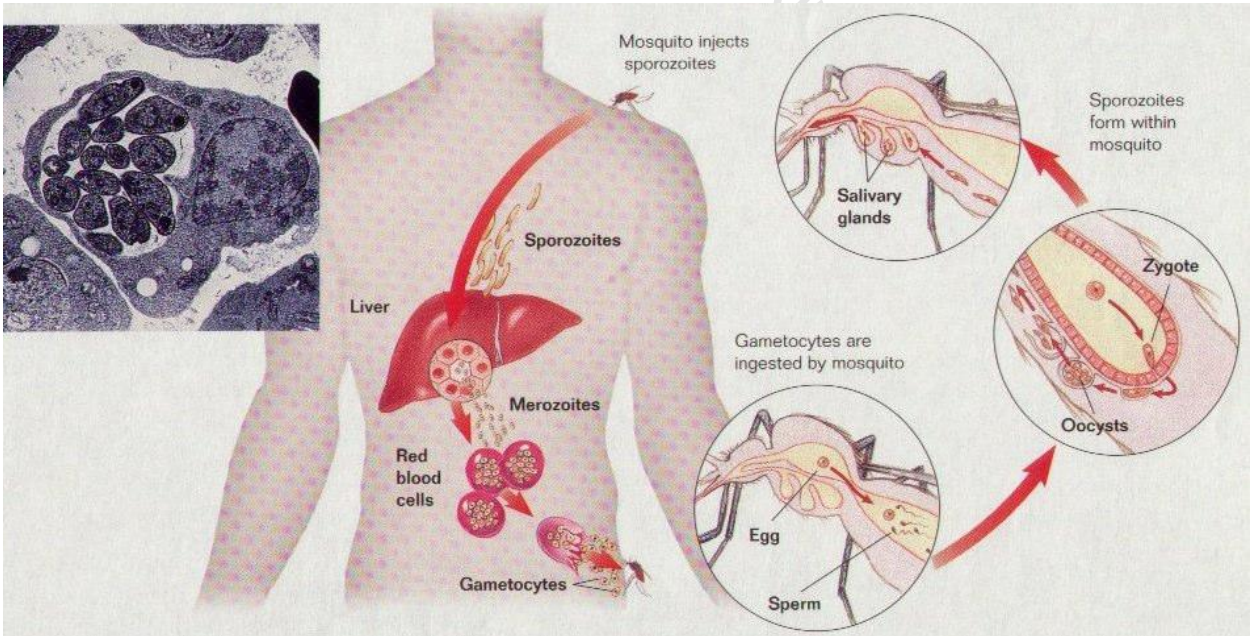


Figure 1.2: Life cycle of malaria[4]

sporozoites travel via the human host's bloodstream to the liver and invade hepatocytic (parenchymal) cells. In the liver, the sporozoites asexually multiply and parasitize the liver cells, forming thousands of another kind of spore called the merozoites. The infected hepatocytes eventually rupture and merozoites are released into the bloodstream. The merozoites invade

the oxygen transporting erythrocytes. Here they devour the hemoglobin, and asexually multiply by a factor of up to 16-fold, destroying the red blood cells. In the asexual reproduction process, the parasites can be first differentiated as a ring stage which develops into trophozoites. The trophozoites become schizonts, and these schizonts eventually turn into merozoites. This round of asexual multiplication can take place in a rapid time of as little as 48 hours. Upon disintegration of the red blood cell, the merozoites are freed, and can either infect other fresh red blood cells and repeat the asexual reproduction cycle, or develop into male and female gametocytes. The gametocytes are taken up by a female *Anopheles* mosquito during feeding, and make their way to the host's stomach. It is here in the gut of the mosquito that another, different cycle of growth and multiplication of the parasite takes place. Sexually active gametocytes come together, and fuse to form a zygote. The zygotes develop into oocytes in the wall of the stomach. The oocytes develop over a period of 10 – 18 days, and contain numerous sporozoites. These sporozoites are released, and migrate to the mosquito's salivary glands, whereby, starting the cycle all over again.

1.2 Symptoms

The fever or flu-like illness associated with malaria is a result of the merozoites invading and destroying red blood cells, and usually starts around 8-30 days after infection. When the red blood cells are destroyed; waste, toxins, and other debris are released into the blood stream. This results in the body producing an immune response (fever) to promote other immune defenses to combat the foreign material. The fever occurs in recurring incidents, initially starting with sudden, violent chills, followed by an intense fever which leads to intense sweating. The typical fever episode lasts approximately 12 hours. Severe anemia (due to the lowered concentration of red blood cells in blood) can result from repeated malaria parasite infections.

Occurrence of fever and other related symptoms is varied according to the species of *Plasmodium* infection. *Plasmodium falciparum, P. vivax* and *P. ovale* produce fevers typically every 48 hours, where *P. malariae* produces fevers every 72 hours. *Plasmodium falciparum* infection is associated with the highest fatality rates. Symptoms of *P. falciparum* can include severe headaches, convulsions and delirium, and in the worst cases, it can actually develop into cerebral malaria. Cerebral malaria occurs when infected red blood cells attach to the walls of tiny blood vessels in the brain, and this leads to the inflammation and blocking of blood and oxygen flow in the brain. In some cases of *Plasmodium vivax* and *Plasmodium ovale*, merozoites can stay dormant in the liver for between three months and five years.

1.3 Diagnosis

Due to the nature of the periodic fever and variable symptoms of malaria, it is difficult to diagnose based on these attributes alone. Instead, a patient's blood sample is usually examined as a blood smear under a microscope to detect if the malaria parasite is present in the red blood cells. It is also possible to tell the species of *Plasmodium* present by observing their appearance under the microscope. However, it is difficult to detect malaria under a microscope in its early stages or if there are only a few parasites present. Recent developments using the polymerase chain reaction makes diagnosis more accurate. The method works by detecting and replicating *Plasmodium* proteins or genetic material in a human host's blood.[5]

1.4 Treatment

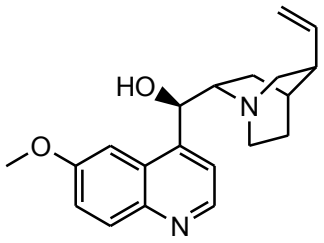
Despite a worldwide effort to eradicate the causative agents or combat the disease, malaria remains the parasitic disease with the greatest threat to mankind. The three main strategies of defense are a combination of vaccination, vector control and drugs. However, parasiticidal drugs remain the current main line choice for disease control.

Quinine, the first known antimalarial drug, is a compound present in the bark of the cinchona tree, and was applied by the 15th century ancient Inca people for the treatment of fever. The antimalarial properties of quinine, and later quinine-type compounds, are believed to be a result of its interference with the parasite's metabolism. However, due to its bitter taste and severe side effects, which includes nausea, headaches, ringing in the ears, temporary loss of hearing, blurred vision, and even death when large doses are administered, it is no longer a frontline choice drug candidate in malaria treatment. Today, quinine is still used in some developing third world countries because of its low cost and effectiveness.

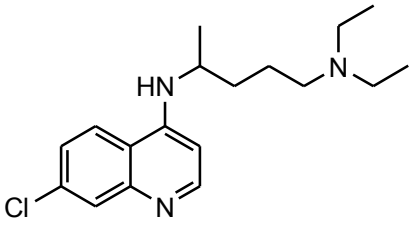
In the 1940's, chloroquine, a synthetic compound with similar chemical properties to quinine, was discovered. It almost immediately became the antimalarial drug of choice due to its high efficacy, ease of manufacture and less severe side effects. However, resistance to chloroquine by the malaria parasite has grown and spread over the last few decades, and has rendered the drug useful to only some parts of Central America and the Middle East.

Mefloquine, another compound related to quinine and chloroquine, is largely used today due to its effectiveness. It, however, has quite severe and adverse permanent side effects, is still too expensive to be used on an effective routine treatment basis in most developing nations and

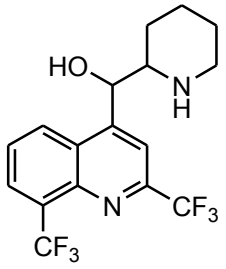
there has also been the emergence of mefloquine-resistant strains in Thailand, Cambodia and Myanmar.[6]



Quinine

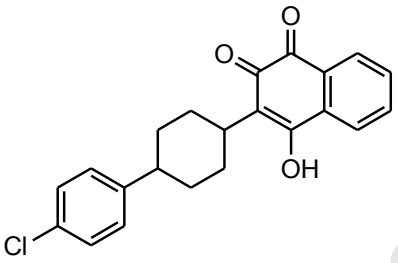


Chloroquine

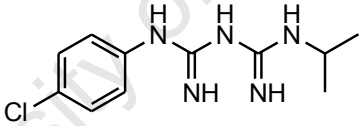


Mefloquine

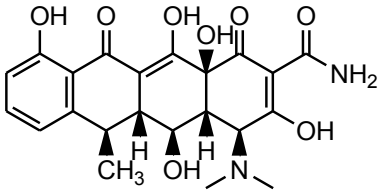
Medicines that are currently prescribed with less risk of severe side-effects, and which are equally effective for malaria prevention are Malarone (combination of atovaquone and proguanil) and doxycycline.



Atovaquone



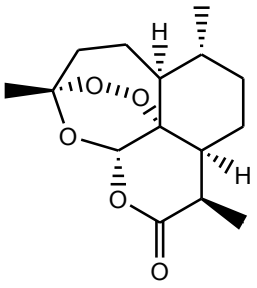
Proguanil



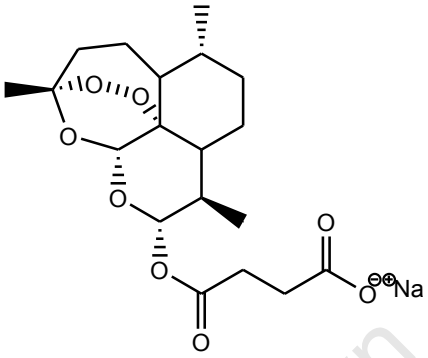
Doxycycline

Drug resistance appears to be more prevalent in south-east Asia. This phenomenon has been studied intensively in Thailand where only after a few years of monotherapy resulted in the emergence of mefloquine-resistant parasites.[7] It was discovered that when artemisinins are combined with previously effective drugs, which are no longer as effective as a result of resistance, the efficacy can be in some areas be restored. It was found that more than 95% of patients were cured by the combination of artesunate and mefloquine, when compared with the 50 - 60% of cases where mefloquine was taken alone.[7,8] Two advantages of using artemisinin in combination therapy is that the cure rates can be considerably increased when

combined with antimalarials from different compound classes, and the development of resistance can be delayed.



Artemisinin



Sodium Artesunate

Due to the emergence of multi-drug-resistant malaria parasite strains and the ever increasing resistance to available drugs, there is a need for the development of new antimalarial drugs. The development of new antimalarial drugs can range from the modification of existing drugs, to the studying and isolating historically known natural product remedies, to the search and development of new leads and novel antimalarial candidates.

1.5 Malaria Drug Targets

Recently, in 2002, the genome of *Plasmodium falciparum* was sequenced, and this has unlocked many opportunities and possibilities of finding and exploring new drug targets.[9] Approximately 5300 protein-encoding genes, of which about 60% function could not be assigned, were identified. This entails a large group of unique malarial proteins which could be potential drug targets. Several other unique features of the parasite were also identified. This includes an apicoplast with 30 genes in its own genome, an unusual electron transport chain, an apparent non-functional F_1F_0 ATP synthase, and a proton-translocating pyrophosphatase which is believed to develop required electrical potential across the plasma membrane.

The identification of purine and nucleoside transporters, as well as purine salvage enzymes hypoxanthine phosphoribosyltranferase (HPRT) and purine nucleoside phosphorylase (PNP), were further assigned from the genome. These are good potential drug targets as all pathogenic protozoan parasites do not synthesize their own purines *de novo*, and rely on the host for their purine provision.[10]

New Permeability Pathways (NPP) (this shall be discussed in detail in Chapter 2) are induced into the host cells membrane, making it more permeable to a variety of required small molecular weight compounds, after invasion of the red blood cells by the parasite. There is much debate of the origin of these transport systems, but they could potentially be a good drug target.[11]

Quinoline derivatives (including chloroquine, quinidine, halofantrine and mefloquine) are main line drugs which are believed to inhibit haem detoxification. The human host haemoglobin is the primary source of amino acids for the malaria parasite. As haemoglobin is consumed (haemoglobin proteolysis) within the food vacuole of the parasite (this process occurs during the intra-erythrocytic stage of the malaria parasite's life cycle), free haem [Fe(II)protoporphyrin IX] is produced. The haem is oxidized to haematin [HO-Fe(III)protoporphyrin IX] which is toxic to the parasite.[12] The parasite detoxifies itself by converting the haematin into haemozoin (known as malaria pigment), which is believed to be a polymer of β-haematin.[13,14] The quinoline anti-malarials are believed to interfere with this process of detoxification. The inhibition of the formation of synthetic crystalline β-haematin has been demonstrated by the presence of several 4-aminoquinolines.[15] The acculmulation of toxic haematin, as a result of the inhibition of haemazoin formation, is believed to kill the parasite.

Artemisinin and its derivatives are increasingly becoming more important and favorable for the treatment of malaria. Artemisinin is derived from *Artemisia annua*, also known as Sweet

Wormwood, which grows throughout the world, and was used in ancient times by Chinese herbalists for the treatment of fever. The effectiveness of these compounds for the treatment of malaria has been attributed to several modes of action. Artemisinin drugs are believed to be converted to dihydroartemisinin by haem [Fe(II)protoporphyrin IX], generating toxic, carbon centered free radicals which are believed to alkylate important parasitic enzymes.[16,17] Artemisinin has also been proposed to be the source of hydroperoxide which may lead to the generation of hydroxyl radicals. These hydroxyl radicals can result in disastrous effects for the parasite by hydroxylating parasitic proteins and possibly leading to the production of reactive oxygen species.[18,19] More recently, artemisinins have been found to inhibit a malarial Ca^{2+}-ATPase, *PfATP6*,[20] as well as, have an anti-inflammatory effect by inhibiting the parasite induced form of a nitric oxide synthase.[21]

Some other important classes of drugs include antifolate agents and protease inhibitors. Pyrimethamine and sulfadoxine (first line therapeutics in some African countries) inhibit two of the five required enzymes (namely dihydrofolate reductase and dihydropteroate synthase respectively) for the synthesis of folate.[22,23,24] Peptidyl fluoromethyl ketones[25] and vinyl sulfones[26] are potent antimalarials that inhibit proteases. Aspartyl proteases (plasmepsin I and II),[27,28,29] cysteine proteases (falcipain 1, 2 and 3)[30-34] and trypanothione reductase[34] are classes of proteases critical for the malaria parasites survival and are potentially good targets for antimalarials.

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Chapter 2

Inhibitors of the New Permeability Pathways

2.1 New Permeability Pathways (NPP)

New permeability pathways (NPP) are induced by the malaria parasite *Plasmodium falciparum* into human host red blood cells (erythrocytes) about 12 - 15 hours after infection.[1] These NPP cause the infected erythrocyte to have a higher permeability to a range of small low molecular weight and structurally unrelated solutes, including anions, cations, amino acids, polyols and nucleosides. It has been proposed that the NPP are predominantly anion-selective channels which also allow the transport of electroneutral and cationic solutes.[1] However, this postulation has recently been challenged.[2]

There is evidence that shows that these NPP may have a bifunctional role. Firstly, they are required for the uptake of essential nutrients, and, secondly, they are required for the removal of metabolic wastes from the infected cells.[3,4,5,6] It has been recently shown that the activity of the NPP increases as the parasite matures.[7] Human erythrocytes do not have high nutritional demands and, hence, are lacking in the ability to take up vast quantities of lipids, amino acids and nucleic acids.[6] This presents problems for the invading parasite, as it requires all the above mentioned nutrients to survive. Additionally, to survive, the parasite requires the uptake of pantothenate,[8] and the removal of lactic acid which forms from the high rate of glucose metabolism.[6] The initial amount of monocarboxylate transporter present in the host red blood cell is not sufficient to cope with extra lactic acid generated by the parasite. The induction of the NPP in the host red blood cell, hence, provides the parasite with means to obtain the

required nutrients and to facilitate the removal of metabolic wastes. Another recent proposal is that the NPP may also assist in the volume regulation of the infected host erythrocyte.[9]

The NPP have only been studied for just over two decades and there is still much confusion and debate about the origin, over the amount and the nature of the NPP.[10] Several research groups have proposed that NPP can be induced by a single channel,[1,11-14] whereas others have hypothesized, as result of their observed and published data, that more than one channel type or pathway is required.[15-20] It is further postulated that the origins of NPP could be both endogenous[13,19,20] and parasite-derived.[10]

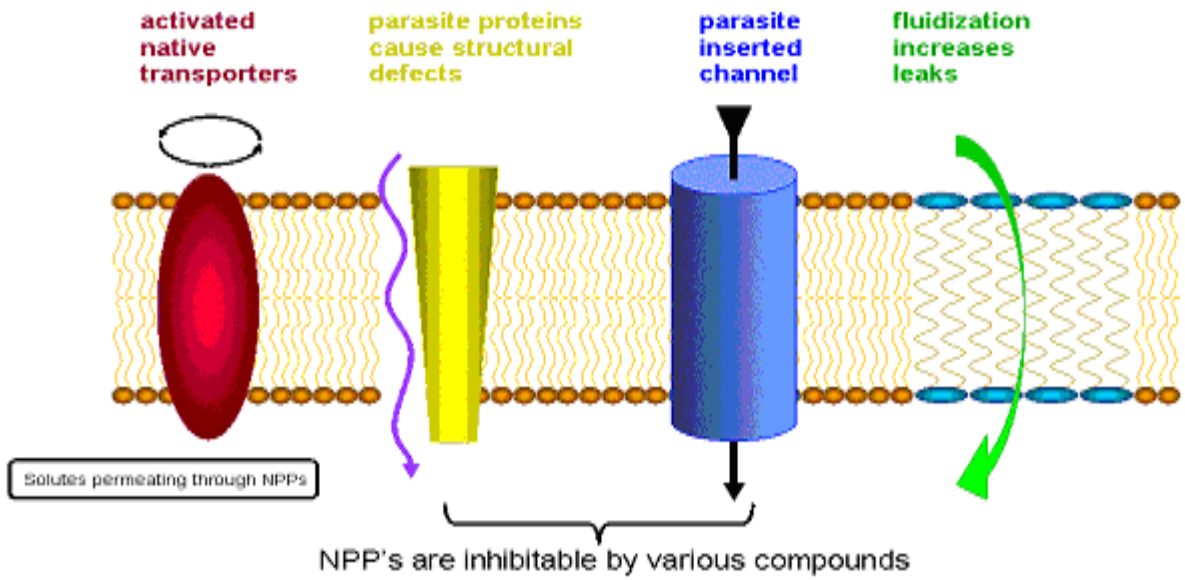


Figure 2.1: Four illustrated possibilities for the increase in membrane permeability by NPP

Four possible reasons for the increased permeability of the host cell membrane induced by the malaria parasite are illustrated in Figure 2.1:

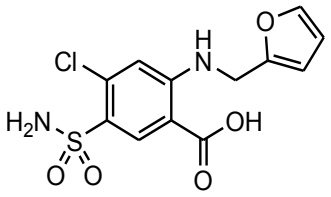
i) existing native transporters and channels in the host cell membrane are activated (**red**)
ii) parasitic proteins cause structural defects to existing transporters and channels (**yellow**)
iii) the parasite inserts channels into the host cell membrane (**blue**)

iv) fluidization in the host cell membrane increasing defects and leaks (**green**)

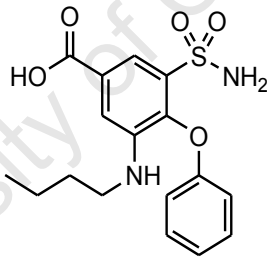
To date, none of the above hypothesis are conclusive, and many discrepant results still present many questions.[2,11,21,22] Continuous work is constantly challenging and attempting to explain these questions. For example, Staines, H. M. *et al.* [2006][10] have recently reported data suggesting that solute transport via NPP is not consistent with a single-channel model.

2.2 Classical Cl^- Transport and NPP Inhibitors

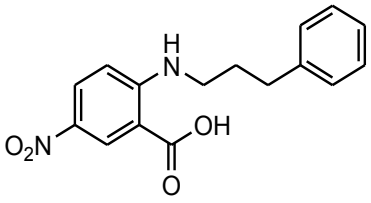
The NPP show the characteristics of 'classical' anion channels, and it has been demonstrated that they can be blocked by a range of anion channel inhibitors including furosemide,[1] phloridzin,[1,23,24] glibenclamide,[25] 5-nitro-2-(3-phenylpropylamino)-benzoic acid (NPPB)[1] and,



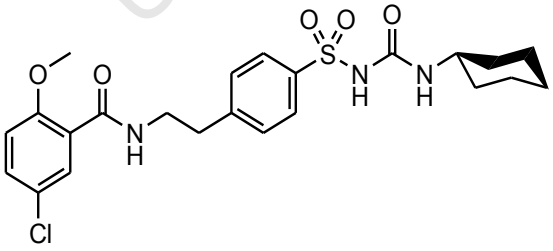
Furosemide



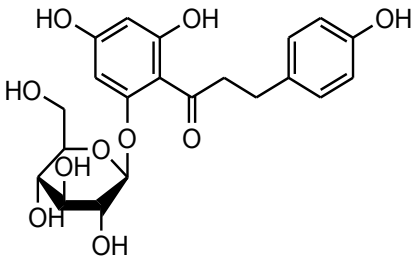
Bumetanide



NPPB



Glibenclamide



Phloridzin

Figure 2.2: Classical Cl^- channel blockers that inhibit NPP and parasite growth in culture

more recently, furosemide and bumetanide analogues.[26] All of these compounds have demonstrated the inhibition of NPP, however, their major downfall is their general lack of specificity over human anion transporters.[27]

To date, not much work has been done on extending the size, and developing libraries, of compounds which are specific NPP inhibitors. Out of a small library of NPPB analogues, Kirk, K. *et al*. [1995][28] reported a NPPB derivative that has shown higher selectivity for parasite NPP over three other human anion transporters. In conclusion, the development of large libraries of analogues of these potent anion and NPP inhibitors could result in the discovery of a drug that is both specific and potent for NPP inhibition.

2.3 Structure-Activity Relationship (SAR) Studies

As mentioned in Chapter 2.2, not much work has been done to extend the size of libraries in search of compounds that show potent NPP inhibition, and, more importantly, inhibitors which are NPP specific. The initial screening of 165 analogues of the NPP inhibitor furosemide, and of the related compound bumetanide (piratinide-like molecule), for their effect on the malaria parasite-induced choline influx resulted in the identification of 13 effective compounds.

Of these, 5 compounds (**H156**, **H157**, **H158**, **H159** and **H161**) (Figure 2.3) showed inhibitory activity *in vitro* against the parasite at a concentration of 10 μM.[1]

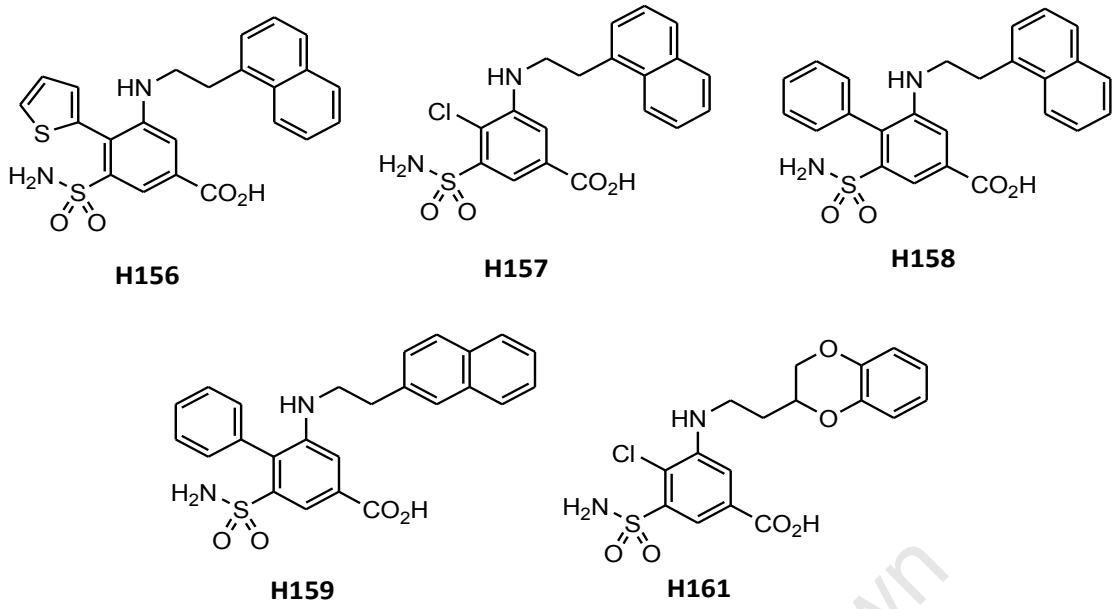


Figure 2.3: Five effective analogues that showed good NPP

Analysis of the data on all the 165 compounds revealed some preliminary structure-activity relationship (SAR) with respect to the importance of the primary sulfonamide and carboxylic acid functional groups. Based on this preliminary data, further SAR studies were carried out and resulted in the identification of other possible derivations that could be investigated (summarized below in Figure 2.4). Compound **H157** was chosen as the foundation of further SAR investigation.

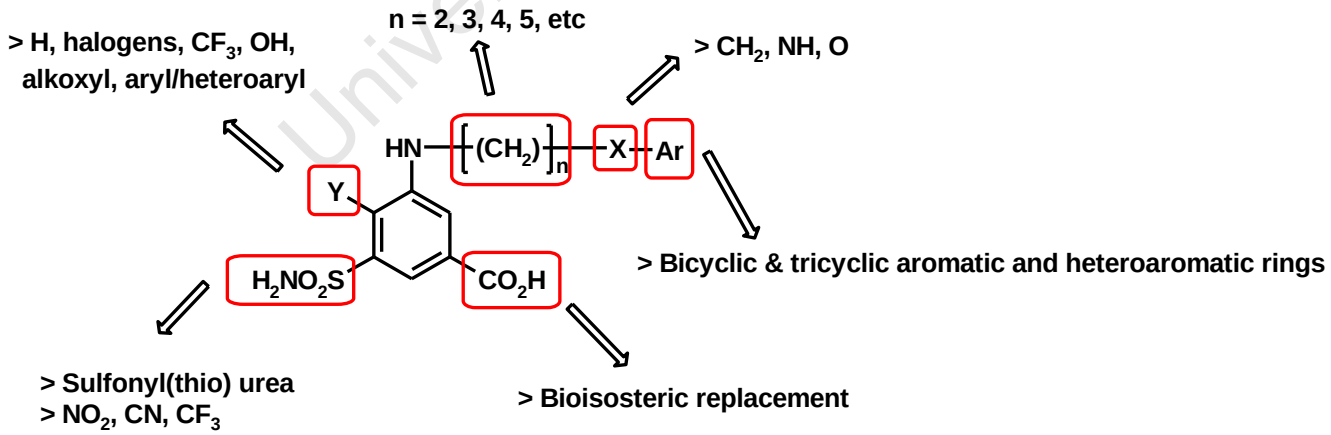


Figure 2.4: Summary of SAR Studies of Piretanide-like molecules

Thus the envisaged SAR for piretanide-like molecules (also applicable to furosemide-like molecules) is summarized as follows:

1) The length of the alkyl (-CH_2-) spacer as well as its nature (X = CH_2, NH, O) would be investigated. The aforementioned 5 compounds (**H156, H157, H158, H159** and **H161**) all have a 2-carbon methylene spacer between the amino benzene and the bicyclic (e.g. naphthyl) group. This spacer has an effect on the lipophilicity, and hence membrane permeability, as well as the solubility properties, of the compounds. From the initial library of 165 compounds, it was noted that compounds with a 1-carbon methylene spacer displayed poorer activity.

2) The carboxylic acid moiety would be transformed into suitable corresponding esters. This could not only enhance the membrane permeability properties of the compounds, but also result in potential prodrugs requiring *in vivo* biotransformation (presumably via hydrolysis mediated by esterases) to give the carboxylic acid moiety.

3) Converting the carboxylic acid moiety into corresponding bioisosteres. Hydroxamic acids and tetrazoles are known carboxylic acid bioisosteres, and may result in alternative and/or additional mechanisms of action. In view of the known metal-chelating property of hydroxamic acids, these compounds may be expected to inhibit metalloenzymes and cysteine proteases (as metal-interactive inhibitors) amongst varied potential targets. This property would have implications for overcoming drug resistance and/or delaying its emergence. Having additional mechanisms of action could effectively result in the development of single agents that provide maximal antimalarial activity by acting against multiple targets within the parasite.
4) The naphthyl group (e.g. in **H156, H157, H158** and **H159**) could be replaced by other bicyclic and tricyclic aromatic and heteroaromatic rings that would be rationally selected.

5) All of the active compounds from the initial library of 165 compounds have a primary sulfonamide moiety. Systematically, it has been concluded that this primary sulfonamide group is vital for the activity of this class of compounds. However, a study of this group was proposed to be undertaken in order to explore its importance for activity. The primary sulfonamide could be replaced by other electron withdrawing groups including the nitro, cyano, and trifluoromethyl moieties. Another rational study would be the conversion of the primary sulfonamide to a secondary/tertiary sulfonyl (thio)urea. This conversion is supported by the known activity of sulfonyl ureas for the inhibition of classical chloride channel inhibitors. Tolbutamide and glibenclamide are examples of such sulfonyl ureas which have also displayed NPP inhibition. As described in point 3 above, this conversion could result in additional mechanisms of action, and potential single agents that address drug resistance by targeting different pathways.

6) A systematic study of the nature of the Y group could be undertaken in order to explore the effect of the absence (Y = H) and presence of electron withdrawing and electron donating groups (Y = F, Cl, Br, I, CF_3, OH and alkoxy).

7) The nature of the aromatic group (Y = aryl/heteroaryl) could be examined by expanding the initial library to include a broader range of substituted aromatic and heteroaromatic (e.g. furan, thiophene, pyridine and quinoline) ring systems.

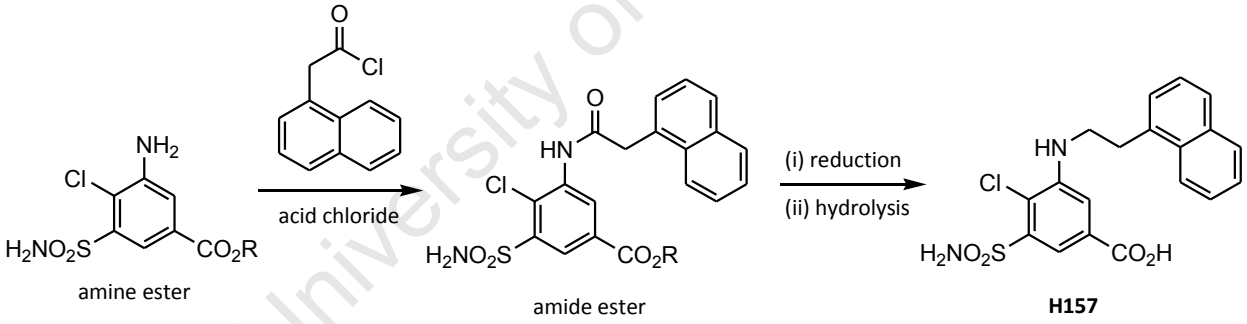
Based on the envisaged SAR studies above, compounds were designed and synthesized in an attempt to answer some of the specific queries with a view to identifying a lead series of compounds, from initial hits, for subsequent optimization. All hits were envisaged to be considered carefully and prioritized according to a number of criteria. The criteria includes drug-like (as defined by Lipinski, C. A. *et al.* [2001][29]), lead-like (as defined by Teague, S. J. *et al.* [1999][30] for compounds with molecular weight less than 350 and clogP less than 3), ease of

chemical synthesis, amenability to chemical library generation, and availability and cost of starting materials.

2.4 Proposed Synthetic Routes

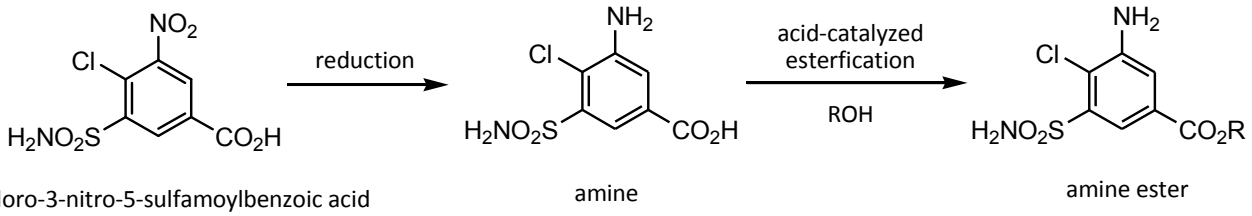
2.4.1 Synthesis of **H157**

Following the protocol described in a patent,[31] **H157** can be synthesized by coupling an appropriate amine ester with an acid chloride. The resulting amide is reduced to its corresponding amine, and the ester is then hydrolyzed to the free carboxylic acid to give **H157** (Scheme 2.4.1).



Scheme 2.4.1: Proposed synthesis of **H157**

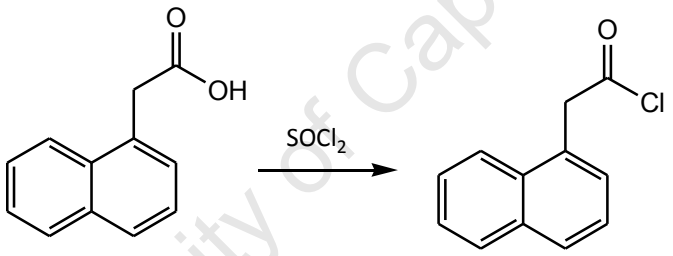
To synthesize the required amine ester (Scheme 2.4.2), commercially available 4-chloro-3-nitro-5-sulfamoylbenzoic acid can be converted to the desired amine by reduction of the nitro functional group. The carboxylic acid can then be converted to an ester by acid catalyzed esterfication with the appropriate alcohol giving the desired amine ester starting scaffold.



4-chloro-3-nitro-5-sulfamoylbenzoic acid | amine | amine ester

Scheme 2.4.2: Proposed synthesis of required amine ester from 4-chloro-3-nitro-5-sulfamoylbenzoic acid

The acid chloride can be obtained by converting the commercially available 1-naphthalene acetic acid to (1-naphthalenyl)-2-ethanoyl chloride with thionyl chloride (Scheme 2.4.3).

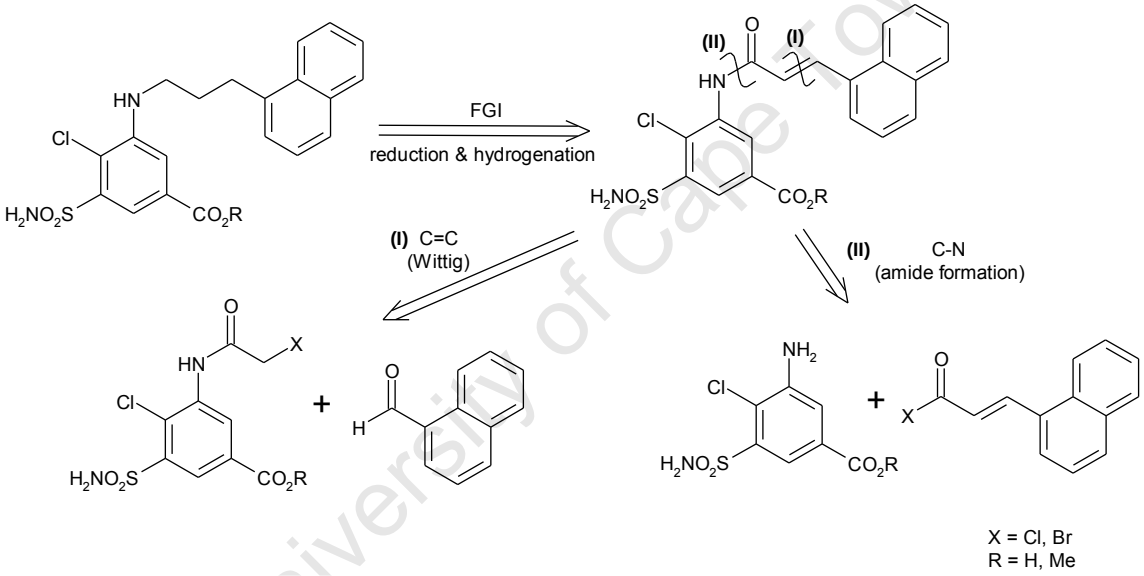


carboxylic acid | acid chloride

Scheme 2.4.3: Conversion of 1-naphthalene acetic acid to (1-naphthalenyl)-2-ethanoyl chloride with thionyl chloride

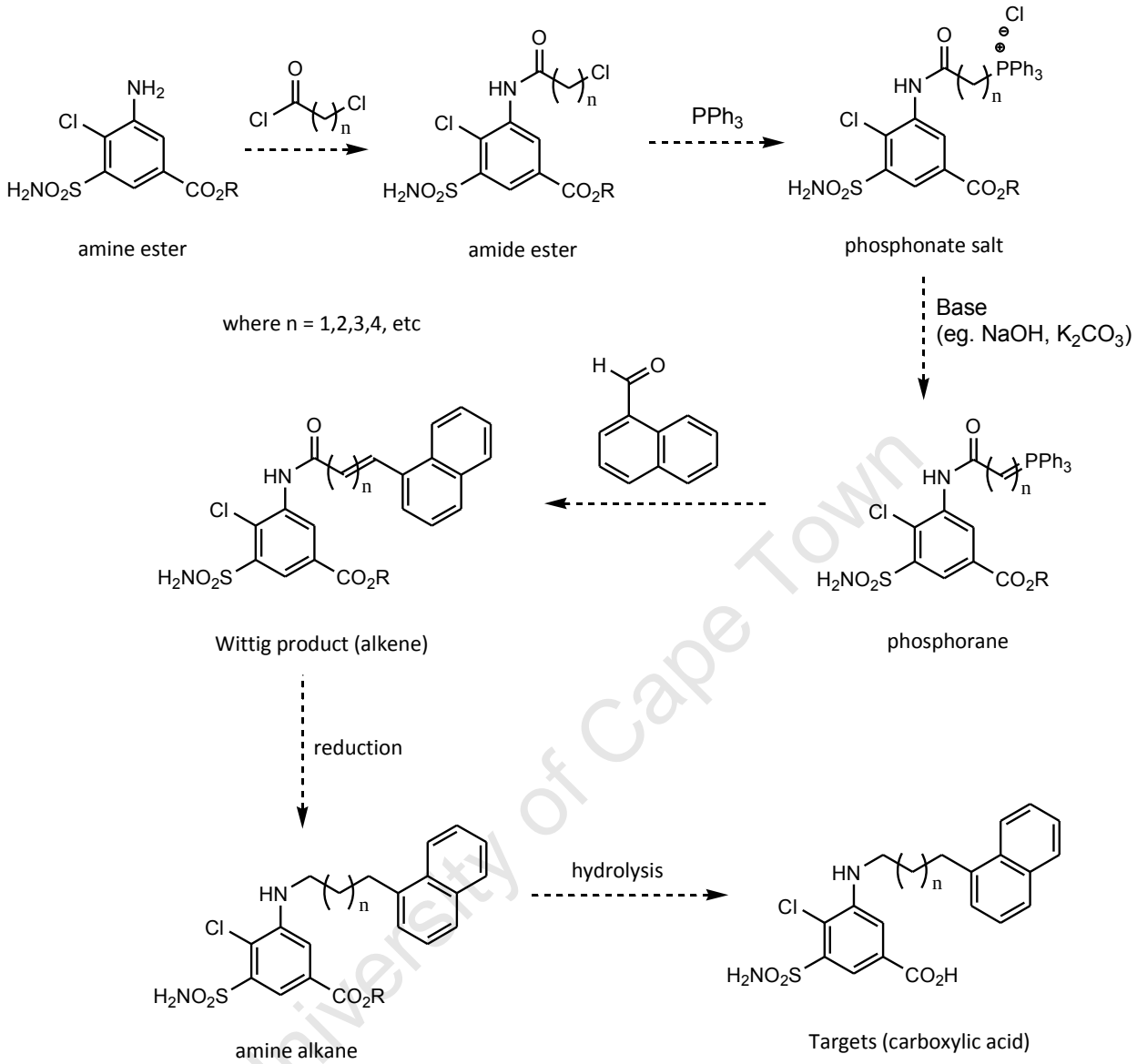
2.4.2 The Length of the Alkyl (-CH_2-) Spacer

To investigate the lengthening of the side chain (spacer between the aromatic moiety and the amine nitrogen), a simple retrosynthetic analysis was proposed based on having three CH_2 units between the nitrogen and aromatic moiety (Scheme 2.4.4). This procedure, however, can be easily adapted to have CH_2 units of more than three as shown below. Two main disconnections were identified: (I) Wittig C=C and (II) amide formation.



Scheme 2.4.4: Retrosynthetic analysis of lengthened "side chain" derivatives of **H157**

Based on the (I) Wittig disconnection the following synthetic scheme (Scheme 2.4.5) was proposed. This synthetic scheme has been further adapted to include derivatives having a "side-chain" length of three or more (-CH_2-) units.

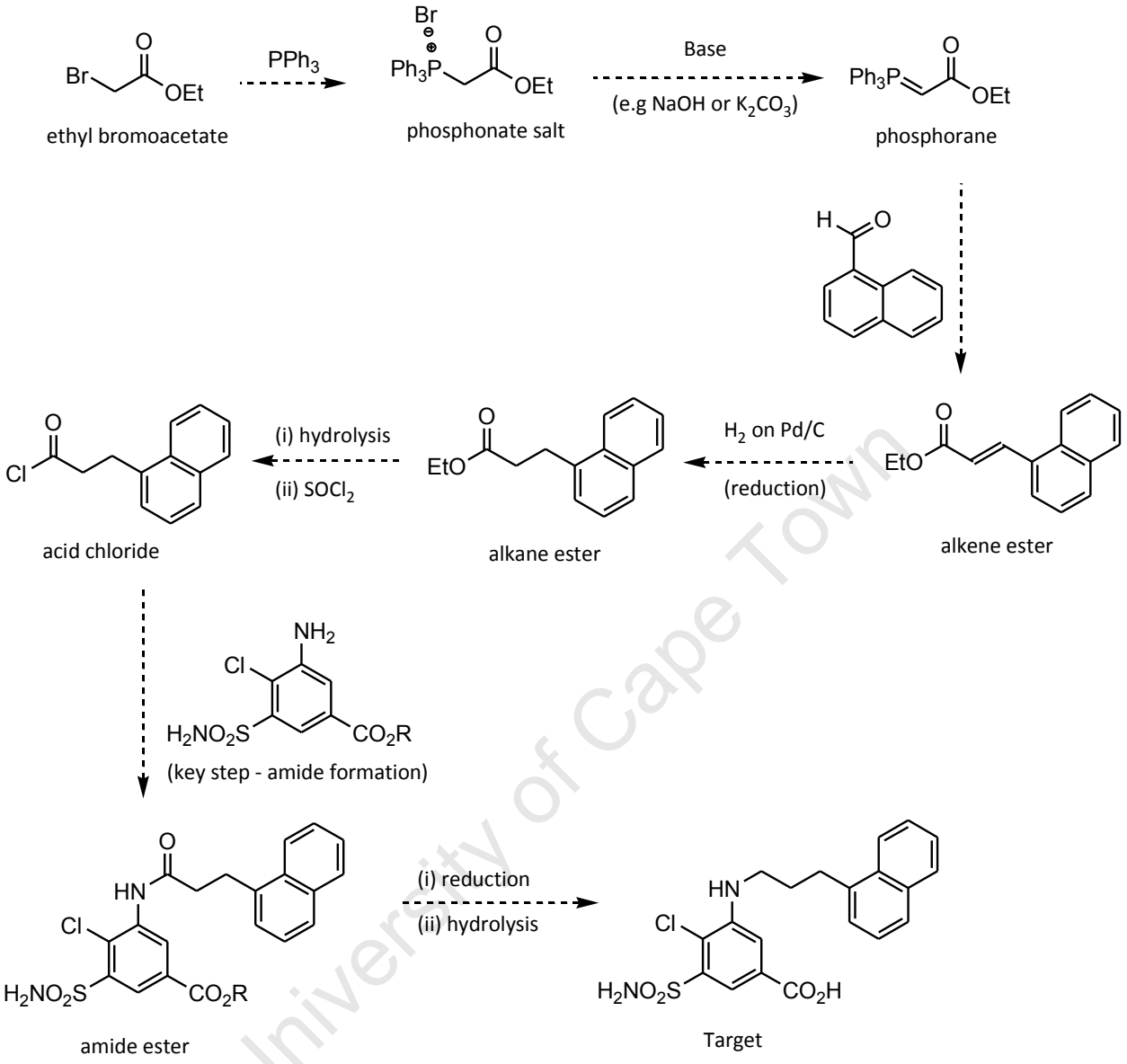


Scheme 2.4.5: Proposed synthesis of lengthened "side chain" derivatives of **H157** based on approach (I) Wittig disconnection

It was envisaged that coupling the amine ester scaffold (synthesis described in Chapter 2.4.1; Scheme 2.4.2) with an appropriate commercially available chloro- acid chloride of variable chain lengths, would give the desired amide. The chloro- amide ester could be converted into a phosphonium salt by the addition of triphenylphosphine, and further converted into the

phosphorane by reaction with an inorganic base. The phosphorane is a prerequisite for a typical Wittig-type reaction, and can be reacted with commercially available 1-naphthaldehyde to give the desired Wittig product (alkene). The amide and alkene functionalities of the Wittig product can be reduced to give the free amine and alkane, and this resulting intermediate product must lastly be hydrolyzed to give the desired target products.

Approach (II) involves the formation of an amide bond as the key step (Scheme 2.4.6). Coupling of the (already described) amine ester with an appropriate acid chloride would give the desired amide intermediate. Synthesis of the acid chloride also involves a typical Wittig reaction. Commercially available ethyl bromoacetate can be converted into a phosphonate salt by reaction with triphenylphosphine. The reaction of the phosphonate salt with an inorganic base gives the desired phosphorane, which can then be coupled with 1-naphthaldehyde under standard Wittig conditions to give the alkene ester. The alkene can be converted to the alkane by standard reduction of alkenes with H_2 on Pd/C. The ester moiety must be converted to the acid chloride, so initially the ester is hydrolyzed to the carboxylic acid, which is then converted to the acid chloride by reaction with thionyl chloride. At this stage, the two key intermediates can be coupled to together to give the desired amide ester. Following approach (I), the amide must be reduced to the amine, and the ester hydrolyzed to the carboxylic acid to give the desired target compound.

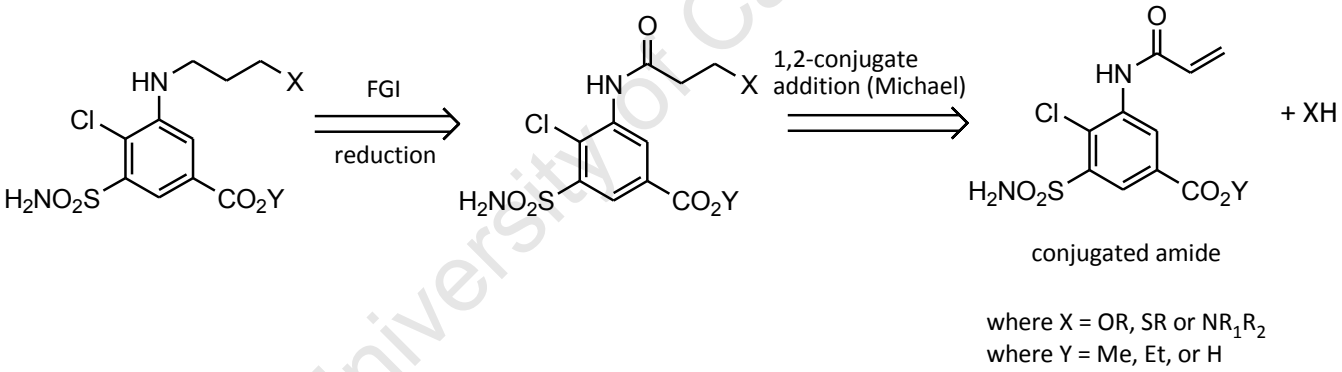


Scheme 2.4.6: Proposed synthesis of lengthened "side chain" derivatives of **H157** based on approach (II) amide formation disconnection

2.4.3 The Nature of the Spacer and Replacement of the Naphthyl Group by Different Cyclic and (Hetero)Aromatic Moieties

In the SAR studies it was envisaged that derivatives of **H157** where the nature of the "side-chain" is modified would be synthesized. These included derivatives in which a heteroatom is introduced and the naphthyl group is replaced with other (hetero)aromatic moieties. The following retrosynthetic scheme (Scheme 2.4.7) was designed to carry this out.

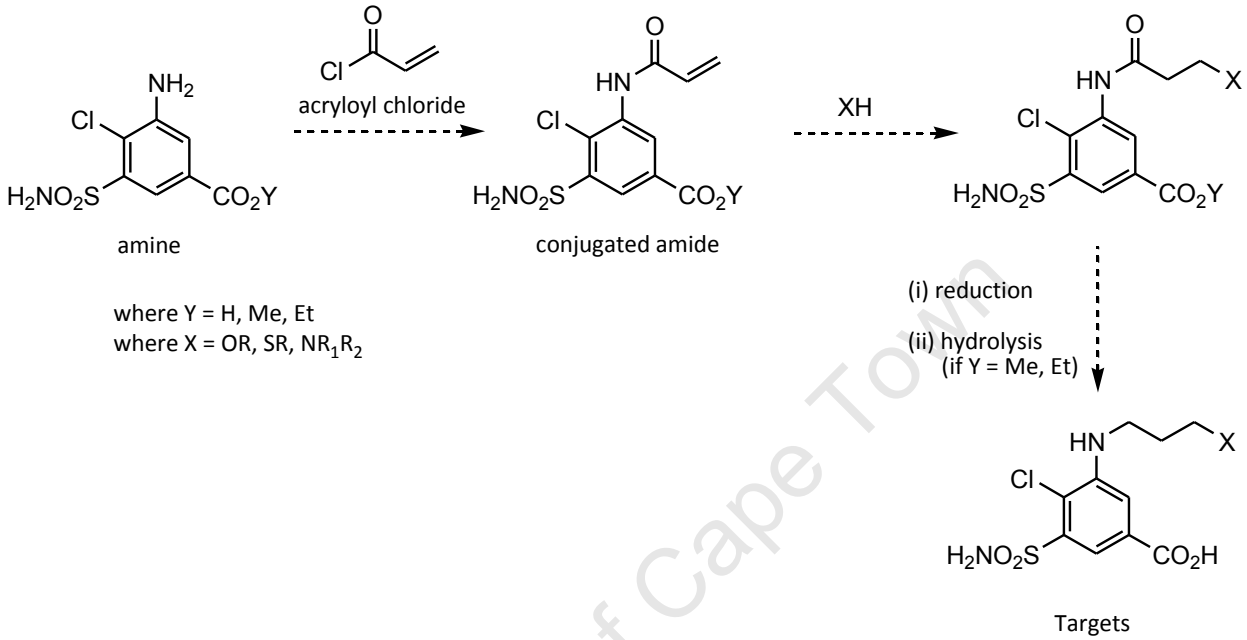
This scheme involves a FGI from an amide to a secondary amine, followed by the 1,2-conjugated addition (Michael addition) disconnection.



Scheme 2.4.7: Retrosynthetic analysis of **H157** derivatives where the naphthyl group is replaced by different cyclic and (hetero)aromatic moieties

The key step of this reaction involves the coupling of the required conjugated amide with an appropriate amine, alcohol or thiol. To make the conjugated amide, the amine ester (or amine acid where Y = H) scaffold (Chapter 2.4.1) can be coupled with commercially available acryloyl chloride. Once the conjugated amide is obtained, it can be coupled with various amines,

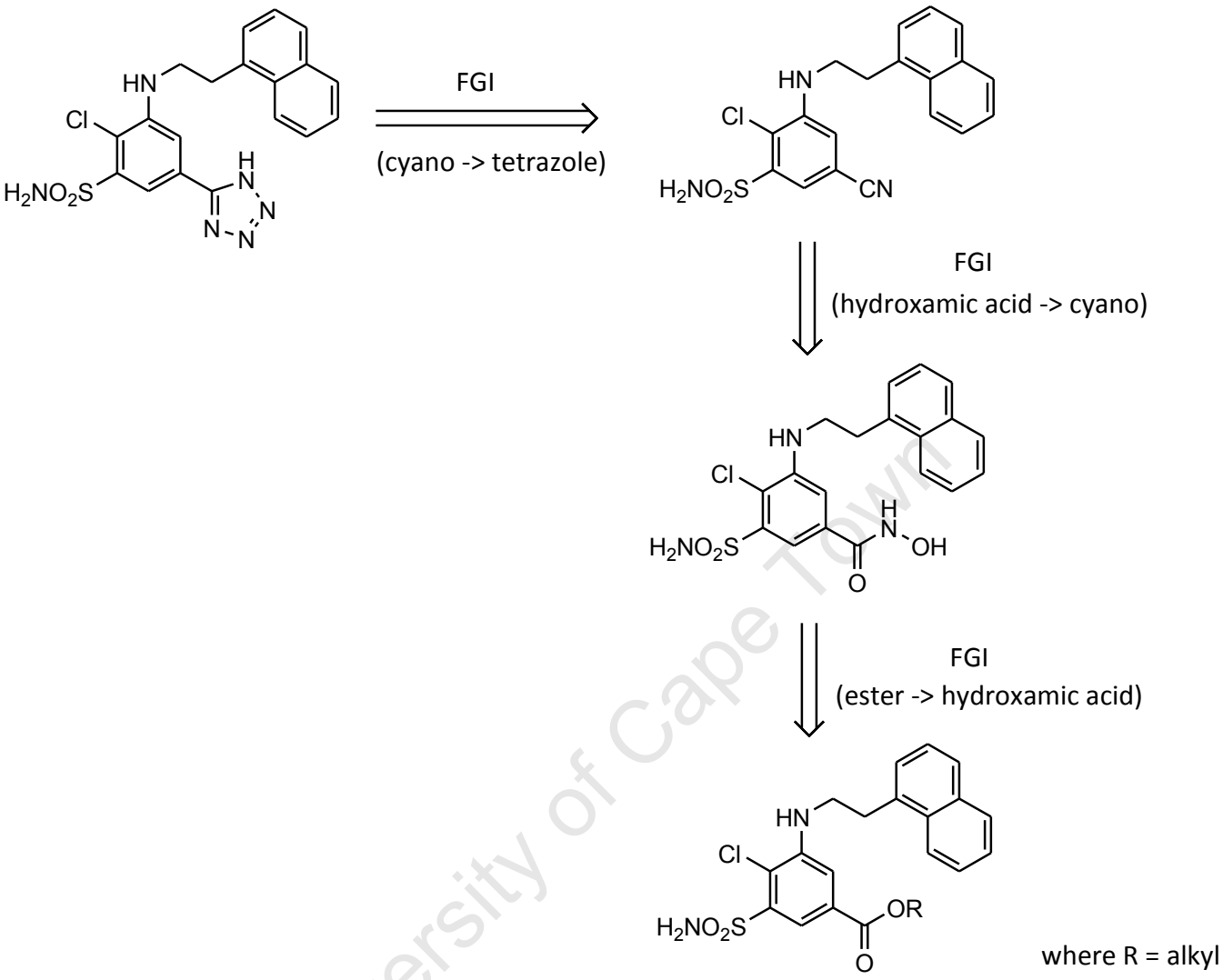
alcohols and thiols. The (Michael) 1,4-conjugate addition products can be reduced (and hydrolyzed if Y = Me or Et) to give the desired target compounds (Scheme 2.4.8).



Scheme 2.4.8: Proposed synthesis of **H157** derivatives where the naphthyl group is replaced by different cyclic and (hetero)aromatic moieties

2.4.4 Bioisosteric Analogues of **H157**

The following retrosynthetic scheme (Scheme 2.4.9) shows the possible functional group interchanges and disconnections that could be used in order to postulate a procedure for the synthesis of both the hydroxamic acid and tetrazole derivatives of **H157** starting from the ester derivative of **H157**. The proposed synthetic scheme will be discussed in detail in Chapter 2.5.4.

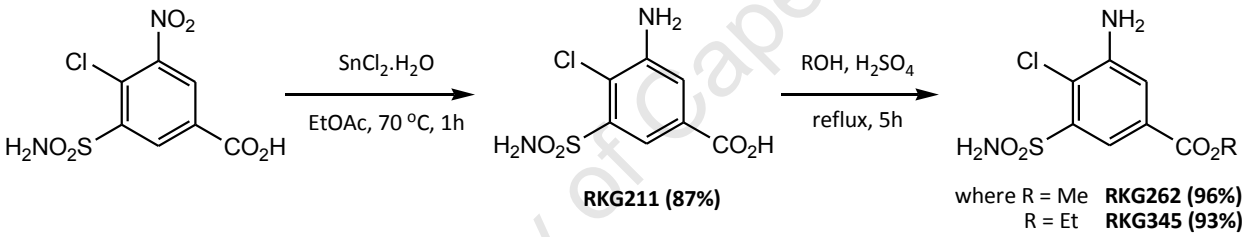


Scheme 2.4.9: Retrosynthetic analysis of bioisosteric derivatives of **H157**

2.5 Methodology and Synthesis of Piretanide-like Analogues

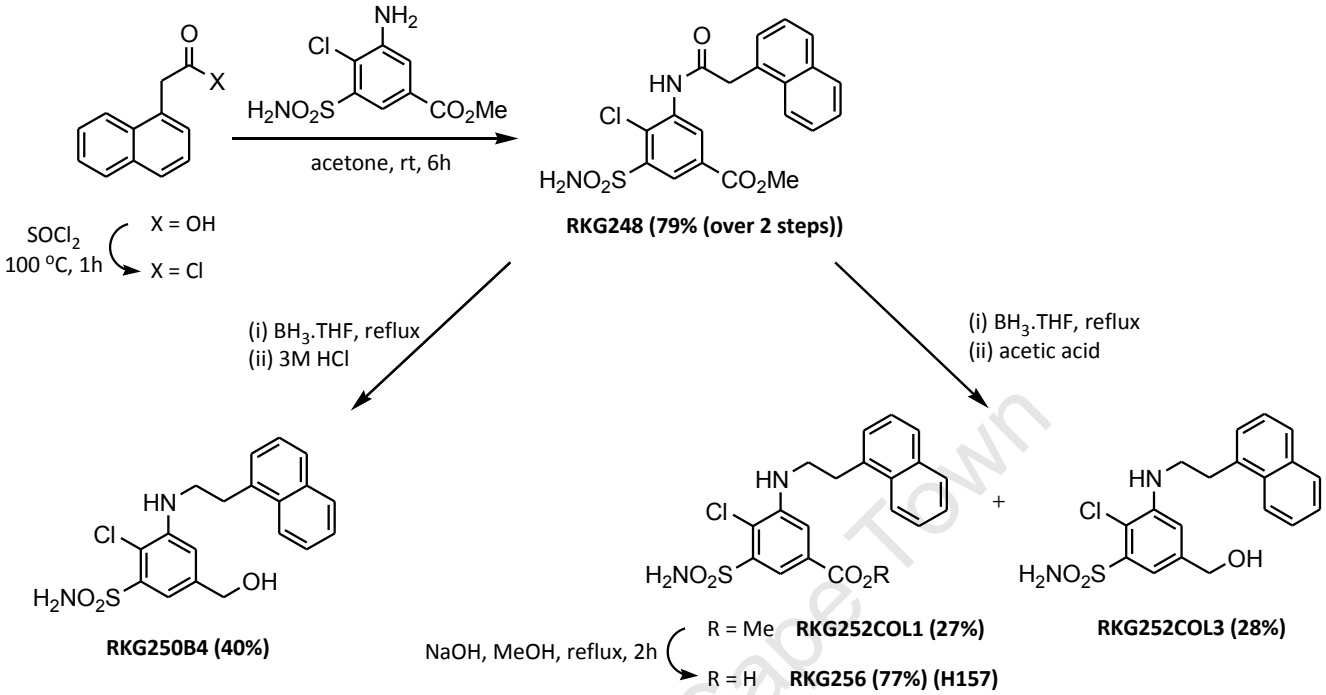
2.5.1 Synthesis of **H157**

Commercially available 4-chloro-3-nitro-sulfamoylbenzoic acid was converted to 3-amino-4-chloro-sulfamoylbenzoic acid (**RKG211**) by the reduction of the nitro group to the corresponding amine using stannous(II) chloride.[32] Acid catalyzed esterfication of the carboxylic acid was carried out using concentrated H_2SO_4 in the appropriate alcohol giving the desired alkyl esters (**RKG262** and **RKG345**) (Scheme 2.5.1).



Scheme 2.5.1: Synthesis of alkyl esters **RKG262** and **RKG345**

1-Naphthalene acetic acid was converted using thionyl chloride ($SOCl_2$) to the corresponding acid chloride. The excess $SOCl_2$ was removed under reduced pressure. The acid chloride was not isolated, but the residue dissolved in acetone and immediately added to the aniline **RKG262**. The amide precipitated out of the acetone solution, and was isolated via filtration and purified by washing with cold acetone. The amide was reduced to the corresponding secondary amine using the commercially available 1M borane-THF complex,[33] and work up in glacial acetic acid. The amine was isolated via column chromatography, and the ester hydrolyzed to the carboxylic acid using sodium hydroxide in a solution of methanol to give the target compound **RKG256** (**H157**) (Scheme 2.5.2).



Scheme 2.5.2: Synthesis of **RKG256** (**H157**)

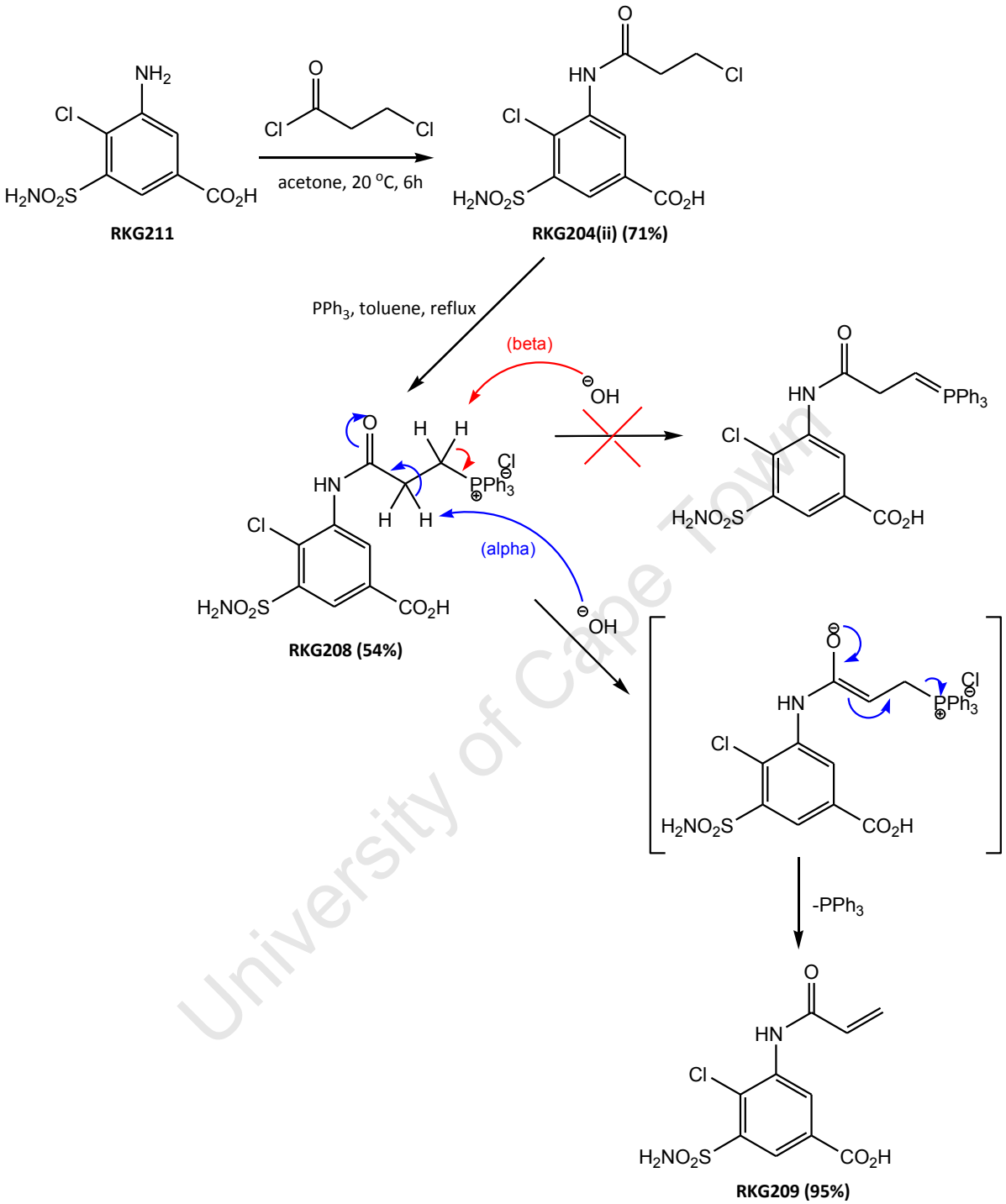
As can be seen in Scheme 2.5.2, when the work-up of the reduction with borane-THF complex was performed using aqueous 3M HCl, only the alcohol **RKG250BF4** was obtained. This demonstrates that the ester may be hydrolyzed in the presence of the aqueous acid, and the resulting carboxylic acid is further reduced by the excess borane, via an acyloxyborane intermediate, resulting in the formation of the alcohol. Based on this conclusion, the reduction was repeated using the weaker glacial acetic acid instead of the aqueous acid. After column chromatography, the target molecule (**RKG252COL1**) was obtained in a low yield of 27% accompanied by the alcohol **RKG252COL3** (28%). This probably was a result of the glacial acetic acid not being dry enough, or due to the slight exposure of the reaction to air (water vapor).

2.5.2 The Length of the Alkyl (-CH_2-) Spacer

Approach (I):

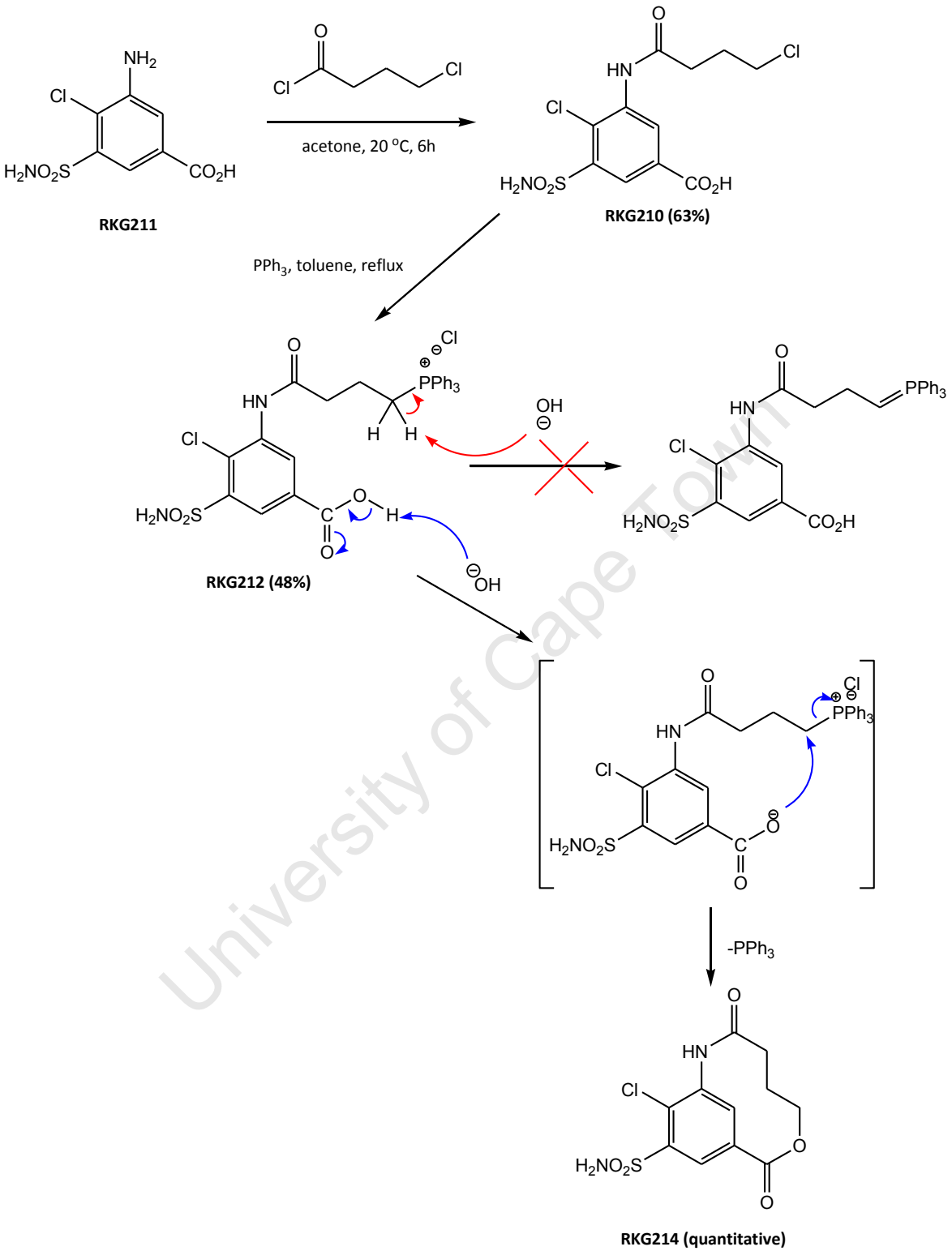
Initially, amine (**RKG211**) was reacted with 3-chloropropionyl chloride to give the amide (**RKG204(ii)**). The corresponding Wittig phosphonium salt was synthesized by refluxing the halogenated **RKG204(ii)** with triphenylphosphine in toluene. Applying the standard protocol for Wittig reactions, the phosphonium salt is converted into the corresponding phosphorane using a base, like NaOH or K_2CO_3, in an aqueous solution. Abstraction of the *β*-hydrogen (in relation to the amide carbonyl) was the desired pathway by the base. However, the apparently more acidic α-hydrogen was instead abstracted, leading to the stable conjugated product **RKG209**. This α-H abstraction led to formation of the intermediate enolate, which underwent conjugate elimination of the triphenylphosphine, a good leaving group. This E1cB-type elimination resulted in the formation of the conjugated product **RKG209** (Scheme 2.5.3).

With hindsight, the failure to obtain the desired product (phosphorane) was not a surprise, but obtaining the conjugated product prompted its use for nucleophilic (Michael) addition reactions, as well as the synthesis of other derivatives of **H157** (piretanide-like) analogues containing heteroatoms in the side-chain.



Scheme 2.5.3: Attempted synthesis of lengthened "side chain" derivatives of **H157**. E1cB-type elimination resulted in the formation of the conjugated product **RKG209**

After the failure to produce the desired phosphorane, a decision was made to attempt a similar protocol using a longer chain acid chloride in which elimination of the triphenylphosphine would not be possible. Thus the amine **RKG211** was reacted with 4-chlorobutyryl chloride to give the halogenated amide (**RKG210**) using the same protocol as discussed with 3-chloropropionyl chloride. The phosphonium salt (**RKG212**) was also synthesized as discussed previously. The phosphonium salt was dissolved in H_2O and after the addition of NaOH, allowing the reaction to stir for 40 hours and acidic work-up (standard procedure), a precipitate formed. This precipitate was isolated, washed and dried *in vacuo*. ^{1}H, ^{13}C NMR and mass spectroscopy analysis showed that the product was again not the desired phosphorane, but suggested the formation of a 10-membered macrocycle (**RKG214**) in a quantitative yield. This may be due to an intra-molecular nucleophilic substitution reaction involving the deprotonated carboxylic acid (carboxylate) as proposed in Scheme 2.5.4:

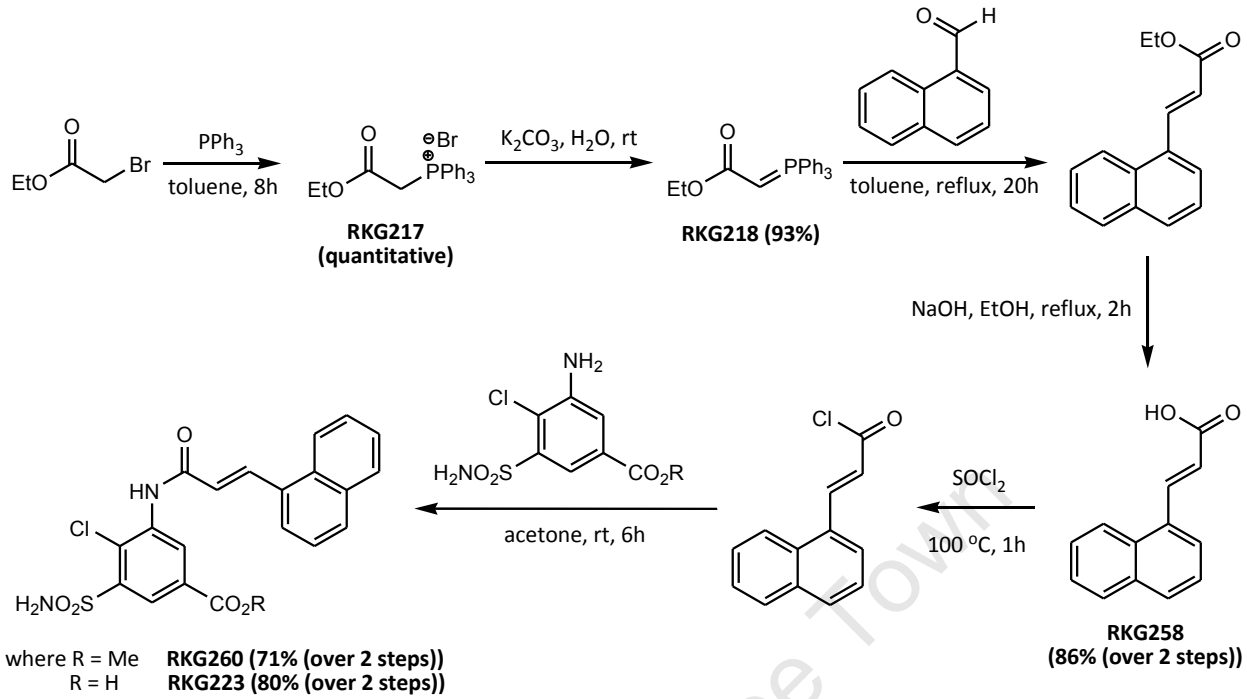


Scheme 2.5.4: Attempted synthesis of lengthened "side chain" derivatives of **H157**. Proposed intra-molecular nucleophilic substitution reaction to give macrocycle **RKG214**

Approach (II):

Due to the failure of approach (I), the second approach via the amide retrosynthetic disconnection was evaluated. This approach would consist of coupling the aniline (**RKG262**) with an appropriate acid chloride synthesized via Wittig chemistry.

Accordingly, ethyl bromoacetate was reacted with triphenylphosphine to give the phosphonium salt (**RKG217**) in quantitative yields. Using either NaOH or K_2CO_3 as the base in the standard Wittig procedure, the desired phosphorane (**RKG218**) was obtained also in comparably high yields. The phosphorane was coupled with 1-naphthaldehyde to give the desired alkene **RKG258**. The mixture of alkene and unreacted aldehyde (which was used in slight excess in coupling step) was isolated via flash chromatography, and subjected to hydrolysis using NaOH. Before acidification of the reaction mixture to obtain the free acid, the intermediate carboxylic anion was easily extracted into an aqueous phase. The pH of the aqueous phase was adjusted to acidic, resulting in the precipitation of the desired carboxylic acid (**RKG259**). The precipitate was filtered off and dried *in vacuo*, giving the carboxylic acid in a high yield (86% over two steps) and purity. The carboxylic acid was converted into the acid chloride with $SOCl_2$, and added to a solution of aniline (**RKG262**) in acetone. After 6 hours of stirring at room temperature (~20 °C) had elapsed, the amide (**RKG260**) had precipitated out, and was filtered off and washed to yield a pure white product (Scheme 2.5.5). The reaction was also performed using aniline (**RKG211**) giving amide (**RKG223**) using the same protocol.



Scheme 2.5.5: Synthesis of lengthened "side chain" alkene derivatives (**RKG260** and **RKG223**) of **H157**

Once amide **RKG260** was obtained, it was noted that borane can reduce both amide and alkene moieties to amines and alkanes respectively. This was accordingly attempted but, as described in the previous chapter, the problem of the ester group being hydrolyzed by the slight presence of water in the acidic work-up, and subsequently being reduced by the excess borane to the alcohol, was a recurring one.

To circumvent this problem, the alkene moiety of **RKG260** was first hydrogenated using H_2/Pd-C in methanol. The reaction was monitored over 5 days via TLC. During this time the alkene and alkane co-ran on the TLC. Excess catalyst (Pd-C) was added and the reaction was left stirring under a positive pressure of hydrogen. The reaction mixture was filtered through Celite©, and

the Celite© was washed thoroughly with methanol. The solvent was removed under reduced pressure, yielding a yellowish/white powder. ^{1}H and ^{13}C NMR analysis revealed that the reaction had not gone to completion, and that a mixture of starting material (alkene) and desired product (alkane) was obtained.

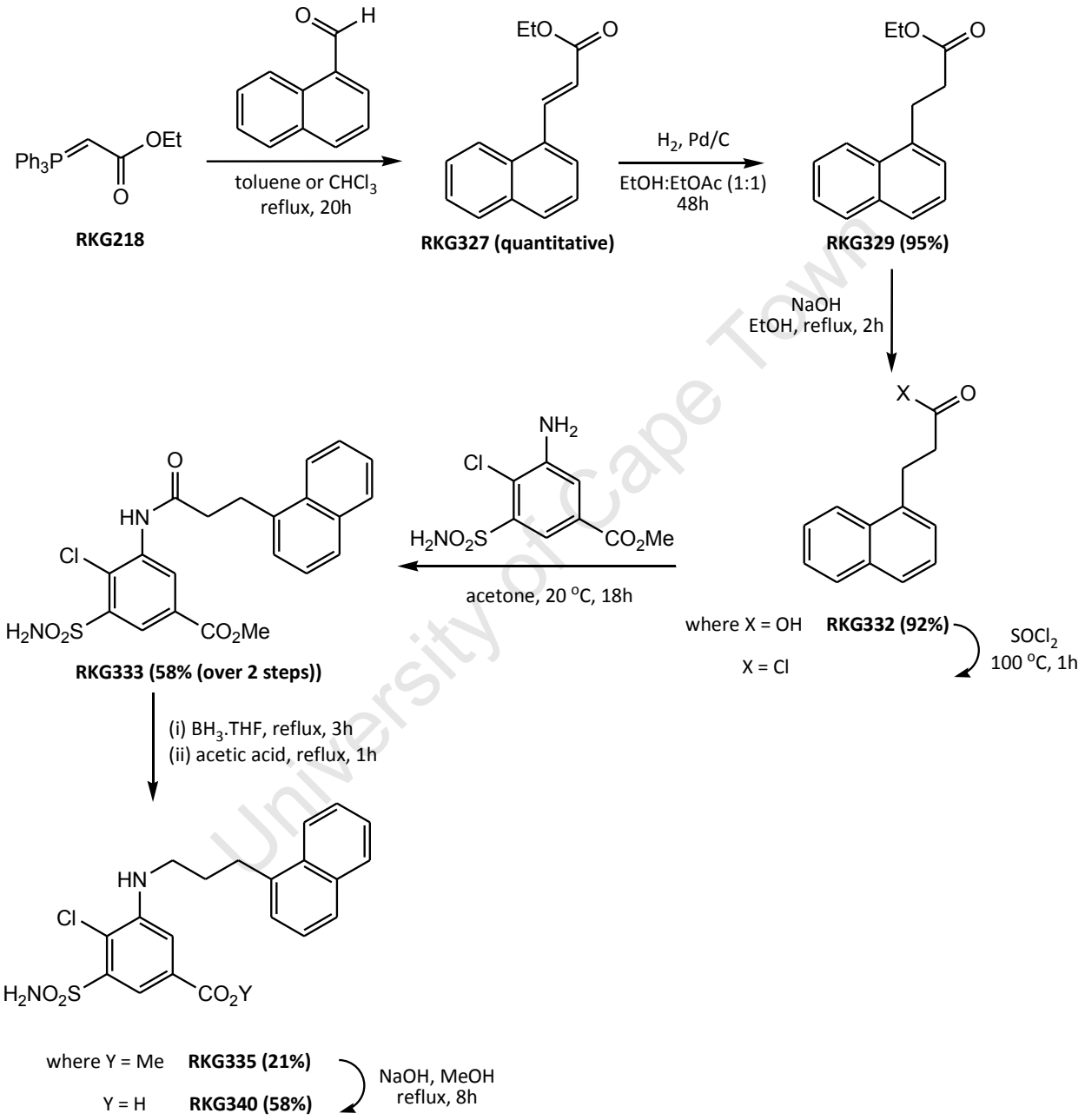
At this point, two alternative approaches were considered in order to obtain the desired analogues of **H157**:

(i) excess styrene, or another volatile alkene, could be used to mop up any excess borane before the acid work-up step is performed, and

(ii) the carboxylic acid (alkene) (**RKG258**) could be hydrogenated to the alkane with H_2 on Pd-C, before converting it to the acid chloride, and clipping it onto the aniline.

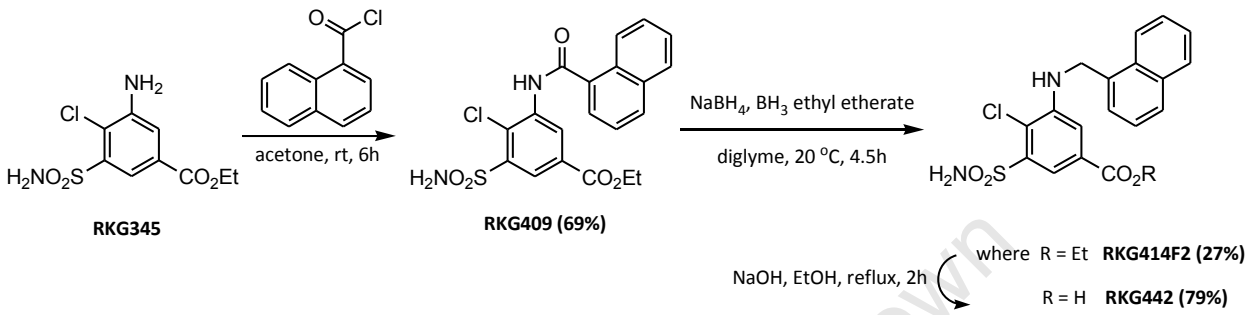
Following approach (II), hydrogenation of the carboxylic acid **RKG258** to the corresponding alkane with H_2 on Pd-C was envisaged. The reaction was attempted using a 1:1 mixture of EtOH and THF, and left to stir for 48 hours under an atmosphere of H_2 gas. Following the general reaction procedure, a white powder was obtained, but ^{1}H and ^{13}C NMR analysis confirmed it to be a mixture of both alkene and alkane. At this juncture, hydrogenation of the ester **RKG327** was considered. The reaction was performed in a 1:1 mixture of EtOH and EtOAc to help facilitate solubility, and left to stir for 48 hours under an atmosphere of H_2 gas. A clear orange oil was obtained following the general reaction procedure, and desired alkane **RKG329** was obtained in a 95% yield. This ester (**RKG329**) was hydrolyzed to the corresponding carboxylic acid **RKG332** using NaOH following the same protocol as discussed above. The carboxylic acid (**RKG332**) was converted to the acid chloride using $SOCl_2$, and immediately reacted with the aniline **RKG262** resulting in amide **RKG333** in a yield of 58% over the two steps. Borane reduction of **RKG333** and an acetic acid work-up gave the desired secondary amine **RKG335**,

which was isolated using column chromatography as a clear brown oil in a low yield of 21%. The methyl ester group of compound **RKG335** was hydrolyzed to the corresponding carboxylic acid using NaOH, and the desired target molecule **RKG340** was obtained in a yield of 58% (Scheme 2.5.6).



Scheme 2.5.6: Synthesis of lengthened "side chain" derivative (**RKG340**) of **H157**

After obtaining the desired target **RKG340** with a three carbon methylene spacer as well as **H157**, the one carbon methylene spacer derivative was next targeted. The compound was synthesized according to the following scheme (Scheme 2.5.7).



Scheme 2.5.7: Synthesis of shortened "side chain" derivative (**RKG442**) of **H157**

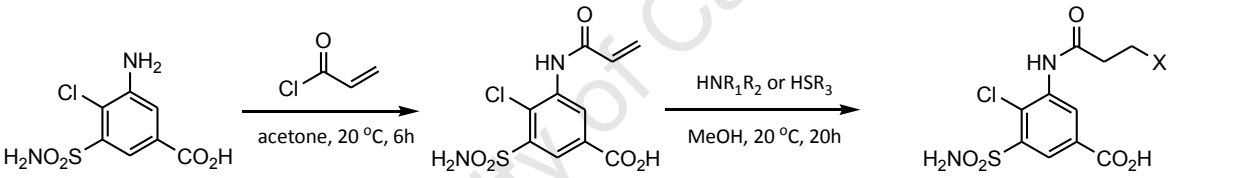
Commercially available 1-naphthoyl chloride was coupled with aniline **RKG345** resulting in the formation of amide **RKG409** in a yield of 69%. Instead of using the borane.THF complex for the reduction of the amide group, as previously performed, sodium borohydride in a solution of BH_3 etherate in diglyme was used. Following the reduction procedure,[31] and after work-up, the desired amine **RKG414F2** was isolated using column chromatography in a yield of 27%. Hydrolysis of the ethyl ester to the free carboxylic acid was carried out using NaOH in ethanol and the desired target compound **RKG442** was isolated in a good yield of 79%.

2.5.3 Synthesis of **H157** Derivatives where the Naphthyl Group is Replaced by Different Cyclic and (Hetero)Aromatic Moieties

As previously noted, an unintentional conjugated product (**RKG209**) was obtained due to a base mediated conjugate elimination of triphenylphosphine while attempting to synthesize a

phosphorane for a Wittig reaction. It was proposed in the SAR studies of piretanide-like molecules that derivatives containing heteroatoms in the side chain would be synthesized and compound **RKG209** was identified as an ideal starting material for such studies.

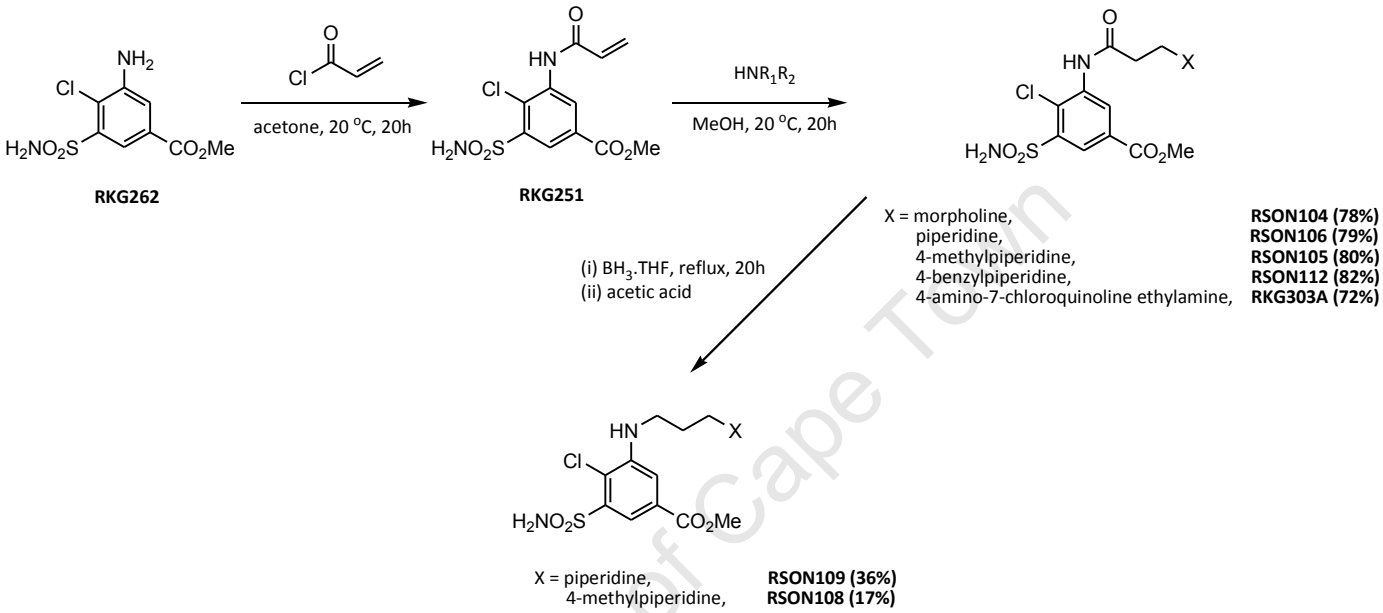
Instead of synthesizing the conjugated amide using the initial method via the phosphonium salt, the aniline **RKG211** was coupled with the available acryloyl chloride to give the desired product. The yield for this coupling (30-58%) was not nearly as high as that obtained from the elimination reaction (95%), but does save a synthetic step. The conjugated amide **RKG209** was reacted with various cyclic amines and *tert*-butyl thiol. The Michael addition reaction was accomplished with varying degrees of success. Yields in the range 8 - 65% were obtained. However, no reaction was observed using the thiol, and neither for the 4-amino-7-chloroquinoline ethyl amine.



Scheme 2.5.8: Synthesis of **H157** derivatives where the naphthyl group is replaced by different cyclic moieties

Due to the low yields obtained above with the aniline and amide, the use of the corresponding methyl ester derivatives was attempted (Scheme 2.5.9). Aniline **RKG262** was coupled with acryloyl chloride to give the conjugated amide **RKG251** in a much improved yield of 84%. Four of the previously used cyclic amines were selected and reacted with the Michael acceptor

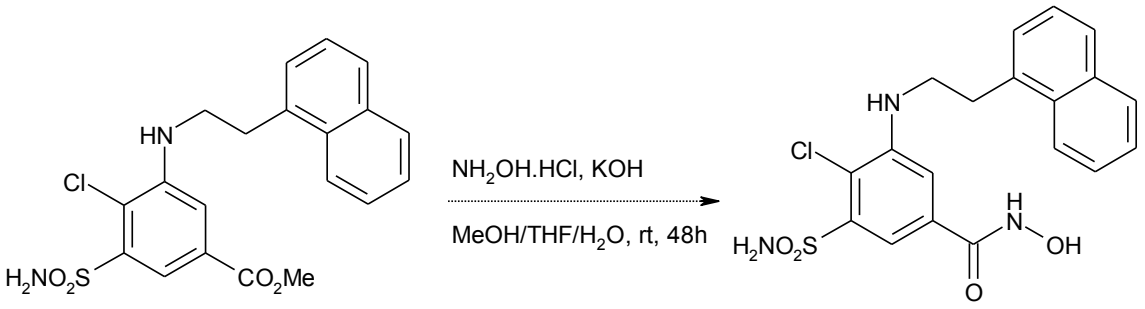
resulting again in vastly improved yields of 72 - 82%. Two amides (namely **RSON106** and **RSON105**) were selected for the borane reduction step to give the corresponding secondary amines. After column chromatography the desired diamine products (**RSON108** and **RSON109**) were isolated in low yields.



Scheme 2.5.9: Synthesis of ester **H157** derivatives where the naphthyl group is replaced by different cyclic and (hetero)aromatic moieties

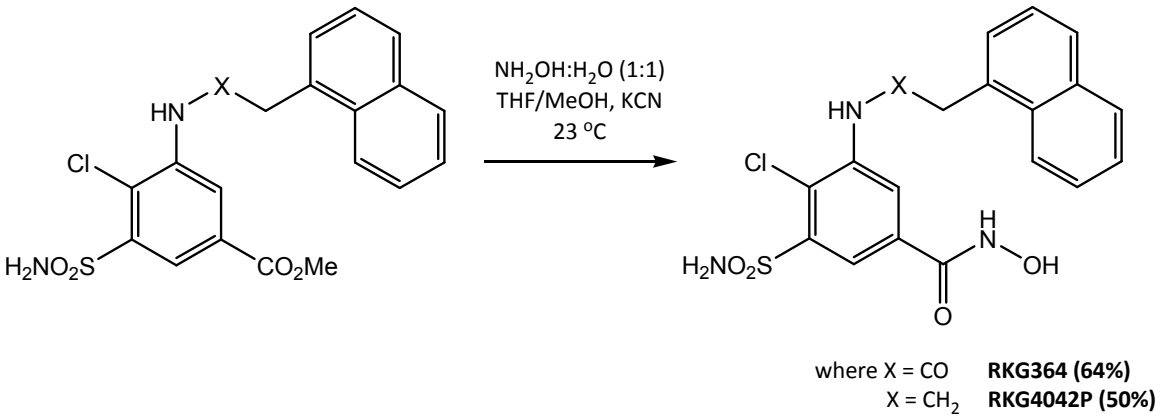
2.5.4 Synthesis of Bioisosteric Analogues of **H157**

Based on the SAR studies summarized earlier, carboxylic acid bioisoteres, namely the tetrazole[34,35,36] and hydroxamic acid,[37,38] are important analogues of **H157**. The conversion of the methyl ester analogue of **H157** to the corresponding hydroxamic acid using the hydroxylamine hydrochloride salt was initially proposed (Scheme 2.5.10).



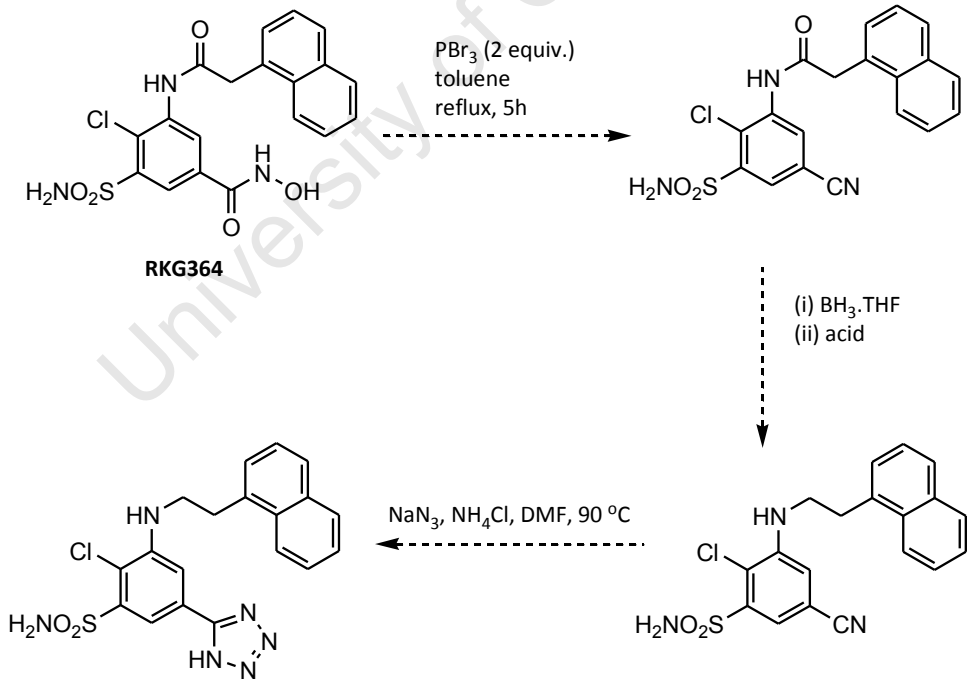
Scheme 2.5.10: Proposed synthesis of hydroxamic acid bioisostere derivative of **H157** using hydroxylamine hydrochloride salt

This reaction was attempted without success. Due to the low yields of the methyl ester analogue of **H157** from the reduction step, an alternative strategy was adopted. Following the literature,[37] **RKG248** (and **RKG346**) were reacted with 50% aqueous hydroxylamine solution in the presence of a catalytic amount of KCN (Scheme 2.5.11). The reaction was monitored by TLC. As described in the literature, KCN is reported to increase the efficiency of transformation form the ester to the hydroxamic acid. The reaction appeared to be progressing as a new product spot was forming (R_f = 0.03; 1:19, MeOH:EtOAc) as the reaction was monitored at 12 hour intervals. In an attempt to drive the reaction to completion, further amounts of 50% aqueous hydroxylamine solution and KCN (approximately half molar equivalents) were added at the 12 hour intervals. The methyl ester reaction appeared to be complete after 3 days (the ethyl ester reaction appeared complete after 6 days). A small amount of precipitate formed and was filtered off. This precipitate was determined to be the hydroxylamine hydroxamic acid salt as confirmed by ^{1}H and ^{13}C NMR in d^6-DMSO. The hydroxamic acid could be isolated via column chromatography as a brown oil, which later crystallized to a white solid. The method was then applied to the methyl ester analogue of **H157** to obtain the hydroxamic acid analogue and the desired target was obtained in a yield of 50%.



Scheme 2.5.11: Synthesis of hydroxamic acid derivatives (**RKG364** and **RKG4042P**) of **H157**

To obtain the tetrazole analogue of **H157**, the following reaction procedure was initially proposed (Scheme 2.5.12) and attempted. This method avoids the borane reduction of the methyl ester amide **RKG248** which had been problematic due to low yields and time consuming column chromatography.



Scheme 2.5.12: Proposed synthesis of tetrazole bioisostere derivative of **H157**

The proposed synthetic route could not be implemented as the first step involving the conversion of the hydroxamic acid moiety to the cyano group using phosphorous tribromide was unsuccessful. Through numerous attempts the reactions were monitored using TLC and the formation of numerous (and co-running) products was observed. Spots were isolated using column chromatography but none of them could be confirmed as the desired cyano product using ^{1}H NMR spectroscopy. Phosphorous tribromide is a very harsh reagent, and studying the protocol as described in the literature,[39] it was further observed that all the aromatic hydroxamic acids that were used were simple aromatic compounds bearing very few or no other functional groups. The solubility of **RKG364** in toluene could have also been a contributing factor to the reaction being unsuccessful as even at a reflux temperature of >110 ^{o}C poor solubility of **RKG364** in toluene was clearly observed.

2.6 Conclusion

Based on the in-depth SAR studies that were initiated at the start of the project, there are many parts of the piretanide-type lead compound **H157** that could be studied. Unfortunately, due to time constraints and the need to focus on other aims of the project, not all of the highlighted SAR studies described in Chapter 2.3 could be carried out. The successful synthesis of **H157** and its derivatives bearing an altered "side-chain" length, numerous carboxylic acid and ester derivatives bearing hetero atoms, as well as heterocyclic and aromatic moieties in place of the naphthyl functionality, and the hydroxamic acid bioisostere of **H157** were achieved. All final target compounds and relevant intermediate compounds that were synthesized were submitted for testing in the sorbitol uptake inhibition (NPP) assay as well as for *in vitro* testing against chloroquine sensitive and chloroquine resistant strains of *Plasmodium falciparum*. The resulting biological data shall be discussed in Chapter 6.

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Chapter 3

Tuberculosis

Tuberculosis (TB) is a chronic or acute bacterial infection caused by *Mycobacterium tuberculosis* (Figure 3.1). It primarily attacks the lungs, but there are cases where the central nervous system, circulatory system, genitourinary system, bones, joints, lymph nodes and skin are also affected. Tuberculosis can also be caused by *Mycobacterium bovis*, *M. africanum*, *M. canetti* and *M. microti*, however, healthy adults are usually not susceptible to infection by these mycobacterium species.[1]

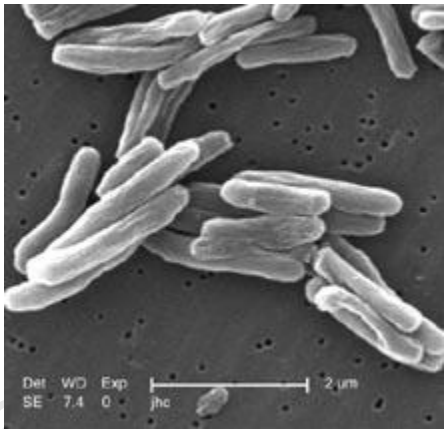


Figure 3.1: Scanning electron micrograph of the rod-shaped *Mycobacterium*[2]

The earliest traces of *Mycobacterium tuberculosis* have been detected in the remains of fossilized bison dating back some 18,000 years.[3] It is still debated whether tuberculosis originated from cattle and at some stage transferred to human, or whether it came from a common ancestor.[4] The earliest tubercular decay observations in prehistoric human remains date back approximately to 4,000 BC. There has also been evidence found in the spines of mummies dating around 3000-2400BC.[5] Scrolls and writings of the ancient Babylonian, Egyptian and Chinese civilizations also refer to and describe the presence of tuberculosis.[6]

Tuberculosis was declared a global emergency in 1993 by the World Health Organization (WHO). It is believed that more than a third of the world's population has been exposed to the

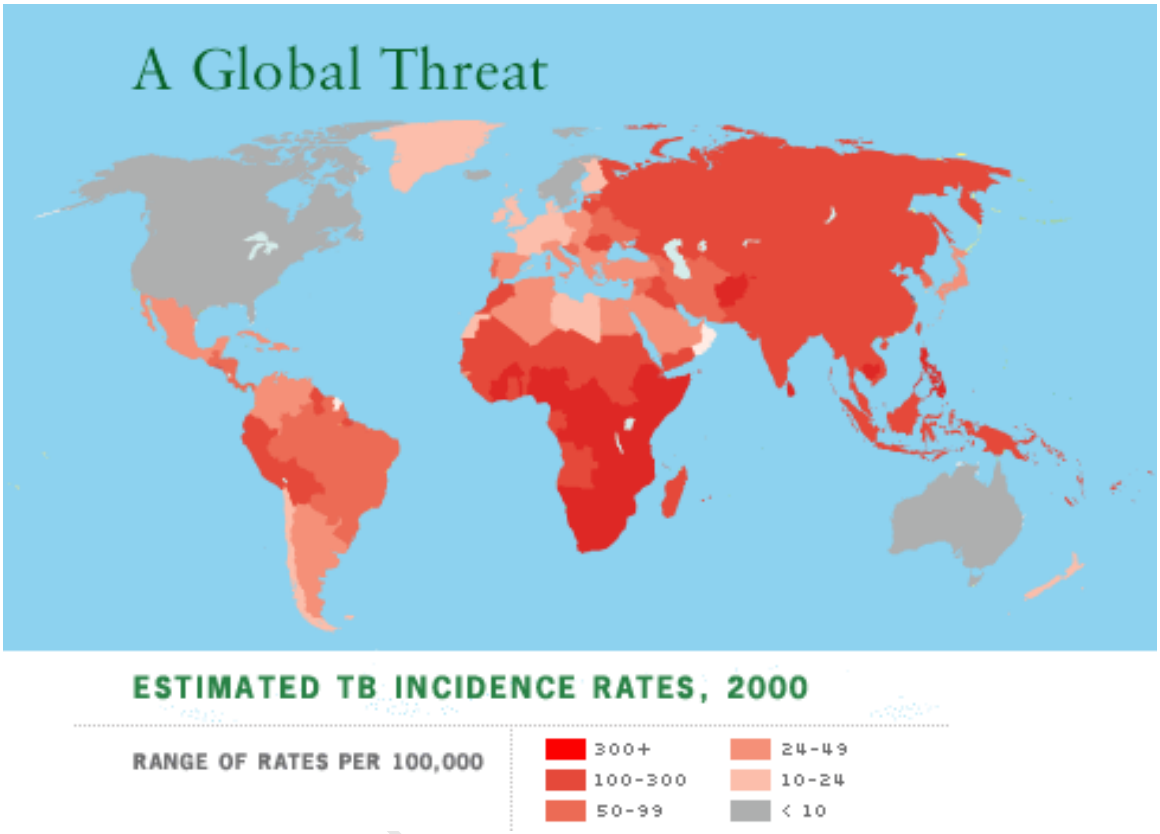


Figure 3.2: WHO estimated global TB incidence rates in 2000[7]

bacterium. Statistics from WHO reveal that a person is infected with TB every second. In the year 2005 alone, 8.8 million people contracted the disease and approximately 1.6 million died as a result.[7] The statistics are overwhelming due to fact that if tuberculosis is detected early, it is curable. However, the prevention of tuberculosis is being continually challenged by the emergence of multi-drug resistant TB (MDR-TB) and extensively drug resistant TB (XDR-TB), the rise in HIV-associated TB, the neglect of TB control programs and poor health systems (mainly in developing nations).[1,7] The WHO further predicted that if something is not done to counter

this growing epidemic, approximately 35 million people will die as a result of tuberculosis in the next 20 years.[7]

3.1 Symptoms and Transmission

Transmittance of TB occurs when a person with active pulmonary TB coughs, sneezes, speaks, or spits, releasing small bacteria-carrying droplets (aerosol) into the air (Figure 1.2.3), and these particles are inhaled by a non-infected individual in close proximity. The risk of contracting the disease is increased in areas where TB is common, unsterilized instruments and needles are used (tattoos, circumcisions and illicit drug abuse), employees and residents abide in highly congested settings, medical services are minimal (low-income populations), patients are immunocompromised by other conditions (e.g. suffering from HIV/AIDS or using immunosuppressant drugs) and health workers are attending to high-risk patients.[8] Another route of transmission of TB is through the skin. This route, however, is less common, and the handling of the bacterium by pathologists and laboratory technicians with open skin wounds presents a risk.

Figure 3.3: A single cough or sneeze can release up to 40,000 infectious aerosol droplets[2,9]

The symptoms of a TB-infected person, include prolonged coughing, coughing up of mucus containing blood, chest pains, difficulty to breathe, fever, chills, night sweats, weight loss, loss of appetite, and fatigue.[10,11]

3.2 Pathogenesis

Approximately 90 percent of all TB infections fall under the classification of asymptomatic, latent TB (LTB). In most of these cases, the human body's immune system may inhibit or destroy the bacteria. Only 5 to 10 percent of people who are infected with TB actually develop the active disease, but of these, more than 50 percent die if the active TB disease is untreated.[6,7] Upon contraction of TB, there are two possible development stages of the disease, namely the primary stage and the secondary stage.

Primary TB:

This primary stage of infection usually passes by unnoticed by the exposed subject as there are no noticeable symptoms, and the TB is not contagious during this phase either. Infection starts when the mycobacteria come in contact with pulmonary alveoli. The body's immune cells, that destroy foreign material, detect and engulf the TB bacteria by aggregating to form literally a "wall" around the inactive bacteria. The contained bacteria are then transported to the local (mediastinal) lymph nodes. The wall is made up of aggregated macrophages, T lymphocytes, B lymphocytes and fibroblasts.[2,12] It is within these tubercles or granuloma (alveolar macrophages), that the mycobacteria can remain inhibited and inactive, and even eventually destroyed as long as the subject's immune system remains strong.[12,13] Over time, the tubercles can gradually gather calcium deposits, and form what is known as a Ghon focus. Permanent scars, which can be seen as dark shadows in the lungs, are a result of the healing of the initial tubercles. The mycobacteria may also remain dormant for many years within these tubercles

awaiting a decline in the immune system, resulting in latent TB (secondary TB). However, within the tubercles, the mycobacteria may multiply resulting in active primary TB. It is at this stage that the symptoms, including coughing, fever, night sweats and weight loss, become prevalent. This stage can be recognized with the use of X-rays (Figure 1.2.4), by observing dark shadows in the lungs, or fluid collection between the lung and the lungs lining.

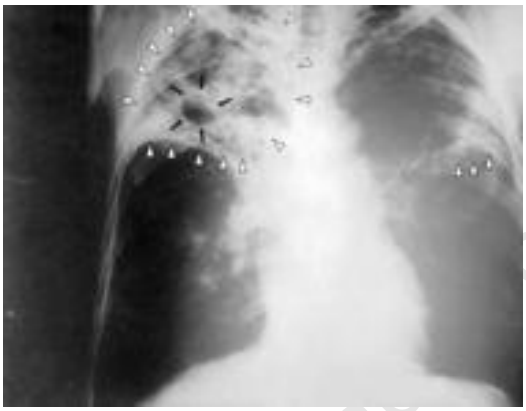


Figure 3.4: Chest X-rays of lungs reveals the presence of bilateral pulmonary infiltrate (white triangles), and "caving formation" (black arrows) present in the right apical region.The diagnosis is far-advanced tuberculosis.[2,14]

Secondary (latent) TB:

As mentioned above, the mycobacteria can remain dormant within the tubercles for many years before becoming active and multiplying. This "awakening" is usually induced by the weakening of the body's immune system. This weakening of the immune system is often a result of HIV/AIDS, or the use of immunosuppressant drugs. The mycobacteria replicate, resulting in the formation of more and more tubercles, and progressively destroy lung tissue. The mycobacteria spread around the body via the bloodstream, and can develop secondary TB lesions in the lung apices, peripheral lymph nodes, kidneys, brain, and bone.[15] At this stage, the coughing up of blood or phlegm is a common symptom, and the disease is contagious to others.

3.3 Diagnosis

Diagnosis of tuberculosis is a not an easy process because the culturing procedure of this slow-growing bacteria is very difficult in a laboratory. There are several different tests that are done in order to diagnose if a person has TB.

To diagnose if an individual is infected with latent tuberculosis, a tuberculin skin test is carried out. This is performed by injecting purified protein derivatives, derived from *M. tuberculosis*, into the skin (Figure 1.2.5). If the subject has been exposed to the infection, swelling would be observed at inspection 48 to 72 hours later. This is possible as the subject would have acquired a hypersensitivity to the mycobacteria, even though the disease is not in an active state. This test, however, is not 100 percent accurate.[6]

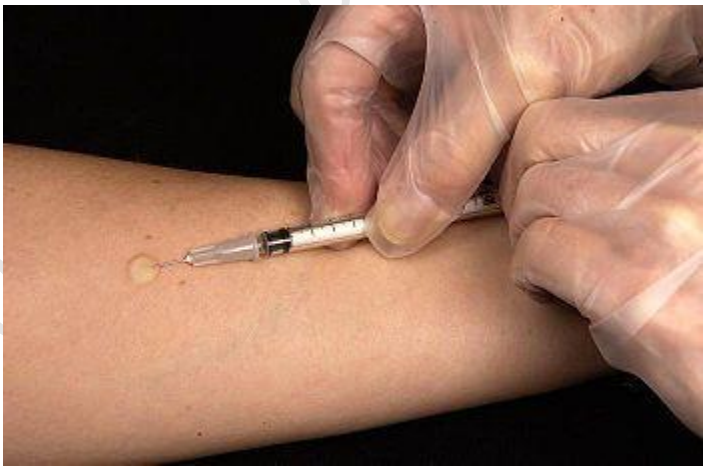


Figure 3.5: The tuberculin skin test is performed to diagnose for latent tuberculosis[2]

For the diagnosis of the active pulmonary tuberculosis, sputum or other bodily fluids and tissue can be analyzed for the bacterium, including staining (Figure 1.2.6), microbiological smears and cultures, in combination with a chest X-ray to show any unusual anomalies in the lungs. Once

diagnosed, further testing is performed in order to determine which strain of the mycobacteria is present. This is done because different drugs are used to treat different strains of TB more effectively, as well as the ever increasing emergence of MDR-TB and XDR-TB.

Figure 3.6: Red-stained *Mycobacterium tuberculosis* in infected human sputum[2]

New TB diagnostic tests are constantly being developed. Tests that are less time consuming, cheaper and more accurate would be the desired attributes, especially in the developing world. The use of genetic engineering and new polymerase chain reaction (PCR) are some of the new techniques being developed to rapidly duplicate any hereditary bacterial material and to identify bacterial DNA.[2,6,16]

3.4 Prevention and Treatment

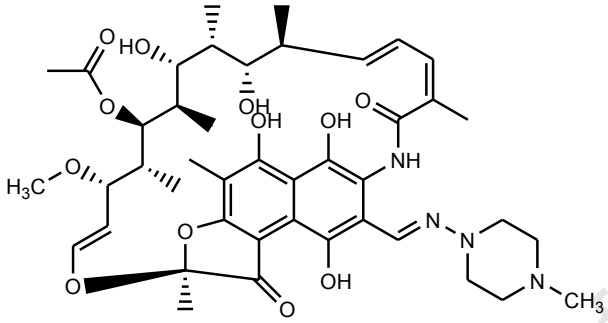
Preventative measures to protect a person from being exposed to the TB bacteria would obviously be first choice in battling TB. Ventilation systems disperse the bacteria, and lower the

risk of being inhaled in public places, and ultraviolet light also can reduce the threat by killing large amounts of the bacteria.

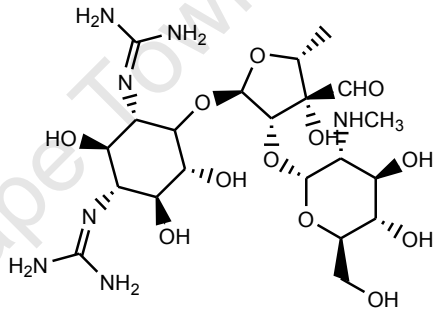
Vaccines, containing weakened bacteria, have been developed as another preventative measure. The *M. bovis*-derived bacillus Calmette Guerin (BCG) vaccine, developed at the Pasteur Institute in France between 1905 and 1921, is still the most effective preventative measure against infection amongst children.[17] However, there is no vaccine currently available that offers adults complete and reliable protection. Recently, there have been concerns about the safety of BCG being used in vaccinating HIV-infected patients as results have varied significantly in trials.[18,19] Newer vaccines that are being developed include a recombinant TB vaccine developed by the National Institute of Allergy and Infectious Disease (NIAID) in the United States which entered clinical trials in 2004,[20] and the TB vaccine, MVA85A, developed by a group from Oxford University which is currently in phase II trials in South Africa.[21]

Drug therapy remains to this day the most effective way of treating tuberculosis. The two most effective first-line antibiotics are rifampicin and isoniazid. Single-drug treatment is susceptible and at risk to the bacteria developing antibiotic resistance,[22] hence, combination or multiple drug therapy is administered to patients with active pulmonary TB. Unlike the typical treatment of other bacterial infections, the course of antibiotic treatment for mycobacteria (regimen) is much longer, and usually ranges between 9 and 12 months. The most commonly prescribed combination of antibiotics for multi drug therapy includes isoniazid, rifampicin (rifampin), pyrazinamide, ethambutol and streptomycin. In some cases, latent TB is still treated by single-drug therapy. The long term treatment of latent TB, with the combination drug therapy of rifampicin plus pyrazinamide, has recently been advised against by the Centers for Disease Control and Prevention (CDC) due to high incidences of hospitalization and death of patients suffering from liver injury.[23]

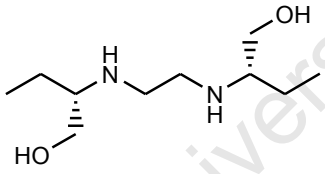
The effective treatment of TB using combination and multiple drug therapy has come under threat by the emergence of MDR-TB and XDR-TB. Drug resistance is believed to be a result of inadequate drug therapy, low quality medication, and the inappropriate use of drugs, for example, patients not taking the prescribed drugs for the total 9 to 12 months treatment course. Tuberculosis strains which are resistant to both first-line antibiotics are classified as MDR-TB. The classification of XDR-TB is used for a TB strain which is also resistant to three or more of the six classes of second-line antibiotics.



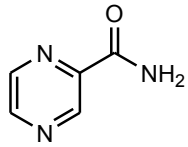
Rifampicin



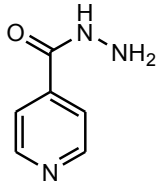
Streptomycin



Ethambutol



Pyrazinamide



Isoniazid

The growing amount of global TB cases, the threat of MDR-TB, and the emergence of XDR-TB highlights the need for new therapies for the treatment of TB.

3.5 Tuberculosis Drug Targets

The mechanisms of action and targets of antituberculosis agents are diverse. The targets of the first-line antituberculosis agents: (i) isoniazid inhibits the synthesis of mycolic acid by targeting primarily InhA, and secondarily KasA and DfrA; (ii) rifampicin inhibits transcription by targeting the RNA polymerase β-subunit; (iii) ethambutol inhibits arabinogalactan synthesis by possibly targeting EmbB; (iv) pyrazinamide's mechanism of action is currently unknown, but it is believed to possibly inhibit FAS-I or alter membrane energetics; and (v) streptomycin inhibits protein synthesis by targeting the 30S ribosomal subunit.[24]

The targets of second-line antituberculosis agents: (i) capreomycin, kanamycin and amikacin all inhibit protein synthesis; (ii) fluoroquinolones inhibits DNA gyrase; (iii) ethionamide inhibits mycolic acid synthesis by targeting InhA; (iv) cycloserine inhibits peptidoglycan synthesis by blocking the synthesis and use of D-alanine (Ala) by targeting Ala racemase and D-Ala-D-Ala ligase; and (v) *para*-aminosalicylic acid by inhibiting folate metabolism by possibly targeting dihydropteroate synthase.[24]

Tuberculosis continues to be a global threat despite all the above mentioned effective antituberculosis agents. The development of new drug candidates and the search for new targets is a continual process and currently several chemical entities are in clinical trials, including two approved fluoroquinolones, moxifloxacin and gatifloxacin,[25,26] rifampicin analogues,[27-29] SQ109 (ethambutol analogue),[30] TMC207 (diarylquinoline),[31] sudoterb (tetra-substituted pyrrole),[32,33] nitroimidazoles[34-38] etc., as well as, many compounds, in the early stages of drug development, are showing good promise, including linezolid (3-aryl-2-oxazolidinone antibiotic) and its analogues,[39-41] *β*-lactams[42,43] and phenothiazines[44-48] etc.. In the near future it is likely that new drugs will become available. However, the problem of

resistance is expected to continually evolve. In this light, a deeper understanding of the disease basic biology, and how small molecules can interact with and modulate its existence, is key to finding and developing new drugs that can offer promise to eventually eradicating this disease.

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Chapter 4

Acetolactate Synthase (ALS) as a Target for Anti-Tuberculosis Agents and Known Inhibitors

Acetolactate synthase (ALS), also known as acetohydroxyacid synthase (AHAS), is the enzyme which catalyses the first step in the biosynthesis of branched chain amino acids (valine, leucine, *iso*leucine) (Figure 4.1).[1]

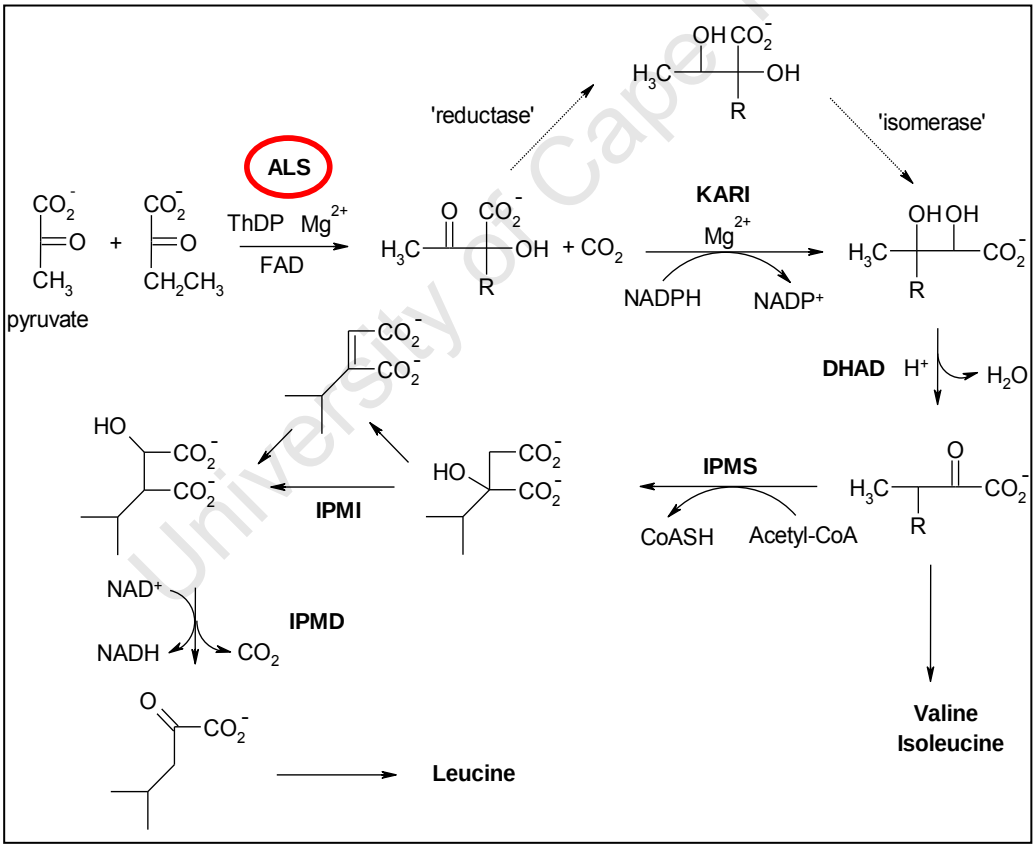


Figure 4.1: The biosynthetic scheme of branched amino acids, demonstating where ALS is needed (circled in red), and that it is important for the first step.[2]

Acetolactate synthase catalyzes the decarboxylation of pyruvate, which is then condensed with either another molecule of pyruvate or 2-ketobutyrate. The condensation results in the formation of the amino acid biosynthetic precursors, namely 2-acetolactate (AL) or 2-aceto-2-hydroxybutyrate (AHB) respectively. With the aid of thiamin diphosphate as the principle cofactor, ALS cleaves the carbon-carbon bonds adjacent to the carbonyl group. Although their role is not currently understood with respect to the catalyzed decarboxylation reaction, a Mg^{2+} cation and flavin adenine dinucleotide (FAD) are also present within the structure.[1,3]

The ALS enzyme is believed to be a biological target for several classes of herbicides (Figures 4.2 and 4.3) including sulfonyl ureas,[4,5] imidazolinones,[5] triazolopyrimidines[4,5] and pyriminidinyl oxybenzoates.[5] Up until 2002 the structure of ALS had not be solved. In 2001, the catalytic subunit of ALS from *Saccharomyces Cerevisiae* was crystallized by Dugglebury, R. G. *et al*. [2001][5] Following soon afterwards, the complete structure of the crystal structure of yeast ALS was published.[6] It was shown that the yeast ALS enzyme shares at least 26% homology with the *Mycobacterium tuberculosis* enzyme.[3]

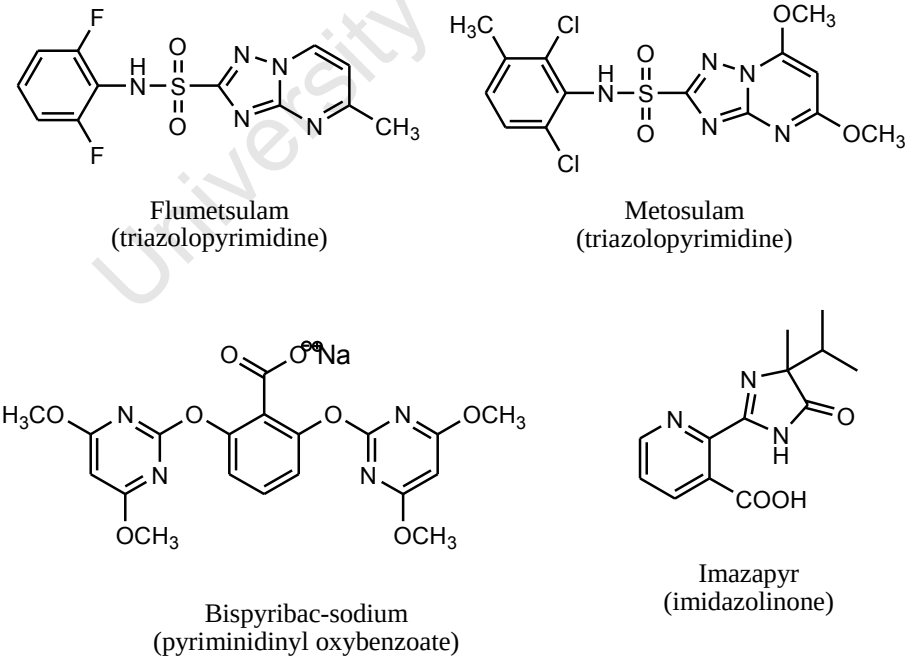


Figure 4.2: Four commercially available herbicides believed to inhibit ALS

The antituberculosis activity of some ALS inhibitors was reported in 1998 by Grandoni, J. A. *et al.* [1998].[2] More recently, the inhibition of ALS from *M*. *avium* by sulfonyl ureas and imidazolinones was published by Zohar, Y. *et al.* [2003][7] (Figure 4.3). The results showed that the inhibition of the *M. avium* enzyme by the tested sulfonyl ureas and imidazolinones was different to that of other plant ALSs previously reported. This highlights and demonstrates the importance of using a mycobacterial enzyme as an inhibition assay in the development of new anti-TB drugs.

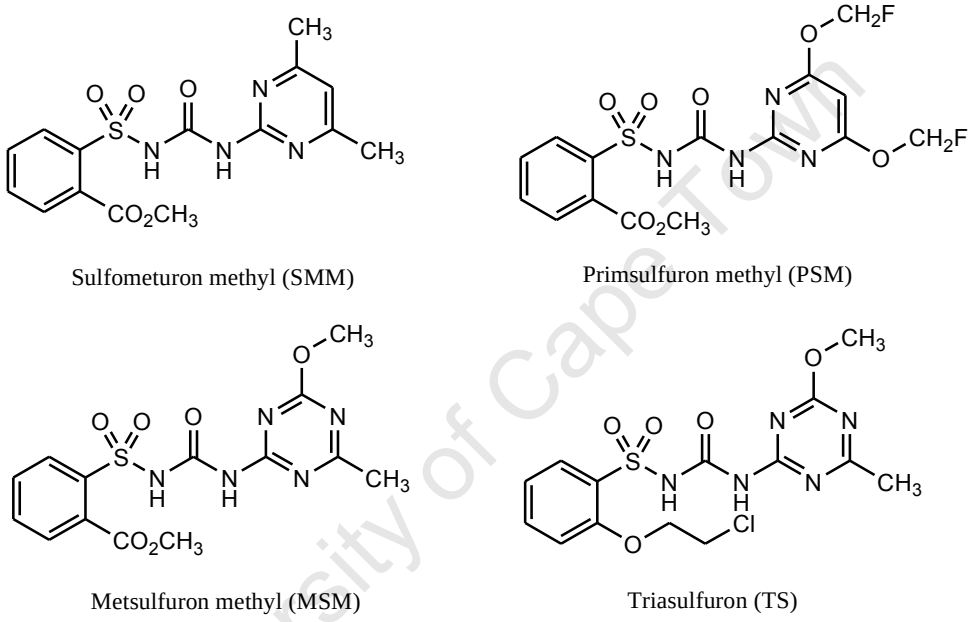


Figure 4.3: Four commercially available sulfonyl ureas that showed potent inhibition of *M. avium* ALS[7]

4.1 Sulfonyl Ureas

Sulfonyl ureas fall into the class of compounds known as herbicides. They were first created in the mid-1970s and have been on the market since 1982. Due to their extraordinary potency, their application dose was one hundred times smaller than the herbicides that they were made to replace. In June 1975, the first sulfonyl urea, chlorosulfuron, was formulated as an insecticide by a DuPont chemist George Levitt. However, it was discovered during testing that the sulfonyl urea had a devastating effect on plants. This observed effect led to an alteration in the development of this compound, and in 1978, in was patented as an herbicide by DuPont.[8,9] Unlike traditional herbicides that kill plants via chemical burn, sulfonyl urea herbicides block the synthesis of essential amino acids by inhibition of acetolactate synthase (ALS).

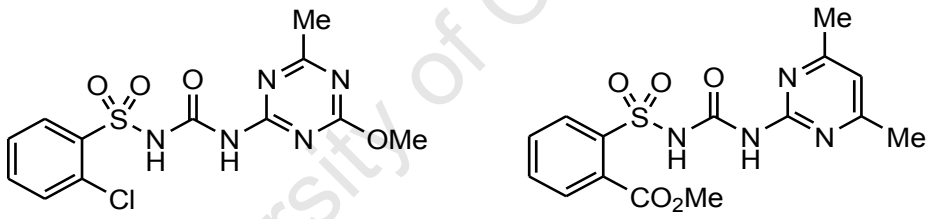


Figure 4.1.1: Commercially available sulfonyl urea herbicides

Another class of sulfonyl ureas has also been developed for the treatment of non-insulin dependent diabetes mellitus (NIDDM).[8] Glibenclamide, and structurally related meglitinide and tolbutamide (Figure 4.1.2), are commercially available anti-diabetic agents, and their mode of action is related to their stimulation of insulin release from the pancreas.[8,10] This mode of action is attributed to their effect on various ion channels. In the literature, glibenclamide hasn't been shown to inhibit ALS, however, recently it has proved to be a potent chloride channel inhibitior.[11]

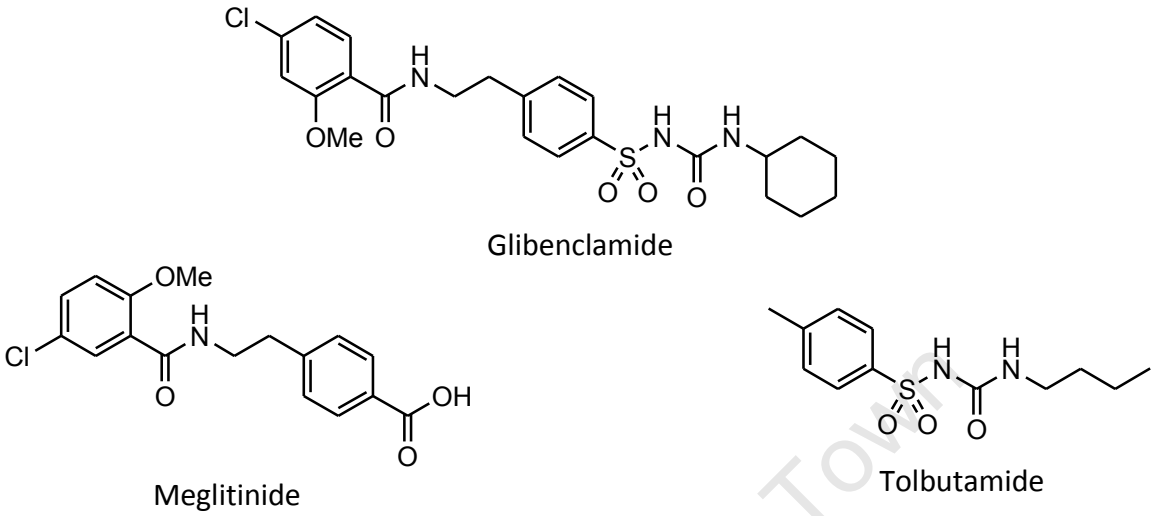


Figure 4.1.2: Glibenclamide and structurally related meglitinide and tolbutamide

Based on the fact that the yeast ALS enzyme shares at least 26% homology with the *Mycobacterium tuberculosis* enzyme, docking studies in collaboration with GlaxoSmithKline (GSK) Stevenage, UK were initiated. These studies have so far resulted in the generation of a virtual library of bis-aryl-based sulfonyl ureas. The docking studies concluded the proposal of a bis-aryl sulfonyl urea moiety template (Figure 4.1.3) as a source and starting point for the development of possible ALS inhibitors.[3]

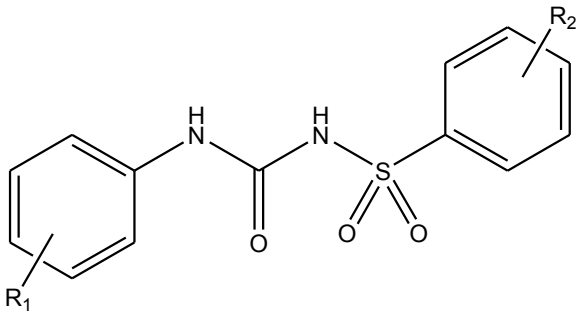
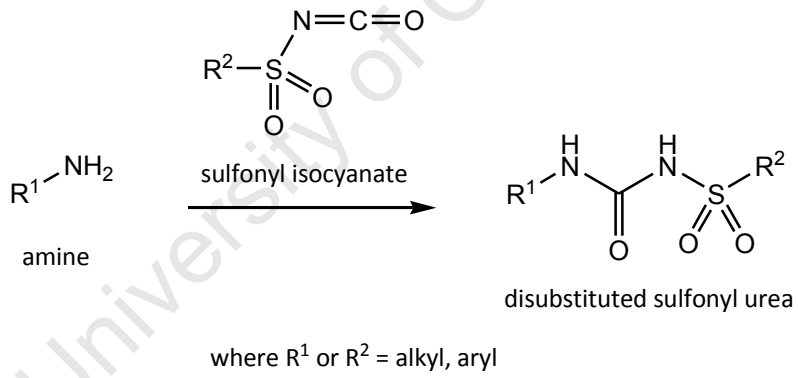


Figure 4.1.3: Basic bis-aryl sulfonyl urea template for ALS inhibition studies

It was proposed that R_1 could be heteroaromatic or aromatic moieties bearing other functional groups, and could be at the *ortho*, *meta* or *para* position on the ring. For simplicity, the docking studies were performed with R_2 being a chloro group in the *ortho* position. It was further proposed that the ring could bear other aromatic groups at R_2, with variable substituents, at the different possible ring positions.

4.1.1 Proposed Synthetic Routes

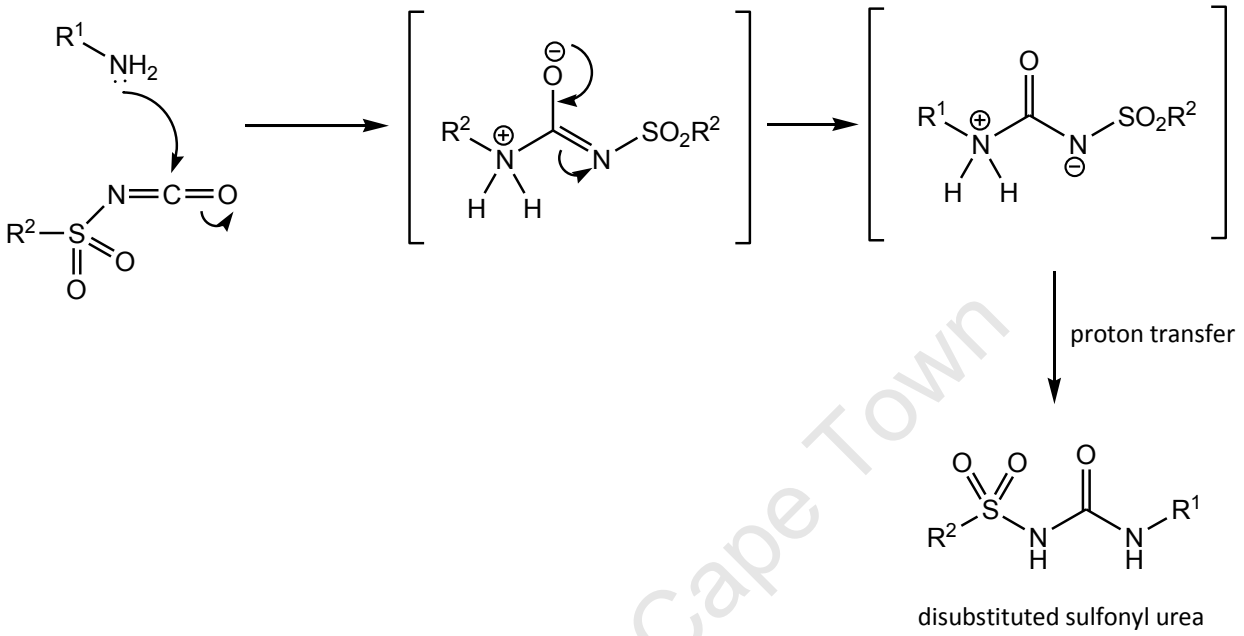
There are several known procedures that can be followed in order to synthesize sulfonyl urea libraries. The most straight forward entails coupling a sulfonyl isocyanate with an amine (Scheme 4.1.1).



Scheme 4.1.1: The coupling of a sulfonyl isocyanate with an amine to produce a sulfonyl urea

The mechanism of this reaction is shown in Scheme 4.1.2. The first step involves the attack of the lone pair of the nitrogen on the amine with the strongly electrophilic carbon of the sulfonyl isocyanate. The π-bond to the oxygen is broken as the electrons move to the more electronegative oxygen atom. The negative charge on the oxygen resonates back towards the

carbon reforming the carbon-oxygen double bond, and pushing the π-electrons onto the nitrogen. Finally, a proton transfer takes place, and the disubstituted sulfonyl urea is formed.



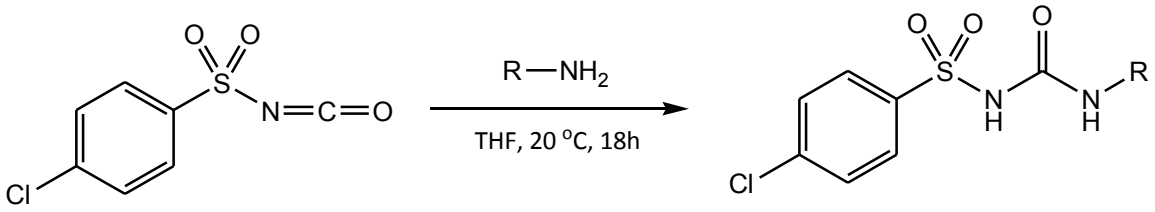
Scheme 4.1.2: The reaction of an amine with a sulfonyl isocyanate to generate a sulfonyl urea

4.1.2 Synthesis of Sulfonyl Ureas

Bromoanilines were (initially) used as the starting primary amine as palladium(0) mediated Suzuki catalyzed coupling reactions were initially envisaged. Suzuki coupling reactions follow the procedure of reacting a bromo-substituted compound with a boronic acid, and, hence, a library of biaryl substituted sulfonyl ureas could be created.

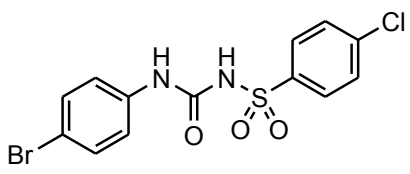
The synthesis of six sulfonyl ureas was initially envisaged. The reaction was achieved by dissolving the amine in dry THF, and then adding an equimolar equivalent of the sulfonyl

isocyanate to the solution in a dropwise manner. The reaction is left to stir at room temperature overnight (18 hours) under an atmosphere of nitrogen. The precipitate which forms is filtered off, yielding the *sulfonyl urea*. The *sulfonyl urea* can be purified further (if TLC shows other impurities) by coloumn chromatography.

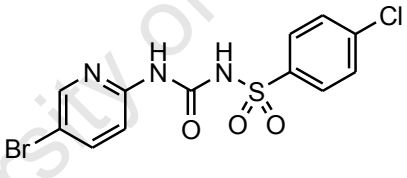


Scheme 4.1.3: Protocol used to synthesize sulfonyl ureas

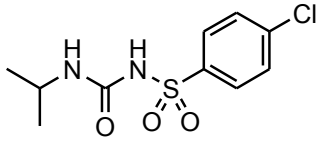
The following six sulfonyl ureas (shown below with their respective yields) were synthesized using the above mentioned protocol.



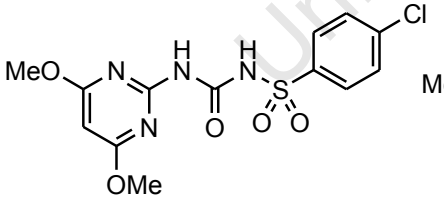
RKG1491, 15%



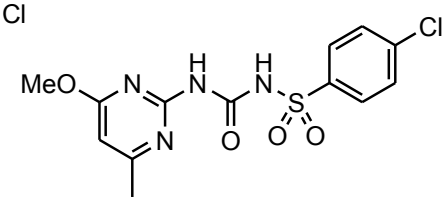
RKG1492, 6%



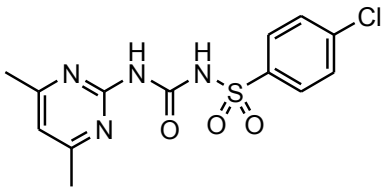
RKG1493, 45%



RKG1531, 80%



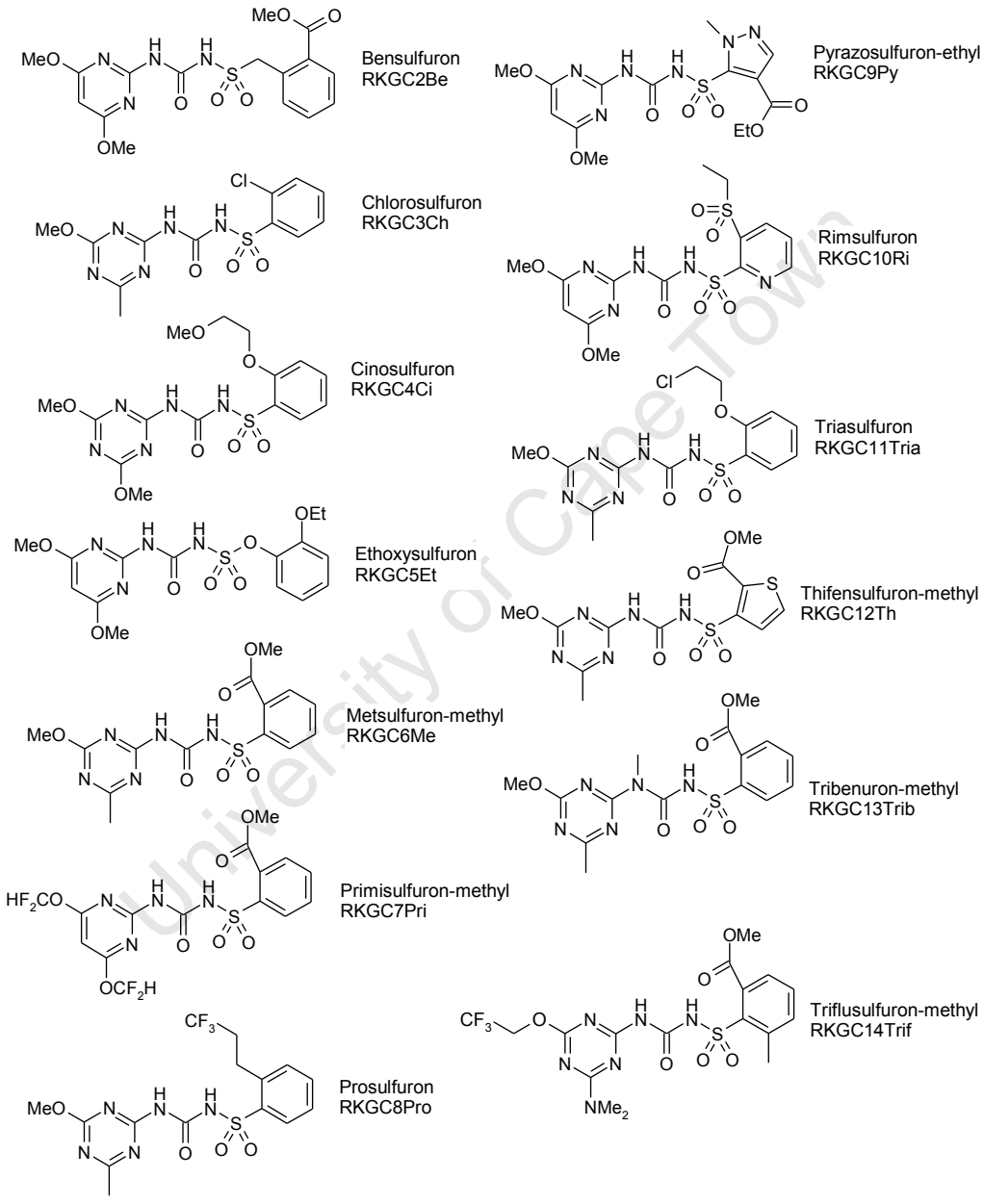
RKG1532, 67%



RKG1533PC, 76%

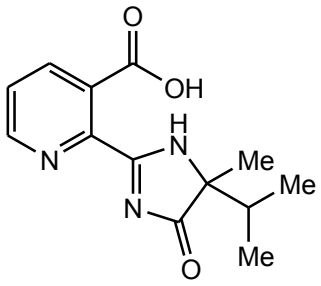
The low yields of the sulfonyl urea **RKG1492** may be attributed to the poor nucleophilicity of the primary aniline due to donation of the lone pair of electrons on the nitrogen into the

pyridinyl ring. To increase the numbers of sulfonyl ureas for preliminary SAR studies, the following 13 commercially available sulfonyl urea herbicides were purchased.

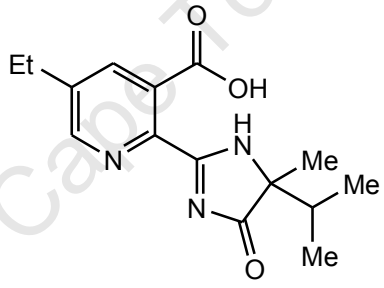


4.2 Imidazolinones

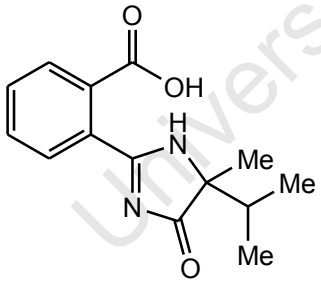
Imazapyr and other imidazolinone compounds were first discovered in 1956 by a BASF chemist named Sydney Upham, and these compounds were later patented due to their anticonvulsant activity. Dr. Marinus Los received much national recognition after discovering the unique and important herbicidal benefits of these compounds, and, today, this class of herbicidal compounds is known as imidazolinones. Imazapyr is a member of this group of compounds, and is also known as Habitat® Herbicide.



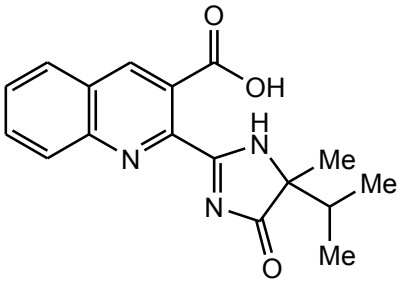
Imazapyr



Imazethapyr



Benzene analogue of Imazapyr



Imazaquin

Imidazolinones are very attractive herbicides as they are very effective at low rates. This is due to the fact that they put less chemical load on the environment when used at the recommended labeled rates (compared with traditional and other commercially available herbicides). Imazapyr, and other related imidazolinones, work as herbicides due to their plant-

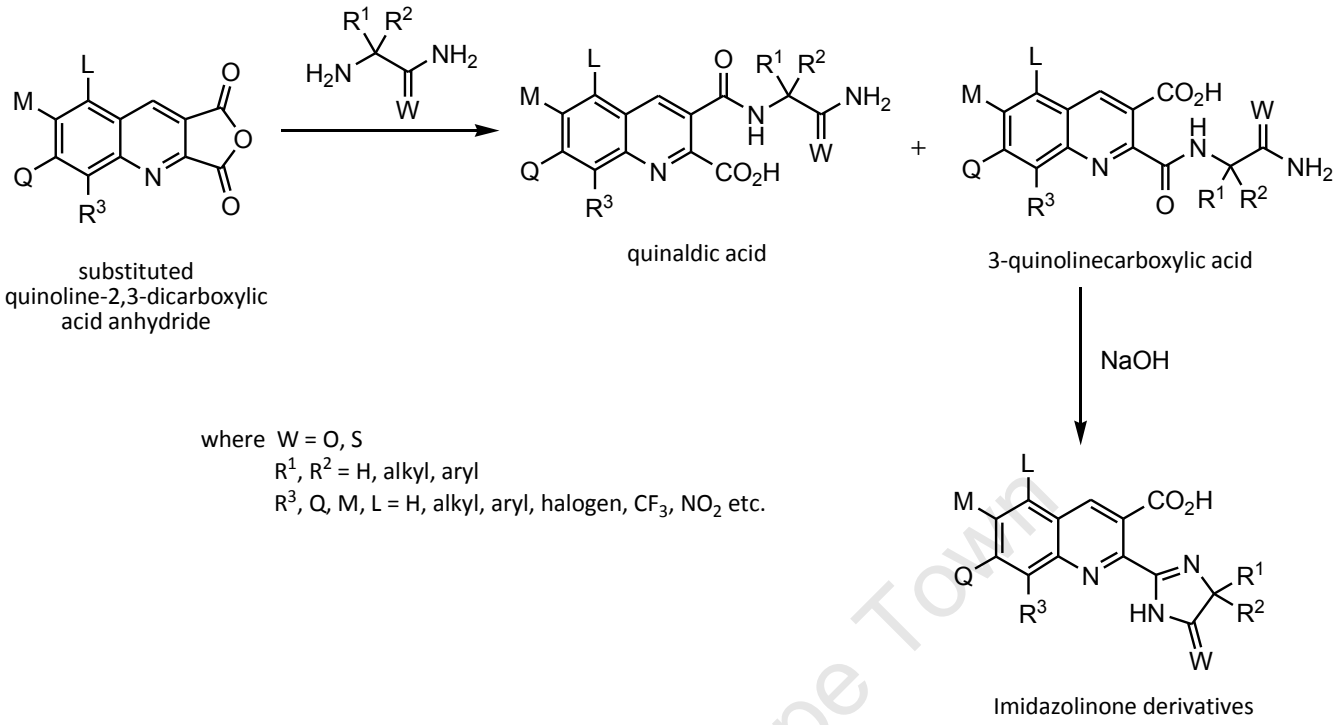
specific inhibition of ALS,[2,6,12,13] and they are considered to be virtually non-toxic (determined by EPA-required testing). Acute human toxicity is measured by its LD_{50} value, and imazapyr (Habitat®) has an oral LD_{50} value of >5,000 mg/kg body weight, and a dermal LD_{50} value of >2,148 mg/kg body weight.[14]

Studying the literature, imidazolinone derivatives have demonstrated a wide range of pharmacological activity, including anticonvulsant,[15,16] anti-Parkinsonian[17] and monoamine oxidase (MAO) inhibition.[18]

4.2.1 Proposed Synthetic Routes

As described in the patents,[19,20] quinoline-type imidazolinone derivatives are synthesized in the following manner (Scheme 4.2.1):

The substituted quinoline-2,3-dicarboxylic acid anhydrides are synthesized from di-, tri- and tetrasubstitued anilines. The quinoline-2,3-dicarboxylic acid anhydride is reacted with an aminocarboxamide or aminothiocarboxamide resulting in the formation of an isomeric mixture of quinaldic acid and 3-quinolinecarboxylic acid. The mixture is reacted with about 2 to 20 molar equivalents of sodium or potassium hydroxide dissolved in water or a mixture of water and alcohol resulting in the formation of the desired 2-(5,5-disubstituted-4-oxo(or thio)-2-imidazolin-2-yl)-3-quinoline-carboxylic acids (imidazolinone derivatives).



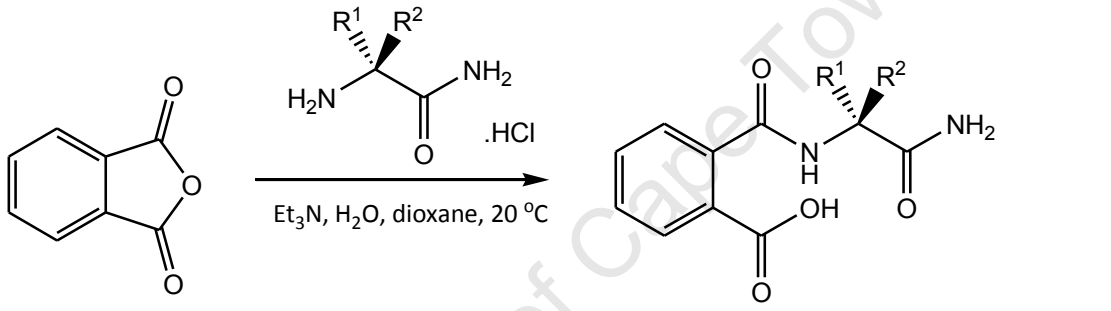
Scheme 4.2.1: The synthesis of imidazolinone derivatives as described in the patents[19,20]

4.2.2 Imidazolinone Analogues

The effective inhibition of ALS by Imazapyr, and other related imidazolinones, makes this class of compounds an excellent candidate for exploration for possible anti-TB activity. The inhibition of ALS (extracted from corn seedings) by Imazapyr, Imazaquin and the benzene analogue of Imazapyr was reported by Shaner, D. L. *et al.* [1984][12] Although several literature reports can be found on the inhibition of ALS by imidazolinones, little can be found on their structurally related benzene analogues. The synthesis and antimycobacterial evaluation of a small exploratory series of imidazolinone benzene analogues was envisaged.

4.2.2.1 Synthesis of Imidazolinone Benzene Analogues

To obtain the imidazolinone benzene analogues, the last two steps described in the patents[19,20] (Scheme 4.2.1) were embarked on. The exception was that commercially available phthalic anhydride would be used as the initial starting material instead of the synthesized substituted quinoline-2,3-dicarboxlyic acid anhydrides. The synthesis of imidazolinone benzene analogues was carried out as described below (Scheme 4.2.2 and 4.2.4):



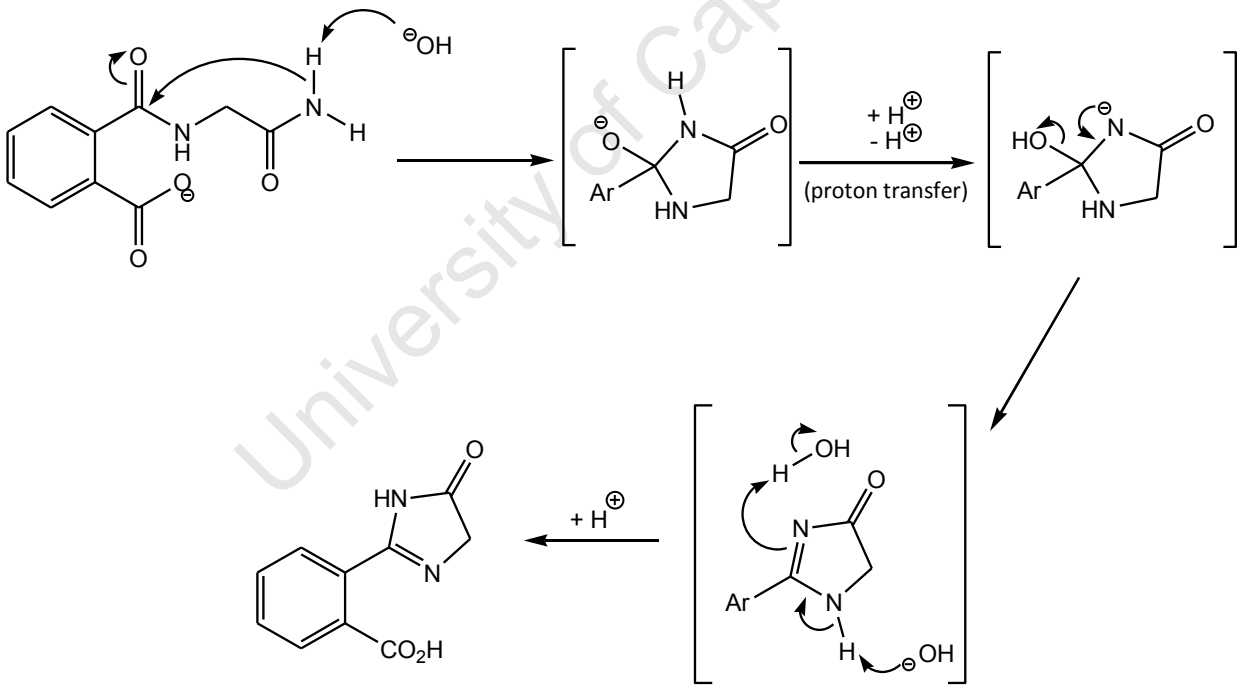
where R^1 = H, R^2 = H (**RKG114A, 77%**)
R^1 = H, R^2 = -CH_2-Ph (**RKG123A, 93%**)
R^1 = H, R^2 = -CH_2-*i*Pr (**RKG125A, 99%**)
R^1 = H, R^2 = -*i*Pr (**RKGANT3, 99%**)

Scheme 4.2.2: The synthesis of the phthalamic acid intermediates

An amino-amide hydrochloride salt was dissolved in a mixture of 2 molar equivalents of triethylamine in deionised water. This mixture was added to a solution of commercially available phthalic anhydride dissolved in dioxane, and the resulting reaction mixture was stirred at room temperature for 30 to 45 minutes. The reaction mixture was concentrated by the removal of the solvent under reduced pressure, and an aqueous solution of 1M HCl added. The solution was placed in the fridge overnight to promote precipitation. The precipitate was

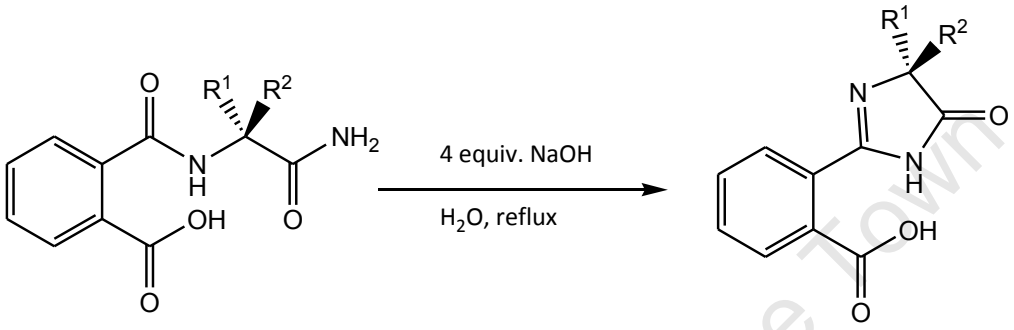
filtered off, washed with cold aqueous 1M HCl and water, and dried *in vacuo* to afford the phthalamic acid in good yield and high purity (Scheme 4.2.2).

The second step involves a base-mediated intramolecular cyclisation of the phthalamic acid to the imidazolinone benzene analogue derivatives (Scheme 4.2.4). The excess base is believed to deprotonate the carboxylic acid group rendering it a carboxylate anion, which prevents it from being accessible for nucleophilic attack as the negative charge is stabilized by resonance within the carboxylate. Under the high reaction temperature, the primary amide nitrogen can attack the secondary amide carbonyl carbon resulting in the intramolecular cyclisation. The formation of an imine occurs, and results in the elimination of a hydroxyl anion. The mechanism of the intramolecular cyclisation is shown in Scheme 4.2.3:



Scheme 4.2.3: Proposed mechanism for the base-mediated intramolecular cyclisation of the phthalamic acid to the imidazolinone benzene analogue derivatives

The phthalic acid is added to a solution of 4 molar equivalents of NaOH dissolved in water. The reaction mixture is stirred and refluxed for 3 hours, then allowed to cool to room temperature.Concentrated HCl was added to adjust the pH to promote precipitation of the product. The precipitate was isolated, purified and dried *in vacuo* to afford the pure imidazolinone derivatives (Scheme 4.2.4).



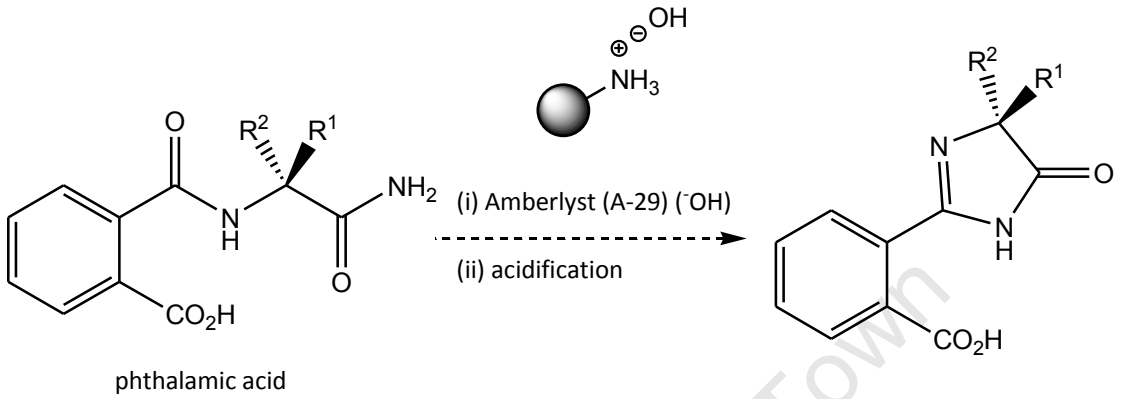
where R^1 = H, R^2 = H (**RKGAS4EGLY, 42%**)
R^1 = H, R^2 = -CH_2-Ph (**RKGAS4EPA, 75%**)
R^1 = H, R^2 = -CH_2-*i*Pr (**RKGAS4ELEU, 54%**)
R^1 = H, R^2 = -*i*Pr (**RKGAS4EVAL, 45%**)

Scheme 4.2.4: Synthesis of imidazolinone benzene analogue derivatives

4.2.2.2 Attempted Polymer-Assisted (Solid Supported) Synthesis in Solution

In order to render the possible synthesis of libraries of this class of compounds, the use of a solid supported base in the last step was considered. Commercially available Amberlyst (A-29) ($^-$OH) resin was chosen to be the solid supported base in an attempt to effect the base-mediated intramolecular cyclisation step. The solid supported base was envisaged to have a bifunctional role; firstly, to be the source of a basic hydroxyl anion which performs the base-mediated cyclisation step, and, secondly, for the deprotonated carboxylic acid group to ionically bond with the positively charged ammonium group on the resin. The resin-bound imidazolinone

benzene analogue could then be easily separated from the reaction mixture by filtration and washing, and subsequent acidification should result in resin detachment yielding the desired target compounds (Scheme 4.2.5).



phthalamic acid

Scheme 4.2.5: Proposed polymer-assisted synthesis of imidazolinone benzene analogue derivatives

Unfortunately, the envisaged solid supported base step did not yield the desired imidazolinone benzene analogues. The glycinamide phthalamic acid (**RKG114A**) (where $R^1 = R^2 = H$) was chosen as the candidate for attempting the base-mediated cyclisation step using the solid supported base. The reaction was attempted using the standard conditions described when using the free base (NaOH).[20] After acidification of the filtered resin, only the starting phthalamic acid was obtained. Since the base-mediated cyclisation was not taking place, the reaction was attempted with the solid supported base at elevated temperatures. As water boils at 100 ^{o}C, NMP was used as the polar solvent to reach reaction temperatures above 100 ^{o}C.

In Table 4.2.1, below, all the reaction conditions that were attempted are shown. Higher reaction temperatures were believed to be what was required to supply adequate activation energy to allow for the base-mediated cyclisation using the solid supported base to occur. Accordingly temperatures were elevated to as high as 160 ^{o}C with no desired effect. It was

observed, however, that when attempting the reaction at 135 ^{o}C, a mixture of the starting phthalamic acid and benzene dicarboxylic acid was isolated. At 160 ^{o}C, no starting phthalamic acid was recovered and only benzene dicarboxylic acid was isolated.

Experiment	Base	Solvent	Temperature (^{o}C)	Yield (%)
417	NaOH (4 equiv.)	H_2O	90	42
418	Amberlyst ($^{-}$OH) (4 equiv.)	H_2O	90	--
419	Amberlyst ($^{-}$OH) (4 equiv.)	H_2O	100	--
420	Amberlyst ($^{-}$OH) (4 equiv.)	NMP	135	--
421	Amberlyst ($^{-}$OH) (4 equiv.)	NMP	160	--

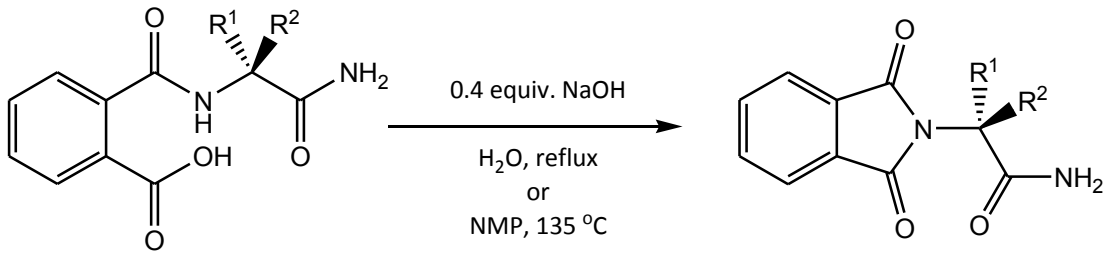
Table 4.2.1: Various reaction conditions used to attempt base-mediated intramolecular cyclisation

4.2.3 Synthesis of Phthalimides Derivatives

Initially when attempting to synthesize the imidazolinone benzene analogues, the base-mediated intramolecular cyclisation step was attempted using a catalytic amount (0.4 molar equivalents) of base (NaOH). Using an acidic work-up and column chromatography, a new product was isolated. The product, however, was not the imidazolinone benzene analogue, but rather the phthalimide derivative (Scheme 4.2.6).

Phthalimides are a class of compounds that are not known for, or have shown, anti-TB activity. However, the phthalimide moiety has been structurally included in several compounds which

have recently shown good promising antimycobacterial,[21] as well anticonvulsant,[22,23] activities. On this basis the phthalimides by-products were considered for testing against *M. tuberculosis*.



phthalamic acids

phthalimides

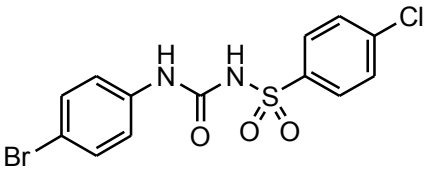
where R^1 = H, R^2 = H (**RKG119A, 67%**)
R^1 = H, R^2 = -CH_2-Ph (**RKG133A, 37%**)
R^1 = H, R^2 = -CH_2-*i*Pr (**RKG131A, 29%**)
R^1 = H, R^2 = -*i*Pr (**RKG145A, 15%**)

Scheme 4.2.6: Synthesis of phthalimide derivatives

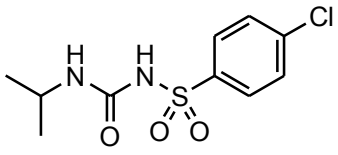
4.3 Sulfonylcyanoguanidines

Sulfonylcyanoguanidines are a class of compounds known for their activity as thromboxane A_2 (TXA_2) receptor antagonists and thromboxane synthase inhibitors,[24-26] and diuretics.[27] Thromboxane A_2 is a potential target for the treatment of cardiovascular, renal and pulmonary diseases.[28-30] Cyanoguanidines are well known to be ATP-sensitive potassium channel openers and inhibitors of insulin release[31,32] and vascular K_{ATP} channel blockers.[33] This activity may lead to the treatment of overactive bladder (OAB)[31] and metabolic and cardiovascular diseases.[34,35] Cyanoguanidines have also been shown to be HIV-1 protease inhibitors,[36] antiproliferate (antitumour) agents[37] and reversible acetylcholinesterase inhibitors (leading to insecticidal activity).[38]

In the literature, Michaux, C. *et al*. [2003][26] conducted a study of sulfonyl ureas and sulfonylcyanoguanidines as thromboxane receptor antagonists and thromboxane synthase inhibitors. Both classes of compounds were identified as dual acting agents with comparable *in vitro* activities. This interesting observation led to the decision to synthesis two sulfonylcyanoguanidines (the analogues of two previously synthesized sulfonyl urea derivatives; **RKG1491** and **RKG1493** respectively) for comparative *in vitro* testing against *M. tuberculosis*.



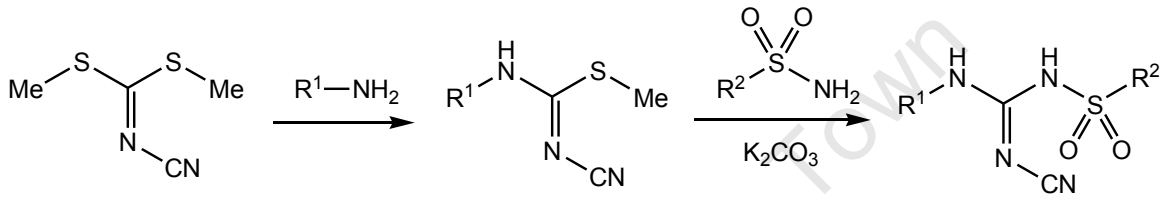
RKG1491



RKG1493

4.3.1 Proposed Synthetic Routes

Following the literature method described by Hantzsch, A. *et al.* [1904],[39] the sulfonylcyanoguanidines were synthesized starting with commercially available dimethyl *N*-cyanodithioimine-carbonate. Carrying out two sequential substitution reactions with an amine and a sulfonamide, respectively, yields the sulfonylcyanoguanidine (Scheme 4.3.1):



dimethyl N-cyanodithioimine-carbonate

sulfonylcyanoguanidine

where R^1, R^2 = Alkyl, aryl

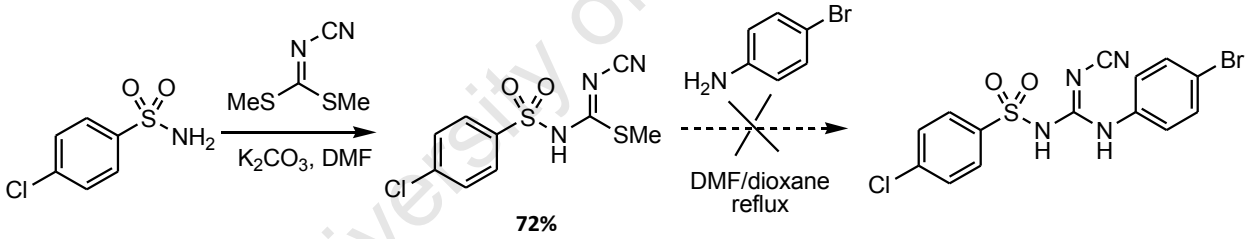
Scheme 4.3.1: Proposed synthesis of sulfonylcyanoguanidines

4.3.2 Synthesis of Sulfonylcyanoguanidines

The synthesis of sulfonylcyanoguanidines was initially attempted by a substitution reaction of dimethyl *N*-cyanodithioiminecarbonate with a sulfonamide in the presence of a weak base (K_2CO_3) to yield the desired intermediate product (N-cyano-N'-alkyl/arylsulfonamido-S-methyl-iso-thiourea). The second step involves the coupling of the iso-thiourea with a primary amine.

First approach:

The first step was performed as outlined by Chibale, K. *et al*. [2002].[40] 4-Chlorobenzenesulfonamide and an equimolar equivalent of both finely ground anhydrous K_2CO_3 and dimethyl *N*-cyanodithioiminocarbonate were added to DMF. The reaction mixture was stirred at room temperature for 15 hours. An aqueous solution of 2M HCl was added, and the precipitate filtered off, and washed successively with the aqueous solution of 2M HCl and diethyl ether, to afford the desired intermediate *iso*-thiourea in good yield (72%). The second step was attempted as described by Dogné, J. M. *et al.* [2001],[24] but using a primary amine (4-bromoaniline) instead of a sulfonamide. The reaction was conducted as described in the literature, but none of the desired product (sulfonylcyanoguanidine) was obtained (Scheme 4.3.2). Only starting material was recovered after the reaction was worked up. This may be a result of the poor nucleophilicity of the primary aniline.

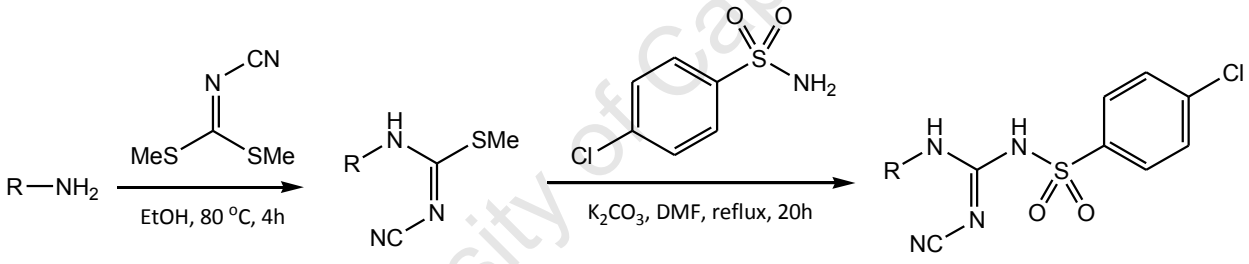


Scheme 4.3.2: Attempted synthesis of sulfonylcyanoguanidines via the first approach

Due to the lack of success using the first approach to obtain the sulfonylcyanoguanidine compounds, an alternate strategy was pursued. In this strategy dimethyl *N*-cyanodithioiminocarbonate was first coupled with the primary aniline. The resulting *iso*-thiourea would then be coupled with the easily deprotonated sulfonamide (using a weak base; K_2CO_3). The deprotonation of the sulfonamide with K_2CO_3 results in a more nucleophilic nitrogen which can substitute the remaining thio-methyl (-SCH_3) leaving group.

Second approach:

The synthesis of the sulfonylcyanoguanidine compounds (**RKG139F3** and **RKG151** respectively) was performed as described by Dogné, J. M. *et al.* [2001][24] and Yokoyama, M. *et al*. [1981].[41] The first step involves the coupling of the primary amine with dimethyl N-cyanodithioiminocarbonate, by refluxing them together in ethanol for 4 hours. After isolation and purification of the *iso*-thiourea (**RKG136A** and **RKG150A**), the intermediate product is added to solution of 4-chlorobenzenesulfonamide and K_2CO_3 dissolved in DMF. The reaction mixture is stirred under reflux for 20 hours, and then allowed to cool to room temperature. The reaction is worked-up as described in the experimental section, and the precipitate is recovered by filtration. The isolated precipitate is dried *in vacuo*, and recrystallized from boiling methanol to afford the pure sulfonylcyanoguanidines (**RKG139F3** and **RKG151A**).



where R = 4-bromophenyl (**RKG136A, 61%**)
R = *iso*-propyl (**RKG150, quantitative**)

where R = 4-bromophenyl (**RKG139F3, 32%**)
R = *iso*-propyl (**RKG151A, 50%**)

Scheme 4.3.3: Synthesis of sulfonylcyanoguanidines via the second approach

4.4 Acylthioureas

Synthetically, acylthioureas have been widely used as the starting block for various transformations; examples include the preparation of four-,[42] five-,[43-49] six-, [45,50,51] and seven-[52] membered heterocyclic ring systems. In industry, the efficient ligand characteristics of *N*,*N*-dialkyl-*N'*-acyl(aroyl)thioureas with platinum group metals, makes them useful for the separation and refinement of platinum, palladium, rhodium, ruthenium, iridium and osmium.[53,54]

From a pharmaceutical perspective, acylthioureas have shown a wide range of promising activities and uses, and have been patented due to their observed antidiabetic,[55] antiarthritic,[56] antineoplastic,[57] local anaesthetic,[58] antihyperlipidemic,[58] antiproliferative[58] (including antitumour platinum acylthiourea complexes,[59] and quinoline- and quinazoline-acylthiourea inhibitiors of PDGF receptor autophosphorylation[60]), anticoagulant[61] (in treatment of cognitive problems[61] and prostate disorder[63,64]) and antiviral (inhibition of Hepatitis C virus replication)[65] activities. Other interesting areas of activity of acylthioureas include; herbicidal,[66,67] fungicidal,[66] bactericidal,[66] insecticidal[68] and plant growth regulator[69] activities.

An exploratory group of four acylthiourea analogues of previously synthesized sulfonyl urea derivatives (**RKG1491**, **RKG1492**, **RKG1493** and **RKG1531**) for comparative *in vitro* testing against *M. tuberculosis* was identified.

4.4.1 Proposed synthetic routes

There are many published routes in the literature which describe the synthesis of acylthioureas. Katritzky, A. R. *et al.* [2004][69] have comprehensively reviewed and summarized the synthesis of mono- and N,N-disubstituted *N*-acylthioureas from a range of precursors (Figure 4.4.1.1).

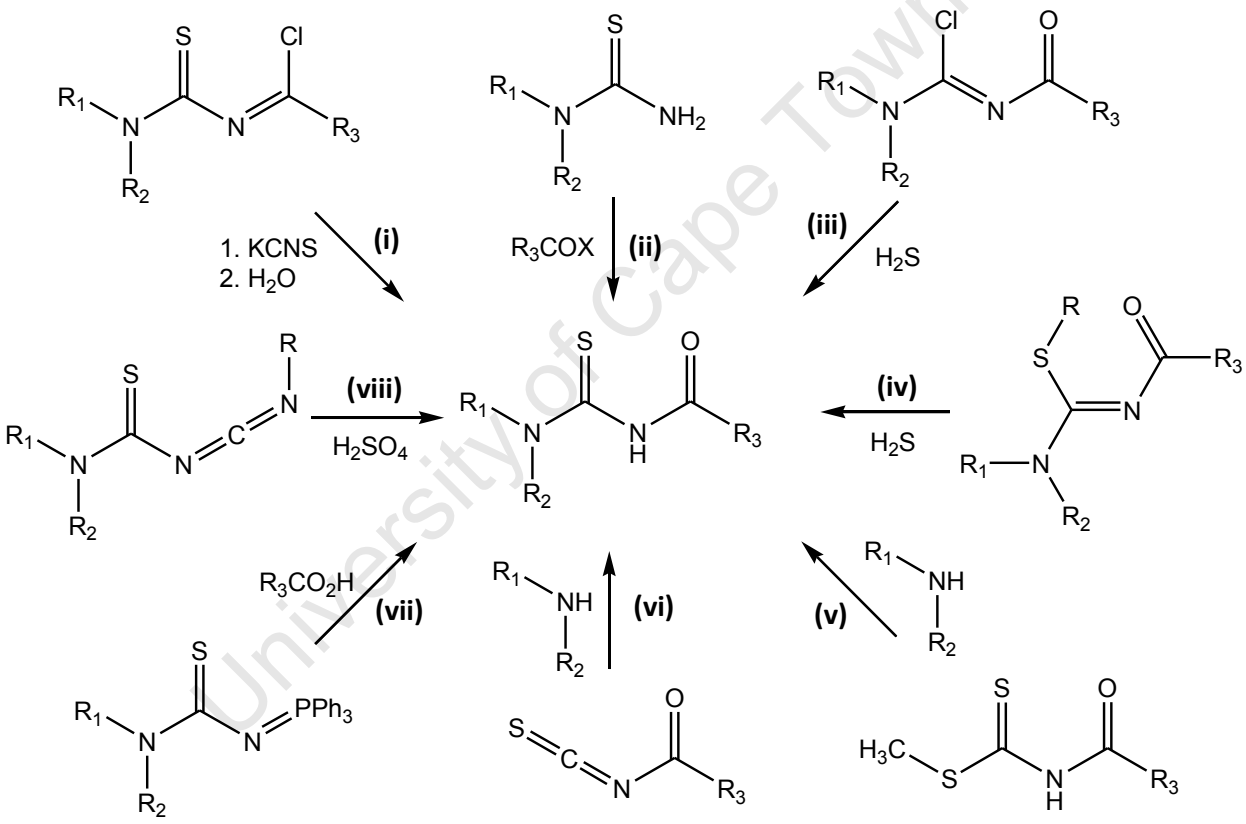
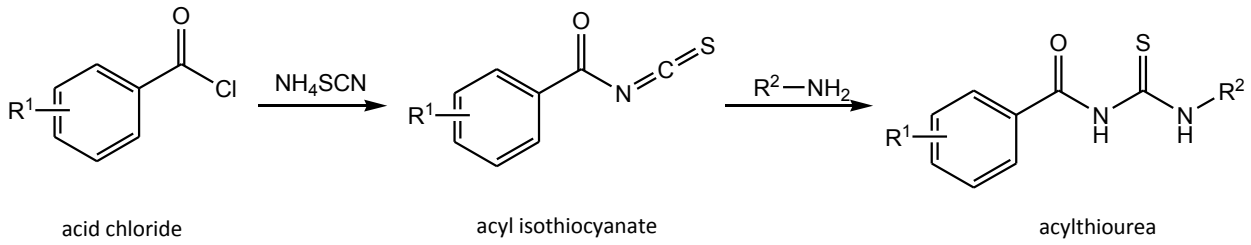


Figure 4.4.1.1: Common routes to synthesize acylthioureas as summarized by Katritzky *et al.* [2004][69]

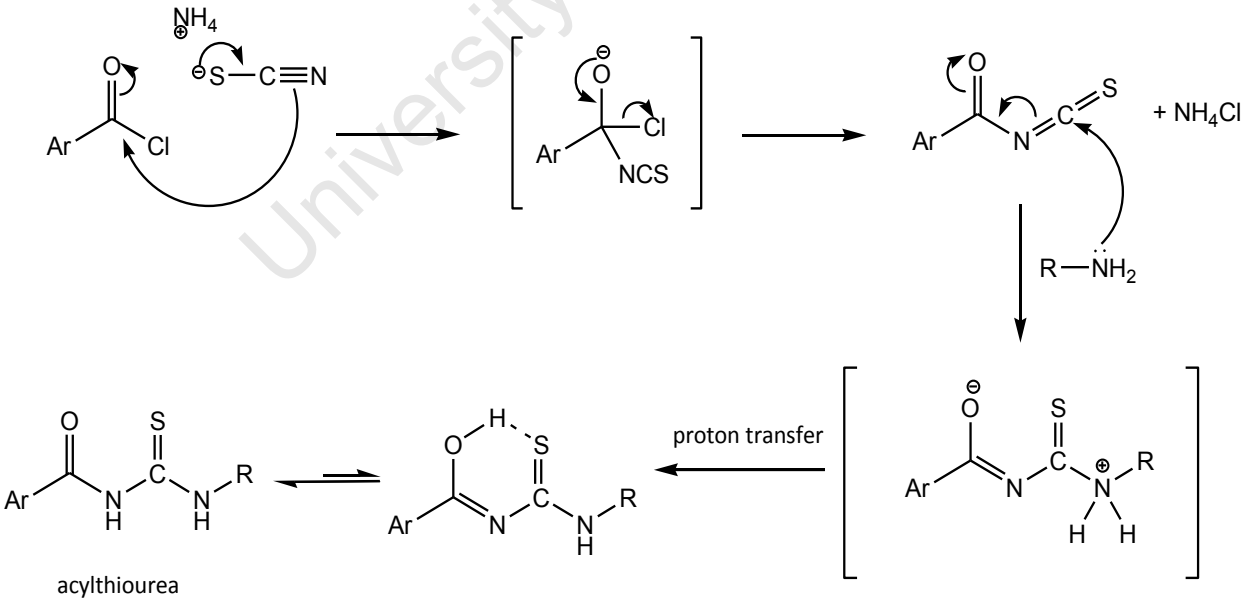
To synthesize the envisaged acylthiourea derivatives, route **(vi)** (from Figure 4.4.1.1) was chosen. This involved reacting an *in situ* generated acyl isothiocyanate, derived from an acid

chloride, with the appropriate primary amine.[70,71] This reaction scheme and mechanism is displayed and detailed below in Scheme 4.4.1 and 4.4.2:



Scheme 4.4.1: The reaction procedure chosen to synthesize acylthioureas

The synthesis is described as a 2 step, 1 pot, protocol. The procedure starts with the conversion of an aryl acid chloride to the reactive acyl isothiocyanate with ammonium thiocyanate. The precipitated salt (ammonium chloride) is filtered off, and the primary amine is added to the filtered reaction solution.[71]

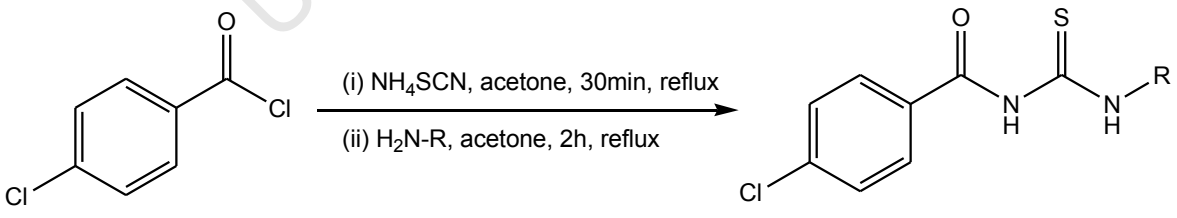


Scheme 4.4.2: The proposed mechanism for the synthesis of acylthioureas

Briefly, the first step, in the mechanism, involves a substitution reaction of the chloride with the thiocyanate to form an acyl isothiocyanate intermediate *in situ*. The acyl isothiocyanate is extremely reactive and not isolated. The addition of an amine, results in a nucleophilic attack of the lone pair of electrons on the amine nitrogen onto the acyl isothiocyanate carbon. The electrons flow though the conjugated π-system, resulting in the formation of the enolate. A proton transfer takes place to form the hydrogen-bond stabilized enol derivative. Tautomerism occurs, and the enol derivative is converted to the more favored amide derivative, the acylthiourea.

4.4.2. Synthesis of Acylthioureas

The synthesis of four acylthioureas was initially envisaged. The reaction proceeded by the addition of the 4-chlorobenzoyl chloride to a solution of ammonium thiocyanate dissolved in acetone. The reaction mixture was stirred under reflux for 30 min, and allowed to cool to room temperature. The precipitated salt (ammonium chloride) was filtered off, and the amine added to the filtered solution of the acyl isothiocyanate dissolved in the acetone. The reaction mixture was stirred under reflux for a further 2 hours and allowed to cool to room temperature followed by removal of acetone under reduced pressure. Deionised water was added to the



where R = 4-bromophenyl (**RKG1541, 72%**)
R = 5-bromopyridin-2-yl (**RKG1542, 80%**)
R = 4,6-dimethoxypyrimidin-2-yl (**RKG1543, 43%**)
R = *iso*-propyl (**RKG162A, 83%**)

Scheme 4.4.3: Synthesis of acylthioureas

residue, resulting in the formation of a precipitate. The precipitate was filtered off and recrystallized from 95% ethanol to afford the pure acylthiourea.

4.5 Conclusion

The envisaged sulfonyl ureas, sulfonylcyanoguanidines, imidazolinones and acylthioureas, as well as phthalimides, were successfully synthesized and characterized. All respective target compounds, including isolated and pure intermediate compounds, were evaluated for anti-TB activity, and the results shall be analyzed, compared and discussed in Chapter 6.

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Chapter 5

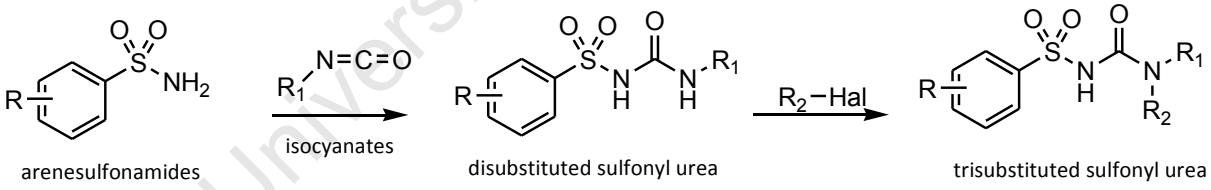
New Synthesis of Sulfonyl Ureas

One of the main aims of this project, besides the synthesis of compounds to target new permeability pathways and acetolactate synthase in the search of new leads in the attempt to treat both malaria and tuberculosis respectively, is to develop efficient synthetic technologies for the synthesis of the target compounds.

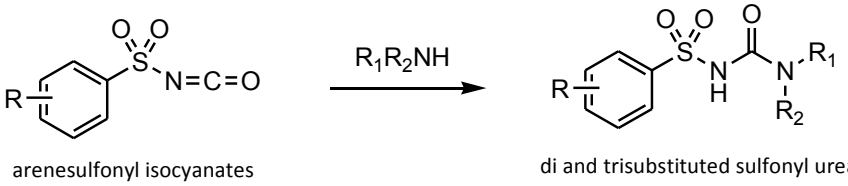
5.1 Commonly used Methodology

There are several approaches that can be followed in order to generate arenesulfonyl urea libraries. In Scheme 5.1, two commonly applied procedures are shown and discussed.

First approach:



Second approach:



Scheme 5.1: Two general approaches to synthesize arenesulfonyl ureas

First approach:

This method is possibly the most common approach for the synthesis of disubstituted arenesulfonyl ureas. The advantage of this method lies in the large number of commercially available isocyanates and sulfonamides. However, a major disadvantage is the need for a second supplementary step in order to access tri- and tetra-substituted arenesulfonyl ureas. Further drawbacks of this approach include the poor solubility of arenesulfonamides in most organic solvents, and the fact that arenesulfonamides are less reactive towards isocyanates than amines. Hence, a catalyst like cuprous chloride,[1] or Lewis acid like boron trifluoride diethyl etherate,[2] or a base is required for this step.

Second approach:

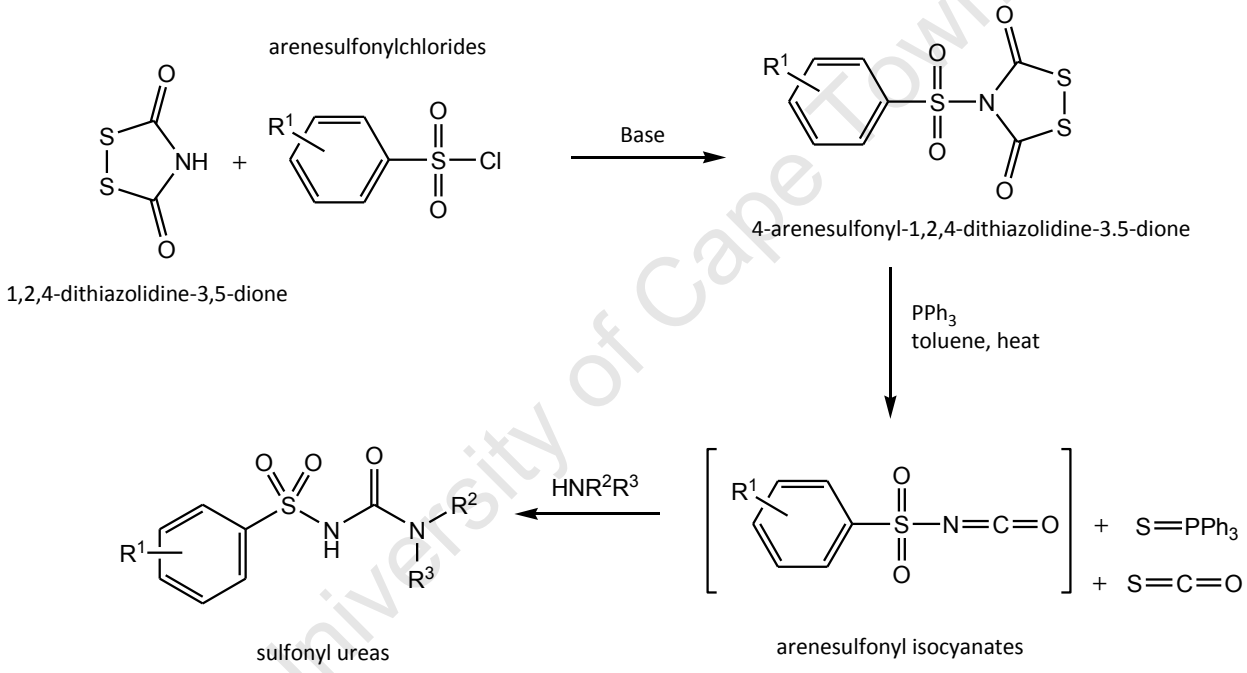
This approach of coupling a highly reactive arenesulfonyl isocyanate with a primary or secondary amine appears to be the most straight forward method of preparing both di- and trisubstituted arenesulfonyl ureas. However, while the technique and chemistry is simple and efficient, this approach is hindered by the limited number of commercially available arenesulfonyl isocyanates.

There are, as highlighted and described, limitations to each approach, and so the need for developing novel synthetic routes to create diverse arenesulfonyl urea libraries should be continually investigated and further developed. A method that generates a vast selection of arenesulfonyl isocyanates from a range of commercially available starting materials could, hence, be very useful

5.2 Proposed Novel Synthetic Route

Another approach to the synthesis of sulfonyl ureas was envisaged. This strategy involves the use of 1,2,4-dithiazolidine-3,5-dione,[3-5] which should provide an alternative route to the arylsulfonyl ureas via stable intermediates. This approach is shown below in Scheme 5.2:

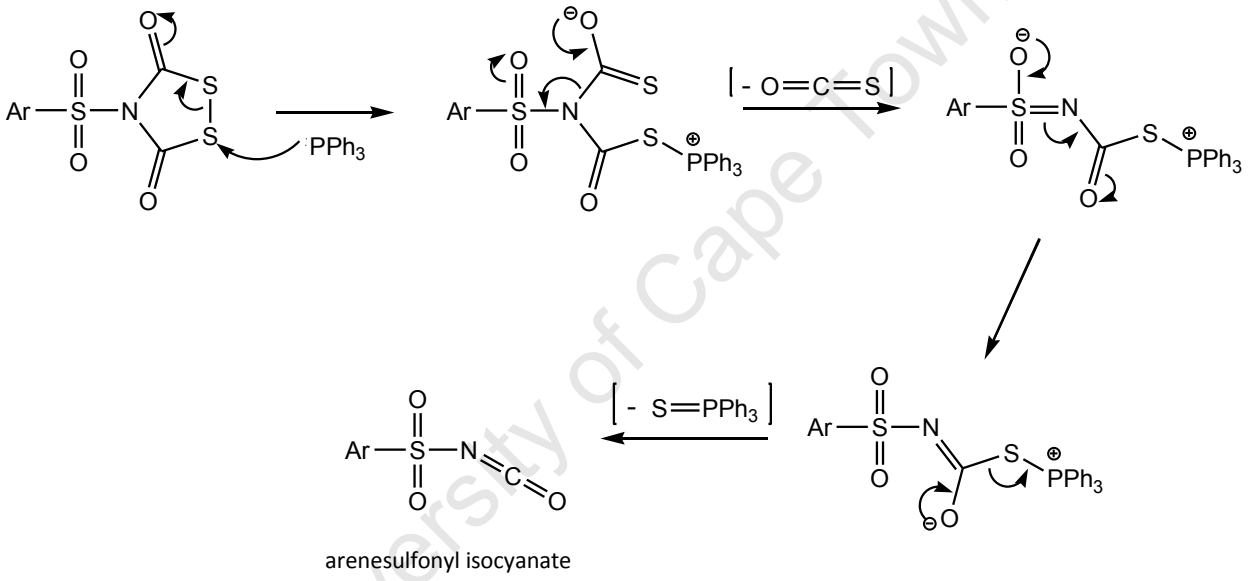
New Approach:



Scheme 5.2: Proposed novel synthetic to generate sulfonyl ureas

The first step involves reacting the 1,2,4-dithiazolidine-3,5-dione with an arenesulfonyl chloride in the presence of a weak base. Wood, M. E. *et al.* [2003][6] observed that the hydrogen on the nitrogen of the 1,2,4-dithiazolidine-3,5-dione scaffold is remarkably acidic with a pK_a of 2.85 ± 0.002. This is attributed to the ability of the negative charge created on deprotonation to be resonance stabilized by the two neighboring carbonyls. The correct conditions and type of base

will have to be determined; $NaHCO_3$ was determined to be the base of choice in acetonitrile for the *N*-alkylation using alkyl halides of 1,2,4-dithiazolidine-3,4-dione, and pyridine was determined to be the base and solvent of choice for *N*-benzoylation with benzoyl chloride (acid chloride).[6] The intermediate product is converted to the corresponding arenesulfonyl isocyanate in the presence of triphenylphosphine. The mechanism of the conversion from the 4-arenesulfonyl-1,2,4-dithiazolidine-3,5-dione into the arenesulfonyl isocyanate is proposed as follows:



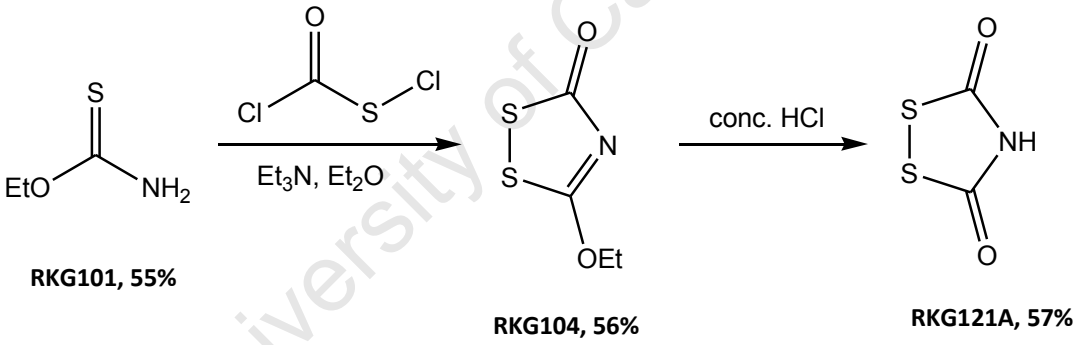
Scheme 5.3: Proposed mechanism for the synthesis of sulfonylcyanoguanidines using the novel approach

The arenesulfonyl isocyanate is not isolated, and is reacted *in situ* with a primary or secondary amine to generate the sulfonyl urea.

5.3 Methodology Development to Synthesize Sulfonyl Ureas

5.3.1 Synthesis of 1,2,4-Dithiazolidine-3,5-dione

O-Ethyl thiocarbamate (**RKG101**) (a. k. a. ethyl xanthamidate) is synthesized as detailed by Davies, W. *et al.* [1951],[3] and then reacted with (chlorocarbonyl)sulfenyl chloride in a solution of triethylamine in diethyl ether to give the cyclized 3-ethoxy-1,2,4-dithiazolin-5-one (**RKG104**). 3-Ethoxy-1,2,4-dithiazolin-5-one is coverted to 1,2,4-dithiazolidine-3,5-dione (**RKG121A**) by reaction with concentrated HCl (Scheme 5.4). The yields obtained are comparable to, if not better than those reported in the literature.[3-5]

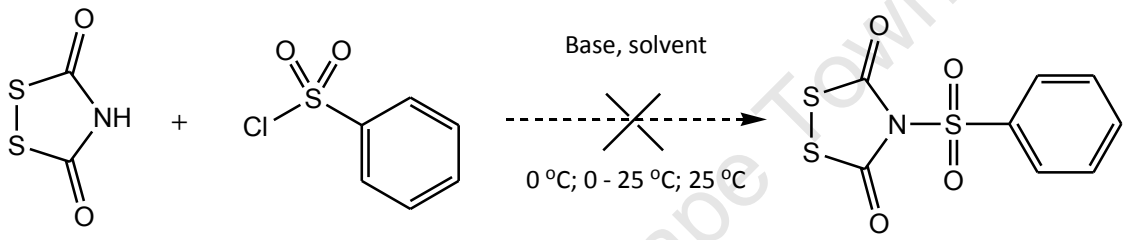


Scheme 5.4: Synthesis of 1,2,4-dithiazolidine-3,5-dione (**RKG121A**)

5.3.2 Attempted Synthesis of 4-Benzenesulfonyl-1,2,4-dithiazolidine-3,5-dione

The reaction of 1,2,4-dithiazolidine-3,5-dione with commercially available benzene sulfonyl chloride was attempted (Scheme 5.5). The reaction was carried out at room temperature (25 ^{o}C) under various combinations of base and solvent, but the desired product was not isolated.

Due to the acidity of the proton of 1,2,4-dithiazolidine-3,5-dione, various weak bases were utilized (namely pyridine, pyridine with a catalytic amount of DMAP, DMAP, $NaHCO_3$ and K_2CO_3) in DCM or DMF. Comparison studies of using both $NaHCO_3$ and pyridine at 0 ^oC was also attempted due to the belief that the desired intermediate product (4-benzenesulfonyl-1,2,4-dithiazolidine-3,5-dione) is unstable at room temperature. Thin layer chromatography investigation of the reactions showed the development of new products. However, upon isolation using preparation thin layer chromatography, none of the new products could be identified or confirmed to be the desired product using ^1H and ^{13}C NMR and mass spectroscopy.



where base = $NaHCO_3$ or K_2CO_3; solvent = CH_3CN, CH_2Cl_2 or DMF
base = pyridine, DMAP or pyridine + $DMAP_{cat}$; solvent = pyridine, CH_2Cl_2 or DMF

Scheme 5.5: Attempted synthesis of 4-benzenesulfonyl-1,2,4-dithiazolidine-3,5-dione

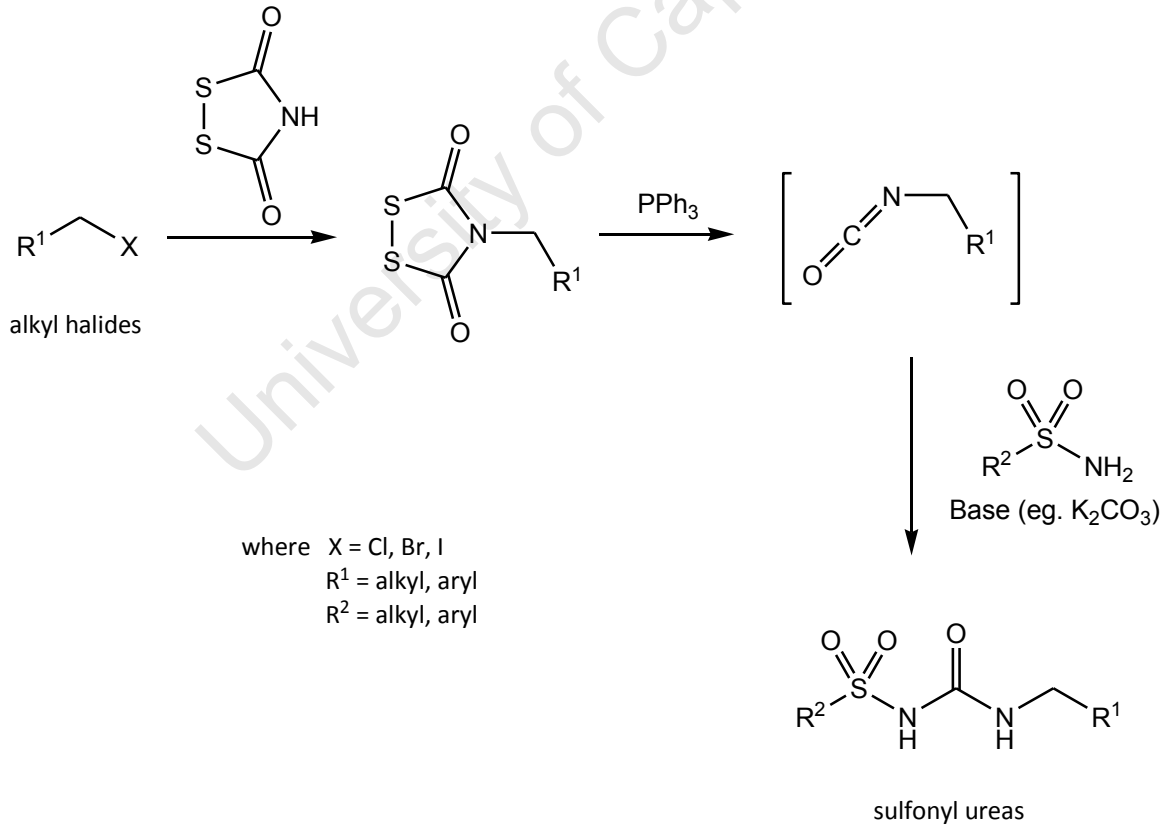
Due the possibility of (further) decomposition of the desired intermediate product while separating using column chromatography, toluene and triphenylphosphine were added to the reaction mixtures and refluxed. The reactions were monitored again via TLC, and a number of new spots appeared. After refluxing for 24 hours, the reactions were quenched and products isolated via column chromatography. However, none of the isolated samples could be identified as the desired sulfonyl urea product.

Other than the proposal that the intermediate 4-arenesulfonyl-1,2,4-dithiazolidine-3,5-dione products may be very unstable and easily decomposed, there are several other observations already recorded in the literature which may explain the failure of some of the reactions.

Decomposition of 1,2,4-dithiazolidine-2,5-dione in reactions with alkyl halides using DMF or DMSO as solvent, as well as at elevated temperatures (40 ^{o}C and higher), was observed and documented by Wood, M. E. *et al.* [2003].[6] The reactivity and decomposition of 1,2,4-dithiazolidine-2,5-diones in the presence of amines (and amine bases), respectively, is also well-documented in the literature by Barany, G. *et al.* [1980].[7]

5.3.3 Alternate New Synthetic Route to Sulfonyl Ureas

During attempts to synthesize sulfonyl ureas (above), another, alternative, route to synthesize sulfonyl ureas was envisaged. The reaction of 1,2,4-dithiazolidine-2,5-dione (**RKG121A**) with alkyl halides was well documented by Wood, M. E. *et al.* [2003],[6] and many examples were

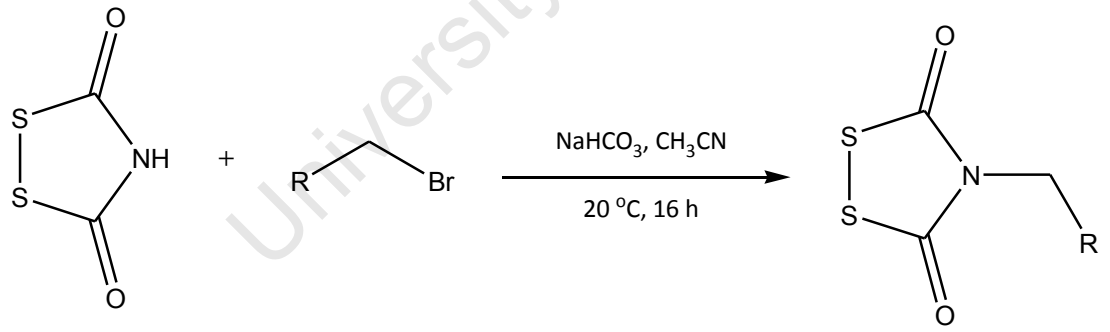


Scheme 5.6: Proposed synthesis of sulfonyl ureas using alternative new method

given. The resulting *N*-alkyl-1,2,4-dithiazolidine-2,5-dione, in the presence of triphenylphosphine, were converted to isocyanates and reacted with amines to give the corresponding ureas. It was, hence, envisaged that these *N*-alkyl-1,2,4-dithiazolidine-2,5-diones would be converted to their corresponding isocyanates, and reacted with sulfonamides in the place of amines.[6] This could provide a new synthesis to sulfonyl ureas, and would provide an alternative route to more diverse sulfonyl urea libraries, as the initial starting reagents are commercially available alkyl halides.

5.3.3.1 Synthesis of Sulfonyl Ureas using Alternate Route

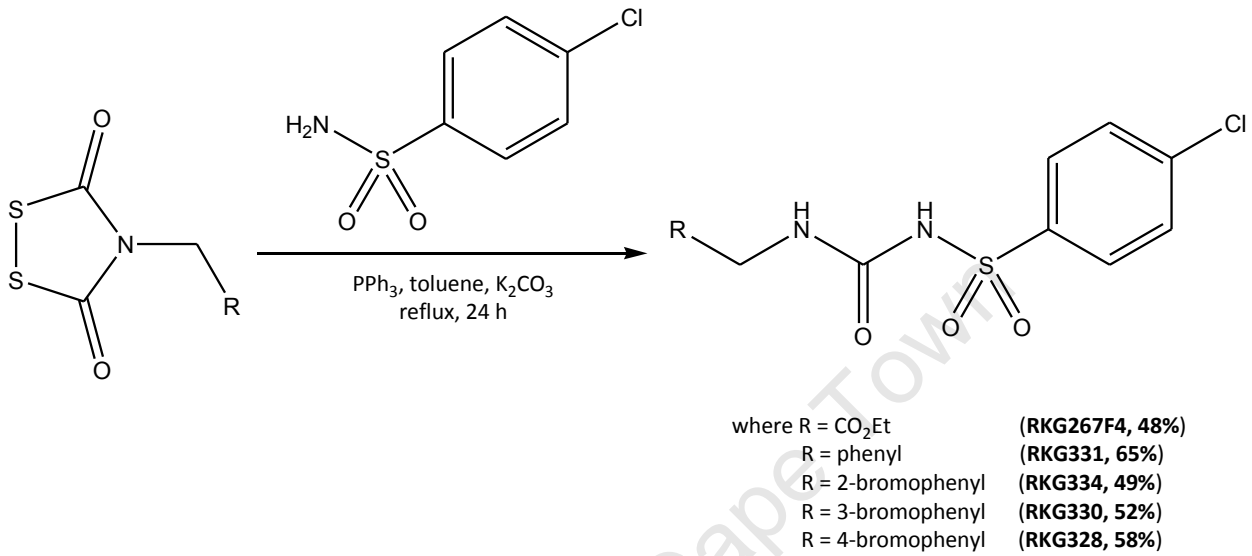
Following the protocol described by Wood, M. E. *et al.* [2003],[6] (3,5-dioxo-1,2,4-dithiazolidin-4-yl)acetic acid ethyl ester (**RKG2661**) was synthesized as described using $NaHCO_3$ in CH_3CN giving a 65% yield (literature: 86%). Another seven alkyl bromides were also reacted with 1,2,4-dithiazolidine-3,5-dione, under the same conditions (Scheme 5.7).



where R = CO_2Et (**RKG2661, 65%**)
R = phenyl (**RKG318, 74%**)
R = 2-bromophenyl (**RKG319, 94%**)
R = 3-bromophenyl (**RKG317, 79%**)
R = 4-bromophenyl (**RKG315, 92%**)
R = $CH{=}CH_2$ (**RKG425B, 68%**)
R = 3-methoxyphenyl (**RKG425C, 76%**)
R = biphenyl-2-yl (**RKG425D, 78%**)

Scheme 5.7: Synthesis of *N*-alkyl-1,2,4-dithiazolidine-3,5-dione intermediates

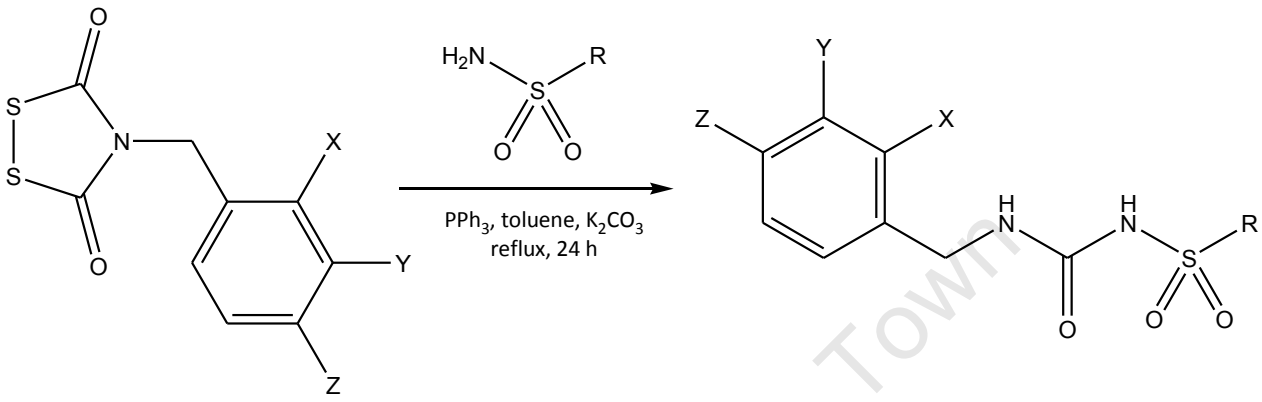
The reaction was attempted using a sulfonamide (4-chlorobenzene sulfonamide), in the presence of PPh_3 and K_2CO_3 in toluene (Scheme 5.8).



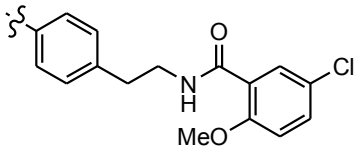
where R = CO_2Et (**RKG267F4, 48%**)
R = phenyl (**RKG331, 65%**)
R = 2-bromophenyl (**RKG334, 49%**)
R = 3-bromophenyl (**RKG330, 52%**)
R = 4-bromophenyl (**RKG328, 58%**)

Scheme 5.8: Synthesis of sulfonyl ureas using the new alternative method

Based on the success of this reaction with 4-chlorobenzene sulfonamide, the reaction was carried out using additional sulfonamides (Scheme 5.9).

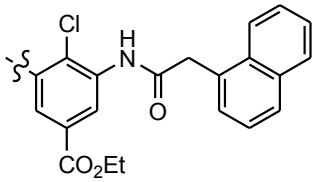


where X = Br, Y = H, Z = H, R = phenyl (**RKG357, 79%**)
X = Br, Y = H, Z = H, R = 2-naphthyl (**RKG360, 67%**)
X = H, Y = OCH_3, Z = H, R = 4-chlorophenyl (**RKG426A, 67%**)
X = H, Y = OCH_3, Z = H, R = 2,4,5-triisopropylphenyl (**RKG426B, 32%**)
X = phenyl, Y = H, Z = H, R = 4-chlorophenyl (**RKG427A, 71%**)
X = Br, Y = H, Z = H, R =



(**RKG359, 80%**)

X = H, Y = H, Z = Br, R =

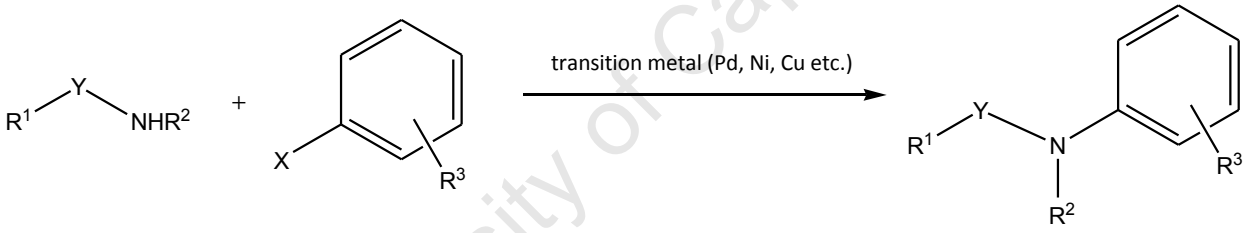


(**RKG351, 47%**)

Scheme 5.9: Synthesis of additional sulfonyl ureas using the new alternative method

5.3.4 Attempted Synthesis of Sulfonyl Ureas using a Key Metal-Catalyzed Step

The efficient amination and amidation of aryl halides using metals, such as palladium,[8-15] nickel[16-18] and copper,[19-33] as a catalyst is extensively represented in the literature. While the palladium-catalyzed C-N bond formation has been studied extensively over the past few years, both the Ullmann and Goldberg reactions (copper-catalyzed *N*-arylation of amines and amides, respectively) predate the palladium-catalyzed amination methodology by almost a hundred years. A simplistic schematic representation of the transition metal catalyzed amidation is presented below (Scheme 5.10):

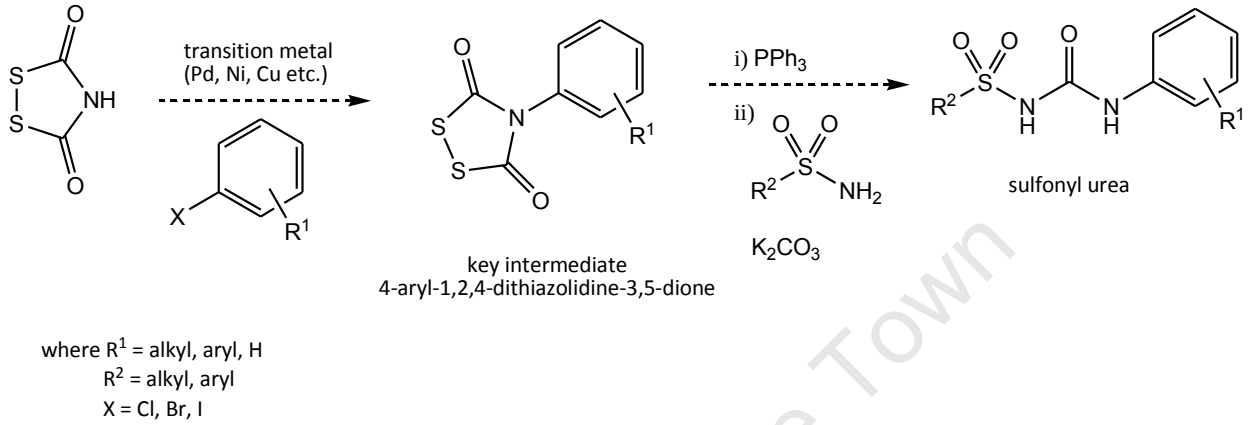


where R^1 = alkyl, aryl, H
R^2 = alkyl, aryl, H
R^3 = alkyl, aryl, H
Y = CH_2, CO
X = Cl, Br, I

Scheme 5.10: Simplistic representation of transition metal catalyzed amidation

The limitation of the alternate new synthetic route to sulfonyl ureas described in Chapter 5.3.3 is the need for the halogen (leaving group) to be attached to a (-CH_2-) carbon unit. Therefore, starting materials and precursors are limited to halogenated alkyl- and benzyl–type moieties. Hence, it was envisaged that an alternative route to sulfonyl ureas using methodology that

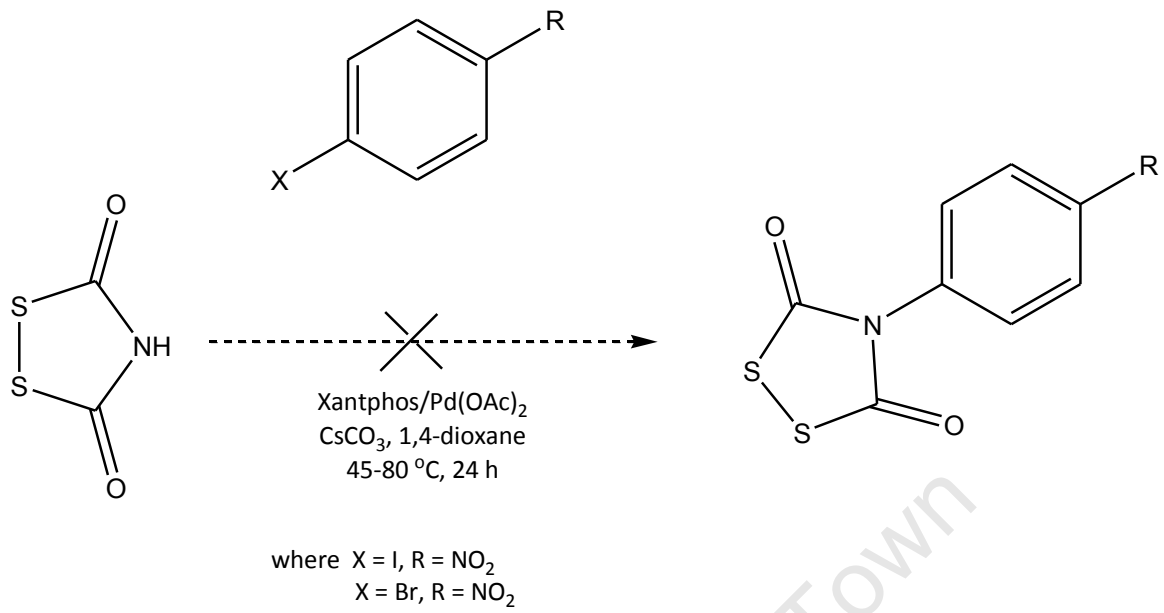
would include aryl halides as starting precursors, would be via a key transition metal catalyzed amidation step. The proposed synthetic scheme is as follows (Scheme 5.11):



Scheme 5.11: Proposed synthesis of sulfonyl ureas via a key transition metal catalyzed amidation step

5.3.4.1 Attempted Pd-Catalyzed Intermolecular Amidation

Based on the observations by Buchwald, S. L. *et al.* [2002],[15] a Xantphos/Pd complex proved to be an active and efficient catalyst (1-4 mol % of Pd catalyst) for the amidation of both activated and unactivated aryl halides. The active catalyst is easily formed *in situ* by the addition of Xantphos ((9,9-dimethyl-9*H*-xanthene-4,5-diyl)bis(diphenylphosphine)), a ligand developed by van Leeuwen, P. W. N. M. *et al*. [1995],[34] to $Pd(OAc)_2$ or $Pd(dba)_3$. Buchwald, S. L. *et al*. [2002][15] established, after a study of a variety of ligands and reaction variables, that the most successful catalytic system was obtained using Xantphos as the ligand, Cs_2CO_3 as the base, THF or 1,4-dioxane as the solvent and temperatures varying from 45 – 100 ^{o}C. Using the optimized protocol described in the literature,[15] the coupling of two different aryl halides with 1,2,4-dithiazolidine-3,5-dione was attempted as detailed below (Scheme 5.12):



where X = I, R = NO_2
X = Br, R = NO_2

Scheme 5.12: Attempted Xantphos/Pd catalyzed coupling of aryl bromides with 1,2,4-dithiazolidine-3,5-dione

Monitoring the reactions at temperatures in the range 45 - 80 ^{o}C with TLC showed varying results. In both cases, a new distinct spot formed as the 1,2,4-dithiazolidine-3,5-dione spot disappeared on reaction. However, upon isolation using column chromatography, NMR analysis of the separated new product did not show the correct desired ^{1}H aromatic signals in conjunction with the desired carbonyl peak in the ^{13}C spectrum. Hence, it could not be concluded that the desired intermediate product had been formed. Further analysis of the NMR spectra seemed to suggest the formation of two inseparable products with similar R_f values.

Not surprisingly it is known that the 1,2,4-dithiazolidine-3,5-dione scaffold decomposes in the presence of triphenylphosphine. Presumably because Xantphos (a phosphorous ligand) was used in our reactions, this may have led to the apparent decomposition. If the desired product did initially form, the present phosphorous ligand could react with it, converting it to the highly reactive and unstable isocyanate. This would also result in no active catalyst being present as required phosphorous ligand would be consumed.

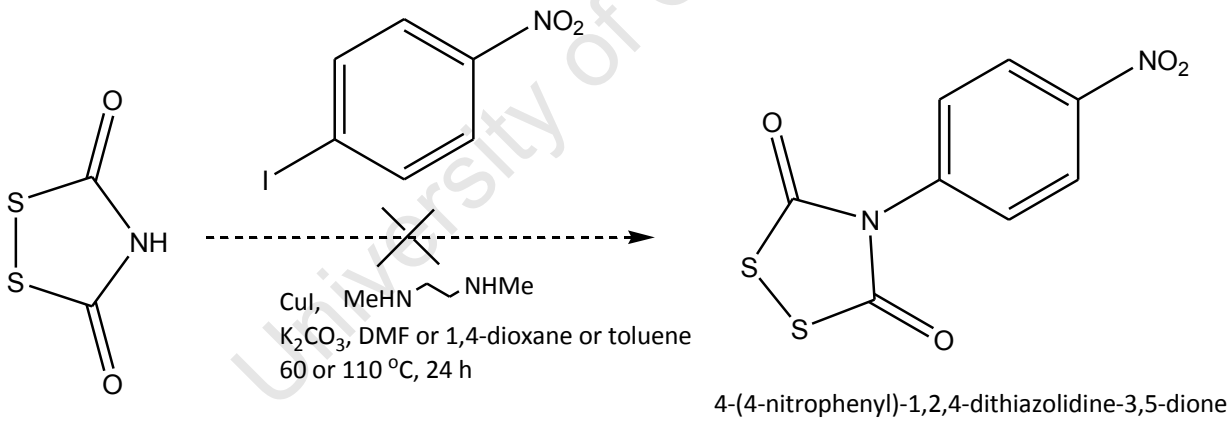
5.3.4.2 Attempted Cu-Catalyzed Intermolecular Amidation

Extensive studies by Klapars, A. *et al.* [2002][33] revealed a simple and inexpensive catalyst system for the amidation of aryl halides using 0.2 – 10 mol % of CuI, 5 – 20 mol % of a 1,2-diamine ligand, and $CsCO_3$, K_2CO_3 or K_3PO_4 as the base. Studying a variety of 1,2-diamine ligands, using *N,N'*-dimethylethylenediamine or *trans-N,N'*-dimethyl-1,2-cyclohexanediamine proved to result in the most active catalyst systems, although several other 1,2-diamine ligands were also sufficiently active. This catalyst system effectively amidated aryl iodides, bromides, and in several cases, aryl chlorides, and showed tolerance to a number of diverse functional groups present on reactants that were not compatible with the Pd-catalyzed amination and amidation protocols. Using the optimized methodology developed by Klapars, A. *et al.* [2002],[33] the coupling of 1-iodo-4-nitrobenzene with 1,2,4-dithiazolidine-3,5-dione was attempted as detailed below (Scheme 5.13):

Commercially available *N,N'*-dimethylethylenediamine (Sigma-Aldrich) was chosen to be the ligand and potassium carbonate was chosen as the base. Initial studies at 60 and 110 ^{o}C using three different solvents, namely 1,4-dioxane, toluene and DMF (all three solvents proved to be effective for the amidation of different aryl halides in the copper catalyzed coupling system[33]), and 1-iodo-4-nitrobenzene as the aryl halide were carried out.

Thin layer chromatography was used to monitor all the systems, and as observed while attempting the Xantphos/Pd reactions, a new product was forming as the 1,2,4-dithiazolidine-3,5-dione was being consumed. At 60 and 110 ^{o}C using both 1,4-dioxane and toluene as the solvent system, the separated new product appeared to be again a mixture of two new products (with corresponding R_f values) with similar ^{1}H NMR profiles (only the intensity of the peaks varied from solvent to solvent). However, for the 60 ^{o}C reaction using DMF as the

solvent, the ^{1}H NMR seemed to suggest the formation of one new product (EI-MS: m/z = 275.98 [M^{+}]). At 110 ^{o}C using DMF as the solvent, again, the ^{1}H NMR seemed to suggest the formation of one new product. However, this time, it appears to be another new product (EI-MS: m/z = 307.95 [M^{+}]), which was previously inseparable for all Pd catalyzed systems, and Cu catalyzed systems using toluene or 1,4-dioxane as the solvent systems. It must be noted that these mass spectra were recorded using EI-MS, and the m/z values recorded may have been either an abundant large molecular fragment or parent ion. Neither of the two compound's mass spectra agree with the desired intermediate product (4-(4-nitrophenyl)-1,2,4-dithiazolidine-3,5-dione) which would be expected to have a m/z value of 255.96. Another possible product which could have formed, and is documented in the literature,[33] is the coupling of the aryl halide to another molecule of aryl halide. In this case, the coupling of two 1-iodo-4-nitrobenzene molecules would result in the formation of 4,4'-dinitrobiphenyl with a m/z value of 244.05.



Scheme 5.13: Attempted Cu catalyzed coupling of 1-iodo-4-nitrobenzene with 1,2,4-dithiazolidine-3,5-dione

Other possible reasons for the failure of the Cu-catalyzed system may be the presence of the 1,2-diamine ligand, or the use of DMF as solvent. As mentioned before in Chapter 5.3.2, it is

documented in the literature by Wood, M, E, *et al.* [2003][6] and Barany, G. *et al.* [1980],[7] that the susceptibility to decomposition of 1,2,4-dithiazolidine-3,5-dione is high in the presence of amines and amine bases, or when using DMF as the solvent.

5.4 Conclusion

Following the literature, the 1,2,4-dithiazolidine-3,5-dione "scaffold" was successfully synthesized. The envisaged new methodology for the synthesis of sulfonyl ureas required the coupling of 1,2,4-dithiazolidine-3,5-dione with a sulfonyl chloride. This coupling step was attempted under a range of conditions, including varying the base, solvent, temperature etc. It was also attempted in order to allow the desired intermediate to remain in solution without isolation, and perform the following step with triphenylphosphine and amine. Presumably the key 4-(arylsulfonyl)-1,2,4-dithiazolidine-3,5-dione intermediate may be too unstable under the experimental conditions attempted. All attempts to synthesize sulfonyl ureas using the initial new methodology were, unfortunately, unsuccessful.

However, another new route to sulfonyl ureas was envisaged using similar chemistry, but starting with *N*-alkyl-1,2,4-dithiazolidine-2,5-dione intermediates. Wood, M. E. *et al.* [2003][6] had already developed this protocol to react the *N*-alkyl-1,2,4-dithiazolidine-2,5-dione with amines in the presence of triphenylphosphine to give ureas. It was, hence, envisaged that the reaction of *N*-alkyl-1,2,4-dithiazolidine-2,5-dione intermediates with sulfonamides in the presence of a weak base (i.e. potassium carbonate) and triphenylphosphine would give the expected products. This alternate method was successful, and eleven sulfonyl ureas were synthesized to demonstrate the success of this method. Yields ranged from 32 - 79%, and the purification of the final sulfonyl ureas was occasionally problematic due to poor solubility of the target sulfonyl urea in a range of solvents.

The alternate new method, too, has its limitations, as only the synthesis *N*-alkyl-1,2,4-dithiazolidine-2,5-dione intermediates has been developed. Thus, the synthesis of *N*-aryl-1,2,4-dithiazolidine-2,5-dione intermediates using transition metal-catalyzed (namely Pd and Cu)

amidation methodology was envisaged. The formation of two new inseparable products under both Pd- and Cu-catalyzed methodology, in low yields, was observed. By varying the temperature of the Cu-catalyzed system, both new products were obtained individually. Their NMR spectra agree with what could be expected for the desired *N*-aryl-1,2,4-dithiazolidine-2,5-dione intermediates, as well as for another bis-aryl byproduct. Mass spectroscopy was obtained on both samples, but the measured molecular ion for both new products do not agree with the *N*-aryl-1,2,4-dithiazolidine-2,5-dione intermediate, nor the bis-aryl byproduct. It is suspected that the *N*-aryl-1,2,4-dithiazolidine-2,5-dione may have decomposed under the conditions of the catalytic systems as phosphine (Pd-catalyzed) and amine (Cu-catalyzed) ligands are required. Although the two "new" unidentified products mass spectra did not agree with the desired target molecule, further investigation of these products could be carried out with x-ray crystallography, as well as, softer MS techniques, including chemical ionization (CI) or fast-atom bombardment (FAB), to determine their structure.

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Chapter 6

Biological Results and Discussion

6.1 New Permeability Pathways

All synthesized potential NPP inhibitors and their intermediates (Chapter 2), as well as, all available potential ALS inhibitors and their respective intermediates (Chapter 4) were submitted in order to test their effect on the uptake of sorbitol, a known NPP substrate. The unpublished assaying method used was developed by N. Spillman (The Australian National University) (experimental details discussed in 6.1.5 Experimental Protocol). The assay involves the monitoring (using a fluorometer) of sorbitol uptake via the NPP of *P. falciparum* infected erythrocytes. The uptake of sorbitol causes haemolysis of the erythrocytes which results in a change in absorbance. The presence of a NPP inhibitor would result in the reduction or prevention of sorbitol uptake. Therefore, if the test compound is an effective NPP inhibitor, the absorbance will remain constant.

6.1.1 Effect of Controls on Transmission

Figure 6.1.1.1 shows the time course of transmission for infected cells with added isosmotic sorbitol. The uptake of sorbitol via the NPP is depicted by the increase in transmission seen over time. As the sorbitol is taken up, haemolysis of the erythrocytes occurs, and once a majority of the cells have lysed, the transmission reaches a plateau. The further addition of saponin (shown by an arrow) causes the haemolysis of all remaining cells, and is observed by the further increase in transmission.

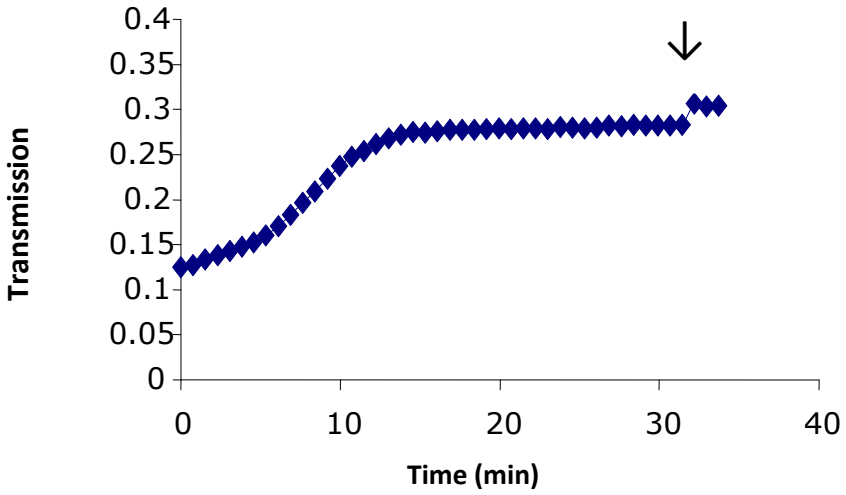


Figure 6.1.1.1: Transmission of solution containing infected cells and sorbitol

Figure 6.1.1.2 shows the time course transmission for infected cells with added furosemide (a potent NPP inhibitor) (a similar graph is observed if **H157** is used as the control). The presence of the NPP inhibitor results in the blocking of sorbitol uptake, and subsequently prevents the haemolysis of the erythrocytes. A relatively constant transmission is the result, and the addition of saponin (shown by an arrow) causes a large increase in transmission due to lysis of the cells.

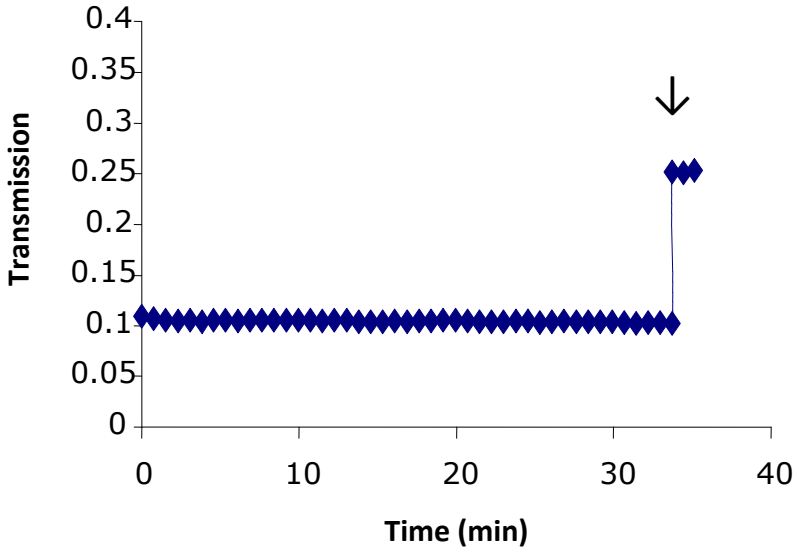


Figure 6.1.1.2: Transmission of solution containing infected cells, sorbitol and furosemide

6.1.2 Effect of Test Compounds on Transmission

The following figures (6.1.2.1 – 6.1.2.8) show all compounds and intermediate products that had little effect, with the exception of the known NPP inhibitor **RKG256** (**H157**), on the haemolysis time course. All samples were tested at a concentration of 50 µM and positive control (furosemide) at a concentration of 0.66 nM.

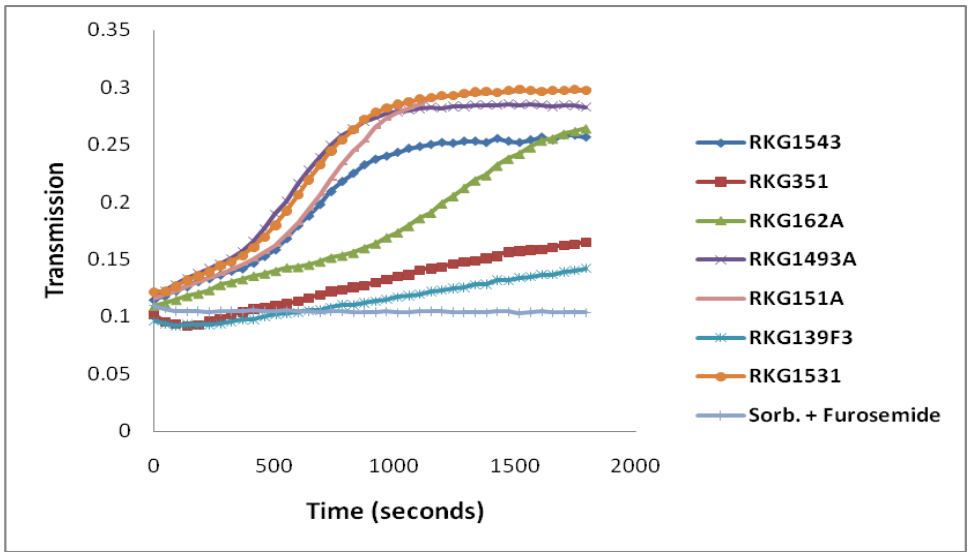


Figure 6.1.2.1: Transmission of solution containing infected cells, sorbitol and test compounds

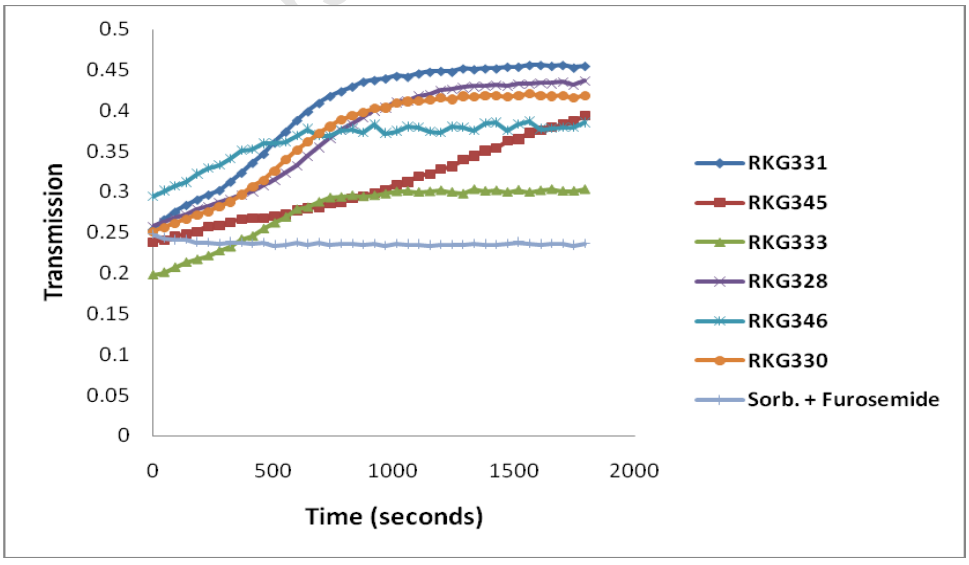


Figure 6.1.2.2: Transmission of solution containing infected cells, sorbitol and test compounds

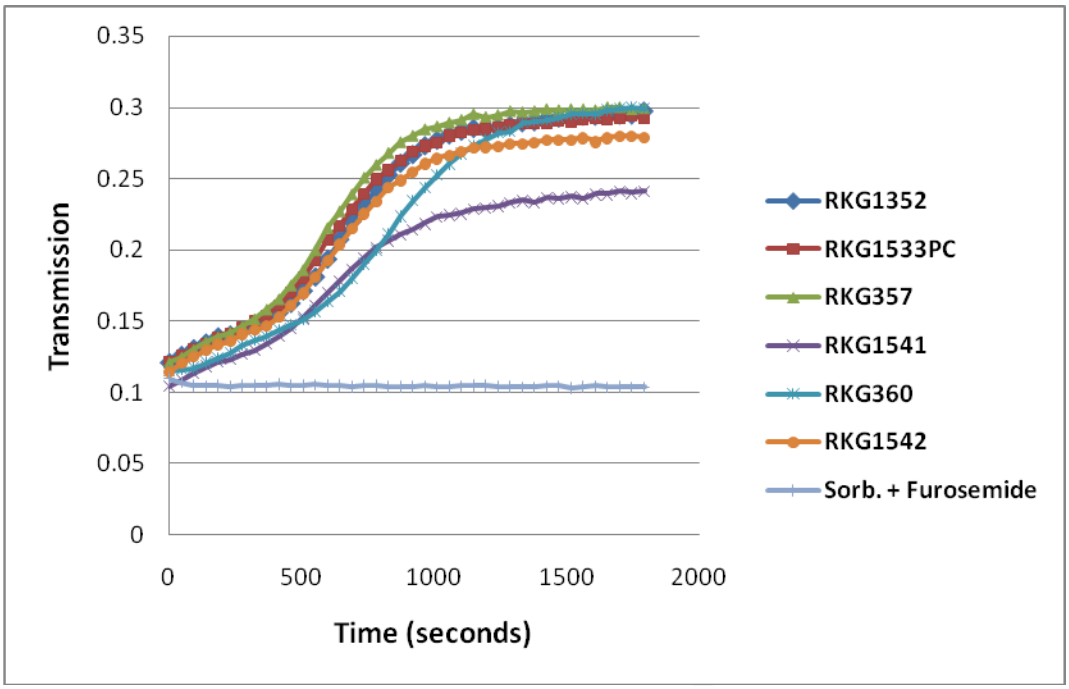


Figure 6.1.2.3: Transmission of solution containing infected cells, sorbitol and test compounds

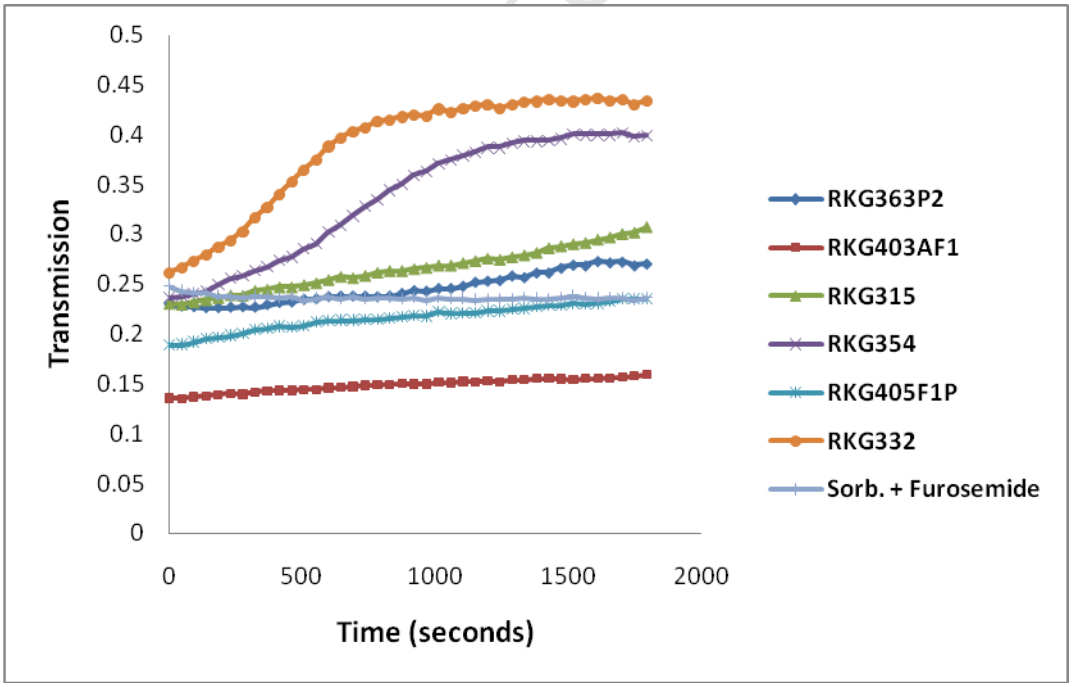


Figure 6.1.2.4: Transmission of solution containing infected cells, sorbitol and test compounds

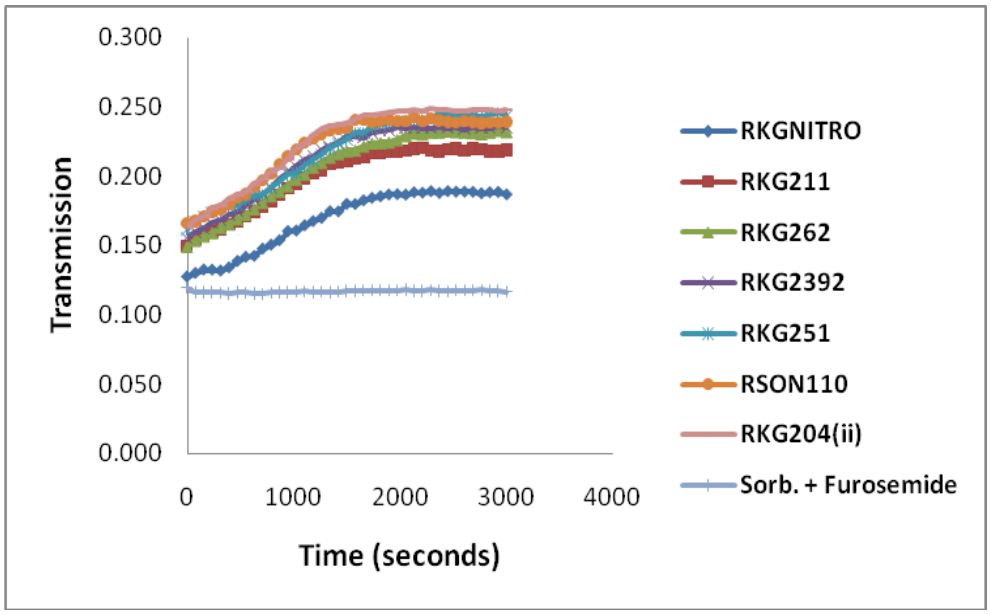


Figure 6.1.2.5: Transmission of solution containing infected cells, sorbitol and test compounds

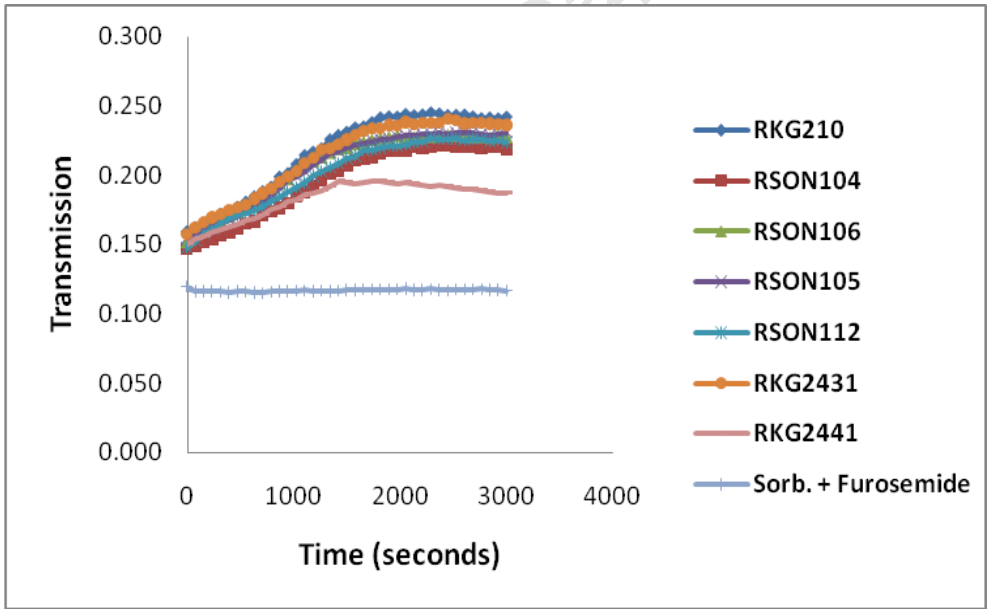


Figure 6.1.2.6: Transmission of solution containing infected cells, sorbitol and test compounds

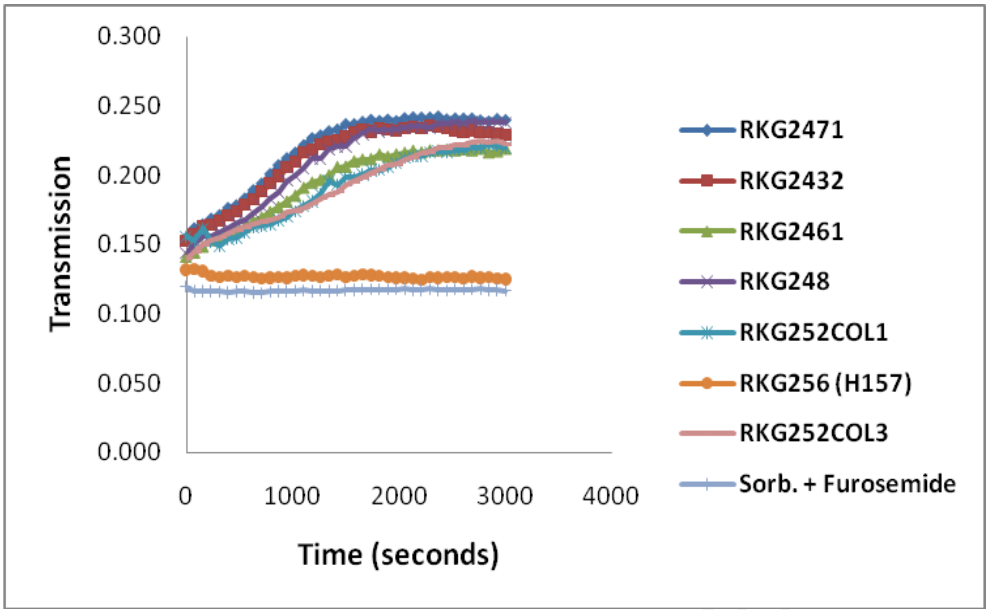


Figure 6.1.2.7: Transmission of solution containing infected cells, sorbitol and test compounds

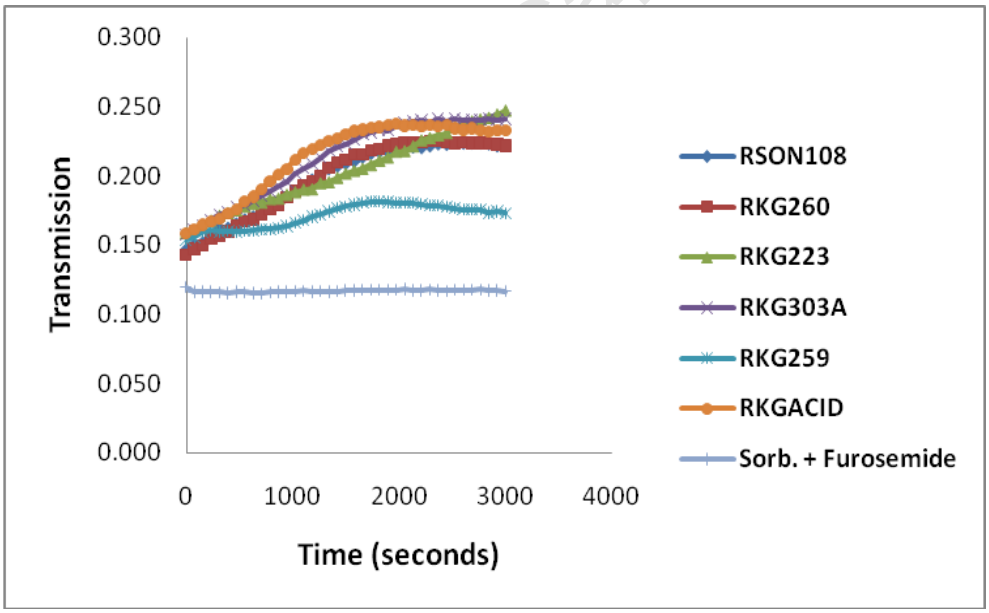


Figure 6.1.2.8: Transmission of solution containing infected cells, sorbitol and test compounds

Figures 6.1.2.1 – 6.1.2.4 represent the assays that were conducted using the trophozoite stage of the FAF6 strain of *P. falciparum* (performed by collaborators from The Australian National

University, June 2007 – K. Easton and K. Kirk). Figures 6.1.2.5 – 6.1.2.8 represent the assays that were conducted using the trophozoite stage of the D10 strain of *P. falciparum* (performed by collaborators from The Australian National University, May 2006 – N. Spillman and K. Kirk).

Of the 55 compounds tested, only four compounds showed pronounced inhibition of the sorbitol-induced haemolysis. One of these four compounds was **RKG256** (**H157**), and effective inhibition was expected, and is graphically represented and confirmed in Figure 6.1.2.7. The other three inhibitory compounds were **RKG4042P**, **RKG340** and **RKG359**. The following three (Figures 6.1.2.9 – 6.1.2.11) represent transmission time courses for each of these inhibitors:

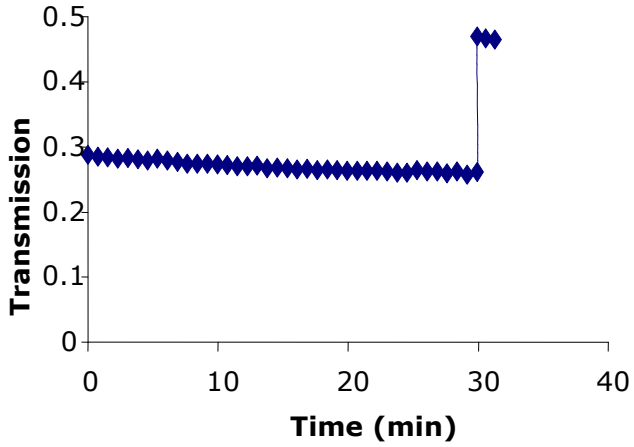


Figure 6.1.2.9: Transmission time course for **RKG4042P**

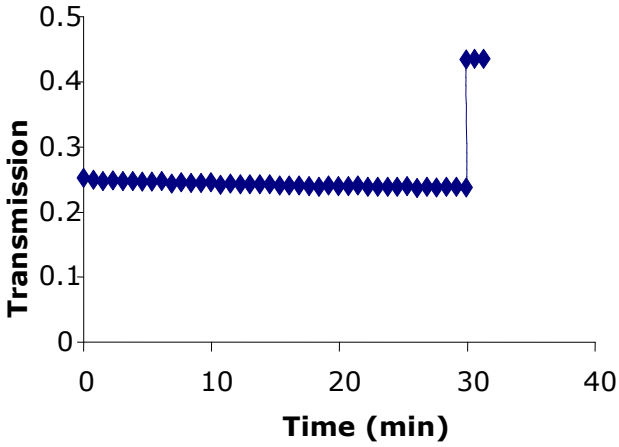


Figure 6.1.2.10: Transmission time course for **RKG340**

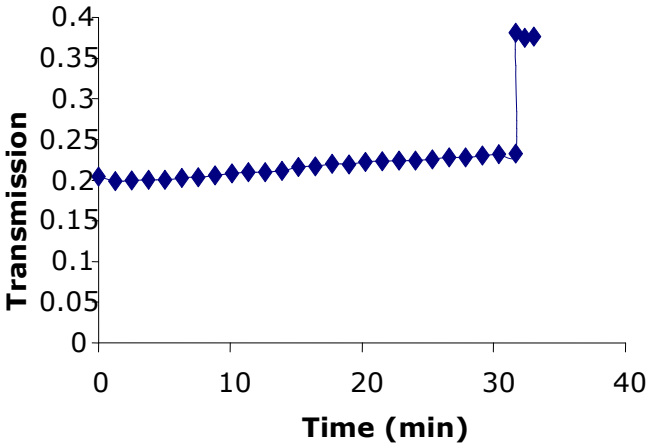


Figure 6.1.2.11: Transmission time course for **RKG359**

Both times courses for **RKG4042P** and **RKG340** show a slight decrease in transmission over time, and this is likely the result of cells settling over time. The small increase in transmission over time observed for **RKG359** is a result of incomplete inhibition of the sorbitol uptake. The large increase in transmission observed for all three compounds at around 30 minutes of elapsed time is due to the addition of saponin.

All three compounds were further tested at concentrations of 22.5, 9 and 4.5 µM. **RKG359** showed good, but incomplete inhibition at all the concentrations. Almost complete inhibition at 9 µM and some inhibition at 4.5 µM was observed for **RKG340**. **RKG4042P** proved be the most effective inhibitor of the three, and proved an approximately constant complete inhibition at all concentrations.

6.1.3 Dose Response for **RKG4042P**

Transmission time courses for concentrations of 45, 22.5, 9, 4.5, 1.8, 0.9, 0.45 and 0.09 µM of **RKG4042P** were recorded, and used to produce a dose response curve (Figure 6.1.3.1).

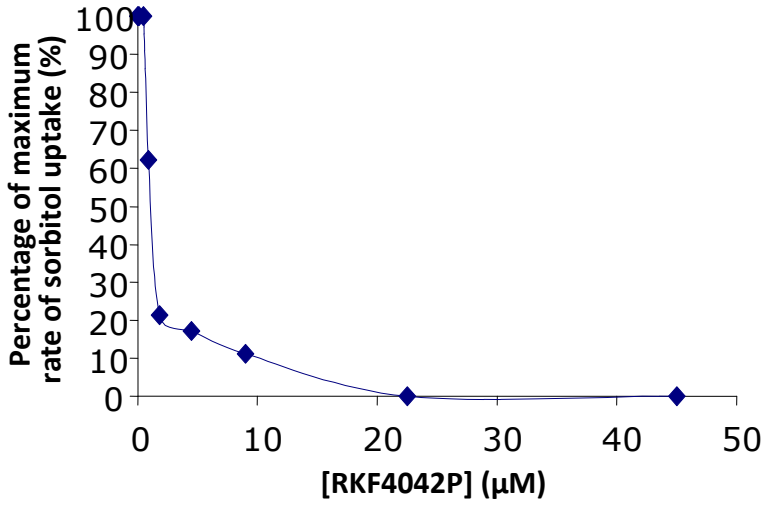


Figure 6.1.3.1: Dose response curve for **RKG4042P**

From the graph (Figure 6.1.3.1), the IC_{50} (concentration required to reduce sorbitol uptake to 50%) of **RKG4042P** calculated to be approximately 1.2 µM.

6.1.4 Conclusion

From the library of 55 compounds that were tested for the inhibition of sorbitol uptake by parasitized erythrocytes, four compounds proved to show a significant inhibitory effect. These four are **RKG256** (**H157**), **RKG4042P**, **RKG359** and **RKG340** (Figure 6.1.4.1).

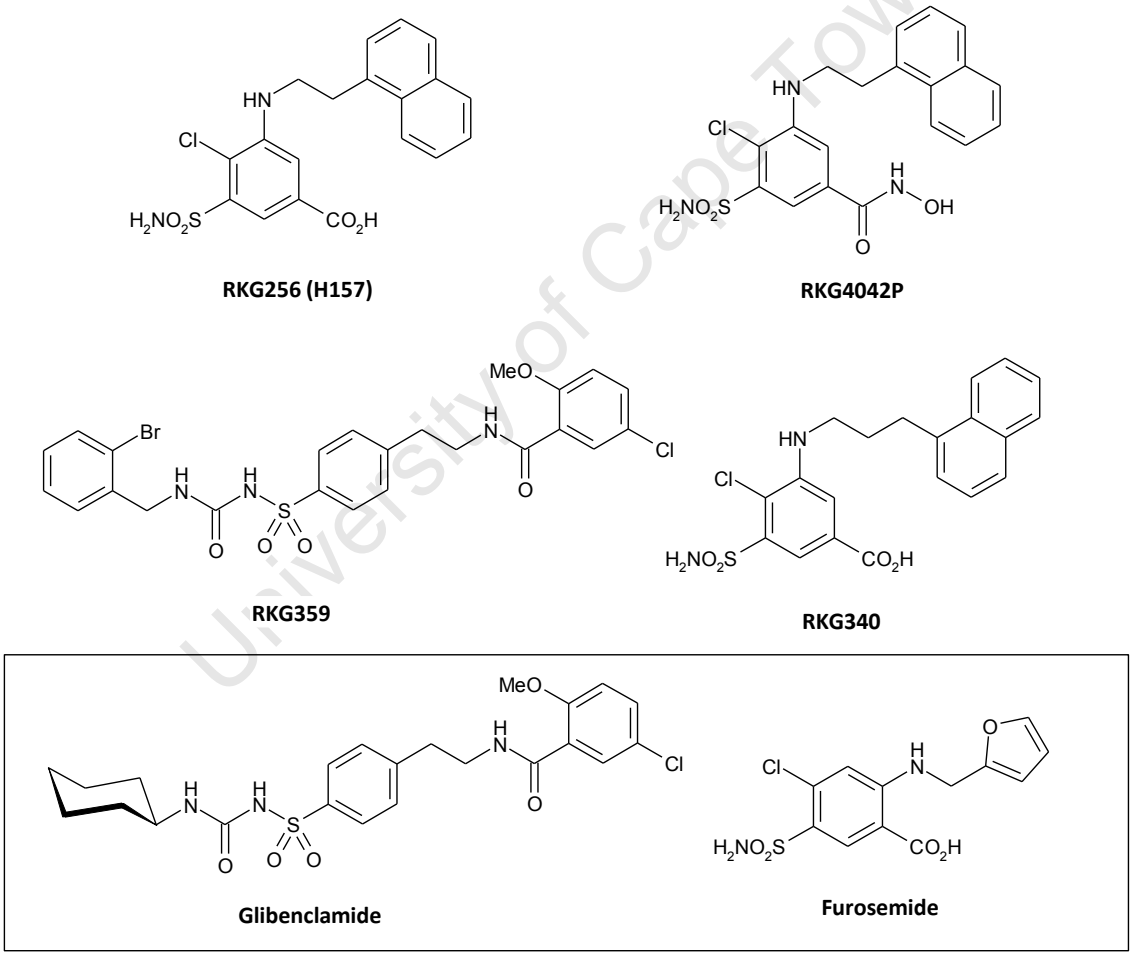


Figure 6.1.4.1: The four compounds that showed significant inhibition of sorbitol uptake by parasitized erythrocytes, and known classical Cl^- channel blockers that inhibit NPP

RKG256 (**H157**) was demonstrated to inhibit parasite-induced choline transport by Staines, H. M. *et al.* [2004][1] and has an IC_{50} of 0.44 µM. It was synthesized for comparison purposes, and to use as a control in the screening of other test compounds. The IC_{50} was, hence, not determined, but it did show significant inhibition of sorbitol uptake as expected. **RKG340** (an extended 'side-chain' derivative of **H157**) showed almost complete inhibition at 9 µM, and although its IC_{50} was not precisely determined, this result is comparable to that of **H157**. It can further be concluded that the extension of the 'side chain' by one methylene unit did not affect the inhibitory effectiveness of this class of piretanide-like compounds. The extension of this 'side chain' by more than one methylene unit may be considered for future SAR studies.

All compounds bearing an amide moiety in the 'side-chain,' ester derivatives of **H157** and other ester intermediates, as well as those bearing heterocyclic and heteroaromatic groups on the 'side-chain' showed little or no inhibition at all. It can be concluded that the secondary amine (anilinic) and carboxylic acid moieties are important for the activity of piretanide-like class of compounds. All acylthioureas, imidazolinone, phthalimide and sulfonylcyanoguanidine analogues showed little or no inhibition of sorbitol uptake.

The IC_{50} of **RKG359** was not determined, but it did show inhibition at concentrations as low as 22.5 and 9 µM. A small collection of eleven sulfonyl ureas (including those synthesized in the sulfonyl urea methodology development) were sent for testing, and only **RKG359** demonstrated any significant inhibition of sorbitol uptake. When analyzing the structure of **RKG359**, it was later found to be an analogue of the known classical Cl^- channel blocker, glibenclamide.[2]

The most active compound tested was **RKG404P2**, which gave an IC_{50} of approximately 1.2 µM. This result is consistent with that obtained for **H157**.[1] Although **RKG404P2** is the hydroxamic acid bioisostere of **H157**, an important observation is that the hydroxamic acid functionality is not as acidic as the carboxylic acid moiety of **H157**. All previously known piretanide-like and

furosemide-like NPP inhibitors are anionic. It would, therefore, be important to synthesize more hydroxamic acid derivatives of known piretanide-like and furosemide-like NPP inhibitors for further investigation. This may lead to the development of novel hydroxamate NPP blockers.

For more conclusive results, the compounds that have shown effective inhibition of sorbitol uptake by parasitized erythrocytes should be investigated for their effect on the uptake of other known substrates believed to enter via the induced NPP. The inhibition of the uptake of substrates like pantothenate or choline could also be assayed.

6.1.5 Experimental Protocol

Parasites cultures:

Assays were conducted using the trophozoite stage of the D10 and FAF6 strains of *Plasmodium falciparum*. *P. falciparum* (strain D10) were cultured in group O erythrocytes by Dr Richard Allen (Postdoctoral research fellow, ANU). Parasites (strain FAF6) were synchronised and provided in human blood (from the Canberra branch of the Australian Red Cross Blood Service) by Natalie Spillman. The parasite sample was washed three times in 50 ml ice cold, bicarbonate-free RPMI.

Washing performed by centrifugation for 5 minutes at 1500 x g, 4°C. 300 µl of uninfected human blood was also obtained and centrifuged for 1 minute at 14000 x g. The supernatant was removed and the cells diluted 1/10 with PBS.

Preparation of cells:

Cells were washed in ice cold RPMI before magnetic enrichment of infected cells using a 21G needle on the Vario MACS column. Enriched infected cells and uninfected RBC were sedimented by centrifugation at 1550rpm (4ºC) and pellets transferred to microcentrifuge tubes. The uninfected RBC were resuspended at a dilution of 1/10, such that 100µL of the pellet was resuspended in 900µL of phosphate-buffered saline using a positive displacement pipette. The infected RBC were resuspended at a concentration of $2.175x10^{8}$ cells/mL. Cell counts were performed using an improved Neubauer counting chamber at an appropriate cell dilution. The parasitemia was calculated using methanol-fixed, Giemsa-stained smear, counting a minimum of 650 cells.

Isosmotic Haemolysis:

A BMG Labtechnologies 96 well plate was loaded with 4uL aliquots of the uRBC and 20uL aliquots of the iRBC, which gave appropriate initial absorbance levels. At time zero, an eight-lane multichannel pipettor was used to add 200uL of the isosmotic sorbitol (supplemented with inhibitor/control) to each well. The change in absorbance (A) corresponding to the lysis of the cells was measured using the HAEMOLYSISEXP-NJS program on the FLUOstar OPTIMA BMG LABTECH reader 413-1042. The experiment was performed at 700nm (37ºC) with orbital or double orbital shaking for up to 5 seconds before each cycle. Cycle times (depending on the number of wells utilised) varied from 34 to 95 seconds. After lysis had stopped for most samples the program was paused and 10µL of the plant-derived detergent Saponin (10%w/v) was added to each well. The absorbance measurements then resumed for another 10 cycles. All data was converted to transmittance (T), using the equation, $T = 10^{-A}$ for further analysis.

6.2 *In Vitro* Testing against *Plasmodium falciparum* Strains

The induction of the NPP into the host erythrocyte is very important for the growth of the malaria parasite *P. falciparum*. Based on work done by Staines, H. M. *et al.* [2004],[1] even when the NPP operate at levels only a fraction of the norm, they still allow sufficient flux of solutes to allow the parasite to survive. Compounds that show the inhibition of NPP at nanomolar concentration ranges lack the potential of becoming antimalarial candidates if they have little effect on the growth of the parasite. In this regard, the antiplasmodial *in vitro* activities of all available synthesized compounds were determined. The antiplasmodial activity against the chloroquine-sensitive D10 strain of *P. falciparum* was determined in the laboratories of P. J. Smith (Division of Pharmacology, University of Cape Town). The antiplasmodial activity against the chloroquine-resistant W2 strain of *P. falciparum* was carried out by the collaborating group of P. J. Rosenthal (Department of Medicine, San Francisco General Hospital, University of California at San Francisco, UCSF)). Table 6.2.1 shows the antiplasmodial activities of compounds that were determined at the two different laboratories against both the chloroquine-sensitive (CQS) D10 and chloroquine-resistant (CQR) W2 strains.

6.2.1 Results and Discussion

Compound **RKG303A**, a chloroquine-based structure, was the only compound active against the (CQR) W2 and (CQS) D10 strains of *P. falciparum* (highlighted in blue in Table 6.2.1) with IC_{50}(W2) = 0.89 ± 0.07 µM and IC_{50}(D10) = 0.693 µM respectively. This activity, however, is more likely attributed to the compound following the 4-aminoquinoline-type mechanism of action, as opposed to the activity being a result of the inhibition of NPP. The rest of the compounds did not show significant activity against the parasite.

Table 6.2.1: *In vitro* activity against both *P. falciparum* (CQR) W2 and (CQS) D10 strains

	W2		D10	
Compound ID	IC_{50} µM	SD	IC_{50} µg/ml	IC_{50} µM
RKG315	**26.19**	0.68	39	128.21
RKG354	**49.26**	2.12	11.7	38.46
RKG332	**>50**	-	>100	-
RKG345	**>50**	-	28.7	102.97
RKG333	**39.39**	0.32	>100	-
RKG346	**20.21**	0.86	>100	-
RKG364	**19.70**	0.26	12.8	29.50
RKG252COL1	**44.31**	5.42	9.8	23.39
RKG404P2	**17.70**	0.29	7.1	16.91
RKG335F2	**36.47**	0.36	>100	-
RKG331	**>50**	-	>100	-
RKG328	**>50**	-	95.3	236.08
RKG330	**>50**	-	>100	-
RKG357	**>10**	-	>100	-
RKG360	**>50**	-	>100	-
RKG359	**>50**	-	53.3	91.76
RKG351	**>10**	-	42.5	64.50
RKG1493A	**>50**	-	>100	-
RKG1531	**>50**	-	>100	-
RKG1532	**>50**	-	>100	-
RKG1533PC	**>50**	-	>100	-
RKG1541	**>50**	-	10.4	28.13
RKG1542	**>10**	-	32.2	86.87
RKG1543	**>10**	-	33.8	95.80
RKG139F3	**>50**	-	86.1	208.13
RKG151A	**>50**	-	>100	-
RKG303A	**0.89**	0.07	0.693	1.28
RKG1491	**-**	-	61.8	158.60
RKG162A	**-**	-	8.9	34.66
RKG340	**-**	-	53.3	127.24
RKG256	**>20**	-	6.34	17.87
RKG414F2	**>20**	-	>10	-
RKG409	**>20**	-	>10	-
RKG442	**>20**	-	>10	-
Chloroquine	0.091	0.024	0.015	0.048

The four compounds that showed effective inhibition of sorbitol uptake (Chapter 6.1) are highlighted in orange in Table 6.2.1. Compounds **RKG359** and **RKG340** were found to have little activity with IC_{50} values of 91.76 and 127.24 µM respectively for the CQS D10 strain, and IC_{50}

higher than the tested limit for the CQR W2 strain. Both **RKG256** (**H157**), IC_{50}(D10) = 17.9 µM, and bioisosteric analogue **RKG4042P** demonstrated the highest activites. The IC_{50} values were constant through both strains for **RKG4042P**, IC_{50}(W2) = 17.7 ± 0.3 µM and IC_{50}(D10) = 16.9 µM. Although, this activity is not as potent when compared to the control chloroquine (which IC_{50} values are in the nanomolar ranges), it still presents the possibility that **RKG4042P** and **RKG256** moderate activities may be a result of NPP inhibition. This can be further concluded as the activities found in both the antiplasmodial assays and sorbitol uptake assays are all in the equivalent micromolar ranges. To be more conclusive, future testing of a bigger range of compounds showing good NPP inhibition should be carried out for antiplasmodial *in vitro* activity.

6.2.2 Experimental Protocol

In vitro testing against chloroquine-sensitive D10 *P. falciparum*

The test compounds were tested in duplicate on one occasion against chloroquine sensitive (CQS) strain of *Plasmodium falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocyte stages of *P.falciparum* were maintained using a modified method of Trager, W. J. and Jensen, B. [1976].[3] Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler, M. T. *et al.* [1993].[4]

The compounds were prepared to a 2 mg/ml stock solution in 10% DMSO or 10% methanol and were sonicated to enhance solubility. Compounds were tested as a suspension if not completely dissolved. Stock solutions were stored at -20 ^{o}C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. A full dose-response was performed for all compounds to determine the concentration

inhibiting 50% of parasite growth (IC_{50} – value). Test compounds were tested at a starting concentration of 10 µg/ml (December 2007) and 100 µg/ml (May 2007), which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2 µg/ml. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/ml. Compound **RKG303A** was tested at a starting concentration of 1000 ng/ml. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via GraphPad Prism v.4.0 software.

In vitro testing against chloroquine-resistant W2 *P. falciparum*

The protocol to carrying out the *in vitro* testing was performed as described by Rosenthal, P. J. *et al.* [1996].[5] Synchronized W2 strain *P. falciparum* parasites are cultured with test compounds (added from 1000x stocks in DMSO) for 48 hours, beginning at the ring stage. After 24 hours, the medium is changed while maintaining appropriate inhibitor concentration. Giemsa-stained smears are made after 48 hours, when control cultures contained nearly all parasites in the ring-stage. The number of new ring forms per 500 erythrocytes are counted, and compared with those of controls cultured in 0.1% DMSO. IC_{50}'s for growth inhibition are determined with GraphPad Prism software from plots of percentages of the level of parasitemia of the control relative to inhibitor concentration.

6.3 *In Vitro* Testing against *Mycobacterium tuberculosis* Strains

In 2005, all sulfonyl ureas, imidazolinones, phthalimides, sulfonylcyanoguanidines, acylthioureas, intermediate compounds, and the commercially available herbicides were sent to the Tuberculosis Antimicrobial Acquisition & Coordianting Facility (TAACF, USA) for antimicrobial assessment against *Mycobacterium tuberculosis*. Compounds were further evaluated at the Department of Medical Biochemistry (UCT) in the *M. tuberculosis* Resazurin assay.

6.3.1 Primary Screening Results (TAACF)

Primary screening results are shown in Table 6.3.1.1. The primary screening is done at the test concentration of 6.25 µg/mL on the Alamar blue assay.[6,7] The Alamar assay makes use of the indicator dye resazurin to measure the metabolic capacity of cells. Viable cells retain the ability to reduce resazurin into the highly fluorescent resorufin. Non-viable cells lose their metabolic capacity to reduce the indicator dye, and thus do not generate a fluorescent signal. Only if compounds show greater than 90% inhibition of the *Mycobacterium tuberculosis* H_{37}Rv strain (hence, prevent the reduction of resazurin to resorufin), are they retested and the cytotoxicity evaluated. If the compound has a determined minimum inhibitory concentration (MIC) of > 6.25 µg/mL it is usually not evaluated further. Table 6.3.1.1 reveals that only **RKGC5Et** showed inhibition of greater than 90% in the initial primary screening. Compounds **RKG162A**, **RKG1541**, **RKGC11Tria**, **RKG1542, RKG1543, RKGC12Th** and **RKGAS4ELEU** showed lower to moderate inhibition ranging from 21 – 41 % at the concentration of 6.25 µg/mL. Compounds **RKGC3Ci**, **RKGAS4EVAL** and **RKGC3Ch** showed only traces of inhibition. Due to the good inhibitory activity of 96% of **RKGC5Et** against the *M. tuberculosis* H_{37}Rv strain at 6.25 µg/mL, cytotoxicity studies were conducted for this compound. Table 6.3.1.2 shows the cytotoxicity results obtained for

Table 6.3.1.1: *In vitro* activity against the *Mycobacterium tuberculosis* H_{37}Rv strain

Compound ID	Assay	MIC (µg/mL)	% Inhibition	Activity
RKGC5Et	Alamar	<6.25	96	+
RKG162A	Alamar	>6.25	41	-
RKG1541	Alamar	>6.25	40	-
RKGC11Tria	Alamar	>6.25	38	-
RKG1542	Alamar	>6.25	37	-
RKG1543	Alamar	>6.25	28	-
RKGC12Th	Alamar	>6.25	24	-
RKGAS4ELEU	Alamar	>6.25	21	-
RKGC4Ci	Alamar	>6.25	7	-
RKGAS4EVAL	Alamar	>6.25	3	-
RKGC3Ch	Alamar	>6.25	1	-
RKGAS4ELGY	Alamar	>6.25	0	-
RKG1492	Alamar	>6.25	0	-
RKG1491	Alamar	>6.25	0	-
RKG1533PC	Alamar	>6.25	0	-
RKGANT3	Alamar	>6.25	0	-
RKGAS4EPA	Alamar	>6.25	0	-
RKGC13Trib	Alamar	>6.25	0	-
RKGC14Trif	Alamar	>6.25	0	-
RKG150A	Alamar	>6.25	0	-
RKGC9Py	Alamar	>6.25	0	-
RKGC10Ri	Alamar	>6.25	0	-
RKG151A	Alamar	>6.25	0	-
RKGC2Be	Alamar	>6.25	0	-
RKG1531	Alamar	>6.25	0	-
RKG1532	Alamar	>6.25	0	-
RKGC1Im	Alamar	>6.25	0	-
RKG114A	Alamar	>6.25	0	-
RKG161A	Alamar	>6.25	0	-
RKG136A	Alamar	>6.25	0	-
RKG147A	Alamar	>6.25	0	-
RKG139F3	Alamar	>6.25	0	-
RKG125A	Alamar	>6.25	0	-
RKG123A	Alamar	>6.25	0	-
RKGC8Pro	Alamar	>6.25	0	-

RKGC5Et, and it was found to have a cytotoxicity IC_{50} of > 62.5 µg/mL, and further concurrent studies proved **RKGC5Et** to have a MIC of > 6.25 µg/mL. It was not evaluated further by TAACF and, therefore, the IC_{50} against *M. tuberculosis* was not determined.

Table 6.3.1.2: Mammalian Cell Cytotoxicity assay summary (TAACF)

Compound ID	% Inh	Assay	MIC (µg/mL)	IC_{50}
RKGC5Et	96	Alamar	>6.25	>62.5

6.3.2 Primary Screening Results (UCT)

The Resazurin assay is based on the ability of living cells to convert a redox dye (resazurin) into a fluorescent end product (resorufin). Resazurin has been identified as the main component of Alamar blue.[8] A color change from blue to pink indicates the growth of the bacteria. The MIC is defined as the lowest concentration of drug or test sample to prevent the color change. The Figures 6.3.2.1 – 6.3.2.4 represents the primary screening of compounds, and depicts the reduction of percentage of resazurin due to inhibition by the compound. Primary-line antituberculosis drugs rifampicin (RIF) and isoniazid (INH) are used as controls. The bar graph representations indicate the percentage of resazurin reduced over a period of 5 days.

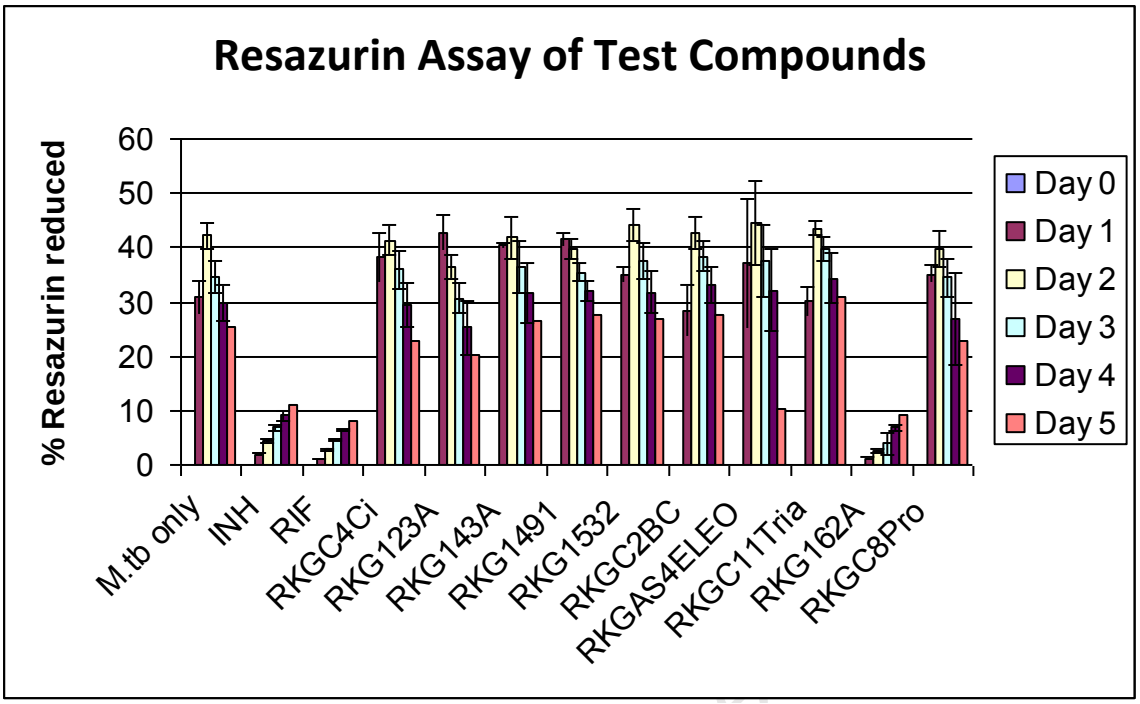


Figure 6.3.2.1: Resazurin assay of test compounds

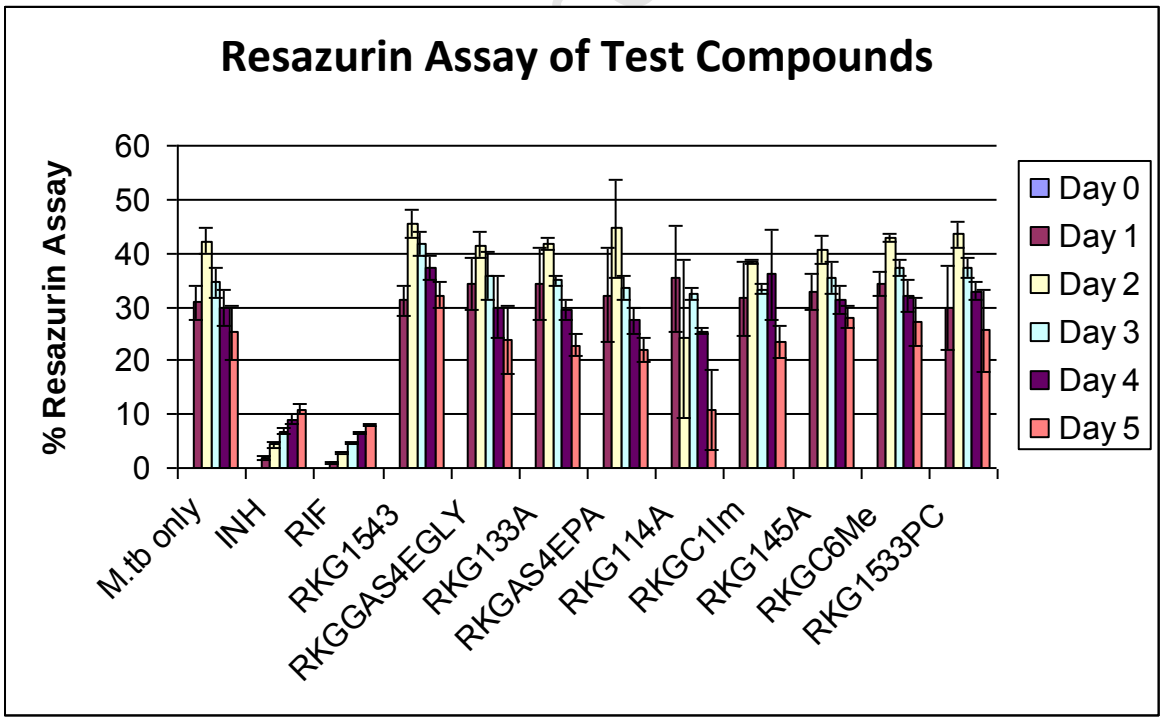


Figure 6.3.2.2: Resazurin assay of test compounds

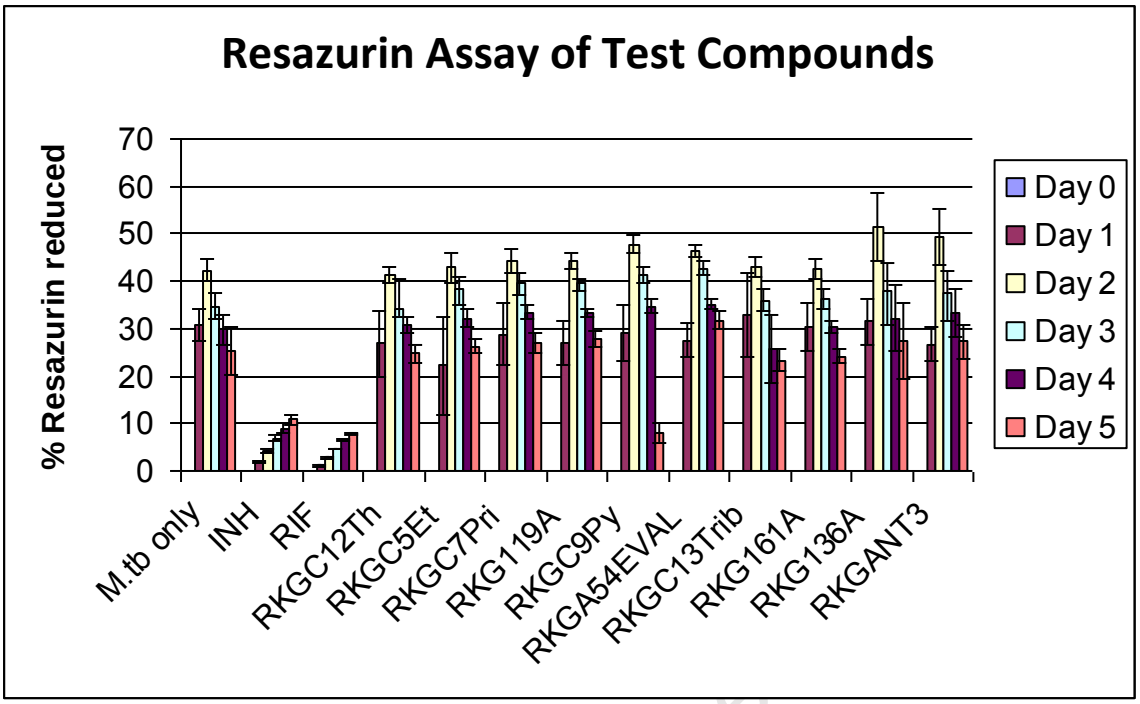


Figure 6.3.2.3: Resazurin assay of test compounds

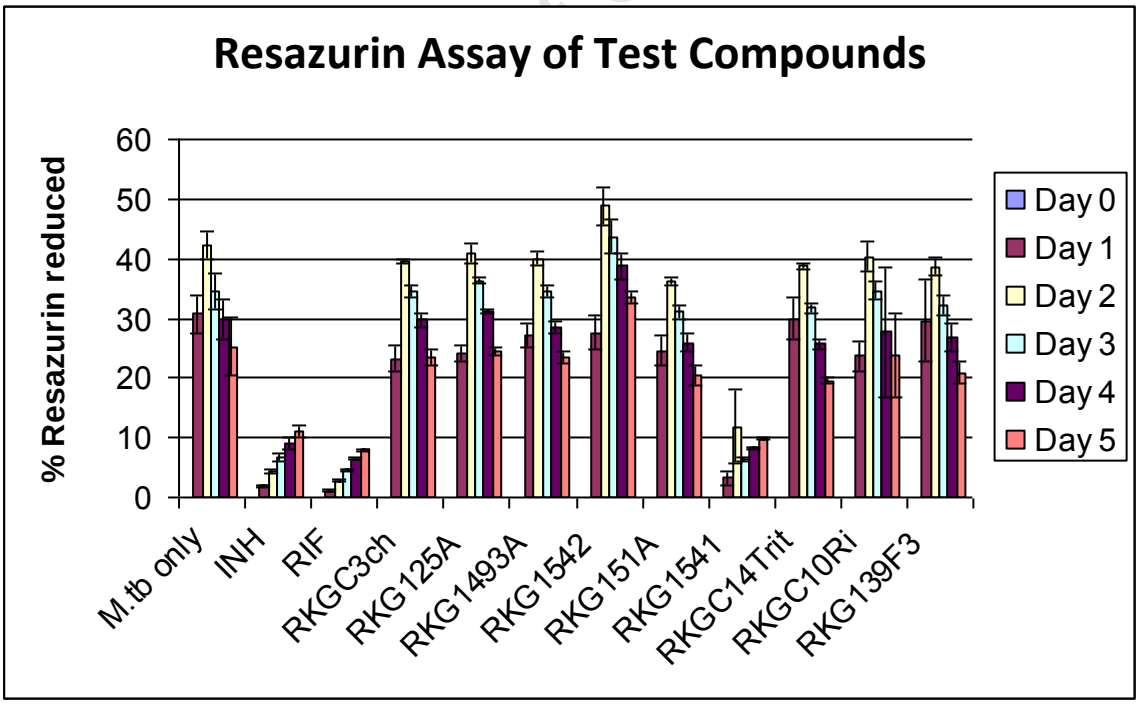


Figure 6.3.2.4: Resazurin assay of test compounds

Good activity is defined by a low percentage of resazurin reduced, and is observed in Figures 6.3.2.1 – 6.3.2.4 for the measurements taken for the controls RIF and INH. Of the compounds submitted for evaluation, only **RKG162A** (Figure 6.3.2.1) and **RKG1541** (Figure 6.3.2.4) were found to show good inhibition for the reduction of resazurin. The minimum inhibitory concentrations (MIC) were then evaluated for each of these two active compounds (Figure 6.3.2.5 and 6.3.2.6).

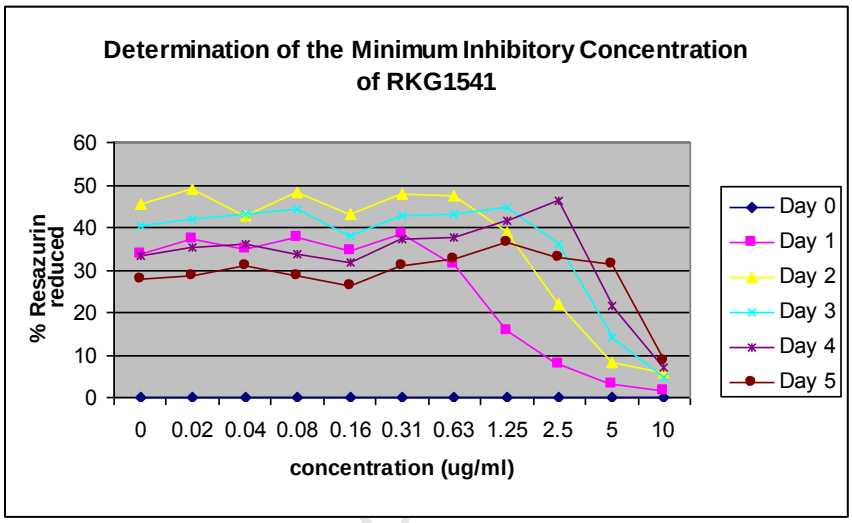


Figure 6.3.2.5: Studies to determine the MIC of **RKG1541**

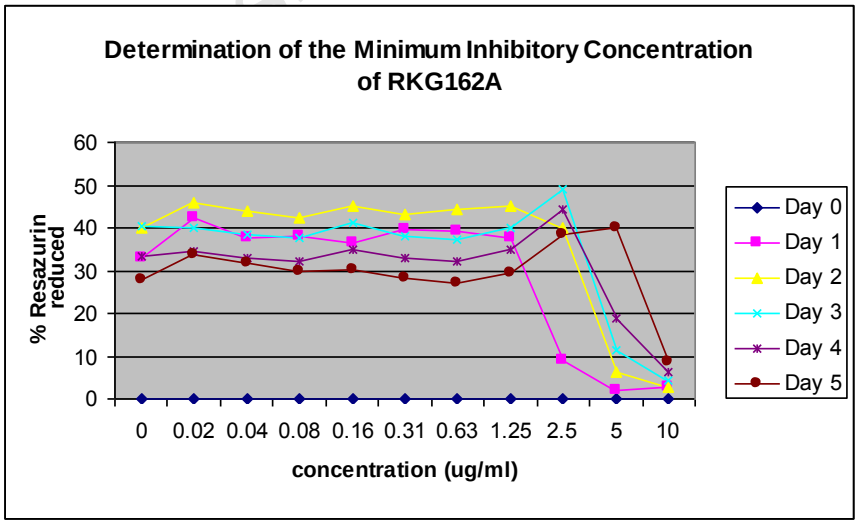


Figure 6.3.2.6: Studies to determine the MIC of **RKG162A**

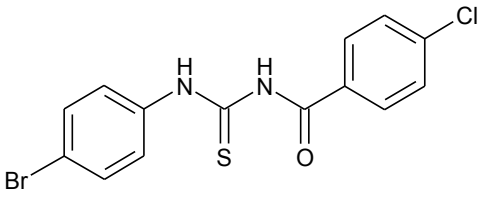
Based on the MIC studies, **RKG1541** was found to have an approximate IC_{50} of 10.7 µM, and RKG162A an approximate IC_{50} of 16.9 µM.

6.3.3 Conclusion

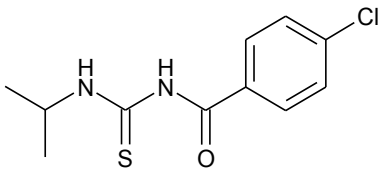
Based on the primary screening by TAACF, the only compound that showed good inhibition of the *Mycobacterium tuberculosis* H_{37}Rv strain was the commercially available sulfonyl urea, **RKGC5Et** (Ethoxysulfuron), and showed an inhibition of 96% in the initially primary screening. It was further evaluated for mammalian cell cytotoxicity studies and found to have an IC_{50} of > 62.5 µg/mL. In further evaluation it was found to have an MIC of > 6.25 µg/mL, and, hence, was not evaluated further. Compounds demonstrating inhibition at the concentration of 6.25 µg/mL include **RKG162A** (41%), **RKG1541** (40%), **RKGC11Tria** (38%), **RKG1542** (37%), **RKG1543** (28%), **RKGC12Th** (24%) and **RKGAS4ELEU** (21%). Compounds **RKGC3Ci**, **RKGAS4EVAL** and **RKGC3Ch** showed only traces of inhibition.

From the screening conducted at UCT, compounds **RKG1541** and **RKG162A** demonstrated moderate inhibition in the resazurin assay, with IC_{50} values of 10.7 µM and 16.9 µM respectively.It is important to note that both compounds are of the acylthiourea class, and were the only two compounds to show activity in both separate (TAACF and UCT) primary screenings. The two active compounds were compared structurally with the two acylthioureas, namely **RKG1542** and **RKG1543**, which only showed low inhibition in the TAACF assay. It is noteworthy that **RKG1542** and **RKG1543** are more hydrophilic compounds, bearing pyridinyl and dimethoxypyrimidinyl moieties respectively, as opposed to the more hydrophobic acylthioureas, **RKG1541** and **RKG162A**, which bear phenyl and alkyl (*iso*-propyl) groups. It may be suggested that the more hydrophobic substituent is important for the compounds activity. However, more informative future SAR should include the synthesis of compounds bearing an

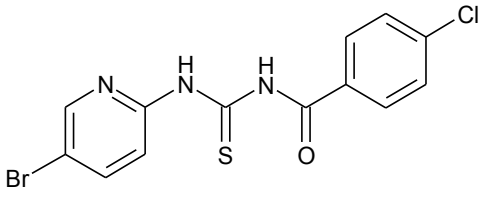
extensive variety of hydrophobic and hydrophilic groups in place of the 4-bromophenyl and *iso*-propyl moieties.



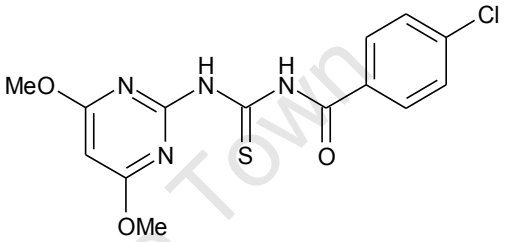
RKG1541



RKG162A



RKG1542



RKG1543

Unfortunately, all synthesized imidazolinones, phthalimides, sulfonylcyanoguanidines, sulfonyl ureas and commercial herbicides (including Imazapyr, **RKGC1Im**) showed little or no inhibitory activity at the concentration ranges used in both primary screenings.

6.3.4 Experimental Protocol

<u>*In vitro* Evaluation at Tuberculosis Antimicrobial Acquisition & Coordinating Facility (TAACF, USA)</u>

1. Primary screening is conducted at 6.25 µg/mL against *Mycobacterium tuberculosis* H_{37}Rv (ATCC 27294) in BACTEC 12B medium using a broth microdilution assay, the Microplate Alamar Blue Assay (MABA).[6] Compounds exhibiting fluorescence are tested in the BACTEC 460 radiometric system.[6] Compounds effecting <90% inhibition in the primary (i.e., MIC > 6.25 µg/mL) are not generally evaluated futher.

2. Compounds demonstrating at least 90% inhibition in the primary screen are retested at lower concentrations against *M. tuberculosis* $H_{37}Rv$ to determine the actual minimum inhibitory concentration (MIC) using MABA. The MIC is defined as the lowest concentration effecting a reduction in fluorescence of 90% relative to controls.

3. Concurrent with the determination of MICs, compounds are tested for cytotoxicity (IC_{50}) in VERO cells at concentrations ≤ 62.5 µg/mL or 10x the MIC for *M. tuberculosis* $H_{37}Rv$ (solubility in media permitting). After 72 hours exposure, viability is assessed on the basis of cellular conversion of MTT into a formazan product using the Promega CellTiter 96 Non-radioactive Cell Proliferation Assay.

In vitro Evaluation at Department of Medical Biochemistry (UCT)

The Resazurin Microtiter Assay plate was carried out as described by Martin, A. *et al.* [2003][9] and Palomino, J.-C. *et al.* [2002].[10]

100 µL of 7H9-S broth (Middlebrook 7H9 supplemented with 0.1% Casitone, 0.5% glycerol, and 10% OADC [oleic acid, albumin, dextrose, and catalase]; Becton-Dickinson) is dispensed in each well of a sterile flat-bottom 96-well. Serial twofold dilutions of each compound were prepared directly in the plate. Isolates were freshly subcultured on LJ medium. A further 100 µL of inoculums standard (prepared in 7H9-S broth, spectrophotometrically adjusted to a no. 1 McFarland tube and diluted to 1:10 in 7H9-S broth for test) is added to each well. Both growth and sterile controls are also included for each isolate. Isolates were freshly subcultured on LJ medium. Sterile water is added to all perimeter wells to prevent evaporation while incubation takes place. The plate is covered, and sealed in a plastic bag. The plate is incubated at 37 ^{o}C for 7 days under a normal atmosphere. 30 µL of resazurin solution (resazurin sodium salt powder is

prepared at 0.02% (wt/vol) in distilled water, sterilized by filtration, and stored at 4 ^{o}C for approximately a week) added, and the plate is reincubated overnight. The change in color from blue to pink indicates the growth of the bacteria. The MIC is defined as the lowest test sample concentration to prevent the color change.

6.4 References

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Chapter 7

Conclusion and Future Work

7.1 Conclusions

All successfully synthesized **H157** derivatives, including relevant intermediate compounds and **H157** respectively, and available potential acetolactate synthase inhibitors, and their respective intermediates, were tested in the sorbitol uptake inhibition (NPP) assay as well as for *in vitro* activity against both chloroquine sensitive and resistant strains of *Plasmodium falciparum*. Four compounds demonstrated significant inhibition of the sorbitol uptake, namely **RKG256** (synthesized **H157**), **RKG340** (an extended 'side-chain' derivative of **H157**), **RKG4042P** (hydroxamic bioisostere of **H157**) and **RKG359** (sulfonyl urea, and analogue of glibenclamide). Almost complete inhibition at the concentration of 9 µM was observed for both **RKG359** and **RKG340**. **RKG4042P** proved to be the most active with an IC_{50} value of 1.2 µM. Against the chloroquine-sensistive D10 and chloroquine-resistant W2 strains, the most active compound was found to be **RKG303A**, a 7-chloroquinoline bearing **H157** derivative [D10 (IC_{50} = 1.28 µM) and W2 (IC_{50} = 0.89 µM)]. From the four compounds that showed good new permeability pathways inhibition, only compounds **RKG256** (**H157**) [D10 (IC_{50} = 17.9 µM)] and **RKG4042P** [D10 (IC_{50} = 16.9 µM) and W2 (IC_{50} = 17.7 µM)] showed notable *in vitro* activity. This activity is not as potent as the chloroquine controls but presents the possibility of the activity being a result of the inhibition of the NPP.

All successfully synthesized sulfonyl ureas, imidazolinones, sulfonylcyanoguanidines, acylthioureas, phthalimides, intermediate compounds, and a collection of 13 commercially available herbicides were tested against the *Mycobacterium tuberculosis* H_{37}Rv strain using the

Alamar blue assay, as well as in the *Mycobacterium tuberculosis* Resazurin assay. Only commercially available ethoxysulfuron (**RKGC5Et**) showed inhibition of greater than 90% in the primary screening against the $H_{37}Rv$ strain at the concentration of 6.25 µg/ml. Other compounds that showed low to moderate inhibition (21-41%) at the concentration 6.25 µg/ml were acylthioureas: **RKG162A**, **RKG1541**, **RKG1542** and **RKG1543**; and commercially available sulfonyl ureas: triasulfuron (**RKGC11Tria**) and thifensulfuron-methyl (**RKGC12Th**). From the primary screening in the resazurin assay, only acylthioureas **RKG162A** and **RKG1541** showed good inhibition of the reduction of resazurin [approximate (IC_{50} = 16.9 µM) and (IC_{50} = 10.7 µM)].

The new synthesis of sulfonyl ureas envisaged using 1,2,4-dithiazolidine-3,5-dione as the starting scaffold, which was proposed to work via a 4-arenesulfonyl-1,2,4-dithiazolidine-3,5-dione intermediate, was unsuccessful. The coupling of 1,2,4-dithiazolidine-3,5-dione with a sulfonyl chloride was attempted under a variety of conditions, however, the 4-arenesulfonyl-1,2,4-dithiazolidine-3,5-dione intermediate could not be isolated or confirmed. Another new route to sulfonyl ureas was envisaged involving the reaction of *N*-alkyl-1,2,4-dithiazolidine-3,5-dione with sulfonamides in the presence of triphenylphosphine and a weak base. This alternate method was successful, and eleven sulfonyl ureas were synthesized to demonstrate the success of the method with yields ranging from 32 – 79%. Due to this new method being initially limited to *N*-alkyl-1,2,4-dithiazolidine-3,5-diones, broadening this new synthetic method to include *N*-aryl-1,2,4-dithiazolidine-3,5-diones was envisaged. Transition metal-catalyzed (Pd and Cu) amidation methodology was used in an attempt to couple 1,2,4-dithiazolidine-3,5-dione with arylbromides. Unfortunately, the methodology using both the Pd- and Cu-catalyzed systems was unsuccessful.

7.2 Future Work

The generation of more **H157** derivatives based on the initial in-depth SAR studies (Chaper 2.3) could be synthesized in order to investigate the activity of these piretanide-like molecules against the new permeability pathways. Due to the observed activity of **RKG4042P** in both the sorbitol uptake NPP assay and both *P. falciparum in vitro* assays, further hydroxamic acid bioisosteres as well as the tetrazole biosisostere of **H157** and other active piretanide-like molecules should be synthesized and biologically evaluated.

Based on the initial studies of the potential acetolactate synthase inhibitors, libraries of acylthioureas using **RKG162A** and **RKG1541** as starting precursors for SAR studies could be initiated. This could be done in order to potentially find a new acylthiourea anti-tuberculosis compound class, as well as determine whether the inhibition of acetolactate synthase is the target of this active class of compounds.

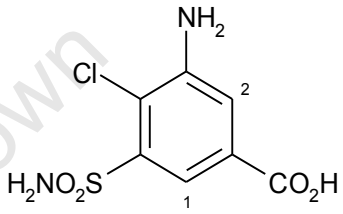
Like the commonly used protocols, the alternate new route to sulfonyl ureas too has its limitations. The inclusion of aryl halides to the starting material repertoire for the new alternate methodology would be most beneficial. Further investigations using alternate reactions conditions and catalyst ligands could be performed in order to potentially develop this method to include aryl halide precursors. X-ray crystallography investigation of the “unknown” products isolated from the Cu and Pd-catalyzed systems would be of interest, and could help in developing this protocol further.

Chapter 8

General Experimental

8.1 Experimental: Chapter 2

Synthesis of *3-amino-4-chloro-sulfamoylbenzoic acid* **(RKG211**)



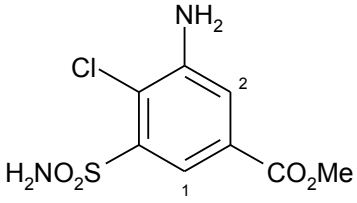
A solution of 4-chloro-3-nitro-5-sulfamoylbenzoic acid (2.82 g, 10.0 mmol) dissolved in EtOAc (120 ml) was treated with $SnCl_2.2H_2O$ (11.3 g, 50.0 mmol), and heated with stirring at 65 – 70 °C for 1 h. The reaction mixture was allowed to cool to room temperature, and diluted further with more EtOAc (100 ml). A 10% Na_2CO_3 aqueous solution (~150 ml) was added until no more precipitation occurred, and the reaction mixture was filtered twice through Celite to remove the gelatinous precipitate. (The Celite® cake was washed thoroughly with more EtOAc and aqueous 10% Na_2CO_3 solution). The aqueous layer of the filtrate was separated from the organic layer, and the organic layer was washed further with 10% Na_2CO_3 aqueous solution (30 ml). The aqueous layer and aqueous washing were combined and the pH was adjusted from basic to acid (pH ~ 1.5) with conc. HCl (10.18M). The acid aqueous phase was extracted with EtOAc (1 x 300 ml, then 1 x 200 ml). The combined organic extractions were washed with 1M HCl (50 ml), dried over anhydrous Na_2SO_4 and filtered. The solvent was then removed under reduced pressure, and the final residue was dried further *in vacuo*.

White powder (2.10 g, 84%); m.p. 244 - 247 °C; IR (NaCl) ν_{max} (cm^{-1}) 3380 (br s) (N-H), 2521 (br w) (O-H), 1687 (s) (C=O), 1632 (s) (N-H), 1530 (m) (C=C), 1325 (s) (S=O), 1270 (s) (C-O) and 1152 (s) (S=O); ^{1}H NMR (300MHz; d^6-DMSO) δ 7.70 (d, 1H, *J* 2.1, Ar-H^1), 7.58 (d, 1H, *J* 2.1, Ar-H^2), 7.48 (s, 2H, -SO_2NH_2) and 5.93 (s, 2H, Ar-NH_2); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 166.96, 147.43, 142.38, 129.95, 119.18, 117.63, and 116.78; LRMS (EI): m/z = 250.0 [M^+]; Anal. Calc. (Found C, 33.2; H, 2.75; N, 10.1; S, 12.1. $C_7H_7ClN_2O_4S$ requires C, 33.5; H, 2.81; N, 11.2; S, 12.8%);

General procedure for the synthesis of esters **(RKG262** and **RKG345)**

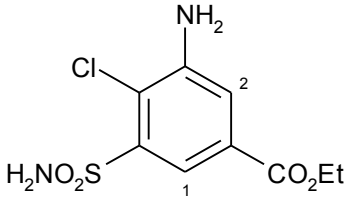
The 3-amino-4-chloro-sulfamoylbenzoic acid (2.00 mmol) was dissolved in a mixture of methanol (6.0 ml) (ethanol in the case of **RKG345**) and conc. H_2SO_4 (3.00 mmol). The mixture was stirred under reflux for 5 hrs, and then allowed to cool to room temperature. The solvent was removed under reduced pressure, and deionised H_2O (10 ml) was added to the residue. The aqueous phase was extracted with EtOAc (1 x 40ml, then 1 x 20 ml). The organic extracts were combined and washed with deionised H_2O (20 ml), saturated $NaHCO_3$ solution (20 ml), deionised H_2O (20 ml), brine (20 ml), deionised H_2O (20 ml), and finally dried over anhydrous Na_2SO_4. The organic phase was filtered, the solvent was removed under reduced pressure, and the residue was dried further *in vacuo*.

1. (*O-Methyl)-3-amino-4-chloro-sulfamoylbenzoate* **(RKG262)**



White solid (0.431g, 81%); m.p. 175 - 178 °C; IR (NaCl) ν_{max} (cm^{-1}) 3402 (br s) (N-H), 1719 (s) (C=O), 1626 (s) (N-H), 1537 (m) (C=C), 1323 (s) (S=O), 1260 (s) (C-O) and 1155 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 7.66 (d, 1H, *J* 2.0, Ar-H^1), 7.56 (d, 1H, *J* 2.1, Ar-H^2), 7.50 (s, 2H, -SO_2NH_2), 6.01 (s, 2H, Ar-NH_2) and 3.80 (s, 3H, -OCH_3); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 165.19, 146.83, 141.83, 127.98, 118.12, 117.53, 115.68 and 52.33; LRMS (EI): m/z = 264.0 [M^+]; Anal. Calc. (Found C, 36.3; H, 3.33; N, 9.70; S, 11.8. $C_8H_9ClN_2O_4S$ requires C, 36.3; H, 3.43; N, 10.6; S, 12.1%);

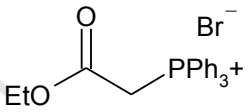
2. (*O-Ethyl)-3-amino-4-chloro-sulfamoylbenzoate* **(RKG345)**



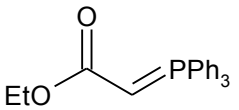
White, fine, needle-like crystals (3.11g, 93%) (12.0 mmol scale); m.p. 169 - 172 °C; ; IR (NaCl) ν_{max} (cm^{-1}) 3361 (s) (N-H), 3272 (N-H), 2985 (w) (C-H), 1705 (s) (C=O), 1593 (m) (C=C), 1565 (m) (C=C), 1531 (s) (C=C), 1481 (w) (C=C), 1327 (s) (S=O), 1304 (s) (C-N), 1264 (s) (C-O), 1146 (s) (S=O) and 769 (C-Cl); ^{1}H NMR (400 MHz; d^6-DMSO) δ 7.69 (d, 1H, *J* 1.9, Ar-H^1), 7.59 (d, 1H, *J* 1.9, Ar-H^2), 7.53 (s, 2H, -SO_2NH_2), 6.04 (s, 2H, Ar-NH_2), 4.30 (q,

2H, *J* 7.1, -OCH_2CH$_3$) and 1.29 (t, 3H, *J* 7.1, -CH$_2$CH_3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 165.47, 147.58, 142.51, 129.02, 118.85, 118.04, 116.43, 61.85 and 14.81; HRMS (EI): m/z = 278.01235 [M$^+$]; Anal. Calc. (Found C, 38.7; H, 3.81; N, 9.83; S, 11.6. $C_9H_{11}ClN_2O_4S$ requires C, 38.8; H, 3.98; N, 10.1; S, 11.5%);

Synthesis of phosphorane (**RKG218)** via the phosphonium salt (**RKG217**)



Ethyl bromoacetate (0.333 ml, 3.00 mmol) was added dropwise to a cooled solution of PPh_3 (944 mg, 3.60 mmol) dissolved in toluene (8.0 ml). The reaction mixtures temperature was allowed to warm up to room temperature (~19 °C), and the mixture was allowed to stir for 8 hrs under N_2. The reaction mixture was placed in the fridge over night, and the white precipitate was collected via filtration, washed with cold toluene, and dryed *in vacuo*.
White solid (1.30 g, quantitative);



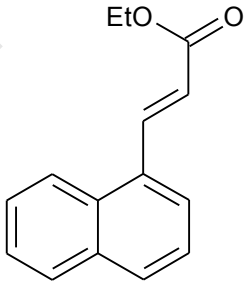
Method A: The phosphonium salt (750 mg, 1.75 mmol) was crushed into a fine powder and added to a solution of K_2CO_3 (2.42 g, 17.5 mmol) dissolved in deionised H_2O (15ml). The reaction was stirred overnight (20 hrs) at 18 °C (room temperature), and the white precipitate that formed was filtered off, washed with deionised H_2O, and dryed *in vacuo*.
White solid (566 mg, 93%);

Method B: The phosphonium salt (1.31 g, 3.00 mmol) was dissolved in deionised H_2O (5 ml), and was titrated with an aqueous NaOH solution (120 mg NaOH dissolved in 5 ml deionised H_2O). As soon as the mixture turned basic, it was placed in the fridge and left overnight. The white precipitate was filtered off, washed with deionised H_2O, and dryed *in vacuo*.
(NOTE: in both methods; if a sticky syrupy oil forms, the product can be extracted with chloroform).

White solid (961 mg, 92%); ^{1}H NMR (300MHz; d^{1}-$CDCl_3$) δ 7.69-7.42 (m, 15H, Ar-*H*), 3.96 (q, 2H, *J* 7.1, CH_2), 2.87 (s, 1H, P=C*H*) and 1.04 (m, 3H, CH_3); ^{13}C NMR (75.5MHz; d^{1}-$CDCl_3$) δ 133.06, 132.93, 131.86, 128.75, 128.58, 57.82 and 14.78;

Synthesis of *(2E)-3-(1-naphthalenyl)-2-propenoic acid ethyl ester* **(RKG327)**

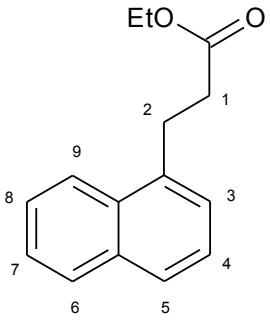
The phosphorane (**RKG218**) (2.79 g, 8.01 mmol) was dissolved in toluene (25 ml). 1-Naphthaldehyde (1.02 ml, 7.50 mmol) was added dropwise to the mixture, and the reaction mixture was refluxed at 120 °C for 20 hrs. The reaction was allowed to cool to room temperature, and the toluene was removed under reduced pressure. The resulting orange residue was flash column chromatographed (5:95, EtOAc:hexane) to isolate the alkene as a clear oil.



Clear, pale yellow oil (1.79 mg, quantitative); R_f = 0.18;

Synthesis of *3-(1-naphthalenyl)-2-propenoic acid ethyl ester* **(RKG329**)

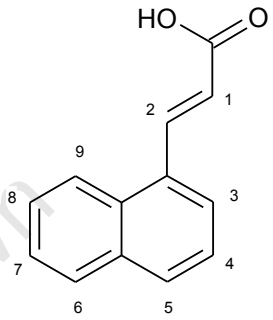
Ester (**RKG327**) (1.00 g, 4.42 mmol) was dissolved in a 1:1 mixture of EtOH and EtOAc (10 ml). A catalytic amount of 10% Pd/C (~20 mg) was added, and H_2 gas was bubbled through the solution for 20 min. The reaction was left to stir for a further 48 hrs under an atmosphere of H_2 gas. The reaction mixture was filtered through Celite®, and the Celite® cake was washed thoroughly with EtOAc (~200 ml). The filtrate solvent was removed under reduced pressure, and an oil was obtained.



Clear, pale orange oil (959 mg, 95%); ^{1}H NMR (400 MHz; d^{1}-$CDCl_3$) δ 8.05 (d, 1H, *J* 8.1, Ar-H^{9}), 7.86 (d, 1H, *J* 7.3, Ar-H^{6}), 7.74 (d, 1H, *J* 8.0, Ar-H^{5}), 7.53 (t, 1H, *J* = 8.1, Ar-H^{8}), 7.49 (t, 1H, *J* 8.1, Ar-H^{7}), 7.40 (t, 1H, *J* 7.1, Ar-H^{4}), 7.36 (d, 1H, *J* 7.0), Ar-H^{3}), 4.16 (quar, 2H, *J* 7.1, -OC$\underline{H}_2$CH$_3$), 3.43 (t, 2H, *J* 8.1, CH_2^{2}), 2.76 (t, 2H, *J* 8.1, CH_2^{1}) and 1.25 (t, 3H, *J* 7.1, -CH$_2$C$\underline{H}_3$); ^{13}C NMR (100.6 MHz; d^{1}-$CDCl_3$) δ 173.03, 136.60, 133.91, 131.66, 128.87, 127.11, 126.04, 125.93, 125.57, 125.55,

123.42, 60.49, 35.29, 28.15 and 14.22; LRMS (EI): m/z = 228.1 [M^+]; Anal. Calc. (Found C, 78.7; H, 7.01. $C_{15}H_{16}O_2$ requires C, 78.9; H, 7.06%);

Synthesis of *(2E)-3-(1-naphthalenyl)-2-propenoic acid* **(RKG259)**



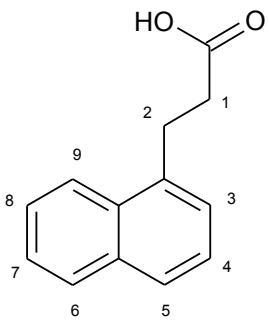
Sodium hydroxide (353 mg, 8.83 mmol) was dissolved in a mixture of EtOH (5.0 ml) and deionised H_2O (1.0 ml). The basic solution was added with stirring to a solution of ester (**RKG327**) (799 mg, 3.53 mmol) dissolved in EtOH (15 ml). The mixture was stirred under reflux (~85 °C) for 2 hrs [OBSERVATION: reaction mixture turned from yellow to dark orange in colour]. The reaction was allowed to cool to room temperature, and chloroform (20 ml) and deionised H_2O (20 ml) were added. The layers were separated, and the organic layer was washed with aqueous 10% Na_2CO_3 solution (20 ml). The aqueous layers were combined and washed with chloroform (20 ml). The aqueous layer pH was adjusted from basic to acidic (pH ~ 1.5) with 6M HCl (added dropwise). A pale orange precipitate formed, and was filtered off and washed with deionised H_2O.

Light pale orange solid (601 mg, 86%); m.p. 173 - 177 °C; IR (NaCl) ν_{max} (cm^{-1}) 3046 (m) (C-H), 2835 (br m) (O-H), 1682 (s) (C=O), 1617 (s) (C=C), 1296 (s) (C-O), 790 and 766 (s) (C-H); ^{1}H NMR (300MHz; d^6-DMSO) δ 8.37 (d, 1H, *J* 15.8, C=C*H*^{2}), 8.18 (d, 1H, *J* 8.1, Ar-*H*^{9}), 8.01-7.90 (m, 3H, Ar-*H*$^{6+7+8}$), 7.64-7.52 (m, 3H, Ar-*H*$^{3+4+5}$) and 6.57 (d, 1H, *J* 15.7, C=C*H*^{1}); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 167.23, 140.02, 133.18, 130.92, 130.64, 130.20, 128.58, 126.99, 126.14, 125.58, 125.09, 122.85 and 121.88; LRMS (EI): m/z = 198.1 [M^+]; Anal. Calc. (Found C, 78.4; H, 5.01. $C_{13}H_{10}O_2$ requires C, 78.8; H, 5.09%);

Synthesis of *3-(1-naphthalenyl)-2-propenoic acid* **(RKG332)**

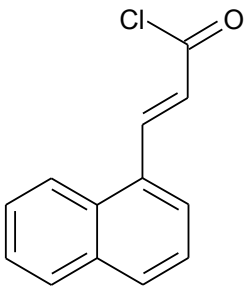
Sodium hydroxide (394 mg, 9.85 mmol) was dissolved in deionised H_2O (2.0 ml). The basic solution was added with stirring to a solution of ester (**RKG329**) (900 mg, 3.94 mmol) dissolved in EtOH (15 ml). The mixture was stirred under reflux (~85 °C) for 16 hrs. The reaction was

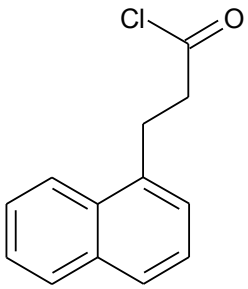
allowed to cool to room temperature, and chloroform (35 ml) and deionised H_2O (40 ml) were added. The layers were separated, and the organic layer was washed with aqueous 2M NaOH solution (20 ml). The basic aqueous layers were combined and washed with chloroform (20 ml). The basic aqueous layers pH was adjusted from basic to acidic (pH ~ 1.5) with 6M HCl (added dropwise), and a white precipitate formed. The precipitate was filtered off and washed with aqueous 1M HCl solution and deionised H_2O.



White powder (728 mg, 92%); m.p. 143 - 146 °C; IR (NaCl) ν_{max} (cm^{-1}) 3048 (m) (C-H), 2924 (m) (C-H), 2638 (m) (O-H), 1704 (s) (C=O), 1598 (m) (C=C), 1508 (m) (C=C), 1438 (s) (C=C) and 1225 (s) (C-O); ^{1}H NMR (d^1-$CDCl_3$) δ 7.83 (dd, 1H, *J* 8.2, 1.3, Ar-H^9), 7.63 (dd, 1H, *J* 7.9, 1.6, Ar-H^6), 7.50 (dd, 1H, *J* 7.4, 2.0, Ar-H^5), 7.30 (dt, 1H, *J* 6.8, 1.5, Ar-H^8), 7.25 (dt, 1H, *J* 6.8, 1.3, Ar-H^7), 7.16 (m, 2H, Ar-H^{3+4}), 3.19 (t, 2H, *J* 8.2, CH_2^2) and 2.50 (t, 2H, *J* 8.1, CH_2^1); HRMS (EI): m/z = 200.08318 [M^+]; Anal. Calc. (Found C, 77.1; H, 5.93. $C_{13}H_{12}O_2$ requires C, 78.0; H, 6.04%);

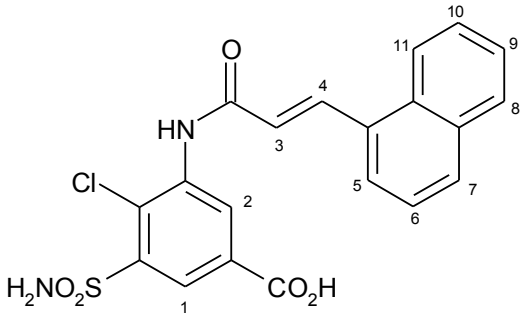
Synthesis of *(2E)-3-(1-naphthalenyl)-2-propenoyl chloride and 3-(1-naphthalenyl)-2-propenoyl chloride*





An excess of freshly distilled $SOCl_2$ was added in a dropwise manner to the carboxylic acid (**RKG259** or **RKG332**) (700 mg, 3.53 mmol) with stirring. The mixture was heated gently for 1 hr at 100 °C under N_2 (a further 1ml of $SOCl_2$ was added after the first 20 min to make a clear solution). The reaction was allowed to cool to room temperature (~20 ^{o}C), and then distilled at atmospheric pressure to remove the excess $SOCl_2$, affording a dark brown residue. The acid chloride was not isolated or purified.

Synthesis of *4-chloro-3-(3-naphthalen-1-yl-acryloylamino)-5-sulfamoyl-benzoic acid* **(RKG223)**



Acetone (1.5 ml) was added to the unpurified acid chloride, and the resulting solution was added dropwise to a solution of 3-amino-4-chloro-sulfomoylbenzoic acid (**RKG211)** (875 mg, 3.53 mmol) (assuming that the formation of the acid chloride was quantitative) dissolved in acetone (4.0 ml). The reaction mixture was stirred at room temperature (19 °C) for 6 hrs. The reaction vessel was cooled down on ice, and the cooled reaction mixture was added to 1M HCl (30 ml). A white precipitate formed, and was filtered off. The precipitate was recrystallized from MeOH to give a pure white product.

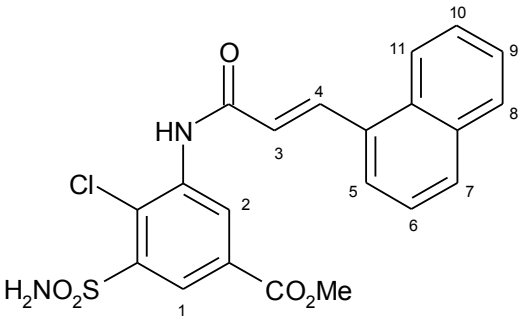
White solid (1.21 g, 80% over two steps); m.p. 298 - 302 °C; IR (NaCl) ν_{max} (cm^{-1}) 3359 (s) (N-H), 3112 (m) (C-H), 2866 (br m) (O-H), 1696 (s) (C=O), 1677 (s) (C=O), 1619 (s) (N-H), 1529 (s) (C=C), 1347 (s) (S=O), 1251 (s) (C-O) and 1160 (s) (S=O); ^{1}H NMR (300MHz; d^6-DMSO) δ 10.09 (s, 1H, CON*H*), 8.74 (d, 1H, *J* 2.0, Ar-H^1), 8.45 (d, 1H, *J* 15.6, C=CH^4), 8.36 (d, 1H, *J* 2.1, Ar-H^2), 8.28 (d, 1H, *J* 8.4, Ar-H^{11}), 8.02 (m, 2H, 2 x Ar-H^{7+8}), 7.92 (d, 1H, *J* 7.2, Ar-H^5), 7.80 (s, 2H, -SO_2NH_2), 7.72-7.58 (m, 3H, 3 x Ar-H^{6+9+10}) and 7.23 (d, 1H, *J* 15.5, C=CH^3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 165.49, 164.23, 142.19, 138.03, 137.22, 133.34, 131.44, 130.79, 130.18, 129.33, 128.68, 128.57, 127.34, 127.06, 126.28, 125.69, 125.01, 124.98, 124.04 and 123.09; LRMS (EI): m/z = 430.2 [M^+]; Anal. Calc. (Found C, 55.1; H, 3.51; N, 6.11; S, 6.88. $C_{20}H_{15}ClN_2O_5S$ requires C, 55.8; H, 3.51; N, 6.50; S, 7.44%);

Synthesis of *4-chloro-3-(3-naphthalen-1-yl-acryloylamino)-5-sulfamoyl-benzoic acid methyl ester* **(RKG260)**

(NOTE: the acid chloride was synthesized as discussed above, except on a 3.00 mmol scale)

Acetone (2.0 ml) was added to the unpurified acid chloride, and the resulting solution was added dropwise to a solution of (*O*-methyl)-3-amino-4-chloro-sulfomoylbenzoate (**RKG262)** (730 mg, 2.75 mmol) (assuming that the formation of the acid chloride was quantitative)

dissolved in acetone (3.0 ml). The reaction mixture was stirred at room temperature (19 °C) for 6 hrs (more acetone was added periodically to aid stirring due to the formation of a precipitate). The reaction mixture was added to 1M HCl (30 ml). A white precipitate formed, and was filtered off and washed with 1M HCl and *cold* acetone, to afford a white product.

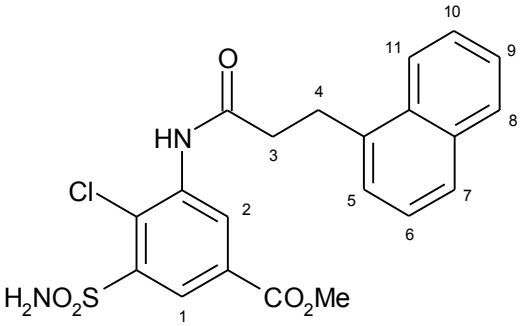


White solid (869 mg, 71% over two steps); m.p. 271 - 275 °C; IR (NaCl) ν_{max} (cm^{-1}) 3267 (s) (N-H), 3015 (m) (C-H), 1715 (s) (C=O), 1656 (s) (C=O), 1628 (s) (N-H), 1524 (s) (C=C), 1343 (s) (S=O), 1253 (s) (C-O) and 1160 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 10.12 (s, 1H, CON*H*), 8.78 (d, 1H, *J* 2 .1, Ar-H^1), 8.45 (d, 1H, *J* 15.6, C=CH^4), 8.35 (d, 1H, *J* 2.1, Ar-H^2), 8.26 (d, 1H, *J* 8.4, Ar-H^{11}), 8.01 (m, 2H, 2 x Ar-H^{7+8}), 7.91 (d, 1H, *J* 7.1, Ar-H^5), 7.84 (s, 2H, -SO_2NH_2), 7.67-7.52 (m, 3H, 3 x Ar-H^{6+9+10}), 7.23 (d, 1H, *J* 15.5, C=CH^3) and 3.92 (s, 3H, -CO_2CH_3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 164.52, 164.27, 142.37, 138.14, 137.40, 133.34, 131.43, 130.79, 130.20, 128.68, 128.19, 128.04, 127.60, 127.06, 126.27, 125.68, 124.98, 124.69, 123.99, 123.10 and 52.76; LRMS (EI): m/z = 444.1 [M^+]; Anal. Calc. (Found C, 56.5; H, 3.77; N, 5.51; S, 6.66. $C_{21}H_{17}ClN_2O_5S$ requires C, 56.7; H, 3.85; N, 6.30; S, 7.21%);

Synthesis of *4-chloro-3-(3-naphthalen-1-yl-propionylamino)-5-sulfamoyl-benzoic acid methyl ester* **(RKG333)**

(NOTE: the acid chloride was synthesized as discussed above, except on a 1.50 mmol scale)

Acetone (2.0 ml) was added to the unpurified acid chloride, and the resulting solution was added dropwise to a solution of (*O*-methyl)-3-amino-4-chloro-sulfomoylbenzoate (**RKG262)** (384 mg, 1.45 mmol) (assuming that the formation of the acid chloride was quantitative) dissolved in acetone (2.0 ml). The reaction mixture was stirred at room temperature (21 °C) for 6 hrs (more acetone was

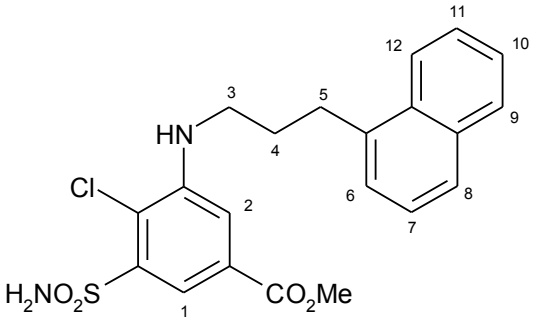


added periodically to aid stirring due to the formation of a precipitate). The reaction mixture was added to 1M HCl (20 ml). A white precipitate formed, and was filtered off and washed with 1M HCl and acetone, to afford a white product.

White solid (378 mg, 58% over two steps); m.p. 237 - 239 °C; IR (NaCl) ν_{max} (cm^{-1}) 3399 (s) (N-H), 3274 (s) (CON-H), 3006 (w) (C-H), 1733 (s) (C=O), 1669 (s) (C=O), 1597 (m) (C=C), 1567 (m) (C=C), 1521 (s) (C=C), 1335 (s) (S=O), 1241 (s) (C-O), 1164 (s) (S=O) and 769 (s) (C-Cl); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 9.87 (s, 1H, CON*H*), 8.46 (d, 1H, *J* 2.0, Ar-H^{1}), 8.31 (d, 1H, *J* 2.0, Ar-H^{2}), 8.14 (d, 1H, *J* 8.3, Ar-H^{11}), 7.92 (d, 1H, *J* 7.9, Ar-H^{8}), 7.78 (m, 3H, Ar-H^{7} and $-SO_2NH_2$), 7.57 (t, 1H, *J* 8.1, Ar-H^{10}), 7.52 (t, 1H, *J* 7.9, Ar-H^{9}), 7.43 (m, 2H, 2 x Ar-H^{5+6}), 3.90 (s, 3H, $-CO_2CH_3$), 3.42 (t, 2H, *J* 8.0, CH_2^{4}) and 2.89 (t, 2H, CH_2^{3}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 172.10, 165.22, 143.06, 138.11, 137.62, 134.14, 132.01, 129.74, 129.31, 129.15, 128.65, 127.41, 126.79, 126.51, 126.31, 126.28, 125.60, 124.29, 53.47, 37.34 and 28.44; HRMS (EI): m/z = 446.06974 [M^{+}]; Anal. Calc. (Found C, 55.9; H, 4.15; N, 5.77; S, 7.16. $C_{21}H_{19}ClN_2O_5S$ requires C, 56.4; H, 4.29; N, 6.27; S, 7.17%);

Synthesis of *4-chloro-3-(3-naphthalen-1-yl-propylamino)-5-sulfamoyl-benzoic acid methyl ester* (**RKG335F2)**

RKG333 (250 mg, 0.56 mmol) was added to dried (anhydrous) THF (2.5 ml). The resulting suspension was cooled to 0 °C, and the borane-THF complex (1.0M in THF) (1.2 ml, 1.19 mmol) was added dropwise and was stirred for 15 minutes. The mixture was heated and refluxed for 21 hrs under an atmosphere of N_2. After cooling to room temperature, the reaction mixture was quenched with glacial acetic acid (~10 ml). The apparatus was rearranged for distillation, and the THF was distilled off (distillation was aided by the application of a slight vacuum). The resulting acidic solution was cooled to 0 °C and aqueous 10% Na_2CO_3 solution was added until a pH of 6-7 was obtained. A brown sticky residue formed, and the aqueous solution was extracted with EtOAc

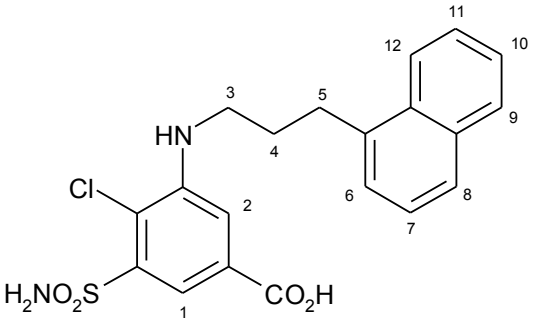


(2 x 20 ml). The organic layer was washed with deionized H_2O (20 ml), and dryed over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The brown residue was dryed further *in vacuo* and column chromatographed (2.5:97.5, MeOH:DCM), yielding **RKG355F2**.

Clear, pale yellow oil (51.3 mg, 21%); R_f = 0.56; ^{1}H NMR (400 MHz; d^4-MeOD) δ 8.01 (m, 2H, 2 x Ar-H^{1+12}), 7.86 (d, 1H, *J* 8.3, Ar-H^9), 7.74 (d, 1H, *J* 8.1, Ar-H^8), 7.49 (m, 2H, 2 x Ar-H^{10+11}), 7.47 (d, 1H, *J* 1.8, Ar-H^2), 7.41 (t, 1H, *J* 8.1, Ar-H^7), 7.34 (d, 1H, *J* 7.0, Ar-H^6), 3.91 (s, 3H, -CO_2CH_3), 3.36 (t, 2H, *J* 7.0, N-CH_2^3), 3.24 (t, 2H, *J* 7.4, Ar-CH_2^5) and 2.18 (quin, 2H, *J* 7.0, CH_2^4); ^{13}C NMR (100.6 MHz; d^4-MeOD) δ 165.68, 145.40, 140.05, 136.94, 134.03, 131.70, 129.56, 128.95, 127.10, 126.11, 126.03, 125.56, 123.76, 123.42, 117.54, 115.30, 52.59, 43.58, 30.27 and 29.77; LRMS (EI): m/z = 432.0 [M^+]; Anal. Calc. (Found C, 58.1; H, 4.65; N, 6.17; S, 7.67. $C_{21}H_{21}ClN_2O_4S$ requires C, 58.3; H, 4.89; N, 6.47; S, 7.41%);

Synthesis of *4-chloro-3-(3-naphthalen-1-yl-propylamino)-5-sulfamoyl-benzoic acid* **(RKG340)**

Sodium hydroxide (18.9 mg, 0.47 mmol) was dissolved in a mixture of MeOH (1.1 ml) and deionized H_2O (0.2 ml), and added to a stirring solution of **RKG335F2** (51.3 mg, 0.12 mmol) dissolved in MeOH (1.0 ml). The reaction mixture was stirred under reflux (~80 °C) for 2 hrs. The reaction was allowed to cool to room temperature, and chloroform (10 ml) and deionized H_2O (5.0 ml) were added, followed by 10% Na_2CO_3 aqueous solution (5.0 ml). The layers were separated, and the organic phase was washed with 10% Na_2CO_3 aqueous solution (10 ml). The aqueous layers were combined and washed with chloroform (10 ml). The aqueous layer pH was adjusted from basic to neutral (6/7). A white precipitate formed and was filtered off, washed with deionized H_2O, and finally dried *in vacuo*.



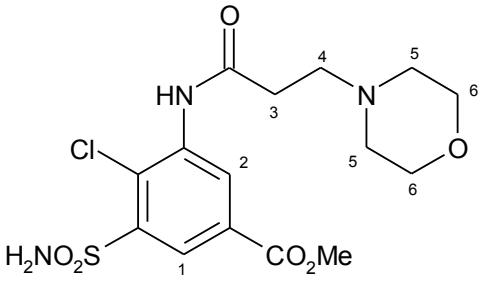
White powder (28.6 mg, 58%); m.p. 248 - 250 °C; IR (NaCl) ν_{max} (cm^{-1}) 3379 (m) (N-H), 2891 (w) (C-H), 1697 (s) (C=O), 1590 (s) (C=C), 1568 (m) (C=C), 1479 (m) (C=C), 1446 (s) (C=C), 1343 (s)

(S=O), 1246 (m) (C-O), 1154 (s) (S=O) and 795 (s) (C-Cl); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 8.04 (m, 1H, Ar-H^{12}), 7.89 (m, 1H, Ar-H^{9}), 7.76 (d, 1H, J = 1.9, Ar-H^{1}), 7.74 (m, 1H, Ar-H^{8}), 7.54 (s, 2H, -SO_2NH_2), 7.48 (m, 2H, 2 x Ar-H^{10+11}), 7.40 (m, 2H, 2 x Ar-H^{6+7}), 7.38 (d, 1H, J 2.0, Ar-H^{2}), 6.05 (t, 1H, J 5.6, NH), 3.35 (m, 2H, N-CH_2^{3}), 3.14 (t, 2H, J 7.6, Ar-CH_2^{5}) and 1.99 (quin, 2H, J 7.4, CH_2^{4}); ^{13}C NMR (75.5 MHz; d^{6}-DMSO) δ 166.33, 145.55, 141.71, 137.67, 133.40, 131.23, 129.84, 128.49, 126.36, 125.79, 125.74, 125.49, 125.43, 123.50, 118.14, 115.65, 113.59, 42.68, 29.50 and 29.16; HRMS (EI): m/z = 418.07491 [M^{+}]; Anal. Calc. (Found C, 57.0; H, 4.44; N, 6.22; S, 6.96. $C_{20}H_{19}ClN_2O_4S$ requires C, 57.3; H, 4.57; N, 6.69; S, 7.65%);

General procedure for the synthesis of amides **(RSON104**, **RSON105**, **RSON106**, **RSON112** and **RKG303A)**

A mixture (suspension) of amide (**RKG251**) (500 mg, 1.57 mmol) in methanol (20 ml) was placed in a reaction tube. The amine (3.5 equivalents, 5.50 mmol) was added dropwise with stirring (if the amine is a liquid), and the reaction was left to stir for 20 hrs at room temperature (~19 °C) [OBSERVATION: the suspension of amide disappeared over time, later followed by the formation of another precipitate]. The precipitate was filtered off, washed with cold methanol and dried *in vacuo*.

1. *4-Chloro-3-(3-morpholin-4-yl-propionylamino)-5-sulfamoyl-benzoic acid methyl ester* **RSON104**

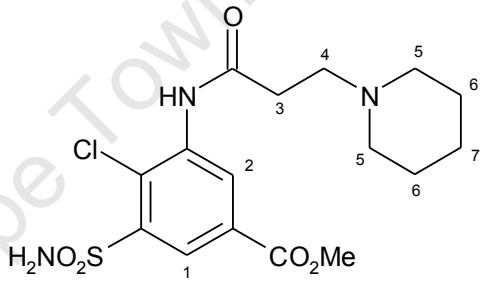


White solid (394 mg, 62%) (0.31 mmol scale: 78%); m.p. 195 - 199 °C; IR (NaCl) ν_{max} (cm^{-1}) 3341 (m) (N-H), 2811 (m) (C-H), 1722 (s) (C=O), 1678 (s) (C=O), 1574 (s) (N-H), 1531 (s) (C=C), 1348 (s) (S=O), 1229 (s) (C-O) and 1161 (s) (S=O); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 10.48 (s, 1H, CONH), 8.77 (d, 1H, J 2.2, Ar-H^{1}), 8.27 (d, 1H, J 2.0, Ar-H^{2}), 7.77 (s, 2H, -SO_2NH_2), 3.89 (s, 3H, -CO_2CH_3), 3.63 (t, 4H, J 4.7, 2 x CH_2^{6}-O), 2.63 (m, 4H, 2 x CH_2^{3+4}) and 2.48 (m, 4H, 2 x CH_2^{5}); ^{13}C NMR (75.5 MHz; d^{6}-DMSO) δ 171.10,

164.50, 142.11, 137.51, 128.02, 126.87, 126.09, 123.91, 65.92 (2C), 53.73, 52.76 (2C), 52.66 and 32.67; LRMS (EI): m/z = 405.1 [M^+]; Anal. Calc. (Found C, 44.0; H, 4.79; N, 10.2; S, 7.24. $C_{15}H_{20}ClN_3O_6S$ requires C, 44.4; H, 4.97; N, 10.4; S, 7.90%);

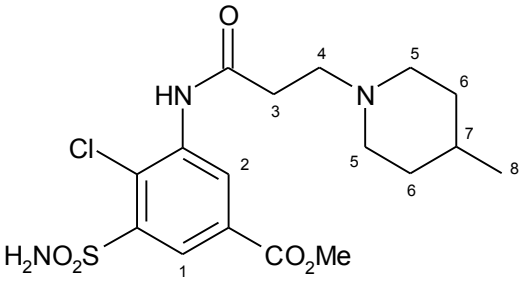
2. *4-Chloro-3-(3-piperidin-1-yl-propionylamino)-5-sulfamoyl-benzoic acid methyl ester* **(RSON106)**

White solid (503 mg, 79%); m.p. 200 - 202 °C; IR (NaCl) ν_{max} (cm^{-1}) 3357 (m) (N-H), 2783 (m) (C-H), 1728 (s) (C=O), 1662 (s) (C=O), 1574 (s) (N-H), 1533 (s) (C=C), 1351 (s) (S=O), 1229 (s) (C-O) and 1159 (s) (S=O); ^{1}H NMR (300 MHz; d^6-DMSO) δ 10.85 (s, 1H, CON*H*), 8.85 (d, 1H, *J* 2.1, Ar-H^1), 8.25 (d, 1H, *J* 2.1, Ar-H^2), 7.77 (s, 2H, -SO_2NH_2), 3.89 (s, 3H, -CO_2CH_3), 2.60 (m, 4H, 2 x CH_2^{3+4}), 2.45 (t, 4H, *J* 5.0, 2 x CH_2^5-N), 1.56 (quin, 4H, *J* 5.1, 2 x CH_2^6) and 1.42 (quin, 2H, CH_2^7); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 171.32, 164.53, 142.06, 137.63, 128.03, 126.40, 125.52, 123.66, 53.88, 53.39 (2C), 52.64, 32.81, 25.16 (2C) and 23.79; LRMS (EI): m/z = 403.1 [M^+]; Anal. Calc. (Found C, 47.1; H, 4.95; N, 10.4; S, 8.25. $C_{16}H_{22}ClN_3O_5S$ requires C, 47.6; H, 5.49; N, 10.4; S, 7.94%);



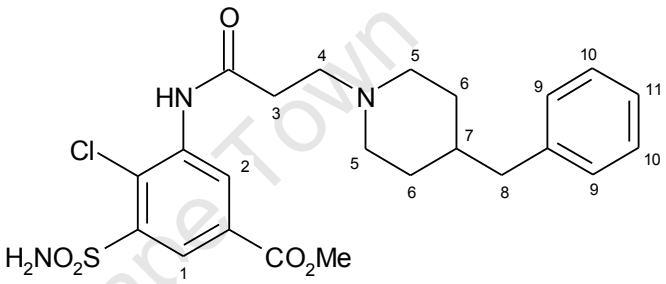
3. *4-Chloro-3-[3-(4-methyl-piperidin-1-yl)-propionylamino]-5-sulfamoyl-benzoic acid methyl ester* **(RSON105)**

White solid (527 mg, 80%); m.p. 207 – 209 °C; IR (NaCl) ν_{max} (cm^{-1}) 3357 (m) (N-H), 2835 (m) (C-H), 1720 (s) (C=O), 1671 (s) (C=O), 1575 (s) (N-H), 1539 (s) (C=C), 1348 (s) (S=O), 1233 (s) (C-O) and 1160 (s) (S=O); ^{1}H NMR (300 MHz; d^6-DMSO) δ 10.83 (s, 1H, CON*H*), 8.85 (d, 1H, *J* 2.0, Ar-H^1), 8.25 (d, 1H, *J* = 2.0, Ar-H^2), 7.79 (s, 2H, -SO_2NH_2), 3.89 (s, 3H, -CO_2CH_3), 2.99 (d, 2H, *J* 11.2, 2 x CH^5H-N), 2.61 (m, 4H, 2 x CH_2^{3+4}), 1.94 (t, 2H, *J* 11.5, 2 x CHH^5-N), 1.62



(d, 2H, *J* 12.3, 2 x CH*H*^{6}), 1.37 (m, 1H, C*H*^{7}), 1.19 (quar, 2H, *J* 12.1, C*H*^{6}H) and 0.90 (d, 3H, C*H*$_3$^{8}); ^{13}C NMR (100.6 MHz; *d*^{6}-DMSO) *δ* 171.39, 164.59, 142.10, 137.70, 128.07, 126.44, 123.71 (2C), 53.56, 52.84 (2C), 52.71, 33.58 (2C), 32.96, 30.12 and 21.69; LRMS (EI): m/z = 417.1 [M$^+$]; Anal. Calc. (Found C, 48.5; H, 5.62; N, 9.84; S, 6.88. $C_{17}H_{24}ClN_3O_5S$ requires C, 48.9; H, 5.79; N, 10.1; S, 7.67%);

4. *3-[3-(4-Benzyl-piperidin-1-yl)-propionylamino]-4-chloro-5-sulfamoyl-benzoic acid methyl ester* **(RSON112)**

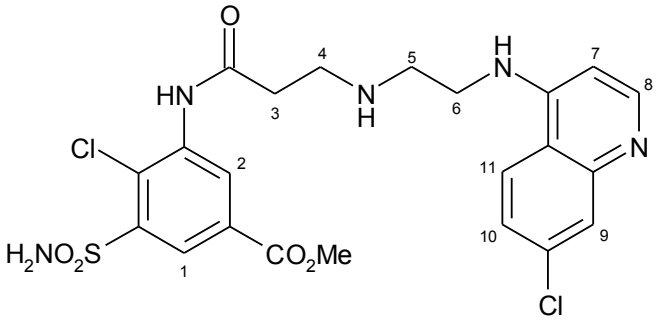


White solid (633 mg, 82%); m.p. 127 - 131 °C; IR (NaCl) ν_{max} (cm^{-1}) 3316 (m) (N-H), 2832 (m) (C-H), 1722 (s) (C=O), 1678 (s) (C=O), 1575 (s) (N-H), 1533 (s) (C=C), 1347 (s) (S=O), 1240 (s) (C-O) and 1181 (s) (S=O); ^{1}H NMR (300 MHz; *d*^{6}-DMSO) *δ* 10.78 (s, 1H, CON*H*), 8.84 (d, 1H, *J* 2.1, Ar-*H*^{1}), 8.26 (d, 1H, *J* 2.1, Ar-*H*^{2}), 7.79 (s, 2H, -SO_2NH_2), 7.29-7.14 (m, 5H, 5 x Ar-*H*), 3.89 (s, 3H, -CO_2CH_3), 2.97 (d, 2H, *J* 11.5, 2 x CH*H*^{5}-N), 2.60 (m, 4H, 2 x C*H*$_2$$^{3+4}$), 2.51 (d, 2H, *J* 6.4, C*H*$_2$^{8}), 1.90 (t, 2H, *J* 11.7, 2 x C*H*^{5}H-N), 1.57 (m, 3H, C*H*^{7} and 2 x CH*H*^{6}) and 1.25 (quar, 2H, 2 x C*H*^{6}H); ^{13}C NMR (100.6 MHz; *d*^{6}-DMSO) *δ* 171.36, 164.56, 142.08, 140.23, 137.66, 128.82 (2C), 128.06 (2C), 126.48, 125.67, 125.57, 123.70, 53.53, 52.74 (2C), 52.69, 42.27, 37.24, 33.00 and 31.46 (2C); LRMS (EI): m/z = 493.1 [M$^+$]; Anal. Calc. (Found C, 55.8; H, 5.70; N, 8.21; S, 5.93. $C_{23}H_{28}ClN_3O_5S$ requires C, 55.9; H, 5.71; N, 8.51; S, 6.49%);

5. *4-Chloro-3-{3-[2-(7-chloro-quinolin-4-ylamino)-ethylamino]-propionylamino}-5-sulfamoyl-benzoic acid methyl ester* **(RKG303A)**

White powder (246 mg, 72%) (0.63 mmol scale); m.p. 127 - 131 °C; IR (NaCl) ν_{max} (cm^{-1}) 3394 (m) (N-H), 2837 (m) (C-H), 1717 (s) (C=O), 1671 (s) (C=O), 1575 (s) (C=C), 1534 (s) (C=C), 1334 (s) (S=O), 1220 (s) (C-O), 1160 (s) (S=O) and 764 (s) (C-Cl); ^{1}H NMR (300 MHz; *d*^{6}-DMSO) *δ* 8.80 (d, 1H, *J* 2.1, Ar-*H*^{1}), 8.41 (d, 1H, *J* 5.4, Ar-*H*^{8}), 8.29 (d, 1H, *J* 2.1, Ar-*H*^{2}),

8.24 (d, 1H, *J* 9.1, Ar-H^{11}), 7.78 (d, 1H, *J* 2.2, Ar-H^{9}), 7.42 (dd, 1H, *J* 9.0, 2.3, Ar-H^{10}), 7.23 (t, 1H, *J* 4.8, N*H*), 6.54 (d, 1H, *J* 5.5, Ar-H^{7}), 3.92 (s, 3H, -CO_2CH_3), 3.45 (quar, 2H, *J* 6.0, CH_2^{6}-N), 2.96 (m, 4H, 2 x CH_2^{4+5}) and 2.60 (t, 2H, *J* 6.0, CH_2^{3}); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 171.68, 164.58, 151.85, 150.10, 148.95, 142.13, 137.72, 133.33, 128.00, 127.37, 126.58, 126.04, 123.95 (2C), 123.78, 117.39, 98.66, 52.69, 46.72, 44.45, 42.19 and 35.93; LRMS (EI): m/z = 539.03 [M^+]; Anal. Calc. (Found C, 48.1; H, 4.27; N, 12.8; S, 5.63. $C_{22}H_{23}Cl_2N_5O_5S$ requires C, 48.9; H, 4.29; N, 13.0; S, 5.93%);



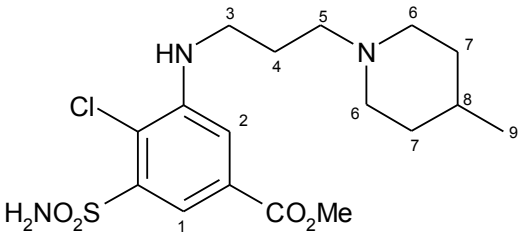
General procedure for the synthesis of amines **(RSON108** and **RSON109)**

The amide (400 mg) is added to anhydrous THF (2.5 ml). The suspension was cooled to 0 °C and the borane-THF complex (3.0 equiv) was added dropwise under N_2. The reaction mixture was stirred for a further 15 min at 0 °C, and then heated and stirred under reflux for 20 hrs under N_2. After cooling to room temperature, the reaction mixture was quenched with dried glacial acetic acid (~10 ml). The apparatus was rearranged for distillation, and the THF was distilled off. The pH of the reaction mixture was adjusted to 6-7 using a 10% Na_2CO_3 aqueous solution. A brown residue formed, and was extracted from the aqueous solution with EtOAc. The organic extracts were combined and dried over anhydrous Na_2SO_4, filtered and dried under reduced pressure. The brown residue was dried further *in vacuo* and column chromatographed (1:9, MeOH:EtOAc).

1. *4-Chloro-3-[3-(4-methyl-piperidin-1-yl)-propylamino]-5-sulfamoyl-benzoic acid methyl ester* **(RSON108)**

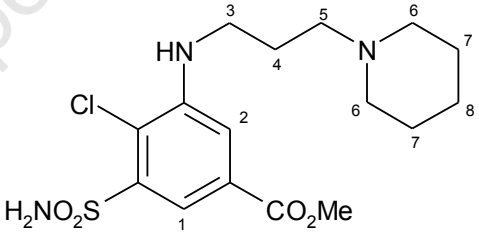
   White powder (68 mg, 17%); ^{1}H NMR (400 MHz; d^4-MeOD) δ 7.93 (d, 1H, *J* 1.9, Ar-H^1), 7.47 (d, 1H, *J* 1.8, Ar-H^2), 3.91 (s, 3H, -CO_2CH_3), 3.33 (t, 2H, *J* 6.4, N-CH_2^3), 2.96 (d, 2H, *J*

12.0, 2 x CHH^6), 2.49 (t, 2H, *J* 7.1, N-CH_2^5), 1.99 (td, 2H, *J* 11.9, 2.1, 2 x CH^6H), 1.88 (quin, 2H, *J* 7.0, CH_2^4), 1.66 (d, 2H, *J* 12.9, 2 x CHH^7), 1.40 (m, 1H, CH^8), 1.28 (quar-d, 2H, *J* 12.1, 3.6, 2 x CH^7H) and 0.94 (d, 3H *J* 6.3, CH_3^9); ^{13}C NMR (100.6 MHz; d^4-MeOD) δ 167.46, 147.50, 142.82, 130.34, 117.60, 115.11, 58.05, 55.14 (2C), 53.00, 43.58, 35.03 (2C), 31.92, 26.36 and 22.16; LRMS (EI): m/z = 403.1 [M^+]; Anal. Calc. (Found C, 50.1; H, 6.48; N, 9.79; S, 7.31. $C_{17}H_{26}ClN_3O_4S$ requires C, 50.6; H, 6.49; N, 10.4; S, 7.94%);



2. *4-Chloro-3-(3-piperidin-1-yl-propylamino)-5-sulfamoyl-benzoic acid methyl ester* **(RSON109)**

White powder (141 mg, 36%); ^{1}H NMR (400 MHz; d^4-MeOD) δ 7.73 (d, 1H, *J* 2.0, Ar-H^1), 7.32 (d, 1H, *J* 2.0, Ar-H^2), 3.85 (s, 3H, -CO_2CH_3), 3.24 (quar, 2H, *J* 6.5, N-CH_2^3), 2.48 (quin, 2H, *J* 1.9, 2 x CHH^6), 2.37 (t, 2H, *J* 6.3, N-CH_2^5), 2.34 (m, 2H, 2 x CH^6H), 1.75 (quin, 2H, *J* 6.3, CH_2^4), 1.53 (m, 4H, 2 x CH_2^7) and 1.39 (m, 2H, CH_2^8); ^{13}C NMR (100.6 MHz; d^4-MeOD) δ 165.35, 146.01, 141.75, 128.44, 118.48, 115.15, 113.00, 57.33, 54.22 (2C), 52.44, 42.92, 25.36 (2C), 24.27 and 24.01; LRMS (EI): m/z = 389.1 [M^+]; Anal. Calc. (Found C, 48.9; H, 6.13; N, 9.98; S, 8.01. $C_{16}H_{24}ClN_3O_4S$ requires C, 49.3; H, 6.20; N, 10.8; S, 8.22%);

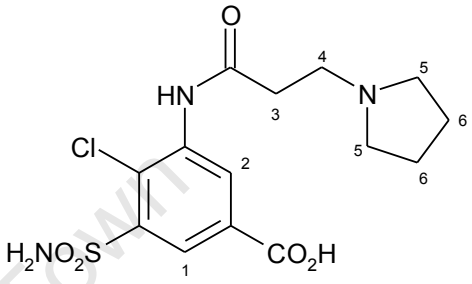


General procedure for the synthesis of amides **(RKG2432**, **RKG2431**, **RKG2441**, **RKG2471**, **RKG2461** and **RKG2462)**

A mixture (suspension) of amide (**RKG209**) (200 mg, 0.66 mmol) in methanol (5.0 ml) was placed in a reaction tube. The amine (3.5 equivalents, 2.30 mmol) was added dropwise with stirring, and the reaction was left to stir for 20 hrs at room temperature (~19 °C). The solvent

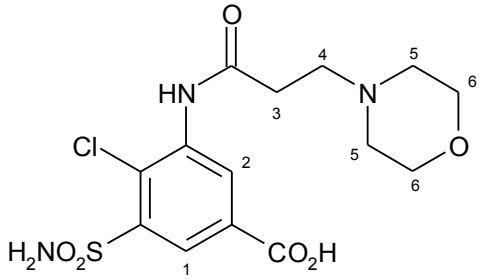
was removed under reduced pressure, and the obtained residue was dissolved in deionized H_2O (~10 ml). The pH of the aqueous solution was adjusted to neutral, and a precipitate formed. The precipitate was filtered off, washed with deionized H_2O and dried *in vacuo*.

1. *4-Chloro-3-(3-pyrrolidin-1-yl-propionylamino)-5-sulfamoyl-benzoic acid* **(RKG2432)**



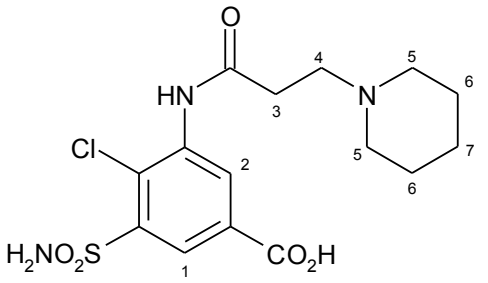
White solid (29.1 mg, 12%); m.p. 202 - 206 °C; IR (NaCl) ν_{max} (cm^{-1}) 3295 (m) (N-H), 2883 (m) (C-H), 2434 (br m) (O-H), 1676 (s) (C=O), 1562 (s) (N-H), 1540 (s) (C=C), 1373 (s) (S=O), 1282 (s) (C-O) and 1154 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 10.3 (s, 1H, CON*H*), 8.50 (d, 1H, *J* 1.9, Ar-H^1), 8.29 (d, 1H, *J* 2.0, Ar-H^2), 7.62 (s, 2H, -SO_2NH_2), 3.27 (t, 2H, *J* 6.8, CH_2^4), 3.12 (m, 4H, 2 x CH_2^5), 2.89 (t, 2H, *J* 7.0, CH_2^3) and 1.88 (m, 4H, 2 x CH_2^6); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 168.89, 165.76, 141.53, 136.25, 130.87, 129.49, 127.76, 125.70, 53.65 (2C), 49.81, 31.40 and 22.42 (2C); LRMS (EI): m/z = 375.0 [M^+]; Anal. Calc. (Found C, 44.3; H, 4.73; N, 10.5; S, 8.00. $C_{14}H_{18}ClN_3O_5S$ requires C, 44.7; H, 4.83; N, 11.2; S, 8.53%);

2. *4-Chloro-3-(morpholin-4-yl-propionylamino)-5-sulfamoyl-benzoic acid* **(RKG2431)**



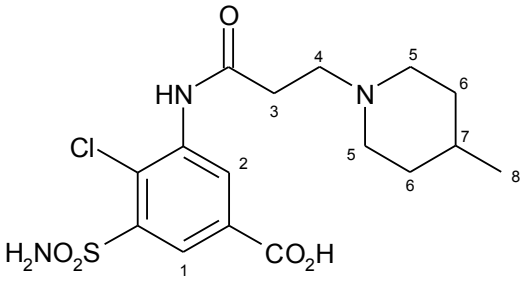
White solid (53.1 mg, 21%); m.p. 172 - 176 °C; IR (NaCl) ν_{max} (cm^{-1}) 3429 (m) (N-H), 3015 (m) (C-H), 2425 (br m) (O-H), 1682 (s) (C=O), 1558 (s) (N-H), 1528 (s) (C=C), 1375 (s) (S=O), 1265 (s) (C-O) and 1160 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 10.2 (s, 1H, CON*H*), 8.53 (d, 1H, *J* 1.8, Ar-H^1), 8.31 (d, 1H, *J* 1.9, Ar-H^2), 7.71 (s, 2H, -SO_2NH_2), 3.59 (t, 4H, *J* 4.6, 2 x CH_2^6), 2.60 (m, 4H, 2 x CH_2^{3+4}) and 2.49 (m, 4H, 2 x CH_2^5); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 170.97, 165.56, 141.86, 137.27, 129.79, 127.28, 125.60, 124.26, 65.89 (2C), 53.74, 52.75 (2C) and 32.65; LRMS (EI): m/z = 391.2 [M^+]; Anal. Calc. (Found C, 41.1; H, 4.84; N, 9.67; S, 7.60. $C_{14}H_{18}ClN_3O_6S$ requires C, 42.9; H, 4.63; N, 10.7; S, 8.18%);

3. *4-Chloro-3-(3-piperidin-1-yl-propionylamino)-5-sulfamoyl-benzoic acid* **(RKG2441)**



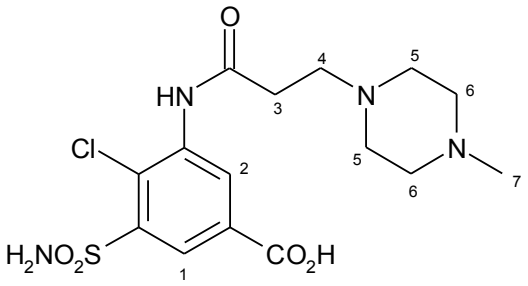
White solid (108 mg, 42%); m.p. 221 - 224 °C; IR (NaCl) ν_{max} (cm^{-1}) 3390 (m) (N-H), 2949 (m) (C-H), 2309 (br m) (O-H), 1682 (s) (C=O), 1638 (m) (C=O), 1563 (s) (N-H), 1368 (s) (S=O), 1268 (m) (C-O) and 1156 (s) (S=O); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 10.0 (s, 1H, CON*H*), 8.40 (d, 1H, *J* 2.0, Ar-H^{1}), 8.30 (d, 1H, *J* 2.1, Ar-H^{2}), 7.64 (s, 2H, -SO_2NH_2), 3.37 (t, 2H, *J* 10.8, 2 x CHH^{5}), 3.31 (t, 2H, *J* 7.1, CH_2^{4}), 2.93 (t, 2H, *J* 7.1, CH_2^{3}), 2.86 (t, 2H, *J* 10.6, 2 x CH^{5}H), 1.76-1.66 (m, 5H, 2 x CH_2^{6} and CHH^{7}) and 1.37 (m, 1H, CH^{7}H); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 168.82, 165.26, 141.99, 136.61, 132.72, 129.22, 128.26, 125.54, 52.45 (2C), 51.59, 29.99, 22.34 (2C) and 20.93; LRMS (EI): m/z = 389.0 [M^{+}]; Anal. Calc. (Found C, 45.8; H, 5.16; N, 10.4; S, 7.81. $C_{15}H_{20}ClN_3O_5S$ requires C, 46.2; H, 5.17; N, 10.8; S, 8.22%);

4. *4-Chloro-3-[3-(4-methyl-piperidin-1-yl)-propionylamino]-5-sulfamoyl-benzoic acid* **(RKG2471)**



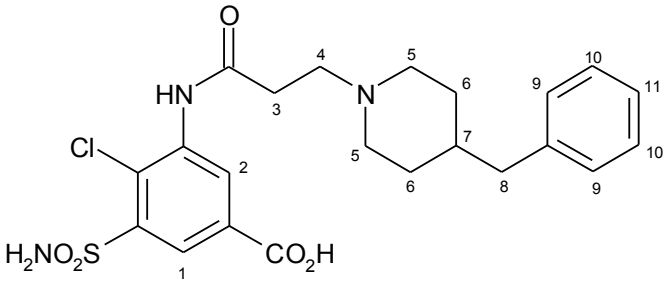
White solid (87.3 mg, 65%) (0.33 mmol scale); m.p. 219 - 222 °C; IR (NaCl) ν_{max} (cm^{-1}) 3388 (m) (N-H), 2925 (m) (C-H), 2350 (br m) (O-H), 1686 (s) (C=O), 1637 (m) (C=O), 1565 (s) (N-H), 1373 (s) (S=O), 1275 (m) (C-O) and 1157 (s) (S=O); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 10.6 (s, 1H, CON*H*), 8.66 (d, 1H, *J* 2.0, Ar-H^{1}), 8.23 (d, 1H, *J* 2.0, Ar-H^{2}), 7.69 (s, 2H, -SO_2NH_2), 3.05 (d, 2H, *J* 11.6, 2 x CHH^{5}), 2.75 (t, 2H, *J* 6.4, CH_2^{4}), 2.63 (t, 2H, *J* 6.5, CH_2^{3}), 2.12 (t, 2H, *J* 11.5, CH^{5}H), 1.62 (t, 2H, *J* 12.4, 2 x CHH^{6}), 1.39 (m, 1H, CH^{7}), 1.21 (dquar, 2H, *J* 12.5, 2 x CH^{6}H) and 0.86 (d, 3H, *J* 6.5, CH_3^{8}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 169.70, 166.39, 141.01, 136.03, 134.82, 129.03, 125.47, 52.16 (3C), 31.48 (2C), 31.09, 28.41 and 20.86; LRMS (EI): m/z = 403.1 [M^{+}]; Anal. Calc. (Found C, 46.9; H, 5.49; N, 10.1; S, 6.95. $C_{16}H_{22}ClN_3O_5S$ requires C, 47.6; H, 5.49; N, 10.4; S, 7.94%);

5. *4-Chloro-3-[3-(4-methyl-piperazin-1-yl)-propionylamino]-5-sulfamoyl-benzoic acid* **(RKG2461)**



White solid (41.8 mg, 31%) (0.33 mmol scale); m.p. 179 - 183 °C; IR (NaCl) ν_{max} (cm^{-1}) 3311 (m) (N-H), 2900 (m) (C-H), 2427 (br m) (O-H), 1698 (s) (C=O), 1689 (m) (C=O), 1565 (s) (N-H), 1520 (s) (C=C), 1373 (s) (S=O), 1286 (m) (C-O) and 1156 (s) (S=O); ^{1}H NMR (400 MHz; d^{6}-DMSO, d^{2}-D_2O wash) δ 10.0 (s, 1H, CON*H*), 8.52 (d, 1H, *J* 1.8, Ar-H^{1}), 8.25 (d, 1H, *J* 1.9, Ar-H^{2}), 7.73 (s, 2H, -SO_2NH_2), 2.73 (t, 4H, *J* 6.5, 2 x CH_2^{6}), 2.67 (s, 3H, CH_3^{7}), 2.59 (t, 4H, *J* 6.5, 2 x CH_2^{5}) and 2.49 (m, 4H, 2 x CH_2^{3+4}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 170.83, 165.45, 141.75, 136.90, 136.26, 129.57, 128.58, 124.99, 52.52 (2C), 52.26, 49.12 (2C), 42.38 and 32.88; LRMS (EI): m/z = 404.0 [M^{+}]; Anal. Calc. (Found C, 44.0; H, 5.00; N, 12.3; S, 7.78. $C_{15}H_{21}ClN_4O_5S$ requires C, 44.5; H, 5.23; N, 13.8; S, 7.92%);

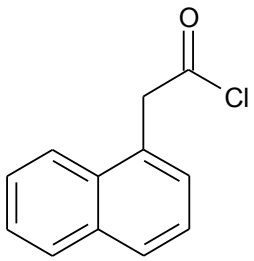
6. *3-[3-(4-Benzyl-piperidin-1-yl)-propionylamino]-4-chloro-5-sulfamoyl-benzoic acid* **(RKG2462)**



White solid (8.8 mg, 6%) (0.33 mmol scale); m.p. 179 - 183 °C; IR (NaCl) ν_{max} (cm^{-1}) 3311 (m) (N-H), 2900 (m) (C-H), 2427 (br m) (O-H), 1698 (s) (C=O), 1689 (m) (C=O), 1565 (s) (N-H), 1520 (s) (C=C), 1373 (s) (S=O), 1286 (m) (C-O) and 1156 (s) (S=O); ^{1}H NMR (300 MHz; d^{6}-DMSO, d^{2}-D_2O wash) δ 10.19 (s, 1H, CON*H*), 8.45 (d, 1H, *J* 2.0, Ar-H^{1}), 8.27 (d, 1H, *J* 2.1, Ar-H^{2}), 7.74 (s, 2H, -SO_2NH_2), 7.24 (t, 2H, *J* 7.8, 2 x Ar*H*), 7.14 (t, 3H, *J* 7.7, 3 x ArH^{+11}), 3.33 (m, 2H, CHH^{5}), 2.88, (m, 4H, 2 x CHH^{6} and CH_2^{4}), 2.52 (m, 4H, 2 x CH^{6}H and CH_2^{8}), 1.75 (m, 2H, 2 x CH^{5}H) and 1.43 (m, 1H, CH^{7}); ^{13}C NMR (75.5 MHz; d^{6}-DMSO) δ 169.30, 165.71, 142.21, 139.65, 136.89, 130.21, 129.46, 129.26 (2C), 128.54 (2C), 126.31, 125.69, 52.08 (2C), 51.89, 43.33, 34.84, 30.62 (2C) and 28.80; LRMS (EI): m/z = 479.1

[M^+]; Anal. Calc. (Found C, 54.6; H, 5.00; N, 8.32; S, 6.07. $C_{22}H_{26}ClN_3O_5S$ requires C, 55.1; H, 5.46; N, 8.75; S, 6.68%);

Synthesis of (1-Naphthalenyl)-2-ethanoyl chloride



Freshly distilled $SOCl_2$ (0.560 ml, 7.62 mmol) was added in a dropwise manner to 1-naphthalene acetic acid (1.18 g, 6.35 mmol) with stirring. The mixture was heated gently for 1 h at 100 °C under N_2 (a further 1.0 ml of $SOCl_2$ was added after the first 20 min to make a clear solution). The reaction was allowed to cool to room temperature, and then distilled at atmospheric pressure to remove the excess $SOCl_2$, affording a dark brown residue. The acid chloride was not isolated or purified.

Synthesis of **RKG248** and **RKG346**

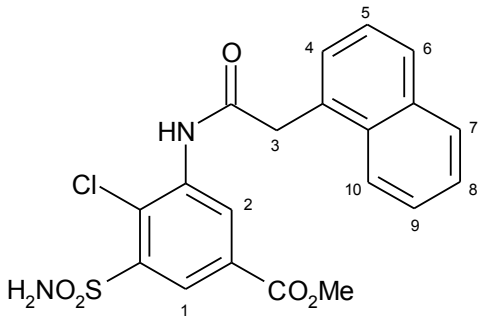
(NOTE: the acid chloride was used as obtained above)

Acetone (4.0 ml) was added to the unpurified acid chloride, and the resulting solution was added dropwise to a solution of *(O-alkyl)-3-amino-4-chloro-sulfomoylbenzoate* (**RKG262** and **RKG345**) (1.40 g, 5.29 mmol) (assuming that the formation of the acid chloride was quantitative) dissolved in acetone (6.0 ml). The reaction mixture was stirred at room temperature (19 °C) for 6 hrs (more acetone was added periodically to aid stirring due to the formation of a precipitate). The precipitate was filtered off and washed with *cold* acetone and deionized H_2O, and dried *in vacuo*.

4-Chloro-3-(2-naphthalen-1-yl-acetylamino)-5-sulfamoyl-benzoic acid methyl ester **(RKG248)**

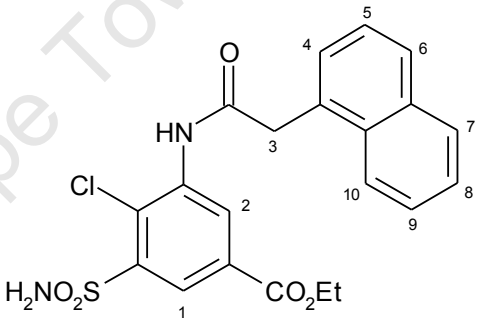
Pale pink solid (1.80 g, 79% over two steps); m.p. 222-225 °C; IR (NaCl) ν_{max} (cm^{-1}) 3240 (s) (N-H), 3009 (m) (C-H), 1734 (s) (C=O), 1669 (s) (C=O), 1599 (s) (N-H), 1520 (s) (C=C), 1335 (s) (S=O), 1239 (s) (C-O) and 1163 (s) (S=O); ^{1}H NMR (300 MHz; d^6-DMSO) δ 10.08 (s, 1H, CON*H*), 8.47 (d, 1H, *J* 2.1, ArH^1), 8.30 (d, 1H, *J* 2.1, ArH^2), 8.15 (d, 1H, *J* 7.6, ArH^{10}), 7.94 (d, 1H, *J* 7.5, ArH^7), 7.85

(d, 1H, *J* 7.9, Ar*H*^{6}), 7.77 (s, 2H, -$SO_2N\mathit{H}_2$), 7.59-7.46 (m, 4H, 4 x Ar*H*$^{4+5+8+9}$), 4.31 (s, 2H, CH_2^{3}) and 3.85 (s, 3H, -$CO_2C\mathit{H}_3$); ^{13}C NMR (75.5 MHz; *d*^{6}-DMSO) *δ* 169.93, 164.37, 142.32, 137.20, 133.33, 131.85, 131.80, 128.47, 128.37, 128.18, 128.01, 127.98, 127.36, 126.08, 125.67, 125.46, 124.87, 124.04, 52.66 and 39.98; LRMS (EI): m/z = 432.0 [M^+]; Anal. Calc. (Found C, 55.2; H, 3.86; N, 6.01; S, 6.91. $C_{20}H_{17}ClN_2O_5S$ requires C, 55.5; H, 3.96; N, 6.47; S, 7.41%);



4-Chloro-3-(2-naphthalen-1-yl-acetylamino)-5-sulfamoyl-benzoic acid ethyl ester **(RKG346)**

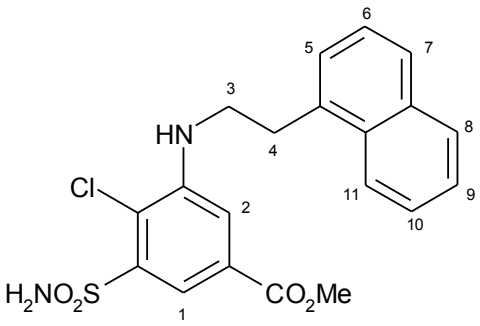
White solid (2.03 g, 76% over two steps); m.p. 222 - 225 °C; IR (NaCl) ν_{max} (cm^{-1}) 3353 (s) (N-H), 3262 (s) (CON-H), 2975 (w) (C-H), 1714 (s) (C=O), 1674 (s) (C=O), 1595 (m) (C=C), 1568 (m) (C=C), 1512 (s) (C=C), 1464 (w) (C=C), 1359 (s) (S=O), 1300 (s) (C-N), 1249 (s) (C-O), 1162 (s) (S=O) and 783 (s) (C-Cl); ^{1}H NMR (400 MHz; *d*^{6}-DMSO) *δ* 10.10 (s, 1H, CON*H*), 8.46 (d, 1H, *J* 2.1, Ar-*H*^{1}), 8.30 (d, 1H, *J* 2.1, Ar-*H*^{2}), 8.14 (d, *J* 8.3, Ar*H*^{10}), 7.93 (d, 1H, *J* 7.7, Ar*H*^{7}), 7.85 (d, 1H, *J* 8.0, Ar*H*^{6}), 7.78 (s, 2H, -$SO_2N\mathit{H}_2$), 7.52 (m, 4H, 4 x Ar*H*$^{4+5+8+9}$), 4.31 (m, 4H, CH_2^{3} and –OCH_2-) and 1.28 (t, 3H, *J* 7.1, -$CH_2C\underline{\mathit{H}}_3$); ^{13}C NMR (100.6 MHz; *d*^{6}-DMSO) *δ* 170.73, 164.64, 143.03, 137.97, 134.10, 132.62, 132.57, 129.31, 129.16, 129.05, 129.01, 128.83, 128.15, 126.86, 126.46, 126.25, 125.68, 124.81, 62.31, 40.75 and 14.74; HRMS (EI): m/z = 446.06977 [M^+]; Anal. Calc. (Found C, 55.2; H, 4.24; N, 5.76; S, 6.53. $C_{21}H_{19}ClN_2O_5S$ requires C, 56.4; H, 4.29; N, 6.27; S, 7.17%);



Synthesis of *4-chloro-3-(2-naphthalen-1-yl-ethylamino)-5-sulfamoyl-benzoic acid methyl ester* **(RKG252COL1)**

RKG248 (422 mg, 1.00 mmol) was added to anhydrous THF (2.5 ml). The resulting suspension was cooled to 0 °C, and the borane-THF complex (1.0M in THF) (2.5 ml, 2.5 mmol) was added

dropwise [OBSERVATION: color change from pink to yellow] and was stirred for 15 minutes. The mixture was heated and refluxed for 21 hrs under an atmosphere of N_2. After cooling to room temperature, the reaction mixture was quenched with glacial acetic acid (~10 ml). The apparatus was rearranged for distillation, and the THF was distilled off (distillation was aided by the application of a slight vacuum). The resulting acidic solution was cooled to 0 °C and aqueous 10% Na_2CO_3 solution was added until a pH of 6-7 was obtained. A brown sticky residue formed, and the aqueous solution was extracted with EtOAc (2 x 20 ml). The organic layer was washed with deionized H_2O (20 ml), and dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The brown residue was dried further *in vacuo* and column chromatographed (2:3, EtOAc:hexane), yielding **RKG252COL1** (R_f = 0.42; 2:3, EtOAc:hexane) and **RKG252COL3** (R_f = 0.13; 2:3, EtOAc:hexane);

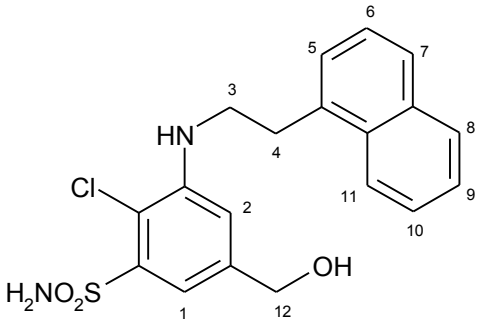


4-Chloro-3-(2-naphthalen-1-yl-ethylamino)-5-sulfamoyl-benzoic acid methyl ester **(RKG252COL1)**

Pale yellow solid (115 mg, 27%) (larger 2.0 mmol scale (132 mg, 16%)); m.p. 119 - 123 °C; IR (NaCl) ν_{max} (cm^{-1}) 3267 (s) (N-H), 2950 (m) (C-H), 1707 (s) (C=O), 1595 (s) (N-H), 1571 (s) (C=C), 1344 (s) (S=O), 1263 (s) (C-O) and 1158 (s) (S=O); ^{1}H NMR (300 MHz; d^6-DMSO) δ 8.20 (d, 1H, *J* 7.5, Ar-H^{11}), 7.92 (dd, 1H, *J* 7.8, 1.7, Ar-H^8), 7.79 (dd, 1H, *J* 7.2, 2.2, Ar-H^7), 7.74 (d, 1H, *J* 1.9, Ar-H^1), 7.55 (m, 4H, 2 x Ar-H^{9+10} and -SO_2NH_2), 7.43 (m, 2H, Ar-H^{5+6}), 7.36 (d, 1H, *J* 1.9, Ar-H^2), 6.17 (t, 1H, *J* 5.8, -N*H*-), 3.84 (s, 3H, -CO_2CH_3), 3.57 (m (quar), 2H, *J* 6.6, 5.8, N-CH_2^3) and 3.37 (t, 2H, *J* 6.5, Ar-CH_2^4); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 165.21, 145.47, 141.90, 135.15, 133.41, 131.47, 128.52, 128.36, 126.79, 126.59, 126.03, 125.57, 125.49, 123.49, 118.59, 115.40, 113.24, 52.40, 43.82 and 31.51; LRMS (EI): m/z = 418.0 [M^+]; Anal. Calc. (Found C, 57.5; H, 4.77; N, 5.98; S, 6.65. $C_{20}H_{19}ClN_2O_4S$ requires C, 57.4; H, 4.57; N, 6.69; S, 7.65%);

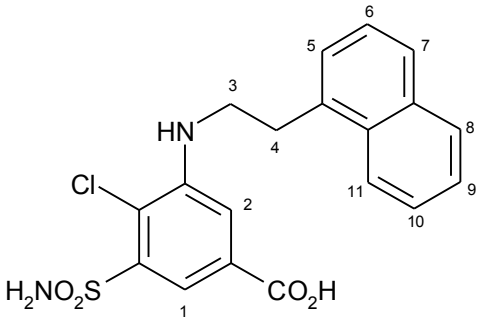
2-Chloro-5-hydroxymethyl-3-(2-naphthalen-1-yl-ethylamino)-benzenesulfonamide

(RKG252COL3)



White solid (110 mg, 28%) (larger 2.0 mmol scale (444 mg, 57%)); m.p. 119 - 123 °C; IR (NaCl) ν_{max} (cm^{-1}) 3267 (s) (N-H), 2950 (m) (C-H), 1707 (s) (C=O), 1595 (s) (N-H), 1571 (s) (C=C), 1344 (s) (S=O), 1263 (s) (C-O) and 1158 (s) (S=O); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 8.21 (d, 1H, *J* 8.1, Ar-H^{11}), 7.93 (dd, 1H, *J* 7.7, 1.7, Ar-H^{8}), 7.80 (m, 1H, Ar-H^{9}), 7.59-7.43 (m, 4H, 4 x Ar-$H^{5+6+7+10}$), 7.34 (s, 2H, -SO_2NH_2), 7.20 (d, 1H, *J* 1.9, Ar-H^{1}), 6.93 (d, 1H, *J* 1.8, Ar-H^{2}), 5.75 (t, 1H, *J* 5.6, -N*H*-), 5.28 (s (not well resolved t), 1H, -O*H*), 4.45 (s, 2H, CH_2^{12}-O), 3.51 (m (quar), 2H, *J* 6.5, 5.5, N-CH_2^{3}) and 3.36 (t, 2H, *J* 6.6, Ar-CH_2^{4}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 144.96, 142.05, 141.11, 135.40, 133.43, 131.54, 128.54, 126.78, 126.60, 126.05, 125.61, 125.54, 123.68, 113.37, 112.10, 111.60, 62.31, 43.99 and 31.64; LRMS (EI): m/z = 390.1 [M^{+}]; Anal. Calc. (Found C, 58.0; H, 5.06; N, 6.80; S, 7.61. $C_{19}H_{19}ClN_2O_3S$ requires C, 58.4; H, 4.90; N, 7.17; S, 8.20%);

Synthesis of *4-chloro-3-(2-naphthalen-1-yl-ethylamino)-5-sulfamoyl-benzoic acid* (**RKG256) (H157)**

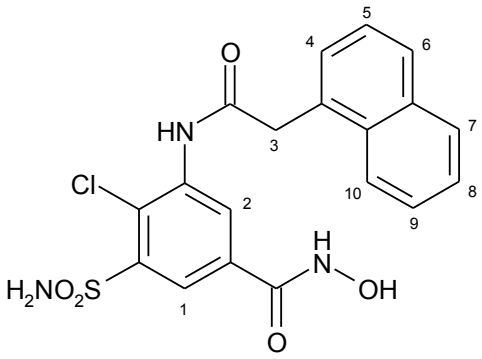


Sodium hydroxide (20 mg, 0.48 mmol) was dissolved in a mixture of MeOH (1.0 ml) and deionized H_2O (0.2 ml), and added to a stirring solution of **RKG252COL1** (50 mg, 0.12 mmol) dissolved in MeOH (1.0 ml). The reaction mixture was stirred under reflux (~80 °C) for 2 hrs. The reaction was allowed to cool to room temperature, and chloroform (10 ml) and deionized H_2O (5 ml) were added, followed by 10% Na_2CO_3 aqueous solution (5 ml). The layers were separated, and the organic phase was washed with 10% Na_2CO_3 aqueous solution (10 ml). The aqueous layers were combined and washed with chloroform (10 ml). The

aqueous layer pH was adjusted from basic to neutral (6/7). A white precipitate formed and was filtered off, washed with deionized H_2O, and finally dried *in vacuo*.

White solid (37.2 mg, 77%); m.p. 266 - 270 °C; IR (NaCl) v_{max} (cm^{-1}) 3378 (s) (N-H), 2950 (m) (C-H), 1642 (s) (C=O), 1557 (s) (N-H), 1490 (m) (C=C), 1367 (s) (S=O), 1269 (s) (C-O) and 1159 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 8.23 (d, 1H, *J* 8.3, Ar-H^{11}), 7.92 (d, 1H, *J* 7.9, Ar-H^8), 7.80 (m, 2H, Ar-H^{1+7}), 7.60-7.41 (m, 5H, 5 x Ar-$H^{2+5+6+9+10}$), 7.29 (s, 2H, -SO_2NH_2), 5.56 (t, 1H, *J* 5.7, Ar-N*H*-), 3.52 (m (quar), 2H, *J* 6.7, 5.60, N-CH_2^3) and 3.36 (t, 2H, *J* 6.6, Ar-CH_2^4); ^{13}C NMR (100.6 Mz; d^6-DMSO) δ 167.61, 144.14, 140.22, 138.03, 135.45, 133.44, 131.55, 128.54, 126.78, 126.48, 126.14, 125.62, 125.56, 123.73, 116.92, 114.67, 114.01, 44.12 and 31.59; HRMS (EI): m/z = 404.05921 [M^+]; Anal. Calc. (Found C, 56.0; H, 4.07; N, 5.95; S, 7.22. $C_{19}H_{17}ClN_2O_4S$ requires C, 56.4; H, 4.23; N, 6.92; S, 7.92%);

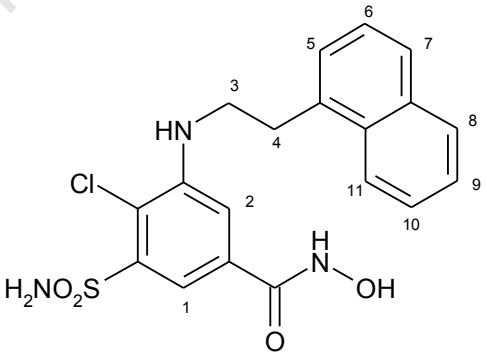
Synthesis of *4-chloro-N-hydroxy-3-(2-naphthalen-1-yl-acetylamino)-5-sulfamoyl-benzamide* **(RKG364)**



Methyl ester (**RKG262**) (500 mg, 1.16 mmol) was dissolved in a 1:1 mixture of THF and MeOH (7.5 ml). An aqueous hydroxylamine solution (50% w/w) (1.07 ml, 17.4 mmol) was added to the mixture, immediately followed by the addition of a catalytic amount of KCN (25 mg, 0.38 mmol). The reaction was allowed to stir at room temperature (~23 ^{o}C), and was monitored by TLC at 12 hour intervals. To drive the reaction to completion, further amounts of aqueous hydroxylamine solution (0.5 ml) and KCN (10 mg) were added at the 12 hour intervals. This technique was pursued until TLC showed no more ester (**RKG262**) to be present. The reaction appeared complete after 3 days. A white precipitate formed (hydroxylamine salt), and aqueous 3M HCl was added until an acidic pH was obtained. The resulting reaction mixture volume was reduced under reduced pressure, and the resulting residue was columned chromatographed (starting MeOH:EtOAc (1:49), gradually increasing to MeOH:EtOAc (3:17), yielding **RKG364**;

Brown oil which crystallized to a white solid (322 mg, 64%); R_f = 0.03 (5:95, MeOH:EtOAc); m.p. 260 - 264 °C; IR (NaCl) ν_{max} (cm^{-1}) 2856 (s) (C-H), 1700 (s) (C=O), 1668 (s) (C=O), 1567 (s) (C=C), 1525 (s) (C=C), 1337 (s) (S=O), 1259 (s) (C-O), 1153 (s) (S=O) and 781 (s) (C-Cl); ^{1}H NMR (400 MHz; d^4-MeOH) δ 8.58 (d, 1H, *J* 1.8, Ar-H^1), 8.48 (d, 1H, *J* 1.9, Ar-H^2), 8.13 (d, 1H, *J* 8.1, Ar-H^{10}), 7.91 (d, 1H, *J* 7.9, Ar-H^7), 7.85 (d, 1H, *J* 8.2, Ar-H^6), 7.71 (m, 2H, Ar-H^{8+9}), 7.56 (m, 2H, Ar-H^{4+5}) and 4.31 (s, 2H, CH_2^3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 170.04, 162.36, 141.44, 138.22, 136.30, 134.07, 132.94, 132.65, 129.10, 128.66, 128.01, 127.13, 126.78, 126.40, 126.23, 124.89, 123.50 and 40.80; LRMS (EI): m/z = 433.01 [M^+]; Anal. Calc. (Found C, 52.1; H, 3.19; N, 10.2; S, 6.89. $C_{19}H_{16}ClN_3O_5S$ requires C, 52.6; H, 3.72; N, 9.69; S, 7.39%);

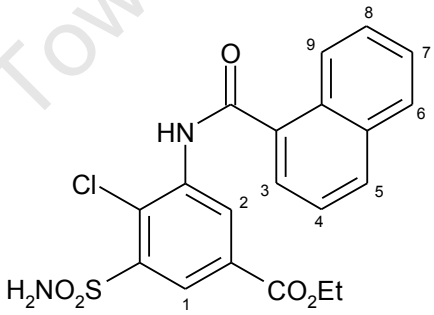
Synthesis of *4-chloro-N-hydroxy-3-(2-naphthalen-1-yl-ethylamino)-5-sulfamoyl-benzamide* **(RKG404P2)**



Methyl ester (**RKG252COL1**) (79.5 mg, 0.190 mmol) was dissolved in a 1:1 mixture of THF and MeOH (1.5 ml). An aqueous hydroxylamine solution (50% w/w) (0.175 ml, 2.85 mmol) was added to the mixture, immediately followed by the addition of a catalytic amount of KCN (3.71 mg, 0.0570 mmol). The reaction was allowed to stir at room temperature (~23 ^{o}C), and was monitored by TLC, at 12 hour intervals, until TLC showed no more ester (**RKG252COL1**) to be present. The reaction appeared complete after five days. The reaction mixture volume was reduced under reduced pressure, and the pH of the resulting aqueous residue was adjusted to neutral/slightly acidic with AcOH:H_2O (1:1). The aqueous solution was extracted with EtOAc (2 x 50 ml). The combined organic extracts were washed with deionised H_2O (2 x 20 ml) and dried over anhydrous Na_2SO_4. The dried organic extract was filtered and the solvent was removed *in vacuo*, resulting in a brown residue. The residue was columned chromatographed (starting 2:98, EtOAc:hexane (1:49), gradually increasing to 100% EtOAc, then changing to MeOH:EtOAc (1:9)), yielding **RKG404P2**;

Brown oil (39.9 mg, 50%); R_f = 0.03 (5:95, MeOH:EtOAc); ^{1}H NMR (400 MHz; d^6-acetone) δ 8.24 (t, 1H, *J* 8.6, Ar-H^9), 8.00 (d, 1H, *J* 1.8, Ar-H^1), 7.92 (d, 1H, *J* 8.1, Ar-H^{11}), 7.81 (m, 2H, Ar-H^8 and N*H*), 7.63 (d, 1H, *J* 1.7, Ar-H^2), 7.58 - 7.43 (m, 6H, Ar-$H^{5+6+7+10}$ and -SO_2NH_2), 3.74 (quar, 2H, *J* 7.3, N-CH_2^3) and 3.52 (t, 2H, *J* 7.3, Ar-CH_2^4); ^{13}C NMR (100.6 Mz; d^6-acetone) δ 166.27, 145.15, 141.63, 135.30, 133.39, 131.61, 131.43, 128.52, 126.79, 126.52, 126.02, 125.57, 123.66, 123.53, 115.79, 113.60, 111.34, 43.86 and 31.38; LRMS (EI): m/z = 419.0 [M^+]; Anal. Calc. (Found C, 53.9; H, 4.09; N, 9.79; S, 7.22. $C_{19}H_{18}ClN_3O_4S$ requires C, 54.4; H, 4.32; N, 10.0; S, 7.64%);

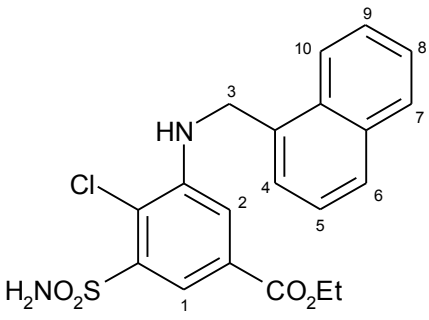
Synthesis of *ethyl 3-(1-naphthamido)-4-chloro-5-sulfamoylbenzoate* **(RKG409)**



Naphthoyl chloride (188 mg, 0.99 mmol) was dissolved in acetone (1.0 ml), and the resulting solution was added dropwise to a solution of *(O-ethyl)-3-amino-4-chloro-sulfomoylbenzoate* (**RKG345**) (150 mg, 0.90 mmol) dissolved in acetone (1.5 ml). The reaction mixture was stirred at room temperature (19 °C) for 48 hrs (more acetone was added periodically to aid stirring due to the formation of a precipitate). The reaction mixture was cooled in an ice bath, and the precipitate was filtered off and washed with *cold* acetone. It was further dried *in vacuo* to yield a pure, white product.

White solid (269 mg, 69%); R_f = 0.28 (2:3, EtOAc:hexane); ^{1}H NMR (400 MHz; d^6-DMSO, d^2-D_2O wash) δ 8.51 (d, 1H, *J* 2.0, Ar-H^1), 8.45 (d, 1H, *J* 2.0, Ar-H^2), 8.34 (d, 1H, *J* 7.6, Ar-H^9), 8.12 (d, 1H, *J* 8.3, Ar-H^3), 8.04 (d, 1H, *J* 7.4, Ar-H^6), 7.93 (d, 1H, *J* 6.9, Ar-H^5), 7.64 (m, 3H, 3 x Ar-H^{4+7+8}), 4.41 (quar, 2H, *J* 7.1, -OCH_2CH$_3$) and 1.36 (t, 3H, *J* 7.1, -CH$_2$CH_3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 168.46, 164.67, 143.31, 138.21, 133.89, 132.08, 131.73, 131.46, 130.46, 129.07, 127.85, 127.14, 126.97, 126.87, 125.82, 125.69, 118.83, 116.41, 62.41 and 14.81; HRMS (EI): m/z = 432.05410 [M^+]; Anal. Calc. (Found C, 55.1; H, 4.23; N, 6.19; S, 7.90. $C_{20}H_{17}ClN_2O_5S$ requires C, 55.5; H, 3.96; N, 6.47; S, 7.41%);

Synthesis of *ethyl 4-chloro-3-(naphthalene-1-ylmethylamino)-5-sulfamoylbenzoate* **(RKG414F2)**



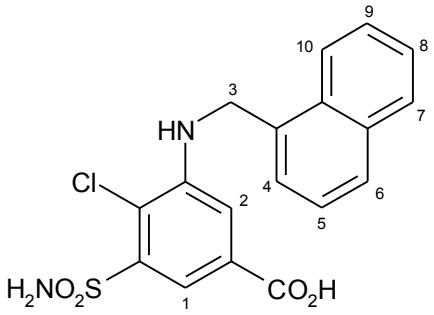
Sodium borohydride (17.7 mg, 0.47 mmol) is added to diglyme (0.9 ml), and the solution is added dropwise to a solution of *ethyl 3-(1-naphthamido)-4-chloro-5-sulfamoylbenzoate* (**RKG409**) (150 mg, 0.38 mmol) in a mixture of BF_3 ethyl etherate (0.080 ml, 89.6 mg, 0.63 mmol) and diglyme (1.4 ml). The reaction was stirred at room temperature (20 ^{o}C) for 4.5 hrs. The reaction progress was monitored by thin-layer chromatography (2:3, EtOAc:hexane). The reaction mixture is poured into ice-water. The oily precipitate is extracted with EtOAc (3 x 20 ml). The combined organic fractions were washed with deionised H_2O (20 ml) and dried over anhydrous Na_2SO_4, filtered, and the solvent was removed under reduced pressure. The brown residue was dried further *in vacuo* and column chromatographed (1:9, EtOAc:hexane), yielding **RKG414F2**.

White solid (43 mg, 27%); R_f = 0.31; (2:3, EtOAc:hexane); ^{1}H NMR (300 MHz; d^6-acetone) δ 8.26 (d, 1H, *J* 8.3, Ar-H^{10}), 7.97 (m, 2H, 2 x Ar-H^{1+7}), 7.87 (d, 1H, *J* 8.2, Ar-H^{6}), 7.58 (m, 4H, 4 x Ar-$H^{2+4+8+9}$), 7.47 (t, 1H, *J* 8.1, Ar-H^{5}), 6.75 (br s, 2H, -SO_2NH_2), 6.19 (m, 1H, N*H*), 5.10 (d, 2H, *J* 5.6, CH_2^{3}), 4.27 (quar, 2H, *J* 7.1, -OCH_2CH_3) and 1.27 (t, 3H, *J* 7.1, -CH_2CH_3); ^{13}C NMR (100.6 MHz; d^6-acetone) δ 164.97, 146.12, 142.04, 134.34, 133.63, 131.65, 129.74, 128.00, 128.25, 126.51, 126.07, 125.65, 125.22, 123.44, 119.37, 117.03, 114.85, 61.27, 45.37 and 13.76; HRMS (EI): m/z = 418.07484 [M^+]; Anal. Calc. (Found C, 57.0; H, 4.19; N, 7.20; S, 7.05. $C_{20}H_{19}ClN_2O_4S$ requires C, 57.3; H, 4.57; N, 6.69; S, 7.65%);

Synthesis of *4-chloro-3-(naphthalen-1-ylmethylamino)-5-sulfamoylbenzoic acid* **(RKG442)**

Sodium hydroxide (10 mg, 0.25 mmol) was dissolved in a mixture of MeOH (0.5 ml) and deionized H_2O (0.2 ml), and added to a stirring solution of **RKG414F2** (25.5 mg, 0.061 mmol) dissolved in MeOH (0.5 ml). The reaction mixture was stirred under reflux (~80 °C) for 2 hrs. The reaction was allowed to cool to room temperature, and the pH was adjusted from basic to acid

(2/3) with 6M HCl. A white precipitate formed and was filtered off, washed with deionized H_2O, and finally dried *in vacuo*.

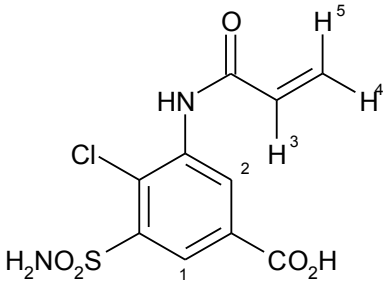


White solid (18.9 mg, 79%); ^{1}H NMR (300 MHz; d^6-DMSO) δ 8.24 (d, 1H, *J* 7.8, Ar-H^{10}), 7.96 (d, 1H, *J* 7.9, Ar-H^7), 7.83 (d, 1H, *J* 7.6, Ar-H^6), 7.78 (d, 1H, *J* 1.9, Ar-H^1), 7.63-7.38 (m, 6H, 4 x Ar-$H^{4+5+8+9}$ and –SO_2NH_2), 7.28 (d, 1H, *J* 1.9, Ar-H^2), 6.66 (t, 1H, *J* 5.7, Ar-N*H*-) and 4.98 (d, 2H, *J* 5.7, CH_2^3); ^{13}C NMR (100.6 Mz; d^6-DMSO) δ 166.12, 145.56, 141.67, 139.52, 133.37, 130.64, 129.54, 128.57, 127.36, 126.17, 125.81, 125.36, 123.60, 123.13, 118.20, 115.94, 114.12 and 44.24; HRMS (EI): m/z = 390.04357 [M^+]; Anal. Calc. (Found C, 55.2; H, 3.96; N, 7.35; S, 7.75. $C_{18}H_{15}ClN_2O_4S$ requires C, 55.3; H, 3.87; N, 7.17; S, 8.20%);

General procedure for the synthesis of amides **(RKG209**, **RKG204(ii)** and **RKG210)**

A mixture of acid chloride (1.1 equiv.) and acetone (1.0 ml) is added dropwise to a solution of amine (**RKG211**) (251 mg, 1.0 mmol) dissolved in acetone (4.0 ml). The reaction mixture is stirred at room temperature (20 ^{o}C) for 6 hrs. The cooled mixture was poured into aqueous 1M HCl (25 ml), and the white precipitate was filtered off, washed with aqueous 1M HCl and deionized H_2O, and dried *in vacuo*.

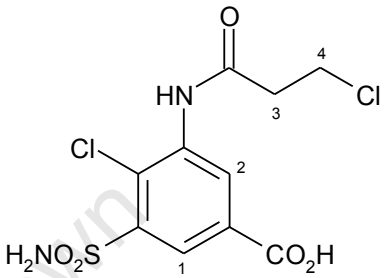
1. *3-Acryloylamino-4-chloro-5-sulfamoyl-benzoic acid* **(RKG209)**



White solid (90.7 mg, 30%) (larger 7.98 mmol scale (1.41 g, 58%)); m.p. 238 - 241 °C; IR (NaCl) ν_{max} (cm^{-1}) 3385 (s) (N-H), 2848 (m) (C-H), 2542 (m) (O-H), 1698 (s) (C=O), 1670 (s) (C=O), 1598 (s) (N-H), 1529 (s) (C=C), 1321 (s) (S=O), 1253 (s) (C-O) and 1159 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 10.0 (s, 1H, CON*H*), 8.52 (d, 1H, *J* 2.0, Ar-H^1), 8.32 (d, 1H, *J* 2.1, Ar-H^2), 7.77 (s, 2H, -SO_2NH_2), 6.65 (dd, 1H, *J* 17.0, 10.2, C=CH^3), 6.32 (dd, 1H, *J* 17.0, 1.8, C=CH^5) and 5.84 (dd, 1H, *J* 10.2, 1.8, C=CH^4); ^{13}C NMR (100.6

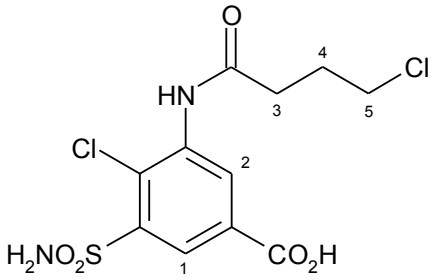
MHz; d^6-DMSO) δ 165.39, 163.83, 142.20, 136.98, 130.87, 129.25, 129.11, 128.21, 125.31 and 103.20; LRMS (EI): m/z = 304.0 [M^+]; Anal. Calc. (Found C, 39.3; H, 2.92; N, 8.40; S, 9.96. $C_{10}H_9ClN_2O_5S$ requires C, 39.4; H, 2.98; N, 9.19; S, 10.5%);

2. *4-Chloro-3-(3-chloro-propionylamino)-5-sulfamoyl-benzoic acid* **(RKG204(ii))**



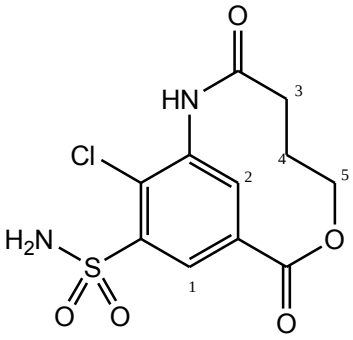
White solid (253 mg, 71%) (1.05 mmol amine scale); m.p. 226 – 230 °C; IR (NaCl) ν_{max} (cm^{-1}) 3385 (s) (N-H), 2849 (m) (C-H), 2540 (m) (O-H), 1702 (s) (C=O), 1647 (s) (C=O), 1596 (s) (N-H), 1523 (s) (C=C), 1355 (s) (S=O), 1242 (s) (C-O) and 1161 (s) (S=O); ^{1}H NMR (300 MHz; d^6-DMSO) δ 9.86 (s, 1H, CON*H*), 8.43 (d, 1H, *J* 2.0, Ar-H^1), 8.34 (d, 1H, *J* 2.1, Ar-H^2), 7.66 (s, 2H, -SO_2NH_2), 3.89 (t, 2H, *J* 6.4, Cl-CH_2^4) and 2.97 (t, 2H, *J* 6.4, CH_2^3); ^{13}C NMR (75.5 MHz; d^6-DMSO) δ 168.95, 165.40, 142.20, 136.92, 129.27 (2C), 128.05, 125.33, 40.34 and 38.59; LRMS (EI): m/z = 340.0 [M^+]; Anal. Calc. (Found C, 35.2; H, 2.88; N, 7.60; S, 8.98. $C_{10}H_{10}Cl_2N_2O_5S$ requires C, 35.2; H, 2.95; N, 8.21; S, 9.40%);

3. *4-Chloro-3-(4-chloro-butyrylamino)-5-sulfamoyl-benzoic acid* **(RKG210)**



White solid (493 mg, 63%) (2.19 mmol amine scale); m.p. 210 - 214 °C; IR (NaCl) ν_{max} (cm^{-1}) 3367 (s) (N-H), 2916 (m) (C-H), 2540 (m) (O-H), 1693 (s) (C=O), 1670 (s) (C=O), 1574 (s) (N-H), 1526 (s) (C=C), 1317 (s) (S=O), 1247 (s) (C-O) and 1160 (s) (S=O); ^{1}H NMR (300 MHz; d^6-DMSO) δ 9.83 (s, 1H, CON*H*), 8.42 (d, 1H, *J* 2.0, Ar-H^1), 8.31 (d, 1H, *J* 2.1, Ar-H^2), 7.74 (s, 2H, -SO_2NH_2), 3.70 (t, 2H, *J* 6.9, Cl-CH_2^5), 2.66 (t, 2H, *J* 7.0, CH_2^3) and 2.05 (quin, 2H, *J* 6.9, CH_2^4); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 171.03, 165.43, 142.14, 137.14, 129.40, 129.17, 128.20, 125.20, 44.80, 32.81 and 27.81; LRMS (EI): m/z = 354.0 [M^+]; Anal. Calc. (Found C, 37.3; H, 3.38; N, 7.47; S, 9.41. $C_{11}H_{12}Cl_2N_2O_5S$ requires C, 37.2; H, 3.41; N, 7.89; S, 9.03%);

Chemical analysis of proposed macrocycle **RKG214**

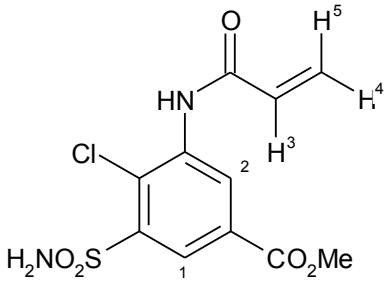


White solid (quantiative); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 8.47 (d, 1H, *J* 2.1, Ar-H^{1}), 8.13 (d, 1H, *J* 2.1, Ar-H^{2}), 7.82 (s, 2H, -SO_2NH_2), 3.72 (t, 2H, *J* 7.0, O-CH_2^{5}), 2.45 (t, 2H, *J* 7.8, CH_2^{3}) and 2.16 (quin, 2H, *J* 7.5, CH_2^{4}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 174.42, 165.08, 142.73, 139.19, 133.68, 133.29, 130.19, 128.23, 48.24, 30.26 and 18.78; LRMS (EI): m/z = 318.0 [M^{+}]; Anal. Calc. (Found C, 41.2; H, 3.49; N, 8.64; S, 9.96. $C_{11}H_{11}ClN_2O_5S$ requires C, 41.5; H, 3.48; N, 8.79; S, 10.1%);

General procedure for the synthesis of amides **(RKG251** and **RSON110)**

A mixture of acid chloride (1.2 equiv) and acetone (1.0 ml) is added dropwise to a solution of amine (**RKG262**) (400 mg, 1.5 mmol) dissolved in acetone (8.0 ml). The reaction mixture is stirred at room temperature (20 ^{o}C) for 20 hrs. The cooled mixture was poured into aqueous 1M HCl (40 ml), and the white precipitate was filtered off, washed with aqueous 1M HCl and deionized H_2O, and dried *in vacuo*.

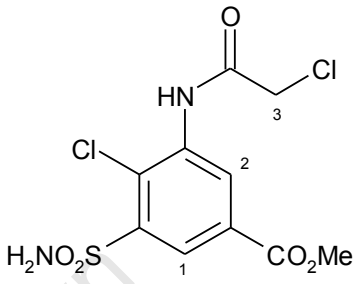
1. *3-Acryloylamino-4-chloro-5-sulfamoyl-benzoic acid methyl ester* **(RKG251)**



White solid (407 mg, 84%); m.p. 211 - 214 °C; IR (NaCl) ν_{max} (cm^{-1}) 3397 (s) (N-H), 2840 (m) (C-H), 1733 (s) (C=O), 1663 (s) (C=O), ~1600 (s) (N-H), 1525 (s) (C=C), 1336 (s) (S=O), 1244 (s) (C-O) and 1156 (s) (S=O); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 10.0 (s, 1H, CONH), 8.58 (d, 1H, *J* 2.1, Ar-H^{1}), 8.34 (d, 1H, *J* 2.1, Ar-H^{2}), 7.80 (s, 2H, -SO_2NH_2), 6.62 (dd, 1H, *J* 17.0, 10.2, C=CH^{3}), 6.33 (dd, 1H, *J* 17.0, 1.9, C=CH^{5}), 5.85 (dd, 1H, *J* 10.2, 1.8, C=CH^{4}) and 3.90 (s, 3H, -CO_2CH_3); ^{13}C NMR (75.5 MHz; d^{6}-DMSO) δ 169.01, 164.43, 163.87, 142.37, 137.14, 130.80, 128.78, 128.37, 127.98, 125.02, and 52.73; LRMS (EI): m/z = 318.0 [M^{+}];

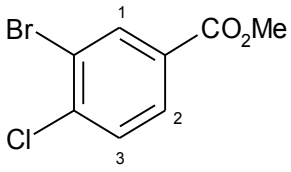
Anal. Calc. (Found C, 40.9; H, 3.42; N, 8.39; S, 9.67. $C_{11}H_{11}ClN_2O_5S$ requires C, 41.4; H, 3.48; N, 8.79; S, 10.1%);

2. *4-Chloro-3-(2-chloro-acetylamino)-5-sulfamoyl-benzoic acid methyl ester* **(RSON110)**



White solid (579 mg, 90%) (1.89 mmol amine scale); m.p. 205 - 209 °C; IR (NaCl) ν_{max} (cm^{-1}) 3397 (s) (N-H), 2840 (m) (C-H), 1733 (s) (C=O), 1663 (s) (C=O), 1600 (s) (N-H), 1525 (s) (C=C), 1336 (s) (S=O), 1244 (s) (C-O) and 1156 (s) (S=O); ^{1}H NMR (400 MHz; d^4-MeOD) δ 8.48 (d, 1H, *J* 2.1, Ar-H^1), 8.36 (d, 1H, *J* 2.1, Ar-H^2), 4.43 (s, 2H, CH_2^3) and 3.91 (s, 3H, -CO_2CH_3); ^{13}C NMR (100.6 MHz; d^4-MeOD) δ 165.77, 164.34, 142.46, 136.72, 128.83, 128.74, 128.15, 125.45, 52.78 and 42.96; LRMS (EI): m/z = 340.0 [M^+]; Anal. Calc. (Found C, 35.5; H, 2.94; N, 8.01; S, 9.03. $C_{10}H_{10}Cl_2N_2O_5S$ requires C, 35.2; H, 2.95; N, 8.21; S, 9.40%);

Synthesis of *(O-methyl)-3-bromo-4-chloro-benzoate* **(RKG236**)



3-Bromo-4-chloro-benzoic acid (1.00 g, 4.25 mmol) was dissolved in a mixture of methanol (12 ml) and conc. H_2SO_4 (0.350 ml, 6.37 mmol). The reaction mixture was stirred under reflux for 5 hrs. The reaction was allowed to cool, and the solvent was removed under reduced pressure. Deionized H_2O (10 ml) was added to the residue, and the resulting aqueous solution was extracted with EtOAc (2 x 30 ml). The organic layer was separated and washed with deionized H_2O (20 ml), saturated aqueous $NaHCO_3$ solution (20 ml), deionized H_2O (20 ml), brine (20 ml) and deionized H_2O (20 ml). After drying over anhydrous Na_2SO_4, the organic layer was filtered and the solvent was removed under reduced pressure. The residue was dried further *in vacuo*, giving a pale orange/white crystalline product.

Pale orange/white solid (1.02 g, 96%); ^{1}H NMR (400 MHz; d^6-DMSO) δ 8.28 (d, 1H, *J* 2.0, Ar-H^1), 7.90 (dd, 1H, *J* 8.4, 2.0, Ar-H^2), 7.52 (d, 1H, *J* 8.4, Ar-H^3) and 3.92 (s, 3H, -CO_2CH_3); $C_7H_6ClBrO_2$

8.2 Experimental: Chapter 4

PLEASE NOTE:

I. When d^6-DMSO is used as the solvent for NMR, spectrums for sulfonyl ureas and acyl thioureas occasionally show duplication of peaks. The first set of peaks (usually the smaller of the pair) is due to strong intramolecular hydrogen bonding of molecule with itself, and the second set of peaks (usually the larger of the pair) is due to strong intermolecular hydrogen bonding of the compound with the DMSO, respectively. For the sake of differentiation between the two, the larger (i.e. has a larger integration) of the duplicated peaks has been marked with an asterisk (i.e. *).

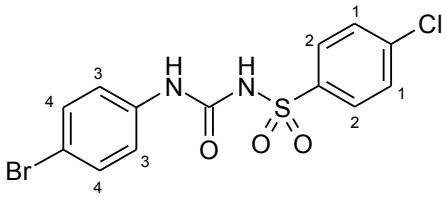
Synthesis of Sulfonyl Ureas

General procedure for the synthesis of *1-(aryl/alkyl)-3-(p-chlorophenylsulfonyl)ureas* **(RKG1491; RKG1492; RKG1493A; RKG1531; RKG1532 and RKG1533PC)**

4-Chlorophenylsulfonyl isocyanate (1.00 mmol) is added dropwise to a solution of amine (1.00 mmol) dissolved in dry THF (2.5 ml). The reaction is left stirring at room temperature (~20 ^{o}C) for 18 h under an atmosphere of N_2. The solvent is then removed under reduced pressure, to afford a residue. The residue was column chromatographed (SiO_2, EtOAc:hexane, 2:3) to give the *sulfonyl urea*.

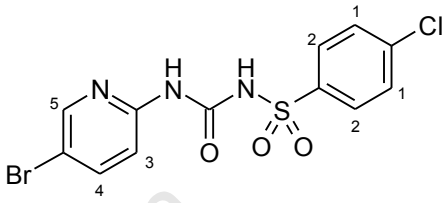
1. *1-(p-Bromophenyl)-3-(p-chlorophenylsulfonyl)urea* **(RKG1491)**

   White powder (60 mg, 15%); m.p. 178 – 182 °C; ^{1}H NMR (400 MHz; d^6-acetone) δ 9.64 (br s, 1H, N*H*), 8.48 (br s, 1H, N*H*), 8.07 (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.66 (2H, d, *J* 8.8, 2 x Ar-H^1) and 7.42 (m, 4H, 4 x Ar-H^{3+4}); ^{13}C NMR (100.6



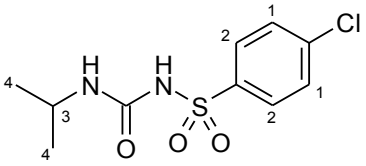
MHz, d^6-acetone) δ 149.24, 139.41, 139.30, 137.00, 131.91 (2C), 129.94 (2C), 129.35 (2C), 121.42 (2C) and 115.79; LRMS (EI): m/z = 390.0 [M^+]; Anal. Calc. (Found C, 40.6; H, 2.57; N, 7.08; S, 8.14. $C_{13}H_{10}N_2O_3SClBr$ requires C, 40.1; H, 2.59; N 7.19; S, 8.23%);

2. *1-(p-Bromo-o-N-pyridinyl)-3-(p-chlorophenylsulfonyl)urea* **(RKG1492)**



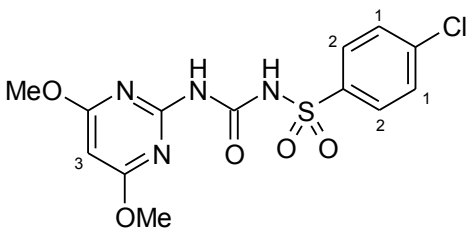
White powder (23 mg, 6%); m.p. 170 – 174 °C; ^{1}H NMR (300 MHz; d^6-acetone) δ 11.52 (br s, 1H, N*H*), 9.15 (br s, 1H, N*H*), 8.41 (d, 1H, *J* 2.6, Ar-H^5), 8.09 (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.96 (dd, 1H, *J* 8.8, 2.6, Ar-H^4), 7.67 (d, 2H, *J* 8.8, 2 x Ar-H^1) and 7.43 (d, 1H, *J* 8.8, Ar-H^3); ^{13}C NMR (75.5 MHz, d^6-acetone) δ 147.76, 142.97, 141.76, 139.58, 139.19, 138.91, 130.19 (2C), 129.33 (2C), 114.50 and 113.41; LRMS (EI): m/z = 389.0 [M^+];

3. *1-(iso-Propyl)-3-(p-chlorophenylsulfonyl)urea* **(RKG1493A)**



White powder (55 mg, 45%); m.p. 140 – 144 °C; ^{1}H NMR (300 MHz; d^6-acetone) δ 9.30 (br s, 1H, N*H*), 8.01 (d, 2H, *J* 8.9, 2 x Ar-H^2), 7.65 (d, 2H, *J* 8.9, 2 x Ar-H^1), 6.16 (d, 1H, *J* 6.6, N*H*), 3.77 (oct, 1H, *J* 6.6, CH^3) and 1.09 (d, 6H, *J* 6.6, 2 x C$H_3{}^4$); ^{13}C NMR (75.5 MHz, d^6-acetone) δ 150.35, 139.74, 139.11, 129.61 (2C), 129.31 (2C), 42.32, 22.06 and 22.04; LRMS (EI): m/z = 276.0 [M^+]; Anal. Calc. (Found C, 43.1; H, 4.70; N, 9.39; S, 11.0. $C_{10}H_{13}N_2O_3SCl$ requires C, 43.4; H, 4.73; N 10.1; S, 11.6%);

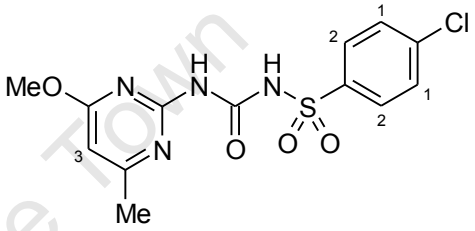
4. *1-(m-Dimethoxy-o-N,N-pyrimidinyl)-3-(p-chlorophenylsulfonyl)urea* **(RKG1531)**



White flakes (298 mg, 80%); m.p. 184 - 187°C; IR (Thin film) v_{max} (cm^{-1}) 3399 (w) (N-H), 3232 (w) (N-H), 3087-2935 (br m) (CH), 1718 (s) (C=O), 1610 (s) (C=C), 1577 (s) (C=C), 1491 (s) (C=C), 1451 (s) (C=C), 1368 (s) (S=O), 1198 (s) (S=O), 1162 (s) (S=O); ^{1}H NMR (400 MHz; d^6-DMSO) δ 12.62* (br s, 1H, N*H*), 10.51* (s, 1H, N*H*), 8.01* (d, 2H, *J* 8.8, 2 x

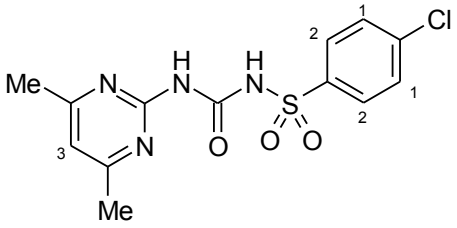
Ar-H^2), 7.81 (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.70* (d, 2H, *J* 9.0, 2 x Ar-H^1), 7.63 (d, 2H, *J* 8.8, 2 x Ar-H^1), 5.96* (s, 1H, Ar-H^3), 5.32 (s, 1H, Ar-H^3), 3.90* (s, 6H, 2 x -OCH_3) and 3.74 (s, 6H, 2 x -OCH_3); ^{13}C NMR (100.6 MHz, d^6-DMSO) δ 171.13 (2C), 155.90, 148.57, 138.75, 137.71, 129.74* (2C), 129.24* (2C), 128.96 (2C), 127.52 (2C), 83.64*, 77.82, 54.53* (2C) and 53.05 (2C); LRMS (EI): m/z = 372.0 [M^+]; Anal. Calc. (Found C, 41.8; H, 3.35; N, 14.2; S, 8.37. $C_{13}H_{13}N_4O_5SCl$ requires C, 41.9; H, 3.51; N 15.0; S, 8.60%);

5. *1-(m-Methoxy-methyl-o-N,N-pyrimidinyl)-3-(p-chlorophenylsulfonyl)urea* **(RKG1532)**



White powder (240 mg, 67%); m.p. 179 – 183 °C; ^{1}H NMR (400 MHz; d^6-DMSO) δ 13.27* (br s, 1H, N*H*), 10.55* (br s, 1H, N*H*), 7.99* (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.81 (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.68* (d, 2H, *J* 8.5, 2 x Ar-H^1), 7.63 (d, 2H, *J* 8.5, 2 x Ar-H^1), 6.51* (s, 1H, Ar-H^3), 5.88 (s, 1H, Ar-H^3), 3.90* (s, 3H, -OCH_3), 3.76 (s, 3H, -OCH_3), 2.34* (s, 3H, -CH_3) and 2.12 (s, 3H, -CH_3); ^{13}C NMR (100.6 MHz, d^6-DMSO) δ 170.25, 166.65, 156.22, 149.55, 138.42, 138.31, 129.60* (2C), 129.14* (2C), 128.96 (2C), 127.53 (2C), 100.74, 54.18 and 22.63; LRMS (EI): m/z = 356.0 [M^+]; Anal. Calc. (Found C, 43.8; H, 3.56; N, 15.7; S, 8.89. $C_{13}H_{13}N_4O_4SCl$ requires C, 43.8; H, 3.67; N 15.7; S, 8.99%);

6. *1-(m-Dimethyl-o-N,N-pyrimidinyl)-3-(p-chlorophenylsulfonyl)urea* **(RKG1533PC)**



White powder (259 mg, 76%); m.p. 196 – 199 °C; ^{1}H NMR (400 MHz; d^6-DMSO) δ 13.27* (br s, 1H, N*H*), 10.51* (s, 1H, N*H*), 8.01* (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.83 (d, 2H, *J* 8.8, 2 x Ar-H^2), 7.70* (d, 2H, *J* 9.2, 2 x Ar-H^1), 7.65 (d, 2H, *J* 8.8, 2 x Ar-H^1), 7.00* (s, 1H, Ar-H^3), 6.33 (s, 1H, Ar-H^3), 2.40* (s, 6H, 2 x -CH_3) and 2.08 (s, 6H, 2 x -CH_3); ^{13}C NMR (100.6 MHz, d^6-DMSO) δ 167.65, 166.68, 156.37, 149.09, 142.95, 136.47, 129.64 (2C), 129.20 (2C), 128.96* (2C), 127.53* (2C), 114.77, 108.62*, 23.20 and 23.16; LRMS (EI): m/z = 340.0 [M^+]; Anal.

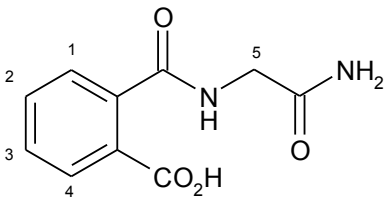
Calc. (Found C, 45.7; H, 3.78; N, 16.0; S, 9.20. $C_{13}H_{13}N_4O_3SCl$ requires C, 45.8; H, 3.85; N 16.4; S, 9.41%);

Synthesis of Imidazolinone Derivatives

General procedure for the synthesis of *N-carbamoyl-alkyl/aryl-phthalamic acids* **(RKG114A; RKGANT3; RKG125A and RKG123A)**

The amino-amide hydrochloride (7.2 mmol) was dissolved in a mixture of triethylamine (14.2 mmol) in deionised water (5 ml) and stirred vigorously for 10 - 15 minutes. This mixture was added to a solution of phthalic anhydride (1.05 g, 7.1 mmol) dissolved in dioxane (15 ml). The resulting solution was stirred at room temperature for 0.5 h, and then concentrated via removal of the solvent under reduced pressure, leaving a syrup/glassy residue. An aqueous solution of 1M HCl (60 ml) was added to the residue, and the mixture was stirred for 5 min. The mixture was placed in the fridge (~5 ºC) overnight, to allow precipitation. The precipitate was collected via filtration, and was washed successively with an aqueous solution of 1M HCl (3 x 5ml), and cold diethyl ether (1 x 5ml). The collected solid was dried further *in vacuo* on a vacuum pump, affording the *phthalamic acid*.

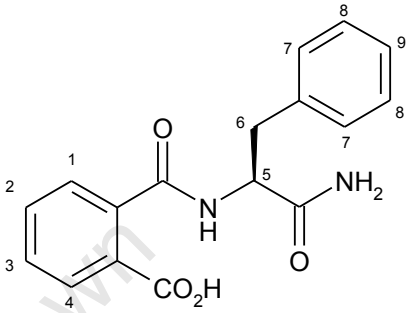
1. *N-Carbamoylmethyl-phthalamic acid monohydrate* **(RKG114A)**



White crystalline flakes (6.22g, 77%) (35.4 mmol scale); m.p. 262 – 265 °C; IR (Thin film) ν_{max} (cm^{-1}) 3536 (w) (COO-H), 3435 (m) (N-H), 3391 (m) (N-H), 3203 (br m) (C-H), 1686 (s) (C=O), 1657 (s) (C=O), 1642 (s) (C=O), 1600 - 1530 (4 x s), (C=C) and 1263 (s) (C-N); ^{1}H NMR (300 MHz; d^6-DMSO) δ 13.18 (br s, 1H, COO*H*), 8.61 (t, 1H, *J* 5.9, CON*H*), 7.84 (dd, 1H, *J* 7.5, 1.1, Ar-H^4), 7.61 (td, 1H *J* 7.5, 1.5, Ar-H^2), 7.53 (td, 1H, *J* 7.5, 1.5, Ar-H^3), 7.48 (dd, 1H, *J* 7.5, 1.1, Ar-H^1), 7.26 (s, 2H, CONH_2), 3.74 (d, 2H, *J* 6.0, CH_2^5) and 3.33 (br s, H_2O); ^{13}C NMR (75.5 MHz, d^6-DMSO) δ 171.27, 168.87, 168.01, 138.51, 131.67,

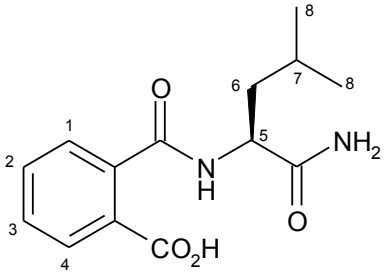
129.59, 129.37, 129.21, 127.82 and 42.73; LRMS (EI): m/z = 222.1 [M^+]; Anal. Calc. (Found C, 50.0; H, 4.77; N, 11.6. $C_{10}H_{10}N_2O_4.H_2O$ requires C, 50.0; H, 5.04; N 11.7%);

2. *N-(1-Carbamoyl-2-phenyl-ethyl)-phthalamic acid* **(RKG123A)**



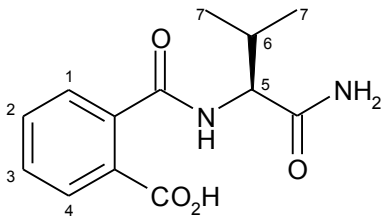
White powder (2.06 g, 93%); m.p. 190 – 193 °C; ^{1}H NMR (400 MHz; d^6-DMSO) δ 8.55 (br d, 1H, *J* 8.5, CON*H*), 7.81 (d, 1H, *J* 7.7, Ar-H^4), 7.53 (t, 1H, *J* 7.5, Ar-H^2), 7.48 (t, 1H, *J* 7.5, Ar-H^3), 7.28 (m, 7H, 5 x Ar-H^{7+8+9} and CONH_2), 6.97 (d, 1H, *J* 7.5, Ar-H^1), 4.55 (m, 1H, CH^5), 3.28 (d, 1H, *J* 14.1, CH^6H) and 2.81 (t, 1H, *J* 14.1, CHH^6); ^{13}C NMR (100.6 MHz, d^6-DMSO) δ 173.79, 169.14, 168.66, 139.48, 139.35, 132.38, 130.10, 130.06, 129.82, 129.73 (2C), 128.76 (2C), 128.12, 126.86, 54.89 and 37.12; LRMS (EI): m/z = 312.1 [M^+]; Anal. Calc. (Found C, 65.4; H, 4.53; N, 8.79. $C_{17}H_{16}N_2O_4$ requires C, 65.4; H, 5.16; N 8.97%);

3. *N-(1-Carbamoyl-3-methyl-butyl)-phthalamic acid* **(RKG125A or RKG166A)**



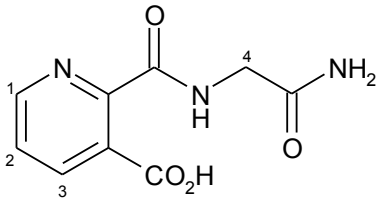
White powder; Yield: 1.95 g, (99%); m.p. 199 – 201 °C; IR (Thin film) ν_{max} (cm^{-1}) 3540 (w) (COO-H), 3457 (m) (N-H), 3333 (m) (N-H), 2949 (m) (C-H), 1700 (m) (C=O), 1642 (s) (C=O), 1628 (s) (C=O), 1530 (s) (C=C), 1451 (s) (C=C) and 1274 (s) (C-N); ^{1}H NMR (300 MHz; d^6-DMSO) δ 13.20 (br s, 1H, COO*H*), 8.45 (d, 1H, *J* 7.9, CON*H*), 7.84 (dd, 1H, *J* 7.9, 1.2, Ar-H^4), 7.61 (td, 1H, *J* 7.3, 1.2, Ar-H^2), 7.52 (td, 1H, *J* 7.3, 1.2, Ar-H^3), 7.40 (dd, 1H, *J* 7.9, 1.2, Ar-H^1), 7.28 (s, 1H, CONH*H*), 7.11 (s, 1H, CON*H*H), 4.31 (m, 1H, CH^5), 1.72 (m, 1H, CH^6H), 1.59 (m, 2H, CHH^6 and CH^7) and 0.90 (dd, 6H, *J* 6.4, 1.9, 2 x CH_3^8); ^{13}C NMR (75.5 MHz, d^6-DMSO) δ 174.31, 168.63, 167.94, 138.88, 131.71, 129.48, 129.37, 129.08, 127.84, 51.44, 39.91, 24.37, 23.20 and 21.19; LRMS (EI): m/z = 278.1 [M^+]; Anal. Calc. (Found C, 60.3; H, 6.47; N, 9.81. $C_{14}H_{18}N_2O_4$ requires C, 60.4; H, 6.52; N 10.1%);

4. *N-(1-Carbamoyl-2-methyl-propyl)-phthalamic acid* **(RKGANT3)**



White flakes (1.95 g, 99%); m.p. 213 – 216 °C; IR (Thin film) ν_{max} (cm^{-1}) 3573 (br w) (COO-H), 3406 (m) (N-H), 3312 (s) (N-H), 2870 (s) (C-H), 1693 (s) (C=O), 1635 (s) (C=O), 1642 (s) (C=O), 1595 (s) (C=C), 1537 (s) (C=C), 1487 (s) (C=C) and 1263 (C-N); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 8.19 (br d, 1H, *J* 8.8, CON*H*), 7.80 (dd, 1H, *J* 7.7, 1.1, Ar-H^{4}), 7.58 (td, 1H, *J* 7.5, 1.3, Ar-H^{2}), 7.50 (td, 1H, *J* 7.7, 1.5, Ar-H^{3}), 7.38 (dd, 1H, *J* 7.7, 1.3, Ar-H^{1}), 7.28 (s, 1H, CONH*H*), 7.09 (s, 1H, CON*H*H), 4.24 (dd, 1H, *J* 8.8, 5.8, CH^{5}), 2.16 (oct, 1H, *J* 6.0, CH^{6}), 0.92 (d, 3H, *J* 6.8, CH_3^{7}) and 0.87 (d, 3H, *J* 6.8, CH_3^{7}); ^{13}C NMR (100.6 MHz, d^{6}-DMSO) δ 172.99, 168.81, 167.83, 138.72, 131.46, 129.73, 129.19, 128.98, 127.86, 58.13, 29.59, 19.36 and 17.67; LRMS (EI): m/z = 264.1 [M^{+}]; Anal. Calc. (Found C, 59.7; H, 6.20; N, 10.7. $C_{13}H_{16}N_2O_4$ requires C, 59.1; H, 6.10; N 10.6%);

Synthesis of *3-(N-Carbamoylmethyl)carboxamide-nicotinic acids* **(RKG164A)**



Glycinamide hydrochloride (1.00 g, 9.05 mmol) was dissolved in a mixture of triethylamine (2.5 ml, 18.1 mmol) in deionised water (5 ml) and stirred vigorously for 10 - 15 minutes. This mixture was added to a solution of 2,3-pyridine dicarboxylic anhydride (1.35 g, 9.05 mmol) dissolved in dioxane (20 ml). The resulting solution was stirred at room temperature (~20 ^{o}C) for 1 h, and then concentrated via removal of the solvent under reduced pressure, leaving a syrup/glassy residue. An aqueous solution of 1M HCl (50 ml) was added to the residue, and the mixture was stirred for 5 min. The mixture was placed in the freezer (~ -12 ºC) overnight, to allow precipitation. The precipitate was collected via filtration, and was washed successively with an aqueous solution of cold 1M HCl (3 x 5ml), and cold diethyl ether (1 x 5ml). The collected solid was dried further *in vacuo* on vacuum pump, affording the *nicotinic acid*.

Small white fluffy crystals; Yield: 0.430 g, (21%); m.p. 159 – 164 °C; IR (Thin film) ν_{max} (cm^{-1}) 3580 (br w) (COO-H), 3470 (br w) (N-H), 3406 (m) (N-H), 2913 (m) (C-H), 1711 (m) (C=O), 1682

(s) (C=O), 1642 (s) (C=O), 1541 (s) (C=C), 1433 (s) (C=C), and 1263 (s) (C-N); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 8.79 (t, 1H, *J* 5.8, CON*H*), 8.71 (dd, 1H, *J* 4.9, 1.5, Ar-H^{1}), 8.07 (dd, 1H, *J* 7.9, 1.5, Ar-H^{3}), 7.64 (dd, 1H, *J* 7.6, 4.6, Ar-H^{2}), 7.37 (br s, 1H, CONH*H*), 7.18 (br s, 1H, CON*H*H) and 3.84 (d, 2H, *J* 5.8, CH_2^{4}); ^{13}C NMR (100.6 MHz, d^{6}-DMSO) δ 170.83, 167.91, 164.86, 149.86, 149.07, 136.95, 128.57, 125.59 and 41.91; LRMS (EI): m/z = 223.0 [M^{+}];

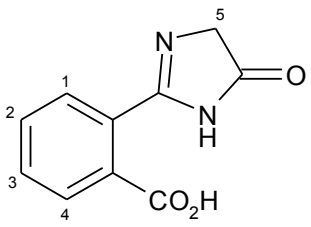
General procedure for the synthesis of *2-[4,5-dihydro-4-alkyl/aryl-5-oxo-1H-imidazol-2-yl]-benzoic acids* **(RKGAS4EGLY; RKGAS4ELEU; RKGAS4EPA and RKGAS4EVAL)**

The *phthalamic acid* (200 mg) is added to deionised water (2.5 ml). Four molar equivalents of NaOH is added to the mixture and stirred. The reaction vessel is sealed under N_2, and the reaction is heated to reflux (~ 115 ºC) for 3 hrs. The reaction was then allowed to stand and cool down to room temperature. Concentrated HCl (10.18M) was added dropwise until a pH of between 2 – 3 was reached. The mixture was left to stand so that precipitation could occur. The precipitate was filtered off, and washed successively with cold aqueous 1M HCl, and cold deionised water. The resulting product was dried further *in vacuo* under vacuum, giving the pure *benzoic acid*.

NOTE: when **RKG123A** and **RKG125A** were used as starting materials, the final product precipitated on the addition of the concentrated HCl. For **RKG114A** and **RKGANT3**, the reaction mixture (at pH of ~2) was placed in the fridge overnight. However, no precipitate was formed. The cooled mixture was left to warm up to room temperature, and a combination of being at room temperature and lots of scratching resulted in the product precipitating out.

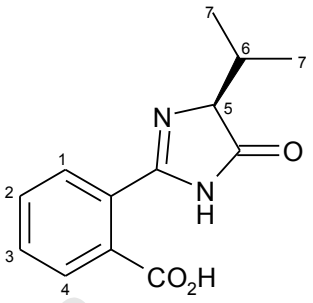
1. *2-[4,5-Dihydro-5-oxo-1H-imidazol-2-yl]-benzoic acid* **(RKGAS4EGLY)**

   White powder (71.2 mg, 42%); m.p. 89 – 93 °C; ^{1}H NMR (400 MHz; d^{4}-MeOD) δ 7.94 (dd, 1H, *J* 7.7, 1.5), Ar-H^{4}), 7.61 (td, 1H, *J* 7.7, 1.5, Ar-H^{2}), 7.55 (m, 2H, *J* 1.5, Ar-H^{1} and Ar-H^{3}) and 4.08 (s, 2H, CH_2^{5}); ^{13}C NMR (100.6 MHz, d^{4}-MeOD) δ 171.74, 171.68, 168.20, 138.16, 131.83, 130.05, 129.77, 129.56, 127.82 and 41.14; LRMS (EI): m/z



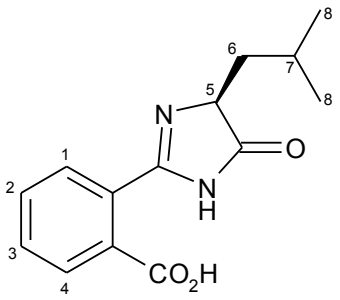
= 204.0 [M^+];

2. *2-[4,5-Dihydro-4S-iso-propyl-5-oxo-1H-imidazol-2-yl]-benzoic acid* **(RKGAS4EVAL)**



White powder (84.7 mg, 45%); m.p. 167 – 171 °C; IR (Thin film) ν_{max} (cm^{-1}) 3409 (br m) (COO-H), 3248 (br m) (N-H), 2961 (m) (C-H), 1709 (s) (C=O), 1640 (s) (C=O), 1600 (s) (C=C), 1545 (s) (C=C) and 1275 (s) (C-N); ^{1}H NMR (300 MHz; d^4-MeOD) 7.95 (dd, 1H, *J* 7.6, 1.5, Ar-H^4), 7.61 (td, 1H, *J* 7.6, 1.6, Ar-H^2), 7.53 (dt, 1H, *J* 7.6, 1.5, Ar-H^3), 7.48 (dd, 1H, *J* 7.6, 1.5, Ar-H^1), 4.52 (d, 1H, *J* 5.8, CH^5), 2.26 (m (oct), 1H, *J* 7.0, 5.8, CH^6), 1.07 (d, 3H, *J* 7.0, C$H_3{}^7$) and 1.04 (d, 3H, *J* 7.0, C$H_3{}^7$); ^{13}C NMR (75.5 MHz, d^4-MeOD) δ 174.70, 172.78, 169.24, 139.68, 132.98, 131.22, 130.80, 130.54, 129.03, 59.70, 31.89, 19.63 and 18.66; LRMS (EI): m/z = 246.1 [M^+];

3. *2-[4,5-Dihydro-4S-iso-butyl-5-oxo-1H-imidazol-2-yl]-benzoic acid* **(RKGAS4ELEU)**

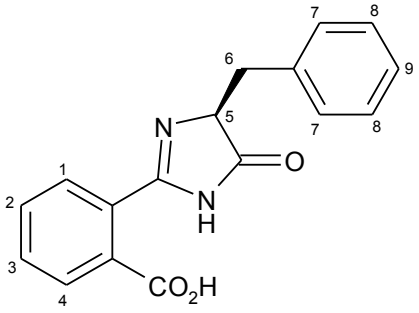


White powder (101 mg, 54%); m.p. 149 – 153 °C; IR (Thin film) ν_{max} (cm^{-1}) 3582 (w) (COO-H), 3248 (m) (N-H), 2955 (m) (C-H), 2866 (m) (C-H), 1700 (s) (C=O), 1643 (s) (C=O), 1600-1551 (3 x s) (C=C) and 1278 (s) (C-N); ^{1}H NMR (300 MHz; d^6-DMSO) δ 12.65 (br s, 1H, CO_2H), 8.53 (s, 1H, N*H*), 7.78 (dd, 1H, *J* 7.6, 1.5, Ar-H^4), 7.62 (td, 1H, *J* 7.6, 1.5, Ar-H^2), 7.54 (dt, 1H, *J* 7.6, 1.5, Ar-H^3), 7.44 (dd, 1H, *J* 7.6, 1.5, Ar-H^1), 4.42 (m, 1H, CH^5), 1.80 (m, 1H, CH^7), 1.69 (m, 1H, CHH^6), 1.55 (m, 1H, CH^6H) and 0.93 (d, 6H, *J* 6.4, 2 x C$H_3{}^8$); ^{13}C NMR (75.5 MHz, d^4-MeOD) δ 173.97, 168.25, 167.82, 137.97, 130.96, 130.77, 129.13, 129.00, 127.70, 50.48, 39.75, 24.12, 22.95 and 21.21; LRMS (EI): m/z = 260.1 [M^+];

4. *2-[4,5-Dihydro-4S-benzyl-5-oxo-1H-imidazol-2-yl]-benzoic acid* **(RKGAS4EPA)**

White powder (141 mg, 75%); m.p. 155 – 159 °C; IR (Thin film) ν_{max} (cm^{-1}) 3409 (w) (COO-H), 3379 (m) (N-H), 3021 (m) (C-H), 2818 (br m) (C-H), 1706 (s) (C=O), 1670 (s) (C=O), 1599 (m) (C=N), 1524 (s) (C=C), 1453 (m) (C=C) and 1290 (s) (C-N); ^{1}H NMR (400

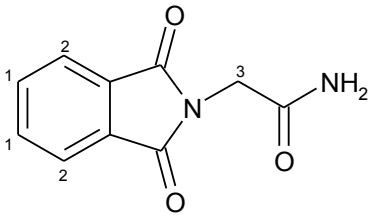
MHz; d^4-MeOD) δ 7.92 (dd, 1H, *J* 7.4, 1.6, Ar-H^4), 7.55 (td, 1H, *J* 7.5, 1.6, Ar-H^2), 7.50 (dt, 1H, *J* 7.4, 1.6, Ar-H^3), 7.30 (m, 5H, 5 x Ar-H^{1+7+8}), 7.21 (m, 1H, Ar-H^9), 4.89 (dd, 1H, *J* 6.0, 7.7, CH^5), 3.26 (dd, 1H, *J* 13.9, 5.9, CHH^6) and 3.12 (dd, 1H, *J* 13.9, 7.7, CH^6H); ^{13}C NMR (100.6 MHz, d^4-MeOD) δ 173.23, 171.03, 168.10, 138.17, 137.23, 131.71, 130.05, 129.71, 129.46, 129.30 (2C), 128.20 (2C), 127.70, 126.58, 54.25 and 37.31; LRMS (EI): m/z = 294.1 [M^+];



General procedure for the synthesis of *1,3-dihydro-1,3-dioxo-α-alkyl/aryl-2H-isoindole-2-acetamides* **(RKG119A; RKG145A; RKG131A and RKG133A)**

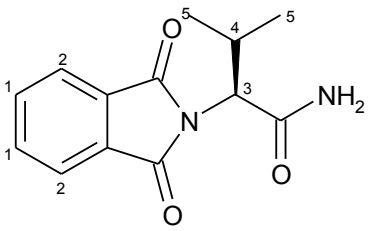
The *phthalamic acid* (0.500 g) and NaOH (0.4 molar equiv) were added to NMP (0.75 ml). The reaction mixture was heated with stirring to 130 - 140 °C for 18 hrs. The reaction mixture was then allowed to cool down to room temperature, and an aqueous solution of 1M HCl was added until a pH of 2 - 3 was obtained. The reaction mixture was column chromatographed (SiO_2, Acetone:DCM, 30:70) to obtain the *2H-isoindole-2-acetamides*. The product was further purified by recrystallization from acetone.

1. *1,3-Dihydro-1,3-dioxo-2H-isoindole-2-acetamides* **(RKG119A)**



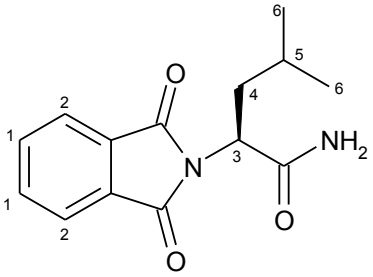
Small white fluffy crystals (1.13 g, 67%) (2.00g scale in deionsied H_20 (3.0ml) at 100 ^oC); m.p. 260 – 262 °C; ^{1}H NMR (400 MHz; d^6-DMSO) δ 7.87 (m, 4H, 4 x Ar-H^{1+2}), 7.65 (br s, 1H, CONH*H*), 7.19 (br s, 1H, CON*H*H) and 4.14 (s, 2H, CH_2^3); ^{13}C NMR (100.6 MHz, d^6-DMSO) δ 168.59 (2C), 168.22, 135.19 (2C), 132.45 (2C), 123.83 (2C) and 40.65; LRMS (EI): m/z = 204.0 [M^+]; Anal. Calc. (Found C, 58.5; H, 4.02; N, 13.8. $C_{10}H_8N_2O_3$ requires C, 58.8; H, 3.95; N 13.7%);

2. *1,3-Dihydro-(αS)-(1-methylethyl)-1,3-dioxo-2H-isoindole-2-acetamides* **(RKG145A)**



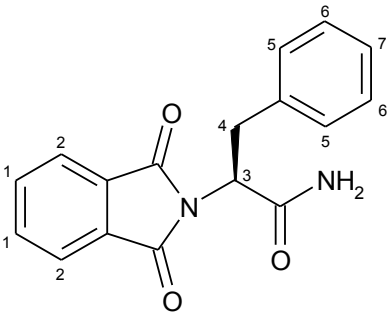
White fluffy crystals (71.2 mg, 15%); m.p. 190 – 191 °C; ^{1}H NMR (300 MHz; d^{6}-acetone) δ 7.89 (m, 4H, 4 x Ar-H^{1+2}), 7.01 (br s, 1H, CONH*H*), 6.45 (br s, 1H, CON*H*H), 4.38 (d, 1H, *J* 9.6, CH^{3}), 2.84 (m, 1H, CH^{4}), 1.12 (d, 3H, *J* 6.7, CH_3^{5}) and 0.85 (d, 3H, *J* 6.7, CH_3^{5}); ^{13}C NMR (75.5 MHz, d^{6}-acetone) δ 169.91, 168.11 (2C), 134.64 (2C), 132.05 (2C), 123.35 (2C), 60.51, 27.74, 20.42 and 19.12; LRMS (EI): m/z = 246.1 [M^{+}]; Anal. Calc. (Found C, 63.6; H, 5.35; N, 11.2. $C_{13}H_{14}N_2O_3$ requires C, 63.4; H, 5.73; N 11.4%);

3. *1,3-Dihydro-(αS)-(2-methylpropyl)-1,3-dioxo-2H-isoindole-2-acetamides* **(RKG131A)**



White powder (134 mg, 29%); m.p. 162 - 165°C; ^{1}H NMR (400 MHz; d^{6}-acetone) δ 7.87 (m, 4H, 4 x Ar-H^{1+2}), 7.09 (br s, 1H, CONH*H*), 6.48 (br s, 1H, CON*H*H), 4.85 (dd, 1H, *J* 11.7, 4.4, CH^{3}), 2.39 (m, 1H, CHH^{4}), 1.97 (m, 1H, CH^{4}H), 1.47 (m, 1H, CH^{5}), 0.93 (d, 3H, *J* 6.6, CH_3^{6}) and 0.91 (d, 3H, *J* 6.8, CH_3^{6}); ^{13}C NMR (100.6 MHz, d^{6}-acetone) δ 170.72, 168.06 (2C), 134.48 (2C), 132.40 (2C), 123.20 (2C), 52.19, 37.27, 25.36, 22.94 and 20.60; LRMS (EI): m/z = 260.1 [M^{+}]; Anal. Calc. (Found C, 65.4; H, 6.33; N, 10.3. $C_{14}H_{16}N_2O_3$ requires C, 64.6; H, 6.20; N 10.8%);

4. *1,3-Dihydro-1,3-dioxo-(αS)-(phenylmethyl)-2H-isoindole-2-acetamides* **(RKG133A)**

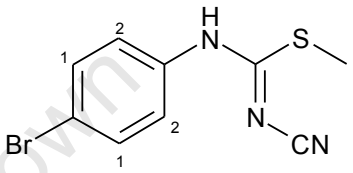


Pale brown/white powder (173 mg, 37%); m.p. 230 – 233 °C; ^{1}H NMR (400 MHz; d^{6}-acetone/d^{6}-DMSO) δ 7.77 (m, 4H, 4 x Ar-H^{1+2}), 7.65 (br s, 1H, CONH*H*), 7.21 (br s, 1H, CON*H*H), 7.11 (m, 5H, 5 x Ar-H^{5+6+7}), 4.93 (dd, 1H, *J* 12.0, 4.5, CH^{3}), 3.53 (dd, 1H, *J* 13.9, 4.5, CHH^{4}) and 3.37 (dd, 1H, *J* 13.9, 12.0, CH^{4}H); ^{13}C NMR (100.6 MHz, d^{6}-acetone/d^{6}-DMSO) δ 170.27, 168.08 (2C), 138.52, 134.91 (2C), 132.07 (2C), 129.22 (2C), 128.77 (2C), 126.93 , 123.50 (2C),

55.03 and 34.45; LRMS (EI): m/z = 294.1 [M^+]; Anal. Calc. (Found C, 69.0; H, 4.79; N, 9.44. $C_{17}H_{14}N_2O_3$ requires C, 69.4; H, 4.79; N 9.52%);

Synthesis of Sulfonylcyanoguanidines

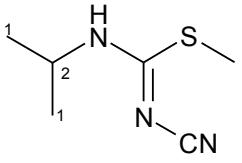
Synthesis of *N-cyano-N'-(4-bromophenyl)-S-methyl-isothiourea* (**RKG136A)**



Dimethyl *N*-cyanodithioiminecarbonate (0.825 g, 5.64 mmol) and 4-bromoaniline (1.087 g, 6.31 mmol) were added to absolute ethanol (2.5 ml), and the mixture was refluxed at 80 ºC for 17 hrs. The reaction mixture was allowed to cool down to room temperature, and then placed in an ice bath. A white precipitate formed, and was filtered off. The white precipitate was recrystallized from absolute ethanol, and washed with cold absolute ethanol.

White powder (0.933g, 61%); m.p. 193 – 195 °C; IR (Thin film) ν_{max} (cm^{-1}) 3406 (br w) (N-H), 3188 (w) (C-S), 3007 (w) (C-H), 2159 (s) (C≡N), 1585 (m) (C=N), 1509 (s) (C=C), 1487 (s) (C=C) and 1285 (m) (C-N); ^{1}H NMR (400 MHz; d^6-DMSO) δ 10.15 (br s, 1H, N*H*), 7.57 (d, 2H, *J* 8.8, 2 x Ar-H^1), 7.42 (d, 2H, *J* 9.0, 2 x Ar-H^2) and 2.70 (s, 3H, -SCH_3); ^{13}C NMR (100.6 MHz, d^6-DMSO) δ 170.87, 137.37, 132.36 (2C), 126.63 (2C), 119.20, 115.24 and 15.59; LRMS (EI): m/z = 271.0 [M^+]; Anal. Calc. (Found C, 40.2; H, 2.69; N, 15.7; S, 11.7. $C_9H_8N_3SBr$ requires C, 40.0; H, 2.98; N 15.6; S, 11.9%);

Synthesis of *N-cyano-N'-iso-propyl-S-methyl-isothiourea* (**RKG150A)**



Dimethyl *N*-cyanodithioiminecarbonate (0.500 g, 3.42 mmol) and isopropylamine (0.298 ml, 207 mg, 3.50 mmol)were added to absolute ethanol (2 ml), and the mixture was refluxed at 80 ^{o}C for 4 hrs. The reaction mixture was allowed to cool down to room temperature, and then the solvent was removed under reduced pressure to give a pale yellow/white residue. An aqueous solution of

0.1M HCl (10 ml) was added, and the resulting solution was extracted with chloroform (3 x 20ml). The combined organic washes were dried over anhydrous Na_2SO_4. The organic phase was filtered, and the solvent was removed under reduced pressure. The resulting product was dried further *in vacuo*.

White powder (0.556 g, quantitative); m.p. 113 – 116 °C; ^{1}H NMR (300 MHz; d^6-DMSO) δ 8.04 (br s, 1H, N*H*), 4.07 (br m, 1H, C*H*^{2}), 2.57 (s, 3H, -SC*H*$_3$) and 1.15 (d, 6H, *J* 6.6, 2 x C*H*$_3$^{1}); ^{13}C NMR (75.5 MHz, d^6-DMSO) δ 168.60, 115.80, 45.57, 21.29 (2C) and 14.11; LRMS (EI): m/z = 157.0 [M^+];

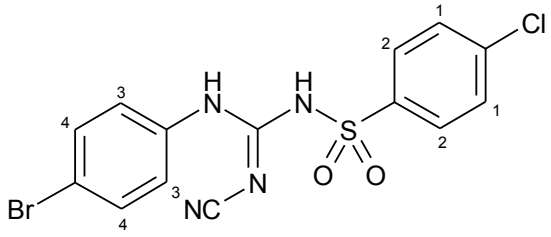
Synthesis of *N-[(cyanoamino)(alkyl/arylamino)methylene]-4-chloro-benzenesulfonamide* (**RKG139F3 and RKG151)**

The *isothiourea* (1.59 mmol) was added to a solution of 4-chloro-benzenesulfonamide (1.59 mmol) and K_2CO_3 (1.59 mmol) in DMF (3 ml). The reaction was refluxed at 120 ºC for 20 hrs. The reaction mixture was allowed to cool down to room temperature and deionised H_2O (12 ml) was added, followed by an aqueous solution of 2.5M NaOH (1 ml). The solution was extracted with diethyl ether (3 x 15ml), and the resulting aqueous phase pH was adjusted to 1 with an aqueous solution of 1M HCl. The precipitate was collected via filtration, and was washed with cold H_2O. The precipitate was dryed *in vacuo*, and was further purified via recrystallization from methanol to obtain the *sulfonamide (sulfonylcyanoguanidine)*.

NOTE: for the synthesis of **RKG139F3**, the final product was obtained via column chromatography (SiO_2, MeOH:DCM, 5:95 to 20:80) of the reaction mixture before the work-up stage as described above.

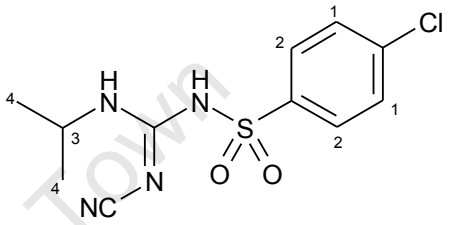
1. *N-[(Cyanoamino)(p-bromophenylamino)methylene]-chloro-benzenesulfonamide* **(RKG139F3)**

   Pale brown/white powder (195.1 mg, 32%) (1.48 mmol scale); m.p. 192 – 195 °C; IR (Thin film) ν_{max} (cm^{-1}) 3319 (br w) (N-H), 2167 (s) (C≡N), 1603 (m) (C=N), 1556 (s) (C=C), 1487 (s) (C=C), 1346 (s)



(S=O) and 1133 (s) (S=O); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 8.91 (br s, 1H, N*H*), 7.76 (d, 2H, *J* 8.5, 2 x Ar-H^{2}), 7.53 (d, 2H, *J* 8.5, 2 x Ar-H^{1}) and 7.39 (m (s), 4H, 4 x Ar-H^{3+4}); ^{13}C NMR (100.6 MHz, d^{6}-DMSO) δ 159.65, 144.85, 139.05, 136.20, 131.87 (2C), 129.33 (2C), 128.25 (2C), 123.47 (2C), 118.88 and 115.23; LRMS (EI): m/z = 412.0 [M^{+}]; Anal. Calc. (Found C, 40.9; H, 2.78; N, 14.0; S, 8.01. $C_{14}H_{10}N_4O_2SClBr$ requires C, 40.7; H, 2.44; N 13.5; S, 7.75%);

2. *N-[(Cyanoamino)(isopropyl)methylene]-chloro-benzenesulfonamide* **(RKG151A)**



White flake-like crystals (237.8 mg, 50%); m.p. 134 – 138 °C; IR (Thin film) ν_{max} (cm^{-1}) 3377 (m) (N-H), 3080 (w) (N-H), 2971 (m) (CH), 2717 (br m) (CH), 2188 (C≡N), 1617 (s) (C-N), 1559 (s) (C=C), 1440 (s) (C=C), 1350 (s) (S=O) and 1162 (s) (S=O); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 7.89 (d, 2H, *J* 8.8, 2 x Ar-H^{2}), 7.72 (d, 2H, *J* 9.0, 2 x Ar-H^{1}), 7.63 (br s, 1H, N*H*), 3.95 (m, 1H, CH^{3}) and 1.14 (d, 6H, *J* 6.6, 2 x C$H_3{}^{4}$); ^{13}C NMR (75.5 MHz, d^{6}-DMSO) δ 154.61, 140.04, 137.59, 129.10 (2C), 128.56 (2C), 115.23, 44.28 and 21.98 (2C); LRMS (EI): m/z = 300.0 [M^{+}]; Anal. Calc. (Found C, 44.1; H, 4.57; N, 18.9; S, 10.8. $C_{11}H_{13}N_4O_2SCl$ requires C, 43.9; H, 4.36; N 18.6; S, 10.7%);

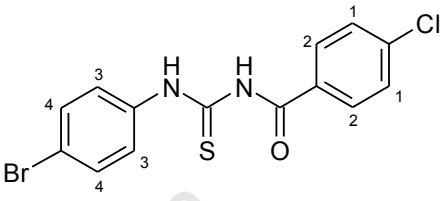
Synthesis of Acylthioureas

General procedure for the synthesis of *1-(p-chlorobenzoyl)-3-(alkyl/aryl)thioureas* **(RKG1541; RKG1542; RKG1543; RKG162A)**

4-Chlorobenzoyl chloride (2.33 mmol) was added dropwise to a solution of ammonium thiocyanate (NH_4SCN) (2.85 mmol) dissolved in acetone (7.5 ml). The mixture was stirred for 30 min under reflux (~60 °C) under N_2, and after the reaction mixture was allowed to cool to room temperature, the inorganic salt (NH_4Cl) was filtered off to yield a solution of 4-chlorobenzoylisothiocyanate in acetone. The alkyl/aryl amine (2.33 mmol) was added to the solution, and the mixture was then refluxed at ~60 °C under N_2 for 2 h. The mixture was left to

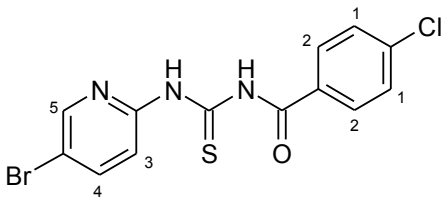
cool to room temperature, and the acetone was removed under reduced pressure. Cold deionised water was added to the residue with stirring, and the solid was collected by filtration. The solid was then recrystallized from 95% ethanol, resulting in the pure *acylthiourea*.

1. *1-(p-Chlorobenzoyl)-3-(p-bromophenyl)thiourea* **(RKG1541)**



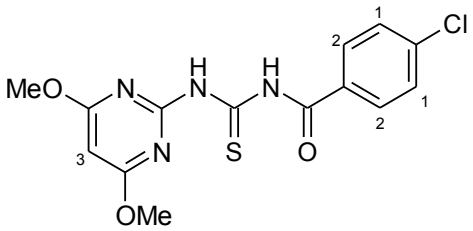
Pale yellow powder (621 mg, 72%); m.p. 194 – 197 °C; ^{1}H NMR (400 MHz; d^{1}-$CDCl_3$) δ 12.52 (br s, 1H, N*H*), 9.06 (br s, 1H, N*H*), 7.84 (d, 2H, *J* 8.8, 2 x Ar-H^{2}), 7.62 (d, 2H, *J* 8.8, 2 x Ar-H^{4}) and 7.53 (m (2d), 4H, *J* 8.8, 4 x Ar-H^{1+3}); ^{13}C NMR (100.6 MHz, d^{1}-$CDCl_3$) δ 178.11, 165.89, 140.54, 136.89, 132.01 (2C), 129.84, 129.61 (2C), 128.89 (2C), 125.51 (2C) and 120.09; LRMS (EI): m/z = 368.0 [M^{+}]; Anal. Calc. (Found C, 45.4; H, 2.52; N, 7.67; S, 8.79. $C_{14}H_{10}N_2OSClBr$ requires C, 45.5; H, 2.73; N 7.58; S, 8.67%);

2. *1-(p-Chlorobenzoyl)-3-(p-bromo-o-N-pyridinyl)thiourea* **(RKG1542)**



Very pale yellow feather-like crystals (692 mg, 80%); m.p. 203 – 205 °C; ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 8.58 (d, 1H, *J* 2.4, Ar-H^{5}), 8.16 (dd, 1H, *J* 9.0, 2.4, Ar-H^{4}), 7.99 (d, 2H, *J* 8.6, 2 x Ar-H^{2}), 7.63 (d, 2H, *J* 8.6, 2 x Ar-H^{1}) and 7.35 (d, 1H, *J* 8.9, Ar-H^{3}); ^{13}C NMR (75.5 MHz, d^{6}-DMSO) δ 177.86, 150.13, 148.84, 140.64, 138.06, 131.02, 130.56 (2C), 128.56 (2C), 128.38, 116.95 and 115.53; LRMS (EI): m/z = 370.9 [M^{+}]; Anal. Calc. (Found C, 42.3; H, 2.13; N, 11.4; S, 8.95. $C_{13}H_9N_3OSClBr$ requires C, 42.1; H, 2.45; N 11.3; S, 8.65%);

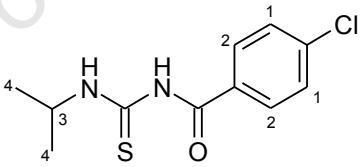
3. *1-(p-Chlorobenzoyl)-3-(m-dimethoxy-o-N,N-pyrimidinyl)thiourea* **(RKG1543)**



Yellow powder (355 mg, 43%); m.p. 178 – 180 °C; IR (Thin film) ν_{max} (cm^{-1}) 3319 (w) (N-H), 3152 (w) (N-H), 2899 (br m) (C-H), 1711 (m) (C=O), 1686 (m) (C=N), 1599 (s) (C=C), 1527 (s) (C=C), 1473 (s) (C=C) and

1245 (br s) (C-O and C=S); ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 12.86* (br s, 1H, N*H*), 12.11* (br s, 1H, N*H*), 11.28 (br s, 1H, N*H*), 9.65 (br d, 1H, N*H*), 8.01* (d, 2H, *J* 8.7, 2 x Ar-H^{2}), 7.94 (d, 2H, *J* 8.7, 2 x Ar-H^{2}), 7.62* (d, 2H, *J* 8.7, 2 x Ar-H^{1}), 7.57 (d, 2H, *J* 8.7, 2 x Ar-H^{1}), 6.06 (s, 1H, Ar-H^{3}) and 3.88 (s, 6H, 2 x -OCH_3); ^{13}C NMR (75.5 MHz, d^{6}-DMSO) δ 181.91, 177.73*, 171.44 (2C), 166.66, 165.25*, 155.67, 137.92*, 137.72, 131.63*, 131.03, 130.42 (2C), 130.21* (2C), 128.62* (2C), 128.33 (2C), 84.62 and 54.61 (2C); LRMS (EI): m/z = 352.0 [M^{+}]; Anal. Calc. (Found C, 47.2; H, 3.57; N, 15.6; S, 9.77. $C_{14}H_{13}N_4O_3SCl$ requires C, 47.7; H, 3.71; N 15.9; S, 9.09%);

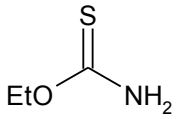
4. *1-(p-Chlorobenzoyl)-3-(iso-propyl)thiourea* **(RKG162A)**



Pale yellow powder (496 mg, 83%); m.p. 98 – 103 °C; ^{1}H NMR (300 MHz; d^{6}-DMSO) δ 11.31* (br s, 1H, N*H*), 10.72* (d, 1H, *J* 7.8), N*H*), 9.67 (br d, 1H, N*H*), 8.26 (br d, 1H, *J* 7.3, N*H*), 7.93* (d, 2H, *J* 8.7, 2 x Ar-H^{2}), 7.86 (d, 2H, *J* 8.3, 2 x Ar-H^{2}), 7.57* (d, 2H, *J* 8.7, 2 x Ar-H^{1}), 7.51 (d, 2H, *J* 8.7, 2 x Ar-H^{1}), 4.43* (m (oct), 1H, *J* 7.8, 6.9, 6.4, CH^{3}), 4.08 (m (oct), 1H, *J* 7.8, 6.9, 6.4, CH^{3}), 1.26 (d, 6H, *J* 6.4, 2 x C$H_3{}^{4}$) and 1.16 (d, 6H, *J* 6.9, 2 x C$H_3{}^{4}$); ^{13}C NMR (75.5 MHz, d^{6}-DMSO) δ 181.92, 178.66*, 167.16*, 166.71, 137.73*, 135.65, 133.49, 131.03*, 130.40* (2C), 129.08 (2C), 128.38* (2C), 128.11 (2C), 46.62*, 41.04, 22.20 (2C) and 21.13* (2C); LRMS (EI): m/z = 256.0 [M^{+}]; Anal. Calc. (Found C, 51.9; H, 5.45; N, 10.7; S, 11.6. $C_{11}H_{13}N_2OSCl$ requires C, 51.5; H, 5.10; N 10.9; S, 12.5%);

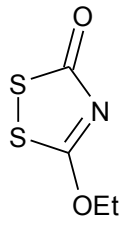
8.3 Experimental: Chapter 5

Synthesis of *O-ethyl thiocarbamate (ethyl xanthamidate)* **(RKG101)**



Ethanol (50 ml) and carbon disulfide (17.6 ml, 0.291 mol) were shaken up with a solution of sodium hydroxide (10 g) in deionised water (150 ml). The solution was then left to stir for 1 h at room temperature [OBSERVATION: the clear solution turned deep orange after the addition of the NaOH solution]. A solution of sodium hydroxide (10 g) in deionised water (100 ml) was neutralized with the slow addition of chloroacetic acid (23.66 g, 0.250 mol) in an ice bath, and the resulting solution was added to the solution obtained above. The mixture was then left to stir overnight at room temperature [OBSERVATION: the deep orange solution changed to deep yellow after the addition of the neutralized chloroacetic acid solution]. Concentrated ammonium hydroxide (46.8 ml, 25% NH_3 in water, ~13.36M, 0.625 mol) was added, and the stirring was continued at room temperature overnight. The solution was neutralized by the addition of glacial acetic acid (14.6 ml), and then extracted with diethyl ether (2 x 150 ml). The organic phase was washed with deionised water (100 ml), followed by 1M HCl (100 ml), and dried over $MgSO_4$. The extract was filtered and concentrated *in vacuo*. The resulting oil (clear, colourless) was placed under n-hexane at -12 ºC, producing a white solid. The purification process was carried out to remove the *S*-ethyl isomer. White flake-like crystals (12.8 g, 49%); m.p. 35 – 37 ^{o}C (lit.[1] 40 - 41 ^{o}C; lit.[2] m.p. 38 - 39 ^{o}C); ^{1}H NMR (300 MHz; d^{1}-$CDCl_3$) δ 4.48 (q, 2H, *J* 7.1, CH_2) and 1.33 (t, 3H, *J* 7.1, CH_3) [no δ 2.90, characteristic of *S*-ethyl isomer];

Synthesis of *3-ethoxy-1,2,4-dithiazolin-5-one* **(RKG104)**



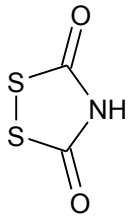
O-Ethyl thiocarbamate (4.00 g, 0.038 mol) and Et_3N (5.3 ml, 0.038 mol) were added to Et_2O (20 ml). The resulting mixture was added dropwise over 1 h to a stirred and chilled solution of (chlorocarbonyl)sulfenyl chloride (3.3 ml, 5.0 g, 0.038 mol) in Et_2O (200 ml). This rate of addition was carried out in order to keep the reaction

temperature below 10 ºC. After the addition was complete, the reaction was allowed to stir for an additional 5.5 h at room temperature (~20 ^{o}C). The white precipitate ($Et_3N.HCl$) was removed via filtration, and the resulting clear pale yellow solution concentrated. The resulting pale yellow oil was cooled to -12 °C providing a light yellow solid. n-Hexane (20 ml) was added, and the mixture was heated to allow the desired product to dissolve into the n-hexane [OBSERVATION: upon heating a yellow oil formed, which was immiscible in the n-hexane (2 layers observed)]. The warm n-hexane layer was separated, and cooled to -12 ^{o}C producing off-white (pale yellow) needle-shaped crystals (2.90 g). The crystals were isolated via filtration, washed with cold n-hexane, and dried *in vacuo*.

Pale yellow, white needle-like crystals (2.90 g, 47%); m.p. 44 – 47 ^{o}C (lit.[3] m.p. 56 – 57 ^{o}C); ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 4.68 (q, 2H, *J* 7.1, CH_2) and 1.46 (t, 3H, *J* 7.1, CH_3);

Synthesis of *1,2,4-dithiazolidine-3,5-dione* **(RKG121A)**

A stirred suspension of 3-ethoxy-1,2,4-dithiazolin-5-one (2.01 g, 12.3 mmol) in concentrated aqueous 32% HCl (12 ml) was heated over 45 min to 110 °C. The solution was stirred for a further 10 min, and filtered through a Buchner funnel while hot to remove elemental sulphur. The filtrate was concentrated and dried to provide a solid, which was then recrystallized from toluene.



White crystals (0.714 g, 43 %); m.p. 132 – 136 ^{o}C (lit.[4] m.p. 141 ^{o}C; lit.[3] m.p. 142 – 144 ^{o}C); ^{13}C NMR (75.5 MHz, d^3-CD_3CN) δ 170.05 (2C);

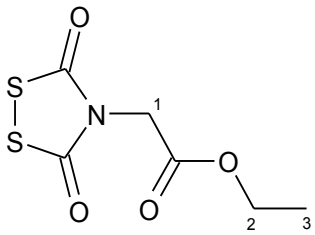
General procedure for the synthesis of compounds **(RKG2661, RKG315, RKG317, RKG318, RKG319, RKG425B, RKG425C** and **RKG425D)**

1,2,4-Dithiazolidine-3,5-dione (**RKG121**) (100 mg, 0.74 mmol) was dissolved in CH_3CN (1.5 ml). Sodium bicarbonate (120 mg, 1.43 mmol) was added with stirring to the solution at room temperature (19 °C). The alkyl bromide (0.87 mmol) was added, and the mixture was left to stir

for 16 hrs. The solvent was removed under reduced pressure with adsorption of the residue onto silica gel for purification by flash chromatography (1:9, EtOAc:hexane);

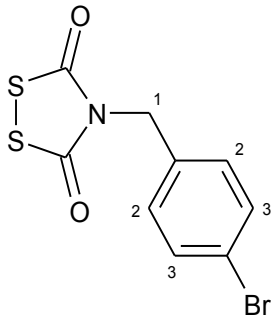
1. *(3,5-Dioxo-[1,2,4]dithiazolidin-4-yl)-acetic acid ethyl ester* **RKG2661**

   Yellow oil (106 mg, 65%); R_f = 0.26; ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 4.46 (s, 2H, CH_2^1), 4.25 (q, 2H, *J* 7.1, CH_2^2) and 1.29 (t, 3H, *J* 7.2, CH_3^3);



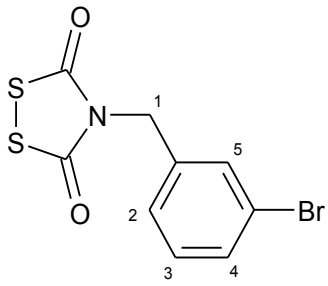
2. *4-(4-Bromo-benzyl)-[1,2,4]dithiazolidine-3,5-dione* **RKG315**

   White crystals (207 mg, 92%); R_f = 0.21; m.p. 93 - 97 °C; IR (NaCl) ν_{max} (cm^{-1}) 2995 (w) (C-H), 1645 (s) (C=O), 1486 (m) (C=C), 1301 (s) (C-N), 724 (s) (C-Br) and 525 (w) (S-S); ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 7.47 (d, 2H, *J* 8.4, 2 x Ar-H^3), 7.29 (d, 2H, *J* 8.30, 2 x Ar-H^2) and 4.85 (s, 2H, CH_2^1); ^{13}C NMR (100.6 MHz, d^1-$CDCl_3$) δ 167.38 (2C), 133.08, 132.03 (2C), 130.86 (2C), 123.02 and 48.65; HRMS (EI): m/z = 302.90176 [M$^+$]; Anal. Calc. (Found C, 35.1; H, 1.96; N, 4.09; S, 19.7. $C_9H_6BrNO_2S_2$ requires C, 35.5; H, 1.99; N, 4.60; S, 21.1%);

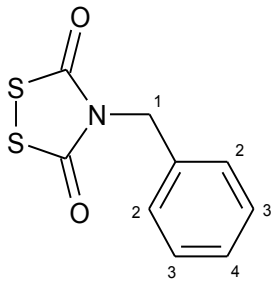


3. *4-(3-Bromo-benzyl)-[1,2,4]dithiazolidine-3,5-dione* **RKG317**

   White needle-like crystals (178 mg, 79%); R_f = 0.30; m.p. 95 - 97 °C; IR (NaCl) ν_{max} (cm^{-1}) 3000 (w) (C-H), 1652 (s) (C=O), 1572 (m) (C=C), 1474 (m) (C=C), 1303 (s) (C-N), 708 (s) (C-Br) and 544 (w) (S-S); ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 7.56 (s, 1H, Ar-H^5), 7.47 (d, 1H, *J* 7.8, Ar-H^4), 7.34 (d, 1H, *J* 7.7, Ar-H^2), 7.21 (t, 1H, *J* 7.8, Ar-H^3) and 4.86 (s, 2H, CH_2^1); ^{13}C NMR (100.6 MHz; d^1-$CDCl_3$) δ 167.34 (2C), 136.17, 132.03, 131.96, 130.38, 127.73, 122.81 and 48.57; HRMS (EI): m/z = 302.90191 [M$^+$]; Anal. Calc. (Found C, 35.0; H, 1.84; N, 3.98; S, 19.8. $C_9H_6BrNO_2S_2$ requires C, 35.5; H, 1.99; N, 4.60; S, 21.1%);

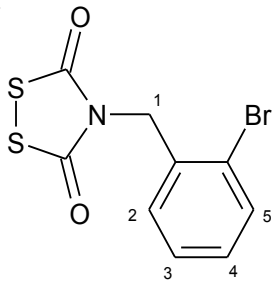


4. *4-Benzyl-[1,2,4]dithiazolidine-3,5-dione* **RKG318**



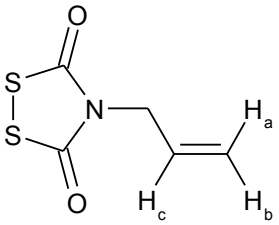
White crystals (124 mg, 74%); R_f = 0.36; m.p. 87 - 91 °C; ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 7.41 (m, 2H, 2 x Ar-H^3), 7.43 (m, 3H, 3 x Ar-H^{2+4}) and 4.91 (s, 2H, CH_2^1); ^{13}C NMR (100.6 MHz; d^1-$CDCl_3$) δ 167.47 (2C), 136.18, 129.06 (2C), 128.82 (2C), 128.71 and 49.38; HRMS (EI): m/z = 224.99127 [M^+]; Anal. Calc. (Found C, 47.8; H, 3.14; N, 6.10; S, 28.0. $C_9H_7NO_2S_2$ requires C, 48.0; H, 3.13; N, 6.22; S, 28.5%);

5. *4-(2-Bromo-benzyl)-[1,2,4]dithiazolidine-3,5-dione* **RKG319**



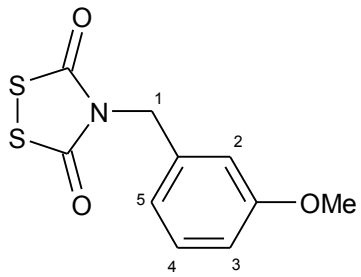
White needle-like crystals (211 mg, 94%); R_f = 0.21; m.p. 80 - 81 °C; IR (NaCl) ν_{max} (cm^{-1}) 3060 (w) (C-H), 1655 (s) (C=O), 1603 (m) (C=C), 1477 (m) (C=C), 1312 (s) (C-N), 749 (m) (C-Br) and 555 (m) (S-S); ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 7.59 (dd, 1H, *J* 7.8, 1.3, Ar-H^5), 7.29 (td, 1H, *J* 7.6, 1.3, Ar-H^3), 7.18 (td, 1H, *J* 7.8, 1.7, Ar-H^4), 7.07 (dd, 1H, *J* 7.7, 1.7, Ar-H^2) and 5.05 (s, 2H, CH_2^1); ^{13}C NMR (100.6 MHz; d^1-$CDCl_3$) δ 162.77 (2C), 133.25, 132.78, 129.62, 127.86, 127.72, 122.85 and 49.21; HRMS (EI): m/z = 302.90180 [M^+]; Anal. Calc. (Found C, 34.9; H, 1.86; N, 4.18; S, 20.0. $C_9H_6BrNO_2S_2$ requires C, 35.5; H, 1.99; N, 4.60; S, 21.1%);

6. *4-Allyl-[1,2,4]dithiazolidine-3,5-dione* **RKG425B**



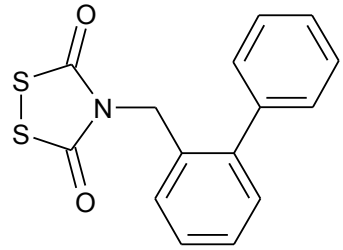
Clear, yellow oil (88 mg, 68%); R_f = 0.31 (1:19, EtOAc:hexane); ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 5.79 (m, 1H, CH^c), 5.28 (m, 2H, CH_2^{a+b}) and 4.33 (dt, 2H, *J* 6.0, 1.4, CH_2); ^{13}C NMR (100.6 MHz; d^1-$CDCl_3$) δ 169.35 (2C), 129.18, 120.20 and 47.96; HRMS (EI): m/z = 174.97561 [M^+];

7. *4-(4-Methoxybenzyl)-[1,2,4]dithiazolidine-3,5-dione* **RKG425C**



White, crystalline solid (143 mg, 76%); R_f = 0.23 (1:19, EtOAc:hexane); ^{1}H NMR (300 MHz; d^1-$CDCl_3$) δ 7.26 (t, 1H, *J* 8.3, Ar-H^4), 6.97 (m, 2H, 2 x Ar-H^{2+5}), 6.87 (d, 1H, *J* 8.3, Ar-H^3), 4.87 (s, 2H, CH_2) and 3.80 (s, 3H, -OCH_3); ^{13}C NMR (75.5 MHz; d^1-$CDCl_3$) δ 167.42 (2C), 159.85, 135.53, 129.85, 121.20, 114.51, 114.28, 55.27 and 49.27; HRMS (EI): m/z = 255.00182 [M^+]; Anal. Calc. (Found C, 46.9; H, 3.60; N, 5.31; S, 24.9. $C_{10}H_9NO_3S_2$ requires C, 47.0; H, 3.55; N, 5.49; S, 25.1%);

8. *4-(Biphenyl-2-ylmethyl)-[1,2,4]dithiazolidine-3,5-dione* **RKG425D**



White, crystalline solid (174 mg, 78%); R_f = 0.38 (1:19, EtOAc:hexane); ^{1}H NMR (400 MHz; d^1-$CDCl_3$) δ 7.43 – 7.14 (m, 9H, 9 x Ar-*H*) and 4.88 (s, 2H, CH_2); ^{13}C NMR (100.6 MHz; d^1-$CDCl_3$) δ 167.12 (2C), 141.87, 140.20, 131.27, 130.41, 129.13 (2C), 128.40 (2C), 127.88, 127.85, 127.49, 126.49 and 47.28; HRMS (EI): m/z = 301.02259 [M^+]; Anal. Calc. (Found C, 59.9; H, 3.61; N, 4.58; S, 21.1. $C_{15}H_{11}NO_2S_2$ requires C, 59.8; H, 3.68; N, 4.65; S, 21.3%);

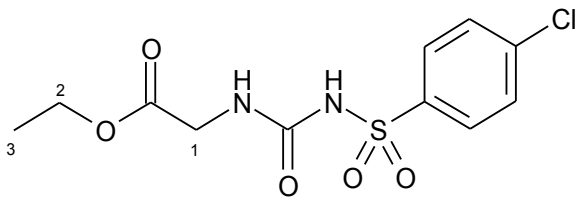
<u>General procedure for the synthesis of compounds</u> **(RKG267F4, RKG328, RKG330** and **RKG331)**

One equivalent of 4-chlorobenzene sulfonamide was added to a solution of alkylated 1,2,4-dithiazolidine-3,5-dione (50 mg) and PPh_3 (1 equiv.) dissolved in toluene (2.0 ml). K_2CO_3 (1 equiv.) was added, and the reaction was stirred under reflux for 24 hrs. The reaction mixture was allowed to cool to room temperature. The suspended material was filtered off and washed with deionised H_2O and toluene.

1. *Ethyl 2-(3-(4-chlorophenylsulfonyl)ureido)acetate* **RKG2672F4**

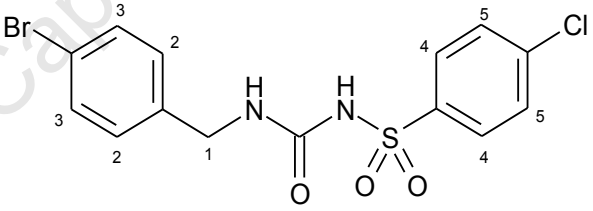
   Isolated using column chromatography (1:9, EtOAc:hexane; adjusting to 2:3, EtOAc:hexane);

   Yellow oil (30.6 mg, 48%); R_f = 0.05 (2:3, EtOAc:hexane); ^{1}H NMR (400 MHz; d^6-DMSO) δ 8.17 (s, 1H, N*H*), 7.58 (m, 4H, 4 x Ar*H*), 4.12 (q and s overlapping, 3H, *J* 7.1, CH_2^2 and N*H*), 4.00 (s, 2H, CH_2^1) and 1.18 (t, 3H, *J* 7.1, CH_3^3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 171.43, 167.37, 131.45 (2C), 128.97, 128.72 (2C), 127.53, 45.99, 38.90 and 13.90; LRMS (EI): m/z = 320.0 [M^+];



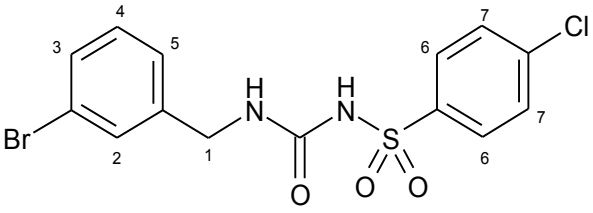
2. *N-(4-Bromobenzylcarbamoyl)-4-chlorobenzenesulfonamide* **RKG328**

   White powder (51.5 mg, 58%); m.p. 243 - 247 °C; IR (NaCl) ν_{max} (cm^{-1}) 3378 (s) (N-H), 3039 (w) (C-H), 2882 (w) (C-H), 1603 (s) (C=O), 1574 (m) (C=C), 1462 (s) (C=C), 1343 (s) (S=O), 1218 (s) (C-O), 1133 (s) (S=O), 755 (s) (C-Cl) and 521 (s) (C-Br); ^{1}H NMR (400 MHz; d^6-DMSO) δ 7.70 (d, 2H, *J* 8.3, 2 x ArH^4), 7.38 (m, 4H, 4 x ArH^{3+5}), 7.10 (d, 2H, *J* 8.0, 2 x Ar-H^2) and 4.00 (s, 2H, CH_2^1); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 160.75, 141.27, 133.70, 130.94, 130.62 (2C), 129.10 (2C), 128.34 (2C), 127.39 (2C), 118.90 and 42.29; LRMS (EI): m/z = 400.9 [M^+]; Anal. Calc. (Found C, 41.2; H, 2.44; N, 6.18; S, 7.57. $C_{14}H_{12}BrN_2O_3SCl$ requires C, 41.7; H, 3.00; N, 6.94; S, 7.94%);



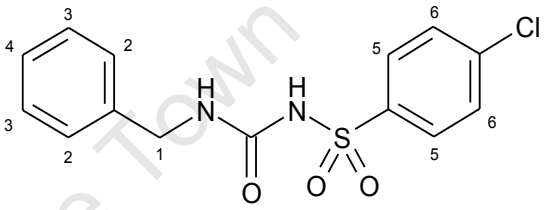
3. *N-(3-Bromobenzylcarbamoyl)-4-chlorobenzenesulfonamide* **RKG330**

   White powder (46.1 mg, 52%); m.p. 240 - 244 °C; IR (NaCl) ν_{max} (cm^{-1}) 3386 (s) (N-H), 3022 (w) (C-H), 2933 (w) (C-H), 1599 (s) (C=O), 1458 (s) (C=C), 1334 (s) (S=O), 1214



(s) (C-O), 1130 (s) (S=O), 783 (s) (C-Cl) and 520 (s) (C-Br); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 7.71 (d, 2H, *J* 8.2, 2 x ArH^{6}), 7.36 (m, 4H, 4 x ArH^{2+3+7}), 7.16 (m, 2H, 2 x Ar-H^{4+5}) and 4.03 (s, 2H, CH_2^{1}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 160.65, 145.08, 133.54, 130.28, 129.94, 129.48, 128.74, 128.30 (2C), 127.35 (2C), 125.83, 121.35 and 42.32; LRMS (EI): m/z = 400.9 [M^{+}]; Anal. Calc. (Found C, 41.2; H, 2.69; N, 6.32; S, 7.18. $C_{14}H_{12}BrN_2O_3SCl$ requires C, 41.7; H, 3.00; N, 6.94; S, 7.94%);

4. *N-(Benzylcarbamoyl)-4-chlorobenzenesulfonamide* **RKG331**

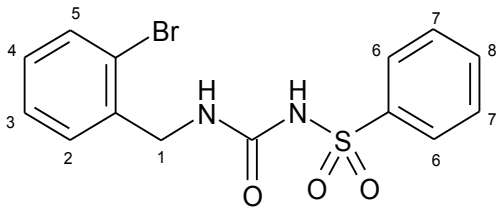


White powder (46.1mg, 65%); m.p. 248 - 252 °C; IR (NaCl) ν_{max} (cm^{-1}) 3378 (s) (N-H), 3028 (w) (C-H), 1603 (s) (C=O), 1476 (w) (C=C), 1458 (s) (C=C), 1344 (s) (S=O), 1220 (s) (C-O), 1134 (s) (S=O) and 728 (s) (C-Cl); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 7.72 (d, 2H, *J* 8.3, 2 x ArH^{5}), 7.37 (d, 2H, *J* 8.4, 2 x ArH^{6}), 7.22 (t, 2H, *J* 7.9, 2 x Ar-H^{3}), 7.14 (m, 3H, 3 x Ar-H^{2+4}) and 4.05 (s, 2H, CH_2^{1}); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 160.50, 146.73, 133.53, 128.35 (2C), 127.79 (2C), 127.33 (2C), 126.83 (2C), 125.95, 123.46 and 43.06; LRMS (EI): m/z = 324.0 [M^{+}]; Anal. Calc. (Found C, 51.3; H, 3.73; N, 8.08; S, 9.24. $C_{14}H_{13}N_2O_3SCl$ requires C, 51.8; H, 4.03; N, 8.63; S, 9.87%);

<u>General procedure for the synthesis of compounds</u> **(RKG357, RKG360, RKG359, RKG351, RKG426A, RKG426B** and **RKG427A)**

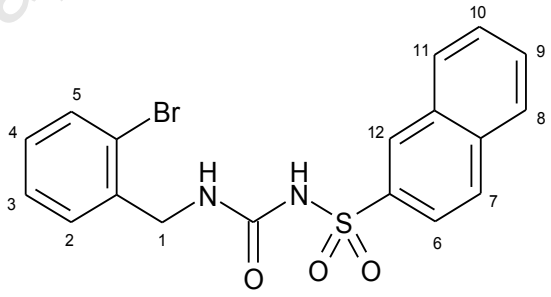
One equivalent of sulfonamide was added to a solution of alkylated 1,2,4-dithiazolidine-3,5-dione (60 mg) and PPh_3 (1 equiv.) dissolved in toluene (2.0 ml). K_2CO_3 (1 equiv.) was added, and the reaction was stirred under reflux for 24 hrs. The reaction mixture was allowed to cool to room temperature. The suspended material was filtered off and washed with deionised H_2O and toluene.

1. *N-(2-Bromobenzylcarbamoyl)benzenesulfonamide* **RKG357**



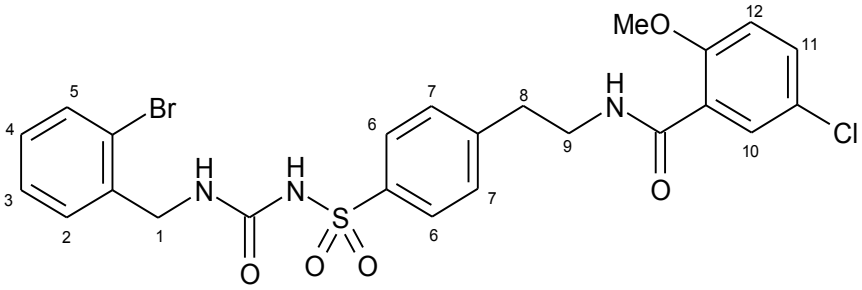
White powder (58.6 mg, 79%); m.p. 214 - 216 °C; IR (NaCl) ν_{max} (cm^{-1}) 3334 (s) (N-H), 3054 (w) (C-H), 2905 (w) (C-H), 1623 (s) (C=O), 1588 (m) (C=C), 1532 (s) (C=C), 1443 (s) (C=C), 1366 (s) (S=O), 1293 (C-N), 1133 (s) (S=O), 753 (s) (C-Cl) and 579 (s) (C-Br); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 7.71 (m, 2H, 2 x ArH^{6}), 7.49 (d, 1H, *J* 7.5, ArH^{5}), 7.32 (m, 3H, 3 x Ar-H^{7+8}), 7.23 (m, 2H, 2 x Ar-H^{2+3}), 7.10 (t, 1H, *J* 7.6), ArH^{4}) and 4.07 (s, 2H, C$H_2{}^{1}$); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 166.42, 141.04, 132.90, 132.50, 129.70, 129.17, 128.71, 128.11 (2C), 127.96, 126.98 (2C), 122.57 and 44.10; LRMS (EI): m/z = 368.0 [M^{+}]; Anal. Calc. (Found C, 45.0; H, 2.96; N, 7.66; S, 8.04. $C_{14}H_{13}BrN_2O_3S$ requires C, 45.5; H, 3.55; N, 7.59; S, 8.68%);

2. *N-(2-Bromobenzylcarbamoyl)naphthalene-2-sulfonamide* **RKG360**



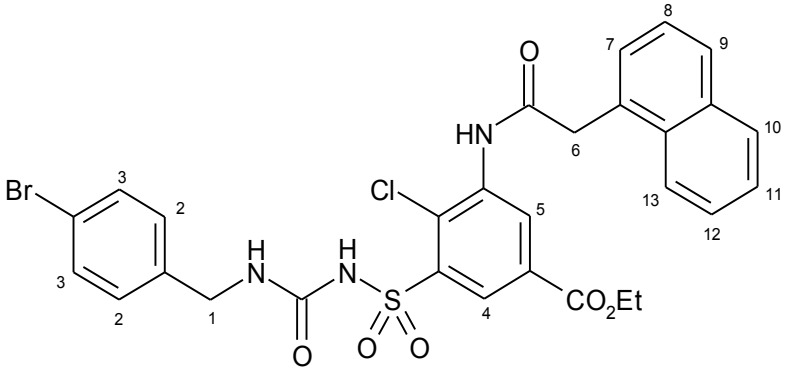
White powder (56.5 mg, 67%); m.p. 160 - 170 °C; IR (NaCl) ν_{max} (cm^{-1}) 3371 (m) (N-H), 1624 (s) (C=O), 1590 (m) (C=C), 1453 (s) (C=C), 1337 (s) (S=O), 1260 (C-N), 1182 (s) (S=O) and 744 (s) (C-Br); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 8.24 (s, 1H, ArH^{12}), 7.92 (m, 2H, 2 x ArH^{6+7}), 7.84 (m, 2H, 2 x ArH^{8+11}), 7.54 (m, 2H, 2 x Ar-H^{9+10}), 7.47 (d, 1H, *J* 8.1, ArH^{5}), 7.18 (m, 2H, 2 x ArH^{2+3}), 7.08 (t, 1H, *J* 8.0, ArH^{4}) and 4.05 (s, 2H, C$H_2{}^{1}$); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 145.74, 133.75, 132.89, 132.62, 132.48, 129.25, 129.13, 128.68, 128.12, 127.90, 127.71, 127.42, 126.99, 126.17, 124.98, 122.55 and 44.10; LRMS (EI): m/z = 418.0 [M^{+}]; Anal. Calc. (Found C, 51.1; H, 3.28; N, 6.72; S, 6.30. $C_{18}H_{15}BrN_2O_3S$ requires C, 51.6; H, 3.61; N, 6.68; S, 7.65%);

3. *N-(4-(N-(2-Bromobenzylcarbamoyl)sulfamoyl)phenethyl)-5-chloro-2-methoxybenzamide* **RKG359**



White powder (92.6 mg, 80%); m.p. 206 - 210 °C; IR (NaCl) ν_{max} (cm^{-1}) 3363 (m) (N-H), 3285 (m) (CON-H), 2902 (w) (C-H), 1638 (s) (C=O), 1615 (s) (C=O), 1535 (s) (C=C), 1508 (s) (C=C), 1462 (s) (C=C), 1342 (s) (S=O), 1295 (s) (C-N), 1227 (s) (C-O), 1163 (s) (S=O), 743 (s) (C-Cl) and 572 (s) (C-Br); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 8.20 (t, 1H, *J* 5.5, N*H*CO), 7.65 (m, 3H, 3 x ArH^{6+10}), 7.48 (m, 2H, 2 x ArH^{5+11}), 7.23 (m, 2H, 2 x ArH^{2+3}), 7.21 (d, 2H, *J* 8.1, 2 x ArH^{7}), 7.12 (m, 2H, 2 x ArH^{4+12}), 4.07 (s, 2H, CH_2^{1}), 3.78 (s, 3H, -OCH_3), 3.51 (m, 2H, CH_2^{9}) and 2.82 (t, 2H, *J* 7.0, CH_2^{8}); ^{13}C NMR (75.5 MHz; d^{6}-DMSO) δ 163.36, 160.44, 155.70, 145.84, 140.35, 132.15, 131.71, 131.43, 129.52, 128.93, 128.47, 127.91, 127.61, 127.16, 126.41, 125.56, 124.28, 122.14, 121.83, 114.11, 56.22, 43.34, 40.45 and 34.52; LRMS (EI): m/z = 579.0 [M^{+}]; Anal. Calc. (Found C, 49.0; H, 3.54; N, 6.25; S, 5.22. $C_{24}H_{23}BrN_3O_5SCl$ requires C, 49.6; H, 3.99; N, 7.23; S, 5.52%);

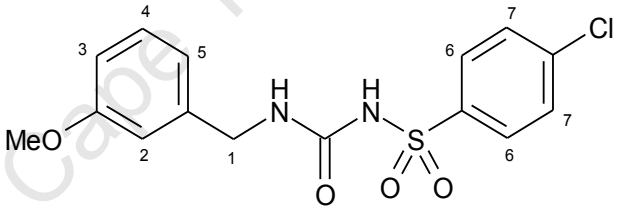
4. *Ethyl 3-(N-(4-bromobenzylcarbamoyl)sulfamoyl)-4-chloro-5-(2-(naphthalene-1-yl)acetamido)benzoate* **RKG351**



White powder (50.7 mg, 47%); m.p. 213 - 217 °C; IR (NaCl) ν_{max} (cm^{-1}) 3366 (s) (N-H), 3262 (m) (CON-H), 2978 (w) (C-H), 1706 (s) (C=O), 1673 (s) (C=O), 1599 (s) (C=O, C=C), 1512 (s) (C=C), 1443 (s) (C=C), 1315 (s) (S=O), 1285 (s) (C-N), 1243 (s) (C-O), 1160 (s) (S=O), 778 (s)

(C-Cl) and 573 (m) (C-Br); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 8.30 (d, 1H, *J* 2.1, ArH^{4}), 8.27 (d, 1H, *J* 2.1, ArH^{5}), 8.16 (d, 1H, *J* 8.3, ArH^{13}), 7.94 (d, 1H, *J* 7.7, ArH^{10}), 7.85 (d, 1H, *J* 8.1, ArH^{9}), 7.53 (m, 3H, 3 x Ar$H^{8+11+12}$), 7.48 (d, 2H, *J* 8.5), 2 x ArH^{3}), 7.38 (d, 1H, *J* 8.3, ArH^{7}), 7.18 (d, 2H, *J* 8.5, 2 x ArH^{2}), 4.29 (m, 4H, C$H_2{}^{6}$ and –OCH_2-), 3.97 (s, 2H, C$H_2{}^{1}$) and 1.28 (t, 3H, *J* 7.1, -OCH$_2$C$\underline{H}_3$); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 170.68, 170.37, 165.51, 147.25, 141.17, 134.10, 132.81, 132.65, 131.70 (2C), 131.33 (2C), 129.88, 129.80, 129.13, 128.79, 128.09, 127.93, 127.60, 126.85, 126.44, 126.24, 124.86, 120.14, 119.57, 62.29, 61.78, 43.06 and 14.83; LRMS (EI): m/z = 657.0 [M^{+}]; Anal. Calc. (Found C, 51.5; H, 3.47; N, 5.76; S, 4.17. $C_{29}H_{25}BrN_3O_6SCl$ requires C, 52.9; H, 3.82; N, 6.38; S, 4.87%);

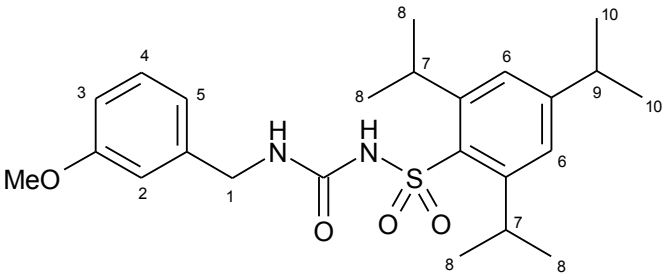
5. *4-Chloro-N-(3-methoxybenzylcarbamoyl)benzenesulfonamide* **(RKG426A)**



White solid (47.6 mg, 67%); ^{1}H NMR (400 MHz; d^{6}-DMSO) δ 7.75 (d, 2H, *J* 8.4, 2 x ArH^{6}), 7.35 (d, 2H, *J* 8.5, 2 x ArH^{7}), 7.13 (t, 1H, *J* 7.8, ArH^{4}), 6.76 (m, 3H, 3 x Ar-H^{2+3+5}), 4.07 (d, 2H, *J* 6.5, C$H_2{}^{1}$) and 3.70 (s, 3H, -OCH_3); ^{13}C NMR (100.6 MHz; d^{6}-DMSO) δ 160.58, 159.07, 146.76, 143.57, 133.45, 128.75, 128.34 (2C), 127.28 (2C), 119.02, 112.31, 111.52, 54.74 and 42.82; LRMS (EI): m/z = 354.0 [M^{+}]; Anal. Calc. (Found C, 50.2; H, 3.87; N, 6.76; S, 8.17. $C_{15}H_{15}ClN_2O_4S$ requires C, 50.8; H, 4.26; N, 7.90; S, 9.04%);

6. *2,4,6-Triisopropyl-N-(3-methoxybenzylcarbamoyl)benzenesulfonamide* **(RKG426B)**

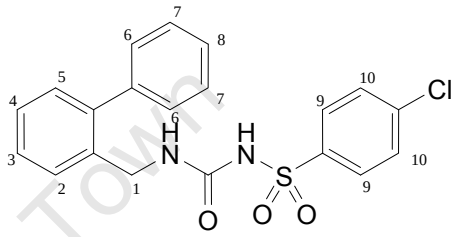
(purified by column chromatography; 1:4, EtOAc:hexane)



White solid (28.4 mg, 32%); R_f = 0.17); ^{1}H NMR (300 MHz; d^{6}-acetone) δ 7.29 (s, 2H, 2 x Ar-H^{6}), 7.13 (t, 1H, *J* 7.8, ArH^{4}), 6.79 (m, 3H, 3 x Ar-H^{2+3+5}), 4.36 (hep, 2H, *J* 6.8, 2 x CH^{7}), 4.29 (s, 2H, C$H_2{}^{1}$), 3.71 (s, 3H,

-OCH_3), 2.96 (hep, 1H, *J* 6.9, CH^9), 2.06 (m, 3H, 3 x CH^{7+9}) and 1.25 (m, 18H, 6 x CH_3^{8+10}); ^{13}C NMR (75.5 MHz; d^6-acetone) δ 160.17, 159.54, 153.19, 150.98 (2C), 141.07, 133.78, 129.56, 123.91 (2C), 119.43, 112.89, 112.74, 54.74, 43.15 (2C), 34.15, 29.28, 24.29 (4C) and 23.15 (2C); HRMS (EI): m/z = 446.22338 [M^+]; Anal. Calc. (Found C, 63.9; H, 6.87; N, 6.76; S, 8.17. $C_{24}H_{34}N_2O_4S$ requires C, 64.5; H, 7.67; N, 6.27; S, 7.18%);

7. *N-(Biphenyl-2-ylmethylcarbamoyl)-4-chlorobenzenesulfonamide* **(RKG427A)**



White solid (56.9 mg, 71%); NMR (400 MHz; d^6-DMSO) δ 7.70 (d, 2H, *J* 8.4, 2 x ArH^9), 7.48 – 7.11 (m, 11H, 11 x Ar$H^{2+3+4+5+6+7+8+10}$), 6.49 (t, 1H, *J* 5.8, CH_2NH), 4.05 (d, 2H, *J* 5.7, CH_2^1) and 3.70 (s, 3H, -OCH_3); ^{13}C NMR (100.6 MHz; d^6-DMSO) δ 160.58, 146.72, 140.41, 140.07, 137.65, 133.48, 129.13, 128.89 (2C), 128.41, 128.30 (2C), 128.07 (2C), 127.31 (2C), 127.03, 126.83, 125.99 and 40.83; LRMS (EI): m/z = 400.0 [M^+]; Anal. Calc. (Found C, 59.2; H, 3.99; N, 6.76; S, 7.17. $C_{20}H_{17}ClN_2O_3S$ requires C, 59.9; H, 4.27; N, 6.99; S, 8.00%);

8.4 References

[1] Davies, W.; MacLauren, J. A. *J. Chem. Soc.* **1951**, 1434-1438.

[2] Goerdeler, J.; Schulze, A. *Chem. Ber.* **1982**, *115*, 1252-1255.

[3] Chen, L.; Thompson, T. R.; Hammer, R. P.; Barany, G. *J. Org. Chem.* **1996**, *61*, 6639-6645.

[4] Dahms, G.; Haas, A.; Klug, W. *Chem. Ber.* **1971**, *104*, 2732-2742.

APPENDIX A

CHEMICALS AND INSTRUMENTATION

Chemicals and Solvents: All commercially available chemicals or reagents were products of Sigma-Aldrich, Fluka or Merck, unless otherwise stated. Bulk solvents (namely dichloromethane, ethylacetate and hexane) used for column chromatography and thin layer chromatography were purchased from Protea Chemicals and Kimix, and were further purified via distillation where necessary.

Thin Layer Chromatography: Reactions were monitored by thin layer chromatography using pre-coated aluminium-backed F_{254} silica gel plates from Merck. UV light was used for visualisation.

Silica Gel Column Chromatography: Merck Silica kieselgel 60: 70-230 mesh was used for all gravity column chromatography.

Melting Points: all melting points were determined using a Reichert-Jung Thermovar hotstage microscope.

IR Spectroscopy: Infrared spectra were recorded on a Thermo Nicolete FTIR instrument in the 4000 – 600 cm^{-1} range. Samples were analyzed as a thin film deposited (samples initially dissolved in chloroform) on NaCl or KBr discs.

Microanalysis: A Fisons EA 1108 CHNO-S instrument was used to determine the elemental composition (C, H, N and S) of samples.

NMR Spectroscopy: ^{1}H spectra were recorded on either a Varian Mercury Spectrometer at 300 MHz or a Varian Unity Spectrometer at 400 MHz.

^{13}C spectra were recorded on either a Varian Mercury Spectrometer at 75 MHz or a Varian Unity Spectrometer at 100 MHz.

All NMR spectra were measured as solutions of sample dissolved in deuterochloroform, deuteromethanol, deuteroacetonitrile, deuterodimethylsulfoxide, deuterated acetone or deuterated water (D_2O) with tetramethylsilane (TMS) as internal standard. All deuterated solvents were purchased products of Sigma-Aldrich.

Note: All ^{1}H NMR coupling constants (*J*) were rounded off to one decimal place.

Mass Spectroscopy: Low resolution mass spectra were recorded with an Applied Systems API 4000 Triple Quadropole with a Turbo Ion Spray source and a Harvard Syringe Pump as solutions in methanol, acetonitrile or deionised water, or mixtures, respectively (Department of Pharmacology, University of Cape Town).

High and low resolution mass spectra were recorded with a VG70-SEQ (EI, operating at 8kV) instrument (School of Chemistry, University of Witwatersrand).

Dried Solvents: Tetrahydrofuran, toluene and diethyl ether were initially dried over lithium aluminium hydride, and subsequently distilled over sodium wire, using benzophenone as indicator, before use.

Thionyl chloride ($SOCl_2$) was freshly distilled, under reduced pressure, prior to use.

Anhydrous NMP, DMAP, methanol and 1,4-dioxane were purchased products of Sigma-Aldrich, and handled under inert conditions (purged with N_2 or Ar gas).

APPENDIX B

In Vitro Testing against *Trypanosoma Brucei* Strain

The primary aims of this research was to synthesize compounds to inhibit new permeability pathways (NPP) induced by the malaria parasite *P. falciparum*, and compounds that inhibit *M. tuberculosis*. There is, however, evidence to suggest that compounds that are active antimalarials also demonstrate potent activity against other protozoan parasites.[1]

Protozoan parasites of the genus *Trypanosoma brucei* are responsible for African Trypanosomiasis in humans, also known as sleeping sickness.[2] The parasite is limited to mainly the sub-Saharan regions of Africa, and is spread by the tsetse fly. It is estimated that 300 000 – 500 000 people in Africa are infected. Infection occurs when a tsetse fly bites a human and the parasite is transmitted into the human hosts' bloodstream. The infective forms of the parasite, known as trypanosomes, travel to the brain via the bloodstream, resulting in multiple neurological symptoms. If the disease remains untreated, the probable outcome is death of the human host. There are two species of *T. brucei* that infect humans, each resulting in different symptoms and illness. If the disease is diagnosed early enough it can be treated. Current drugs, however, are associated with cytotoxicity, and there is no vaccine currently available.

In 2004, a small library was submitted to the laboratories of V. Yardley (London School of Hygiene & Tropical Medicine, UK) for *in vitro* testing against *T. brucei brucei*. The results are shown in Table AC1 (next page). Inhibition studies, to inhibit the activity of cultured *T. brucei brucei*, at four different concentrations were initially determined. The actual ED_{50} was then determined. All compounds, with exception of **RKG136A** and **RKG162A**, did not show any effective inhibition of the growth of the parasite at a concentration lower than 30 µg/ml. Data for the standard control drugs pentamidine (*T. brucei*) and Podophyllotoxin (cytotoxicity) is included for comparative purposes.

Table AC1: *In vitro* activities against *T. brucei brucei*

Compound ID	% inhibition 30	10	3	1	ED_{50} µg/ml +/- 95% CL	ED_{50} µM +/- 95% CL	Cytotoxicity ED_{50} µg/ml	Cytotoxicity ED_{50} µM	TI
RKG 114A	6.36	7.97	8.36	6.12	>30	-	>300	-	-
RKG 119A	5.65	1.39	0	-	>30	-	>300	-	-
RKG 123A	14.4	8.48	4.12	7.2	>30	-	185.1	592.6	-
RKG 125A	6.2	5.12	4.25	0	>30	-	>300	-	-
RKG 131A	9.46	3.07	1.54	0	>30	-	>300	-	-
RKG 133A	15.8	10.7	12.2	9.3	>30	-	>300	-	-
RKG 136A	60.7	8.65	13.7	14.0	25.8 ± 2.96	95.3 ± 11.0	>300	-	>12
RKG 139 F3	14.2	8.53	803	9.31	>30	-	133.4	322.5	-
RKG 145A	4.9	8.15	4.22	3.62	>30	-	>300	-	-
RKG 1491	8.64	2.65	8.42	5.2	>30	-	39.1	100.3	-
RKG 1493A	9.42	6.43	4.07	7.77	>30	-	>300	-	-
RKG 150A	6.43	6.62	10.7	9.15	>30	-	>300	-	-
RKG 151A	47.0	34.3	29.8	29.6	>30	-	>300	-	-
RKG 1531	5.42	0.13	0		>30	-	>300	-	-
RKG 1532	11.2	5.7	2.54	7.75	>30	-	>300	-	-
RKG 1533 PC	3.7	28.7	38.6	31.1	>30	-	>300	-	-
RKG 1541	18.5	37.0	34.3	27.8	>30	-	66.0	178.5	-
RKG 1542	43.7	41.6	35.8	35.0	>30	-	75.3	203.2	-
RKG 1543	18.3	16.8	4.79	4.69	>30	-	62.0	175.7	-
RKG 162A	88.7	55.6	40.1	45.7	3.43 ± 1.20	13.4 ± 4.67	>300	-	>87
RKG ANT 3	35.5	32.2	29.2	26.5	>30	-	26.1	98.8	-
RKG C1 Im	20.0	0	-	-	>30	-	>300	-	-
RKG C2 Bc	16.1	5.8	0	-	>30	-	>300	-	-
RKG C4 Ci	14.4	12.6	4.55	2.35	>30	-	>300	-	-
RKG C10 Ri	9.99	0.71	3.68		>30	-	>300	-	-
RKG C11 Tria	28.0	11.6	9.27	4	>30	-	>300	-	-
RKG C12 Th	17.8	15.9	13.5	10.1	>30	-	>300	-	-
RKG C13 Trib	26.4	3.91	0	-	>30	-	>300	-	-
RKG C3 Ch	16.4	4.14	0	-	>30	-	279.4	780.8	-
RKG C5 Et	44.3	12.6	5.77	4.19	>30	-	123.9	311.0	-
RKG C6 Me	0.54	0.33	0	-	>30	-	147.6	387.0	-
RKG C7 Pri	5.49	0	0.31	1.53	>30	-	182.7	390.0	-
RKG C8 Pro	12.2	7.47	0.35	3.3	>30	-	>300	-	-
RKG C9 Py	16.8	12.4	17.6	15.7	>30	-	>300	-	-
RKG C14 Trif	28.4	3.91	0	0.97	>30	-	>300	-	-
Pentamidine	-	-	-	-	0.00307	0.00902	-	-	-
Podophyllotoxin	-	-	-	-	-	-	0.00024	-	-

Eighteen sulfonyl urea derivatives, five synthesized (**RKG1491**, **RKG1493A**, **RKG1531**, **RKG1532** and **RKG1533PC**) and thirteen commercially available herbicides (**RKGC2 – RKGC14**), were

tested, and found to have had no antiprotozoal activity in the ED_{50} range of below and up to the concentration of 30 µg/ml.

Four acylthiourea derivatives (**RKG1541**, **RKG1542**, **RKG1543** and **RKG162A**) were tested. All four compounds contain the *p*-chlorobenzoyl moiety, however, the three compounds containing a second aromatic or heteroaromatic moiety showed no activity in the ED_{50} range of below and up to the concentration of 30 µg/ml. Compound **RKG162A** (containing an iso-propyl moiety) proved to be the most potent in this limited library, and displayed an ED_{50} of 13.4 µM. The cytotoxicity of **RKG162A** was not determined as it had an ED_{50} concentration greater than 300 µg/ml. This results in a favourable therapeutic index of greater than 87.

Two sulfonylcyanoguanidine derivatives (**RKG139F3** and **RKG151A**) both containing the p-chlorobenzenesulphonamide moiety were tested. Both sulfonylcyanoguanidines showed no activity in the concentration range of below and upto 30 µg/ml. Compound (**RKG136A**), an intermediate compound in the synthesis of sulfonylcyanoguanidines, proved to be active with an ED_{50} value of 95.3 µM, and favourable cytotoxicity ED_{50} of greater than 300 µg/ml, where intermediate compound **RKG150A** showed no activity in the same concentration range.

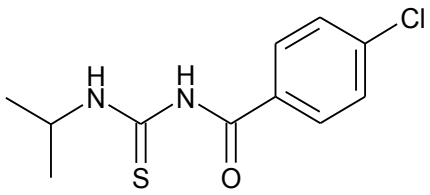
Four phthalamic acid intermediate products (**RKG114A**, **RKGANT3**, **RKG125A** and **RKG123A**) and four phthalimide derivatives (**RKG119A**, **RKG145A**, **RKG131A** and **RKG133A**) were also submitted for testing. None of these compounds, along with the commercially available herbicide, imazapyr (**RKGC1Im**), showed any activity in the concentration range of below and up to 30 µg/ml.

B1 Conclusions and future work

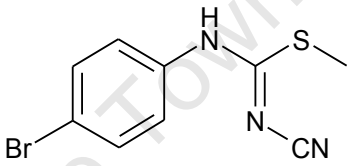
A small library of 35 compounds was tested for *in vitro* activity against *T. t. brucei*. Of these 35 compounds, two proved to be active in the concentration range below 30 µg/ml; **RKG162**, ED_{50} = 13.4 µM and **RKG136A**, ED_{50} = 95.3 µM. In comparison with the standard control drug

Pentamidine, ED_{50} = 0.00902 µM, these two compounds are moderately active against *T. brucei*. These compounds were, however, not synthesized for antiprotozoal activity evaluation, and the activity displayed is interesting in itself.

Future work may include SAR studies of acylthioureas (using **RKG162A** as a starting scaffold) and the synthesis of a library of acylthiourea derivatives where the *iso*-propyl moiety is substituted for other alkyl groups.



RKG162A



RKG136A

B2 Experimental Protocol: *In Vitro* Activities against *Trypanosoma brucei brucei* S427

Trypomastigotes (in bloodstream form) are maintained in MEM medium with Earle's salts supplemented with 25 mM HEPES, 1 g/l additional glucose, 10ml/l MEM nonessential aminoacids (100x), 0.2 mM 2-mercaptoethanol, 2 mM Na-pyruvate, 0.1 mM hypoxanthine, 0.05 mM bathocuprionedisulphonic acid, 0.15 mM L-cysteine and 15% heat inactivated, foetal calf serum. All the cultures and assays are conducted under an atmosphere of 5% CO_2/95% air mixture at 37 °C.

Drug sensitivity assays

The stock drug solutions are prepared in 100% DMSO unless otherwise suggested by the supplier at 20 mg/ml, and ball milled or sonicated if necessary, and stored at 4 °C. For the assays, the compound is further diluted to the appropriate concentration using complete medium. Assays are performed in sterile 96-well microtiter plates, each well containing 100 µl of parasite culture (1 x 104 bloodstream forms) with or without serial drug dilutions at 37°C for 72 hours in 5% CO_2. The highest concentration for the test compounds is 30 µg/ml. Each drug is tested in triplicate. A 3-fold serial dilution is performed down to a suitable concentration to obtain an ED_{50} value. Initial testing is 30, 10, 3 and 0.1 µg/ml. The control drug is Pentamidine and is diluted down to 0.0001 µg/ml (12 dilutions). Control wells are without drug, blanks are medium only. After 72 h of incubation the plates are inspected under an inverted microscope to assure growth of the controls and to determine the minimum inhibitory concentration (MIC): this is the lowest drug concentration at which no trypanosomes with normal morphology and motility as compared to the control wells can be seen. 20 µl of Alamar Blue are added to each well and the plates incubated for another 2 – 4 h. Then the plates are read on a Gemini Plate Reader (Molecular Devices) using an excitation wave length of 530 nm and an emission wave length of 580 nm (cut off 550 nm).

Primary screen

The primary screen uses the *T. b. brucei* strain. The compounds are tested at 7 concentrations (drug concentration range from 30 µg/ml to 0.1 µg/ml in 3-fold dilutions). In this assay Pentamidine had a mean ED_{50} value of 3.07 ng/ml.

Secondary screen

Active compounds (ED_{50} <0.2 µg/ml) are tested again over the appropriate dose range against *Trypanosoma brucei rhodesiense* STIB 900 to confirm IC_{50} and IC_{90} values in comparison to standard Pentamidine and internal chemical group standard if available.

B3 Toxicity Test Protocol

Cell cultures

KB cells (a cell line derived from a human carcinoma of the nasopharynx) is typically used as an assay for antineoplastic agents. KB cells are maintained as monolayers in RPMI 1640 + 10% HIFCS. All cultures and assays are conducted at 37 °C under an atmosphere of 5% CO_2/95% air mixture.

Drug toxicity assays

Stock drug solutions are prepared in 100% DMSO unless otherwise suggested by the supplier at 20 mg/ml, and ball milled or sonicated if necessary, and stored at 4 °C. For the assays, the compound is further diluted to the appropriate concentration using complete medium.

The cytotoxicity test is carried out as detailed in following steps:

Day 1

KB cells are harvested, counted and washed in serum-free medium (2000 rpm, 10 mins, 4 ^{o}C) and resuspended in fresh medium (RPMI 1640 + 10%HIFC) at a concentration of 4 x 10^4/ml. 100µl is added to wells on a 96-well plate (4 x 10^3/well). The plate is incubated overnight at 37 ^{o}C, 5% CO_2/air mix to allow the cells to adhere.

Day 2

Test compounds are prepared in 100% DMSO 20 mg/ml and diluted down to a starting concentration of 600 µg/ml (2X top concentration) with RPMI + 10% HIFCS. Control wells had no drug. A 10-fold serial dilution is performed across the plate – 300, 30, 3 etc. Podophyllotoxin is used as the control drug. The plate is incubated for 72 hours at 37 ^{o}C, 5% CO_2/air.

Day 5

Each well is assessed by microscope observation. 20 µl Alamar Blue™ is then added to each well. Plates are incubated for a further 2 - 4 hours before reading (Gemini), EX/EM 530/580, cut-off 550nm IC_{50} (IC_{90}) values are calculated using sigmoidal regression analysis (MS *xl*fit™).

B4 References

[1] http://www.dpd.cdc.gov/dpdx/HTML/Leishmaniasis.htm

[2] O'Sullivan, M. *Curr. Med. Chem.*: *Anti-infective Agents* **2005**, *4*, 355-378.