Antimalarial and Cysteine Protease Inhibitor Pharmacophores as Scaffolds for New Antimalarial Agents

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Antimalarial and Cysteine Protease Inhibitor Pharmacophores as Scaffolds for New Antimalarial Agents

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Doctor of Philosophy

By

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Dedication:

To my late young brother and friend Brian Mubanga Musonda, who did not live long enough to see me through to my postgraduate studies, (M.Y.S.R.I.P).

DECLARATION

Unless otherwise stated all the work presented in this thesis was carried out in Department of Chemistry, Faculty of Science at the University of Cape Town by Chitalu Christopher Musonda Jr under the supervision of Associate Professor Kelly Chibale.

No part of this thesis has been submitted in the past, or is being or is to be submitted for a degree at this University or any other university.

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Date

Abstract

Malaria, caused by protozoan parasites of the genus *Plasmodium*, remains one of the greater health problems to have faced mankind throughout recorded history. Current estimates are that over 300 million people are afflicted, while between 1.5 and 2.7 million people are killed annually. In sub-Saharan Africa it has been estimated to cause an annual infant mortality of nearly 1 million. Worse still, the malaria parasite has developed resistance to antimalarial drugs where the disease is endemic. Thus, there is an urgent need to develop new chemotherapeutic agents that can escape this resistance.

The work presented in this thesis is threefold:

(i) A new series of antiplasmodial agents were initially designed based on the β-amino alcohol bioactiphore, a subunit that is found in a number of antimalarial agents. The compounds were generally low in activity against a chloroquine-resistant K1 strain of *P. falciparum*, with the most active (**3.49**) having an IC_{50} of 330 nM. The chloroquine potentiation effects of the compounds were also measured, and compound **3.24** was found to reverse chloroquine resistance more than threefold in the same strain. Second generation compounds were designed in such a way as to hybridize the β-amino alcohol, the 4-amino-7-chloroquinoline antimalarial pharmacophore template and different privileged substructural motifs. Upon synthesis, the compounds were evaluated for their abilities to inhibit plasmodial and trypanosomal growth *in vitro*. The two most active compounds from the series inhibited growth in a chloroquine-sensitive *P. falciparum* D10 strain with IC_{50} of 53 nM, while chloroquine inhibited parasite development in the same strain with an IC_{50} of 96 nM. One of the compounds, **3.68**, was able to suppress parasitaemia *in vivo* in an animal model upto 97% 15 days after infection. The same compound also exhibited high antitrypanosomal activity, arresting development of *Trypanosoma brucei brucei* with an ED_{50} of 17 nM. Synthetic

methodology was also developed for the rapid purification and isolation of libraries of β-amino alcohols.

(ii) Various thiosemicarbazones and semicarbazones were designed and synthesized as potential mechanism-based inhibitors of parasitic cysteine proteases. The compounds were then evaluated for the abilities to inhibit parasite growth *in vitro*, and also for their abilities to inhibit the activities of the malarial and trypanosomal cysteine proteases falcipain and rhodesain respectively. The activities of the thiosemicarbazones against a chloroquine-resistant W2 strain of *P. falciparum* ranged from 8 – 210 nM. The most active compounds: **4.7c**, **4.7g** and **4.17** against *T. b. brucei* cultures inhibited parasite development with respective IC_{50} values of 5.7, 5 and 2.4 nM. These did not exhibit significant toxicities to a mammalian cell line.

(iii) Multicomponent reactions offer the advantage of introducing chemical diversity in fewer steps than conventional multistep organic synthesis. New chloroquine-type compounds were designed and synthesized using the Ugi 4 component condensation reaction and it variants. The synthesized compounds ranged from simple peptidic molecules to rigid heterocycles. Of the different classes of compounds synthesized, a 2,4,5-trisubstituted aminooxazole **5.115f** inhibited parasite development in a chloroquine-resistant FCM29 strain with an IC_{50} of 3.5 nM, showing a 46-fold improvement over chloroquine in the same strain. Other compound from the 2-imidazoline series **5.67**, **5.62**, **5.70** and **5.65** were also very potent against a chloroquine-resistant K1 strain with respective IC_{50}s of 33, 84, 16 and 20 nM. Compound **5.67** was also trypanocidal towards *T. b. brucei* with an ED_{50} of 70 nM, and was only cytotoxic with an IC_{50} of 8 μM to a mammalian cell line. Synthetic methodology was also developed for the rapid purification and isolation of 4-aminoquinoline lactams.

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List of Abbreviations

amu	Atomic mass units
µg	Microgram(s)
µM	Micromolar
Ar	Aromatic
Boc_2O	Ditertiarybutyldicarbonate (boc-anhydride)
br s	Broad singlet
Bn	Benzyl
^{13}C NMR	Carbon Nuclear Magnetic Resonance
CD_3OD	Deuteromethanol
$CDCl_3$	Deuterochloroform
CDI	Carbonyl diimidazole
$CHCl_3$	Chloroform
CH_3CN	Acetonitrile
Cp	Cyclopentadienyl
Cys	Cysteine
d	Doublet (in ^1H NMR)
D_2O	Deuterowater
DCE	Dichloroethane
DCM	Dichloromethane
dd	double doublet (in ^1H NMR)
DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DMSO-d_6	Deuterodimethylsulfoxide
ED_{50}	50% Effective Dose
EDC	*N*-(3-Dimethylaminopropyl)-*N'*-ethylcarbodiimide
EI	Electron Impact
eq	Equivalent(s)
Et_3N	Triethylamine
EtOAc	Ethylacetate
EtOH	Ethanol
FAB	Fast Atomic Bombardment
Fc	Ferrocene
^1H NMR	Proton Nuclear Magnetic Resonance

h	Hour(s)
Hex	Hexane
His	Histidine
HOBt	Hydroxybenzotriazole
HPLC	High Performance Liquid Chromatography
HRMS	High Resolution Mass Spectrometry
IC_{50}	50% Inhibitory Concentration
IMCRs	Isocyanide Multicomponent Reactions
K_2CO_3	Potassium carbonate
KF-Al_2O_3	Potassium fluoride on alumina
LRMS	Low Resolution Mass Spectrometry
m	Multiplet
MCR	Multicomponent Reaction(s)
m.p.	Melting point
m/z	Mass to charge ratio
MeOH	Methanol
$MgSO_4$	Magnesium sulfate
min	Minute(s)
mM	Millimolar
mL	Millilitre
Na_2SO_4	Sodium sulfate
nM	Nanomolar
NMP	*N*-Methyl-2-pyrrolidinone
ppm	Parts per million
PFV	Parasitic food vacuole
Ph	Phenyl
R_f	Retention factor
s	Single (in ^{1}H NMR)
t	Triplet (in ^{1}H NMR)
TBAB	Tetrabutylammonium bromide
THF	Tetrahydrofuran
TLC	Thin layer chromatography
t_R'	Retention time
UV	Ultraviolet

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Chapter 2: Parasitic Cysteine Proteases and Their Inhibitors

Chapter 5: 4-Aminoquinoline-containing Compounds *via* Multicomponent Reactions (MCRs)

Publications So Far Arising from This Thesis

- Cailean Clarkson, **Chitalu C. Musonda**, William E. Campbell, Peter J. Smith, Kelly Chibale, "Synthesis of Totarol Amino Alcohol Derivatives and their Antiplasmodial Activity and Cytotoxicity," *Bioorganic and Medicinal Chemistry*, **2003**, *11*, 4417 – 4422.

- Kelly Chibale, **Chitalu C. Musonda**, "The Synthesis of Parasitic Cysteine Protease and Trypanothione Reductase Inhibitors," *Current Medicinal Chemistry*, **2003**, *10*, 1863 – 1889.

- **Chitalu C. Musonda**, Kelly Chibale, "Application of Combinatorial and Parallel Synthesis Chemistry Methodologies to Antiparasitic Drug Discovery," *Current Medicinal Chemistry*, **2004**, *11*, 2519 – 2533.

- **Chitalu C. Musonda,** Dale Taylor, Peter J. Smith, Jiri Gut, Elizabeth Hansell, Phillip J. Rosenthal, Kelly Chibale, "Application of Multicomponent Reactions to Antimalarial Drug Discovery: Part 1. Parallel Synthesis and Antiplasmodial Activity of New 4-Aminoquinoline Ugi Adducts," *Bioorganic and Medicinal Chemistry Letters*, **2004**, *14*, 3901 – 3905.

- **Chitalu C. Musonda,** Jiri Gut, Philip J. Rosenthal, Vanessa Yardley, Renata C. Carvalho de Souza and Kelly Chibale, "Application of Multicomponent Reactions to Antimalarial Drug Discovery. Part 2. New Antiplasmodial and Antitrypanosomal 4-Aminoquinoline γ– and δ Lactams *via* a 'Catch and Release' Protocol," *Bioorganic and Medicinal Chemistry*, **2006**. *In Press*.

Conference Presentations

- 5th COST B9 Congress, London School of Hygiene and Tropical Medicine, (2002), London, UK (Poster presentation)

- South African Chemical Institute Biennial Conference, (2002), Port Elizabeth, South Africa, (Poster presentation)

- Keystone Symposium on Drugs Against Protozoan Parasites: Target Selection, Structural Biology and Medicinal Chemistry (2005), Copper Mountain, Colorado, USA, (Poster presentation)

CHAPTER 1

INTRODUCTION

1.1 History of Malaria

Malaria, caused by protozoan parasites of the genus *Plasmodium*, remains one of the greater health problems to have faced mankind throughout recorded history.[1] The antiquity of the disease is manifest by the host specificity of more than a hundred parasite species found in mammals, birds and reptiles. Early descriptions and documentations of malaria were believed to have been made in ancient Egypt, Greece and China where the disease is believed to have been prevalent. Often associated with wetlands and stagnant waters, malaria was termed *"mal' aria"* by the Romans -a direct Italian translation for "bad air". The association of malaria with stagnant waters later on led to the various drainage methods that were employed by the Greeks and Romans from as early as the 6th century BC, continuing through to the Middle Ages in the greater part of Europe.[2] However, it was only in 1880 that Charles Alphonse Laveran discovered the causal agent of malaria as being a parasite.[3] The pioneering work of Sir Ronald Ross established the role of the mosquito in the transmission of malaria by discovering the complete parasite's life cycle just before the turn of the 19th century. He was awarded the Nobel Prize in 1902 for his tireless efforts in the malaria field.

1.1.1 The Global Eradication Programme

Faced with the ever growing problem of malaria, a massive global campaign to eradicate the disease was initiated in the 1960's following successful eradication in the United States of America. One of the direct results of these efforts was the reduction in the

numbers of those at risk of malaria to approximately 10% of the global population.[4] This approach of attempting to eradicate the disease was aimed at eliminating the mosquito vector by means of mass spraying of insecticides such as DDT, dieldrin etc. However, the global success of this approach has mainly been hampered by the lack of financial resources and political upheavals in developing countries, where malaria is a serious health problem. The problem has been further exacerbated by the emergence of resistance in the mosquitoes towards the insecticides that were used in such programmes.[5] Additionally, evidence suggesting developing resistance of the disease-causing parasite to insecticides was just beginning to emerge before the programme could be declared a success.

1.1.2 The Current Global Distribution of Malaria

Currently, malaria is the most important parasitic disease in the world. In the recent past, the disease has escalated and it has become one of the main causes of deaths in endemic regions. Current estimates are that over 300 million people are afflicted, while between 1.5 and 2.7 million people are killed annually.[6] In sub-Saharan Africa it has been estimated to cause an annual infant mortality of nearly 1 million.[7] Elsewhere in the world, it is estimated that malaria claims 100 000 lives annually in all age groups, mainly amongst those that lack immunity and are infected by *P. falciparum* in areas where appropriate diagnosis and treatment remain unavailable.[7] Figure 1.1 depicts the current global malaria distribution.

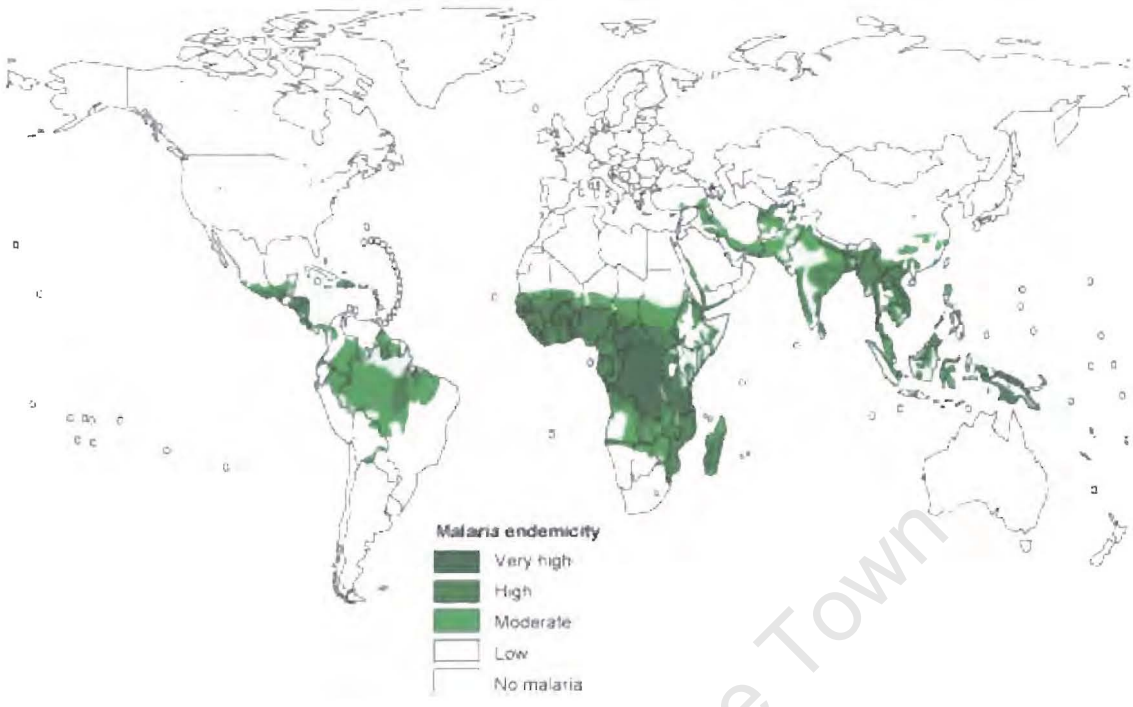

Figure 1.1: Geographic distribution of malaria[8]

In order to understand and appreciate the importance of the disease, it is vital to establish the life cycle of the causative agent at this point.

1.2 The Malaria Parasite's Life Cycle

Today it is known that human malaria is caused by four species of the *Plasmodium* genus, namely (in order of decreasing significance): *P. falciparum, P. vivax, P. malaria* and *P. ovale*. The most severe and fatal malaria, caused by *P. falciparum*, accounts for about 80% of global malaria cases and is most common in tropical Africa. The second most common malaria parasite is *P. vivax*, which is endemic to America and Asia. The other two *Plasmodia* species, *P. malariae* and *P. ovale*, are less common. However, it should be noted that all four species of the malaria parasite may occur together in any endemic region but with different degrees of prevalence.

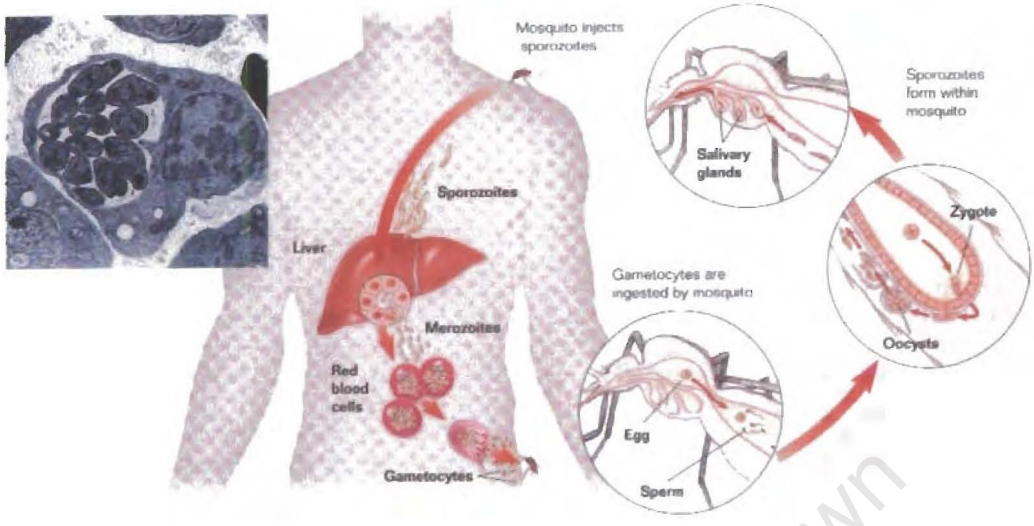

Figure 1.2: Life cycle of the malaria parasite[9]

A summary of the malaria life cycle is illustrated in Fig. 1.2. It is composed of the asexual and sexual phases with the former taking place in the host and the latter part of the cycle in the mosquito. The onset of infection is initiated by the bite of an infected female *anopheles* mosquito during the course of a blood meal. During its blood meal, the mosquito injects saliva into the human blood, which carries the infective forms of the parasite called sporozoites. Upon entry in the blood, the sporozoites are rapidly transferred to the liver where they infect the liver cells (hepatocytes) establishing infection and, in a period of ten days, multiply from a single parasite to ten thousand parasites. Following maturation of the sporozoites to merozoites, the parasites are released from the liver into the blood stream where they invade red blood cells (erythrocytes), generating a food vacuole membrane that contains lipids and proteins derived from both the host and the parasite. In the erythrocytes the parasite embarks on a 48-hour replication cycle, multiplying from a single merozoite to twenty new daughter

merozoites, each of which is capable of invading new erythrocytes. Within the erythrocytes the merozoites develop into trophozoites or 'ring forms' and enlarge in the process. This enlargement of the trophozoites is accompanied by an active metabolism that includes ingestion of host cytoplasm and the proteolysis of hemoglobin into amino acids. Within a few days in a non-immune host, the parasitaemia rises up to 10^{11} parasites! The intermittent fevers that are associated with malaria are due to the synchronous rupture of infected erythrocytes and release of merozoites. Erythrocytes that are infected with these stages of the malaria cycle adhere to endothelial cells and sequester in the microvasculature of vital organs including the brain, heart and lungs. Sequestration in the brain is often a contributing factor to cerebral malaria.

Alternatively, the parasite can differentiate into sexual forms known as macro- or microgametocytes. Ingestion of gametocytes by the mosquito vector induces gametogenesis (production of gametes) and escape from the host erythrocyte. The resulting zygote develops into a motile ookinete, which penetrates the epithelial cells and develops into an oocyst. The oocysts then undergo multiple rounds of asexual replication resulting in the production of sporozoites. Rupture of the mature oocysts releases the sporozoites into the body cavity (homoceal) of the mosquito. The sporozoites migrate to and invade the salivary glands, thus completing the life cycle.

1.3 Classification of Antimalarial drugs

1.3.1 Clinically Established Drugs

By and large, clinically established antimalarial drugs can be categorized by i) the stage of the malaria parasite that they affect and the corresponding clinical objective[10] or ii) the mechanism of action through which they act.[11-13]

1.3.1.1 Classification based on the Stage of the Life Cycle

Depending on the stage of the malaria cycle that they target, antimalarial drugs can be classified as blood schizonticides, tissue schizonticides, gametocides or sporontocides.[10] Blood schizonticides are active against the asexual intraerythrocytic stages of the parasite, where they suppress the replication of *Plasmodia* species within the erythrocytes. Tissue schizonticides act to prevent the development of hepatic schizonts and, as such are more of prophylactic drugs than curative. On the other hand, gametocides destroy the intraerythrocytic sexual forms of the parasite, preventing transmission between hosts. Sporontocides block the development of oocysts and sporozoites in the mosquito.

1.3.1.2 Classification Based on Mode of Action

Antimalarial drugs can also be classified according to the mode of action that they exert in order to achieve their observed effects. The following is a summary of the different subclasses to which most drugs currently on the market belong.

1.3.1.2.1 Compounds Acting on Haem Detoxification

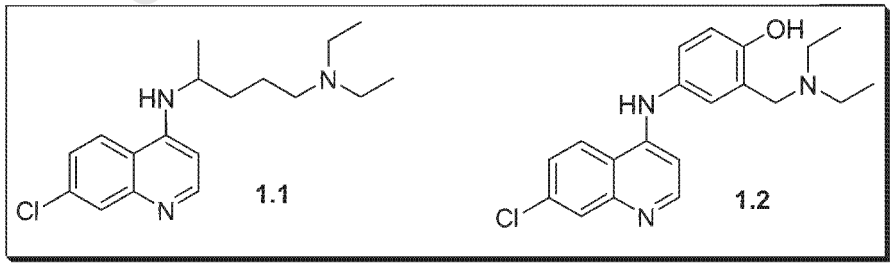

Figure 1.3: Chemical structures of chloroquine (**1.1**) and amodiaquine (**1.2**)

This group of antimalarials includes the most common antimalarial drugs such as the 4-aminoquinolines chloroquine (**1.1**) and amodiaquine (**1.2**, Fig. 1.3). Chloroquine formed the mainstay of malaria treatment for many years until recently when widespread resistance has developed in almost all areas where malaria is endemic.

Other drugs in this category include quinine (**1.3**) and quinidine (**1.4**). Both of these antimalarials are constituents of the cinchona alkaloid *Cinchona ledgenaria* ('fever tree'), whose crushed bark was used to treat malaria in the old days.

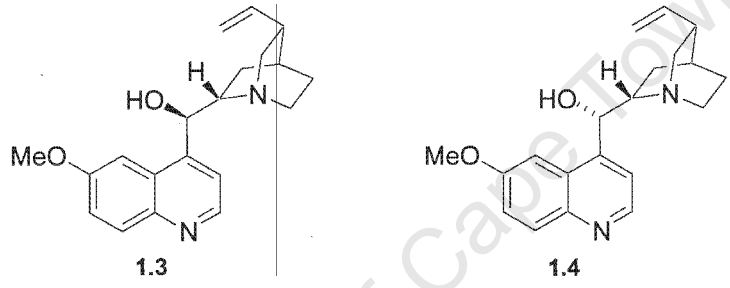

Figure 1.4: Chemical structures of quinine (**1.3**) and quinidine (**1.4**)

Reports of resistance to chloroquine begun to surface with the first cases of chloroquine-resistant strains of *P. falciparum* being reported around the sixties in South America (Brazil and Colombia) and Thailand. This led the World Health Organization (WHO) to initiate efforts to synthesize new antimalarials, which ultimately resulted in the development of mefloquine **1.5** and halofantrine **1.6**,[1] Fig. 1.5.

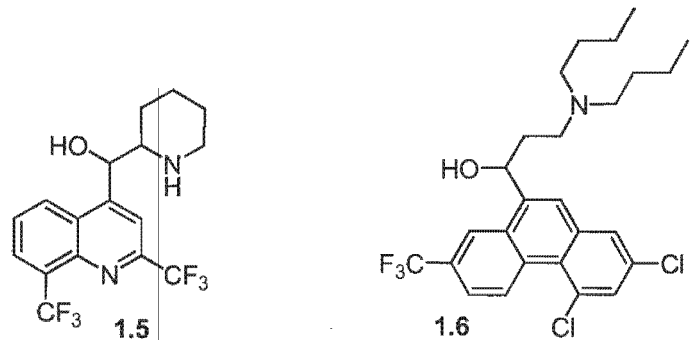

Figure 1.5: Chemical structures of mefloquine (**1.5**) and halofantrine (**1.6**)

1.3.1.2.2 Inhibitors of nucleic acid synthesis

In this group are found the antifolates, sulfonamides and naphthoquinones. Antifolates act by inhibiting dihydrofolate reductase (DHFR), causing a depletion of tetrahydrofolate and consequently DNA synthesis.[14] Single mutations in the gene encoding for DHFR are believed to be the cause of resistance to inhibitors by the parasite. This class of inhibitors is exemplified by pyrimethamine (**1.7**) and proguanil (**1.8**), Fig. 1.6.

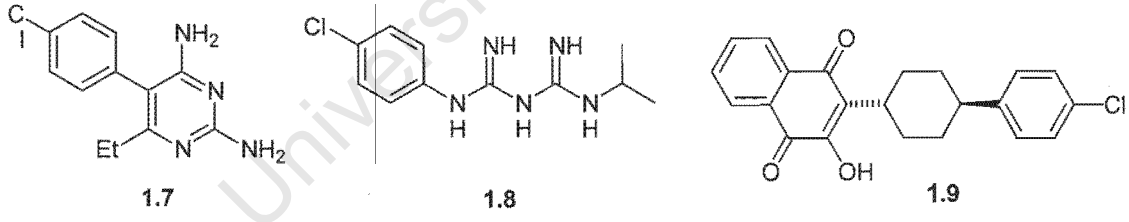

Figure 1.6: Chemical structures of pyrimethamine (**1.7**), proguanil (**1.8**) and atovaquone (**1.9**)

The second class of inhibitors, the sulfonamides, act by mimicking *para*-aminobenzoic acid (PABA) thereby preventing the formation of dihydropteroate from hydroxymethyldihydropterin (a reaction catalyzed by dihydropteroate synthase, DHPS) by competing for the active site of DHPS synthesis. Ultimately, this results in reduced dihydropyrimidine synthesis, and then DNA, serine and methionine formation. As with

antifolates, resistance to sulfonamides in *Plasmodia* is conferred by single mutations of the corresponding gene.[14]

The most successful naphthoquinone antimalarial is perhaps atovaquone **1.9** (Fig 1.6) which is however only administered in a fixed combination with proguanil (Malarone®). This combination is more efficacious than either drug alone and is effective against strains that are resistant to a variety of other antimalarials besides having a favourable safety margin. Whilst it is known that atovaquone acts primarily on mitochondrial functions, its specific mode of action and synergy with proguanil remains to be understood clearly. However, the general agreement is that it acts on the mitochondrial electron transfer chain; recently the activity and synergy of atovaquone has been ascribed to its interference with mitochondrial membrane potential.[14]

1.3.1.2.3 Inhibitors of Protein Metabolism

In this group are found the antibiotics, which have a long history in the treatment of malaria. Renewed interest in the use of antibiotics to treat malaria emerged with the appearance of chloroquine–resistant and multidrug resistant strains of *P. falciparum*. The tetracyclines (usually doxycycline or tetracycline) inhibit parasite protein synthesis in the mitochondria and/or apicoplast.[15]

1.3.1.2.4 Drugs Generating an Oxidative Stress

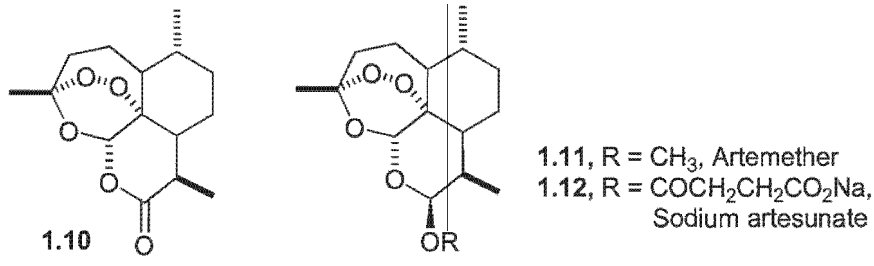

Figure 1.7: Chemical structures of artemisinin **1.10**, artemether (**1.11**) and sodium artesunate (**1.12**)

This class of antimalarials comprises artemisinin (**1.10**) and its family of derivatives (including artemether **1.11** and sodium artesunate **1.12** (Fig. 1.7), which are believed to act by generating an oxidative stress within the parasite.[16] It has been suggested that the endoperoxide group plays a key role in the antimalarial activity of the artemisinins. Indeed, one suggestion argues that the reductive activation of the O-O bond by Fe^{II}-haem should produce heme derivatives that could be alkylated by a C-centred radical derived from QHS-like compounds.[17–19] Further mechanisms of action that have been put forward suggest that these compounds inhibit a Ca^{2+} ATPase inducible nitric oxide synthetase and nuclear factor NF-kB.[20] To date, the actual mechanism of action of artemisinin and related compounds still remains unclear.

1.3.2 Novel Inhibitors of Parasitic Pathways and Enzymes

The rapid development of resistance to antimalarial drugs presents a major threat. The safest and least expensive drugs are rapidly losing their efficacy and there are few drugs to replace them. Resistance to new antimalarials has developed rapidly, in part due to the fact that many drugs are closely related or have the same target enzymes or

biological processes. For example, resistance to chloroquine developed only after the drug had been in extensive use for 20 – 30 years, while resistance to mefloquine, a structurally related compound, developed in less than four years. The evolution and spread of resistance to chloroquine and other quinoline-based drugs means that these compounds are now virtually useless in many endemic areas. There thus arises the need for new chemical entities without cross-resistance to existing antimalarial drugs. One way of achieving this is to identify new biological targets on which to base new treatment strategies. In this vein, greater effort must be devoted to the development of additional novel drugs to those previously known, in the hope that the new drugs would not demonstrate cross-resistance with the current antimalarials.

To date, a number of biosynthetic processes are known to be occurring within the malaria parasite, some of which are unique to the organism. Since some of these processes are essential for the survival of the parasite, their inhibition could lead to the death of the parasites. Indeed, this strategy is currently under exploitation, and several targets have been identified and validated. Some of these novel biological processes, enzymes and organelles include host cell proteins, the mitochondrion, membrane biosynthesis, cell cycle control, the plastid organelle and parasite transporters.[21]

1.4 Mechanism of Action of Quinoline-Containing Antimalarials

Perhaps the most significant and most efficacious group of antimalarials, they include the chloroquine-type 4-aminoquinolines, 8-aminoquinolines, and quinine-type β-amino alcohols. The mechanism of action of chloroquine (**1.1**) remains unresolved despite its many years of field use and study. It is believed that chloroquine is selectively taken up inside the parasitic food vacuole where the parasite actively digests haemoglobin.[22] The weakly basic nature of chloroquine helps to explain its accumulation in the food vacuole;

at neutral pH chloroquine could diffuse across the membranes against a pH gradient using energy and, once in the acidic vacuole, the protonated drug becomes trapped.[23]

The observation that chloroquine is only active against the intraerythrocytic stages of the malaria cycle has provided a clue to its mechanism of action. The currently held hypothesis is that of an interaction of chloroquine with the haemoglobin digestion process in the acidic food vacuole. Thus, chloroquine would interfere with polymerization of haem into nontoxic haemozoin (malaria pigment), thereby causing an accumulation of haem, which is toxic to the parasite. The presence of haem within the parasite does not however seem to be fatal to the parasite, as the excess could presumably be disposed of by diffusion from the cell or possibly by reduced glutathione-mediated degradation.[24] The toxic event, then, would be the formation of a toxic haem-chloroquine complex which would protect haem from being degraded by reduced glutathione.

Like chloroquine, the quinine-type β-amino alcohols act on the intraerythrocytic stages of the malaria cycle. Experiments with proteinase inhibitors have revealed that the interaction of these antimalarials with haem is central to their activity.[25]. Being less basic than chloroquine, it is believed that the uptake of this class of compounds is enhanced by the action of a specific transport system.[26]

1.4.1 Mechanism of Chloroquine Resistance

The development of resistance to chloroquine was gradual, prompting the suggestion that multiple mutations were required to produce the resistant phenotype. In the early years of its development, resistance to chloroquine was localized to South America and South East Asia, but to date the resistance has virtually spread to all areas where malaria is endemic.[27] Following its establishment, the resistant phenotype seems stable, persisting even in the absence of drug pressure.

In order to understand the occurrence of resistance towards the quinoline drugs in *P. falciparum*, we need to look at the problem of this resistance from three fundamental points: i) by what cellular mechanism(s) the parasite escapes the toxic action of these drugs; ii) whether the parasite undergoes genetic mutations or changes in gene expression to develop this resistance, and iii) whether changes to the existing quinoline drugs may circumvent the observed resistance.

Chloroquine-resistant parasites exhibit reduced cellular accumulation of the drug compared to their nonresistant counterparts[28,29] It is argued that this reduced accumulation is either a result of reduced uptake of the drug or an enhanced drug efflux from the infected cells.[30] The use of chemosensitisers – compounds without any inherent cytotoxicity, which can completely reverse the resistance of cells to the cytotoxic action of drugs,[31] e.g. verapamil, in combination with chloroquine to reverse resistance has been observed in the phenomenon of multidrug resistance (MDR) in cancer. MDR is believed to stem from the presence of an ATP-dependent, membrane-bound protein called P-glycoprotein (Pgp).[30,31] Verapamil then, would compete with anti-cancer drugs for Pgp binding thereby preventing efflux from the cell compartments. The similarities that exist between MDR and chloroquine resistance in malaria parasites suggest that a similar energy-dependent efflux protein may be present in malaria parasites.[28,32] *P. falciparum* possesses a transmembrane protein called P-glycoprotein homologue 1 (Pgh 1) coded for by the *Plasmodium falciparum* multidrug resistance 1 (*pfmdr1*) gene, which resembles the mammalian protein Pgp.[33]

Evidence that resistance to chloroquine is associated with a second gene, called *Plasmodium falciparum* chloroquine transporter (*pfcrt*) gene has recently become more compelling.[34,35] The gene codes for the protein PfCRT, a member of the drug

metabolite/transporter superfamily that is located on the food vacuole membrane of intraerythrocytic parasite. PfCRT has been directly linked with chloroquine resistance and, current evidence attests to the function of the protein as a transporter that directly mediates the efflux of chloroquine from the food vacuole.[35] The pattern of mutations in this protein has been observed to be different among isolates emanating from the Old and the New Worlds, correlating with the origin of resistance in South America and Southeast Asia.[34] A common mutation, (K76T) across the different chloroquine resistant strains appears to be the cause of resistance.

Another recent hypothesis that has been put forward to explain the cause of resistance in *P. falciparum* suggests that an increase in glutathione levels is met with increased resistance to chloroquine.[36] In fact, it was found that the increase in glutathione was accompanied by a corresponding increase in the activity of glutathione-S-transferase, an enzyme that catalyzes the synthesis of glutathione.[36]

Other hypotheses that have been put forward to explain chloroquine resistance include reduced Na^+/H^+ exchanger (NHE),[37] increased vacuolar pH[38] and reduced access to haematin.[39]

1.5 Prevention and Cure of Malaria

Prevention of malaria requires observance of a number of measures, some of which are discussed in the following subsection.

1.5.1 Effective Prevention

Effective prevention of malaria may be attained by observing the following measures:

i. An awareness of risk. In order to avoid contracting malaria, it is vital that individuals are aware of the risks that their surroundings pose. The risk of

being bitten by a mosquito and the type of malaria transmitted varies from one location to another and the time of the year. This should also include proper disposal of rubbish in order to minimize standing water close to habitation and spraying of unavoidable standing water within areas of habitation with insecticides.

ii. Bite avoidance. The use of repellent creams containing diethyltoluamide (DEET) is highly recommended for use in areas where mosquitoes are prevalent. DEET has an excellent safety profile in all age groups but should only be used to recommended limits. The use of bed nets impregnated with insecticides also significantly reduces the risk of bites.

iii. Chemoprophylaxis. Taking drugs to prevent malaria infection is an essential aspect of prevention and malaria control where the disease is prevalent. Different antimalarials are available depending on a particular location as well as on individual circumstances. Some of the drugs currently used for prophylactic purposes include proguanil, mefloquine, doxycycline, maloprim and Malarone®.

iv. Diagnosis. When made promptly followed by early treatment of an infection, this serves as a successful preventive measure.

1.5.2 Cure of Malaria

The curative measures discussed in the following section may be considered as novel approaches as opposed to the use of already existing drugs to which the parasite may have become resistant. In this vein, regimens for malaria are not discussed in this section. Although not entirely practical at present in all cases, drug resistant malaria may

be cured by either delaying the emergence of resistance to multiple drugs or reversing it. Alternatively, the emergence of resistance may be reversed by the use of chemosensitisers or modifying already existing drugs.

1.5.2.1 Delaying the Emergence of Drug Resistance

This can be achieved by the introduction of completely new drugs on the market or drugs that are targeted at the resistant organisms. An alternative approach is combination therapy in which more than one drug is administered. This second approach is employed based on the principle that drug resistance to each drug occurs by an independent mechanism and that resistance to multiple drugs will only occur when a single organism or cell contains mutations that lead to resistance to each independent drug.

1.5.2.2 Reversal of Drug Resistance by using Chemosensitizers

Two different mechanisms are known to cause drug resistance in *P. falciparum*, namely the selection of mutations in the target enzymes and the amplification of certain genes associated with drug resistance. An alternate strategy would be direct targeting of the mechanism of the drug resistance and attempt to either prevent it or reverse it once it has appeared. In this regard, compounds that appear to inhibit drug efflux can thus be used to modulate drug resistance or chemosensitize resistant parasites to conventional drugs.

1.6.1 Reversing Resistance: Structural Modifications to Chloroquine

1.6.1.1 Modifications to the Quinoline Ring

Modifications to the quinoline ring have provided SAR studies with interesting results.[40,41] Earlier independent studies on antiplasmodial activity and inhibition of β-haematin formation by De *et al* and Kaschula *et al* established the significance of the nature of the

substituents at the C-7 position of new synthetic chloroquine analogues. These findings are discussed in a later section of this thesis (section 3.6.1.1, p. 66), suffice to say that the findings lead one to surmise that the antiplasmodial activity of chloroquine may be influenced by the negative inductive effect of the group attached at C-7 and/or the lipophilicity of the group.

1.6.1.2 Modifications to the Aminoalkyl Side-Chain

Analogues of chloroquine in which the alkyl chain is either shortened, bearing 2 or 3 carbon atoms, or significantly lengthened up to 10 – 12 carbon atoms, retain their activity against the chloroquine resistant strains, while those that have chain lengths approaching that of chloroquine possess intermediate activities.[41] This finding suggests a structure-specific resistance mechanism at play on the one hand, and a mechanism that seems to recognize the aminoalkyl side chain of chloroquine on the other. Other modifications to the aminoalkyl side-chain that have yielded biologically active compounds, particularly against resistant parasites, include the *bis*-quinolines,[43] also with varying lengths of methylene spacers and incorporation of a ferrocenyl subunit[44] or a phenyl group.[45]

1.7 Other Parasitic Protozoan Diseases

There is evidence at hand to suggest that compounds that are active antimalarials also exhibit potent activity against other parasitic diseases.[46] In this vein, although the primary focus of this research was malaria, a deliberate restriction has been made to the discussion of a disease that is caused by a specific protozoan, and for which compounds intended for screening against the malaria parasite were also screened against this parasite.

1.7.1. African Sleeping Sickness

African Trypanosomiasis, also called 'sleeping sickness' in humans (nagana in cattle), is caused by protozoan parasites within the *Trypanosoma brucei* complex.[47] The parasite is spread to humans through the bite of a tsetse fly and is believed to infect 300 000 – 500 000 people in Africa, mostly in the sub-Saharan regions. If it remains untreated the disease could be fatal. If an infected tsetse fly bites and transmits the parasite to a human, the trypanosomes (infective forms of the parasite) travel through the blood to the brain, causing multiple neurological symptoms that remain even after treatment. Two subspecies of *T. brucei* infect humans, and each causes a different illness. No vaccine is available for African trypanosomiasis. The disease can however be treated if diagnosed early enough, but currently available drugs are generally associated with cytotoxicity.

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CHAPTER 2

PARASITIC CYSTEINE PROTEASES AND THEIR INHIBITORS

2.1 Introduction

Enzymes that catalyze the hydrolysis of peptide bonds are called peptidases (or proteases). Depending on the site of hydrolysis, the peptidases may be categorized as endo- or exopeptidases.[1] The former catalyze cleavage (hydrolysis) of internal amide bonds, while the latter catalyze cleavage of terminal peptide bonds. Further classification of the endo- and exopeptidases divides them into amino and carboxy (endo/exo-) peptidases. Amino exopeptidases catalyze cleavage on the amino terminal of peptide chains; similarly carboxy exopeptidases catalyze hydrolysis on the carboxy terminal of peptide bonds.

Another classification of exo- and endopeptidases, based on the reactive groups at the active site involved in catalysis classifies them as serine, aspartic, threonine and cysteine endopeptidases, and metalloendopeptidases. Cysteine proteases (previously called thiol proteases) have been found in a range of organisms including parasitic protozoa, viruses, bacteria, plants and mammals,[2] and more recently in fungi.[3,4] Since the focus of the work contained herein is on parasitic protozoa, discussion of peptidases will be limited to cysteine proteases found in parasitic protozoa, with strict restriction to plasmodial (falcipains) and trypanosomal (cruzain and rhodesain) cysteine proteases.

2.1.1 Parasitic Cysteine Proteases

Parasitic cysteine proteases contain two principal amino acid residues that are actively involved in the hydrolysis of peptide bonds, namely Cys 25 and His 159. The cysteine proteases utilize the cysteine thiol group for their catalytic activity. Members of the cysteine protease group are broadly classified into two main clans called CA and CD.[5, 6] The CA clan or papain superfamily encompasses the majority of cysteine proteases in which the catalytic residues (Cys 25 and His 159) are highly conserved among all the members.

2.1.1.1 Protease Binding and Classification of Binding Pockets

The active site of an enzyme is a 3D shape that has to be on or near to the surface of the enzyme if the substrates are to reach it. It could be a groove, hollow or gully that allows the substrate to 'sink into' the enzyme. The active site of an enzyme performs the dual function of binding the substrate and catalyzing a reaction. The efficiency of these two processes defines the overall activity of the enzyme towards the particular substrate (i.e. it determines the specificity of the enzyme). Some important factors that affect interactions between the active site and a substrate are size, polarity, accessibility and charge.

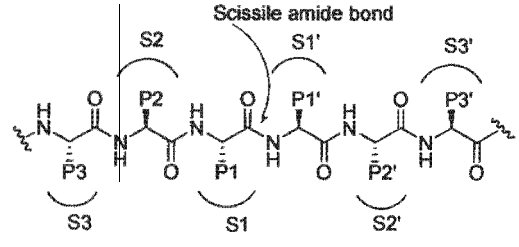


Figure 2.1: Nomenclature of papain active site

The standard nomenclature for designating the substrate-inhibitor residue is based upon that originally described by Schecter and Berger,[7] Fig. 2.1. Papain-like cysteine proteases are believed to have an active site that possesses several 'subsites', each of which has the ability to accommodate a single amino acid residue of the peptide substrate. Basing on the assumption that the amino acids are lined up in such a way that the amide bond serving as the site of hydrolysis occupies a particular location, (herein called the active site), then the amino acids that occupy adjacent sites towards the N-terminal end are termed P_1, P_2, P_3...P_n substituents, whilst those towards the C-terminal are called P_1', P_2', P_3'...P_n'. The corresponding binding sites are termed S_1, S_2, S_3.....S_n and S_1', S_2', S_3'..... S_n' respectively.

2.1.1.2 Mechanism of Proteolysis

The proposed mechanism of peptide hydrolysis catalyzed by cysteine proteases, in which an active site cysteine thiol is involved during hydrolysis, is shown in Fig 2.2. His-159 in close proximity polarizes the enzyme cysteine thiol group allowing deprotonation to occur even at near neutral pH to generate a highly nucleophilic thiolate/imidazolium ion pair,[8,9] (Fig. 2.2A). This strongly nucleophilic pair allows the thiolate ion to attack the carbonyl carbon of the scissile amide bond resulting in a tetrahedral intermediate (Fig. 2.2B). Stabilization of this transition state oxyanion occurs by H bonding to the NH backbone of Cys-25 and to the NH_2 group of the Gln-19 side chain. Acylation of the enzyme makes the imidazolium ion sufficiently acidic to protonate the nitrogen of the leaving group, and liberation of the first product follows immediately (Fig. 2.2C). A second tetrahedral intermediate is then formed *via* base-catalyzed hydrolysis of the acyl-enzyme (Fig. 2.2D). Following collapse of the second tetrahedral intermediate and product release (Fig. 2.2E), the enzyme is regenerated (Fig. 2.2F).

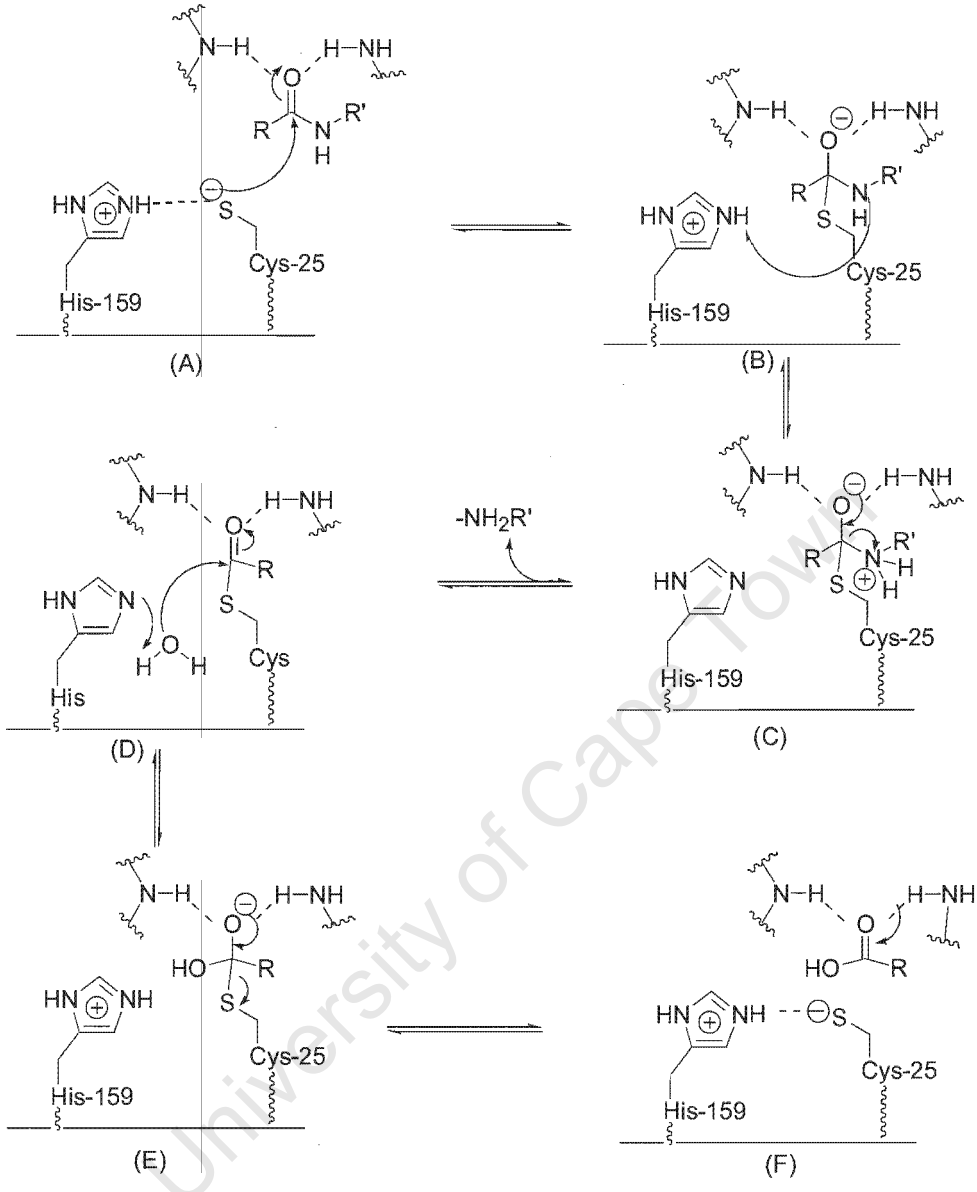


Figure 2.2: Mechanism of peptide hydrolysis catalyzed by cysteine proteases

2.1.2 The Role of Falcipains in Haemoglobin Degradation

2.1.2.1 Utilization of Host Amino Acids by the Parasite

The life cycle of the malarial parasite was discussed in the previous chapter (section 1.2). One of the key organelles of the parasite, hosting important biochemical processes on which parasite survival is dependent, is the food vacuole. These important processes

include acidification, haemoglobin degradation, peptide transport, haem polymerization and quinoline action. Also, several key parasitic enzymes are known to reside in the parasitic food vacuole (PFV) including the cysteine proteases (falcipains), plasmepsins, a metalloprotease (falcilysin), a haem polymerase, etc.

The degree to which the intraerythrocytic stages of the malaria parasite can perform *de novo* synthesis of amino acids is rather limited. Hence, in order to obtain the much needed amino acids for protein biosynthesis, the malaria parasite must rely on degradation of host proteins, as well as import of extracellular nutrients. Evidence attesting to the suggestion that haemoglobin provides this source of amino acids has established that within 12 h of the ring or trophozoite-stage, the parasite degrades 75% of the host haemoglobin.[10-12] This evidence is further supported by the finding that amino acids from radiolabelled haemoglobin are incorporated into parasite proteins.[13,14] Indeed, if the degradation of haemoglobin is somewhat retarded by means of genetic or chemical modifications, parasite growth is directly interfered with; erythrocytes that contain fetal haemoglobin, which is poorly cleaved by proteases, do not support parasite growth.[15,16]

2.1.2.2 The Haemoglobin Degradative Pathway

It seems the degradation of haemoglobin occurs in a semi-ordered pathway according to Fig. 2.3 (17). Accordingly, distinct proteases have been isolated from the parasitic food vacuole and have been shown to be responsible for the degradation of the haemoglobin tetramer. These proteases have been identified as the aspartic proteases (plasmepsins), cysteine proteases (falcipains) and a metalloprotease (falcilysin).

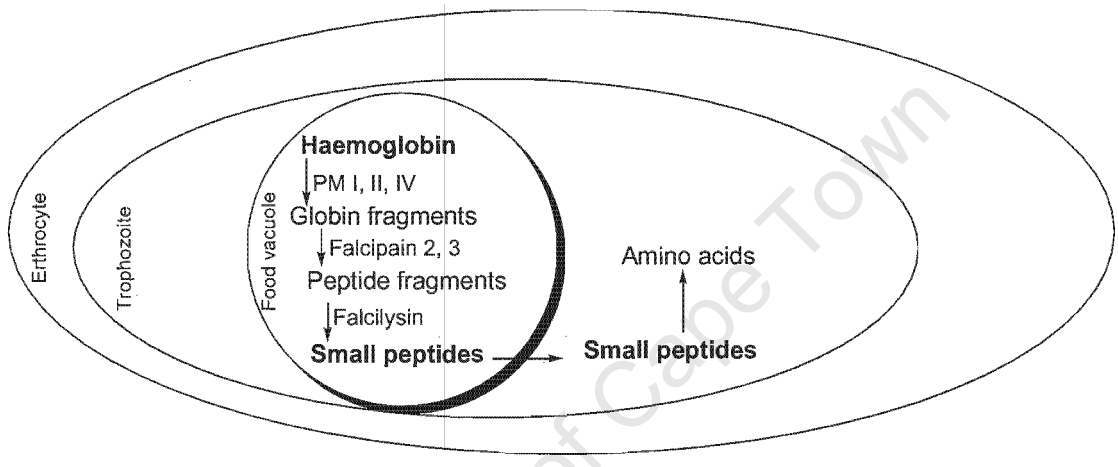


Figure 2.3: The haemoglobin degradation pathway[17]

The sequence of events leading to the liberation of free amino acids from haemoglobin is believed to occur as shown in Fig. 2.3.

(a) Initially, plasmepsins I, II and IV (PM I, II and IV) cleave native haemoglobin at the same site, location 33Phe-34Leu in the α-chain.[18,19] Once the haemoglobin has been denatured, the plasmepsins make different secondary cleavages, with plasmepsins I preferring Phe at the P_1 position and plasmepsins II preferring other hydrophobic residues (especially Leu) at the P_1' position.[19] Bray and coworkers have provided unequivocal data on the degradative pathway by showing that plasmepsin inhibitors prevent haem release during short- or long-term in culture or in extracts.[20]

(b) The falcipains, three of which have been identified as falcipains 2A, 2B (hereafter referred to as falcipain 2) and 3 are involved in the next step of the metabolic pathway. A fourth falcipain, called falcipain 1 is known, although it does not seem to take part in this pathway. Falcipains 2 and 3 are incapable of degrading native haemoglobin, but cleave denatured haemoglobin under non-reducing conditions.[18,19,21] Thus, once the globin fragments have been generated, falcipains 2 and 3 carry out further degradation to peptide fragments that are 10 – 15 amino acids in length. A second role for falcipain 2 was proposed by Hanspal *et al*,[22] implicating the enzyme in assisting cleavage of specific components of the erythrocyte skeleton at the inner surface of the parasite membrane.

Falcipains 2 and 3 prefer substituents with Leu at the P_2 substrate. However, falcipain 3 differs from falcipain 2 in the following respects:[23]

- It is only efficiently processed into the active form at acidic pH;
- It is more stable and active at acidic pH;
- It has a lower specific activity against typical papain-family peptide substrates.

On the other hand, the role of falcipain 1 remains to be resolved. Initially, Greenbaum *et al* proposed that falcipain 1 was involved in host cell invasion,[24] but subsequent studies by Greenbaum and coworkers and other independent researchers revealed this not to be the case.[25,26]

(c) Finally falcilysin, a metalloprotease enzyme that is incapable of cleaving either haemoglobin or denatured globin, comes into play further downstream. It cleaves the short peptide fragments generated by the proteolytic activities of the plasmepsins and falcipains 2 and 3 into even shorter peptides of 6 – 8 amino acids. Falcilysin prefers to cleave at the sites in which the P1, P1′ and/or P4′ residues are polar.

2.1.2.3 Falcipains in Drug Discovery

Cultured malarial parasites that are treated with cysteine protease inhibitors develop swollen food vacuoles and become filled with undegraded globin, implicating a role for the falcipains. This is probably a result of the inability of the falcipains to degrade plasmepsin-generated globins, causing the observed build up to levels that cause osmotic swelling.[18] A number of classes of small molecule inhibitors of cysteine proteases have been shown to inhibit falcipain activity and to cause similar antiparasitic effects. Evaluation of inhibitor-treated parasites showed that they had accumulated native haemoglobin indicating that the entire process of haemoglobin degradation had been blocked.[27] The degree of inhibition of falcipain 2 correlated with the degree of parasite inhibition. Five strains of *P. falciparum* that differed widely in their sensitivities to standard antimalarial agents all showed high sensitivity to fluoromethyl and vinylsulfone cysteine protease inhibitors, providing some argument that multidrug resistant parasites may not be resistant to cysteine protease inhibitors.

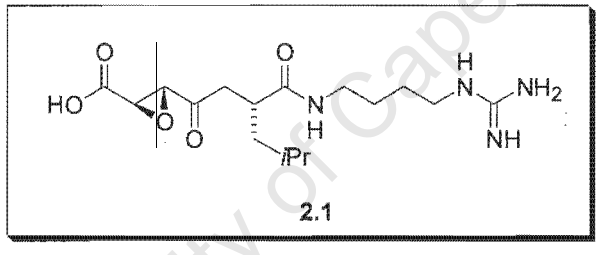
In vivo, a fluoromethyl ketone was capable of curing 80% of *P. vinckei*-infected mice, albeit at high and frequent dosages.[28] Similarly, a vinylsulfone cysteine protease inhibitor was able to clear 40% of *P. vinckei*-infected mice treated orally at 50 – 100 mg/kg twice per day for four days.[29]

Therefore, it is apparently clear that the development of specific, potent falcipain inhibitors may serve as potential therapeutics. Indeed, the development of malaria cysteine protease inhibitors has intensified in the recent past and, hopefully with the recent advances in the characterization of the falcipains, drug discovery efforts in this area may just begin to bear fruit.

2.1.3 Trypanosomal Cysteine Proteases

2.1.3.1 Cruzain

Cruzain, also called cruzipain, is the major cysteine protease of *T. cruzi*, the causative agent of Chagas' disease (section 1.7.2). The enzyme is expressed in all the life cycle stages of the parasite. It's relation to the papain family of cysteine proteases, manifested in its known fragment sequences, specificity of proteolysis and inhibition by the naturally-occurring cysteine protease inhibitor called E-64 (2.1, Fig 2.4) was proven by its crystal structure. Cruzipain may function in nutrition, remodeling of the mammalian cell, or evasion of host defence mechanisms.[30]



2.1

Figure 2.4: Chemical structure of E-64

Experiments have shown that addition of a cruzain inhibitor to cultures of mammalian cells exposed to trypomastigotes or to mammalian cells already infected with *T. cruzi* amastigotes block replication and differentiation of the parasite, thereby arresting the parasite life cycle.[31] This disclosure provides evidence for the essential role of cruzipain in the replication of intracellular parasites.

2.1.3.2 Rhodesain

Rhodesain is the major cysteine protease found in the subspecies *T. brucei rhodesiense*, the causal agent of African sleeping sickness. The enzyme is present in

the parasite during all stages of the life cycle, and has been implicated in regulation of parasite replication.[32] Inhibitors of the enzyme block the parasite life cycle in infected mammalian cells.

2.1.4 Inhibitors of Cysteine Proteases

At present, a whole range of inhibitors of cysteine proteases exists. However, despite the large number of inhibitors known to date, they are broadly categorized into three distinct classes depending on the mechanism of inhibition,[1,33] which are discussed below.

2.1.4.1 Irreversible Inhibitors

This group of inhibitors encompasses a series of active site titrants in which the mechanism of inhibition is dependent upon the alkylation of the thiolate anion of Cys-25 of the active site. These inhibitors are exemplified by the epoxide **2.2**,[34] vinyl sulfone and α,β-unsaturated ester Michael acceptors **2.3** and **2.4**,[35] respectively, Fig. 2.5. Several other examples not included here exist in the literature.[36]

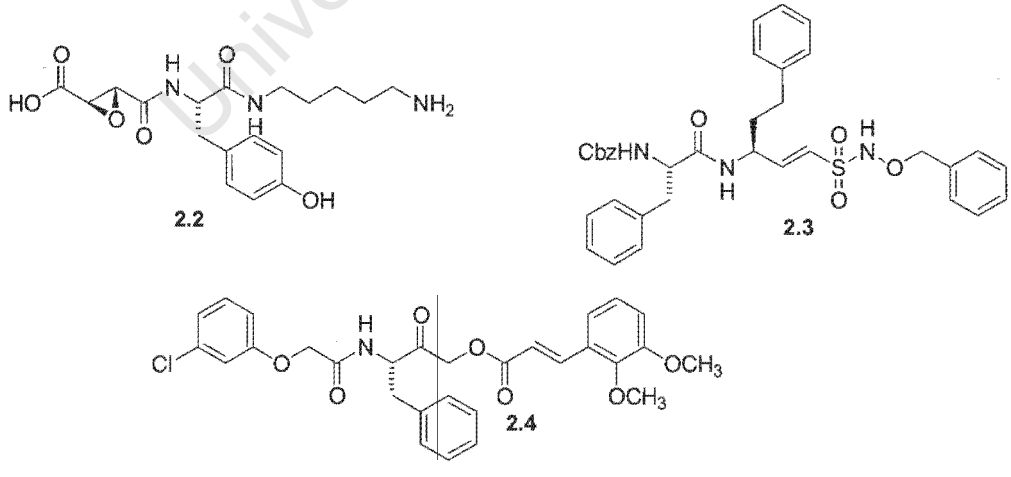


Figure 2.5: Chemical structures of epoxide (**2.2**), vinylsulfone (**2.3**) and α,β-unsaturated ester (**2.4**) irreversible cysteine protease inhibitors

2.1.4.2 Reversible Inhibitors

This group of inhibitors includes compounds that depend on the formation of a covalent, but reversible transition state intermediate with the active site cysteine thiol. Some of the earlier examples included peptidyl aldehydes, which lack selectivity due to their high reactivity. Coupled with the metabolic liabilities associated with them, they are considered not to be ideal candidates for drug development. Otherwise, their less reactive ketone counterparts (exemplified by **2.5**, Fig. 2.6),[37] serve as better examples of reversible inhibitors of cysteine proteases, including thiosemicarbazones (**2.6**) and pyrazolines (**2.7**),[38] and isatins (**2.8**).[39]

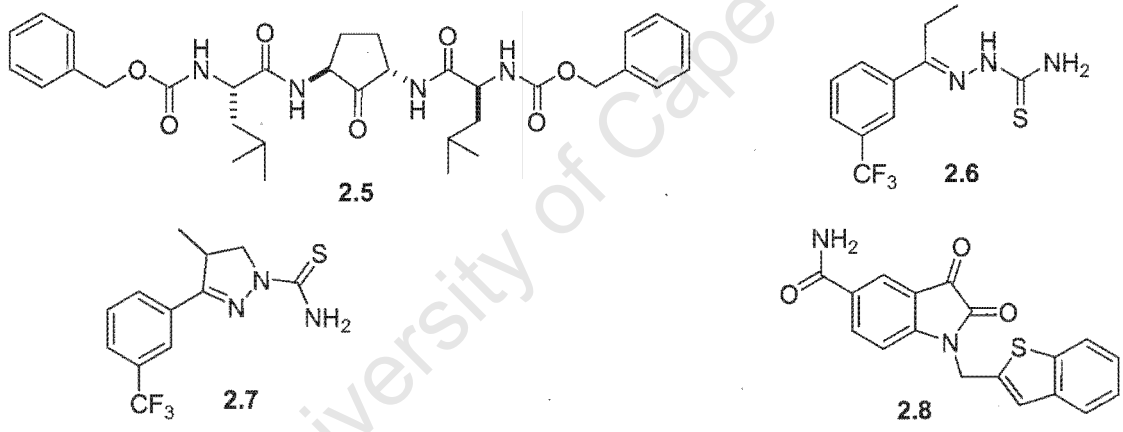
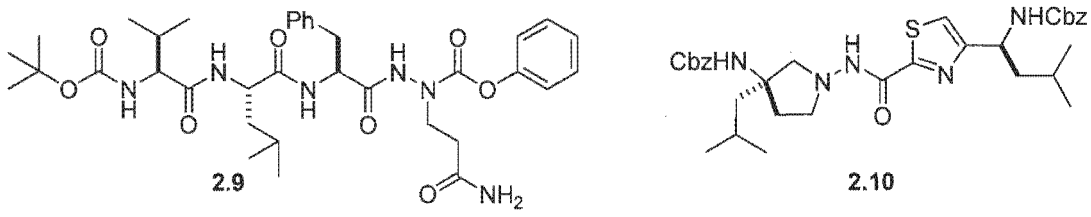


Figure 2.6: Examples of 1,3-diaminoketone **2.5**, a thiosemicarbazone **2.6**, pyrazoline **2.7** and isatin **2.8** reversible parasitic cysteine protease inhibitors

2.1.4.3 Slow Turnover Inhibitors

This class of inhibitors includes a series of compounds that act by forming a stable thioacyl-enzyme complex that is slow to hydrolyze. The group is principally exemplified by aza-substituted peptides, such as the peptidyl carbazate ester **2.9**[40] and the diacyl hydrazide **2.10**,[41] Fig. 2.7.



2.9 2.10

Figure 2.7: Chemical structures of a peptidyl carbazate ester **2.9** and a diacyl hydrazide **2.10**

2.2 Aims and Objectives:

The research question and/or hypothesis we intended to address was whether compounds synthesized based on suitable (known) respective cysteine protease and antimalarial bioactiphores (or pharmacophores), namely the β-amino alcohol, the thiosemicarbazone and the 4-amino-7-chloroquinoline template would be potent as antiplasmodial agents, and if these compounds could be effectively used as antitrypanosomal agents. Therefore we sought to achieve the following general aims and objectives:

- To utilize the "fragment-based"[42,43] and "multiple ligand designed'[44] approaches for the synthesis of novel compounds based on known antimalarial bioactiphores;
- To utilize the 4-aminoquinoline template as a scaffold for generating focused libraries of β-amino alcohol derivatives;
- To utilize the 4-aminoquinoline template as a scaffold for generating focused libraries *via* multicomponent reactions;
- To evaluate the biological activities of the synthetic compounds with a view to establishing antiparasitic activity, and for appropriate compounds enzyme inhibitory activity;
- To explore structure-activity relationships (SARs) for antiplasmodial (or antiparasitic) activities within the different compound libraries;
- To develop efficient and cost-effective synthetic methodologies amenable to the generation of compound libraries.

Specific Objectives

(i) By delineating the SARs of the most promising compounds from the different classes of libraries, we hoped to obtain an insight into the structural requirements needed to optimize lead compounds. This would be achieved by synthesizing new compounds in which substituents at particular positions of the different compounds are varied.

(ii) To synthesize mechanism-based cysteine protease inhibitors by utilizing the known thiosemicarbazone bioactiphore, and to probe the nature of the parasitic cysteine proteases' active sites with respect to the most favourable substituents in terms of inhibitory activities of these compounds.

(iii) In order to synthesize large compound libraries of sufficient purities, we hoped to utilize the polymer-assisted solution phase synthesis strategy in combination with suitable polymer-supported reagents for developing methodologies that would be economical in terms of both time and resources.

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CHAPTER 3

SYNTHESIS AND BIOLOGICAL EVALUATION OF β-AMINO ALCOHOLS

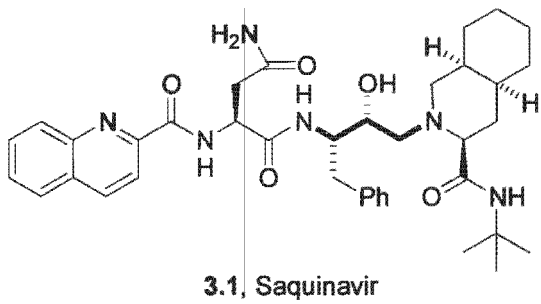
3.1 Introduction

The following chapter describes the synthesis, characterization and biological evaluation of a novel series of small molecules containing pharmacophores that are inherent in some known antimalarial agents. The envisaged molecules were designed to contain the ethanolamine group, also known as the β-amino alcohol group, as well as other appropriate functionalities. Where appropriate, oxidation of the amino alcohols would ultimately lead to aminoketones. All target compounds were designed and synthesized primarily to determine any inherent antiplasmodial and in some cases chloroquine potentiation or chemosensitization of resistant malaria parasites as well as antitrypanosomal activities.

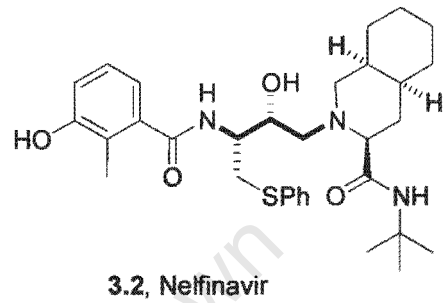
3.1.1 Rationale

The β-amino alcohol group is present as a significant subunit in a number of drugs and drug candidates that are active against a spectrum of pathogenic conditions. As such, β-amino alcohols have found application in a number of research areas including the development of inhibitors of HIV proteases,[1] renin[2] and angiotensin converting enzyme (ACE).[3] In the context of malaria, the β-amino alcohol moiety is found in a number of clinically established drugs including quinine (**1.3**), quinidine (**1.4**) and mefloquine (**1.5**) (section 1.3.1.2.1, pp. 7 & 8).

In terms of HIV research, the β-amino alcohol moiety is a known recognition motif for viral aspartic proteases. Accordingly, the incorporation of this group in potential HIV inhibitors has been exploited to generate drug candidates and drugs that are currently on the market, e.g. saquinavir **3.1** and nelfinavir **3.2** (Fig. 3.1).[4]



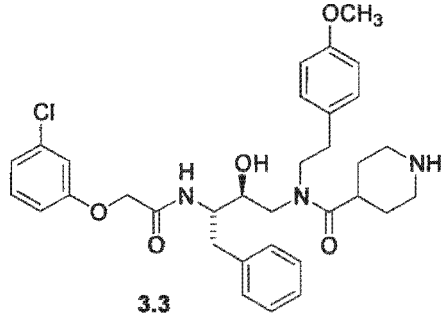
3.1, Saquinavir



3.2, Nelfinavir

Figure 3.1: Chemical structures of anti-HIV drugs, saquinavir (**3.1**) and nelfinavir (**3.2**)

Given the homology that is found in similar enzymes across different organisms, it would not seem unreasonable to assume that the β-amino alcohol moiety would be recognised by malarial aspartic proteases. Studies by Ellman and others have shown that the β-amino alcohol compound **3.3**, was active in *in vitro* assays against the malarial aspartic proteases plasmepsins I and II,[5] and also inhibited parasite growth in cultured parasite-infected human erythrocytes.[5]



3.3

Figure 3.2: Chemical structure of a chloroquine-resistant and chloroquine-sensitive *P. falciparum* active β-amino alcohol

Moreover, structural requirements for antiplasmodial activity of amino alcohol antiplasmodial agents include the presence of an aromatic portion and the amino alcohol portion in such a way that the groups are separated by two to three carbon atoms.[6] It should be pointed out at this juncture that the design of the target compounds in this part of the project was not aimed at evaluating them against *P. falciparum* plasmepsins (or any other specific enzymes), but rather against whole cell parasites. Only after if the compounds showed reasonable activity would it be necessary to identify the biological targets against which they would act. This approach is a "lead-to-target" approach. Accordingly, two groups of *β*-amino alcohols were designed for exploratory studies:

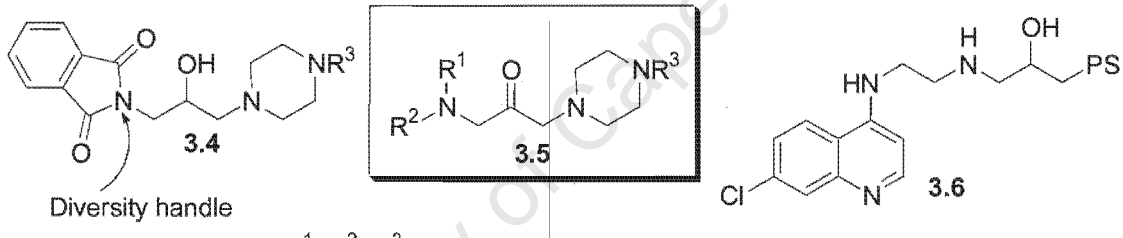


Figure 3.3: General chemical structures of designed *β*-amino alcohols and ketones

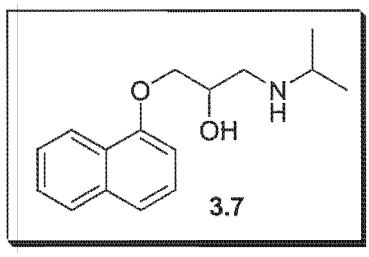
i) the first group, exemplified by **3.4** (Fig 3.3), incorporated a tertiary amino group at one end and a masked primary amino group on the other terminal, at which diversity could be introduced with a view to incorporating other antimalarial pharmacophores within the structures. A deliberate restriction was made to using piperazine-derived amines from the outset. The arylpiperazine moiety is one of the most occurring frameworks present in a number of drug candidates. A 2001 report listed a total of 2271 arylpiperazines, 65 of which were undergoing phase 2 clinical trials or higher over 23 therapeutic areas.[7] Therapeutic applications of arylpiperazines include use as

antibacterials, α_1-adrenergic blockers,[8] phosphodiesterase III inhibitors, antivirals, antimycobacterials,[7] dihydrofolate reductase inhibitors,[9] cysteine protease (human rhinovirus 3C protease) inhibitors[10] etc. As such, the arylpiperazine substructure is a significant group for drug discovery purposes.

Oxidation of these β-amino alcohols would ultimately lead to amino ketones **3.5**, whose keto group is a potential electrophilic site of attack by the thiolate anion of cysteine proteases. Studies have indeed shown that cysteine protease inhibitors of the type **3.5** are active both *in vitro* and *in vivo* against resistant strains of *P. falciparum*.[11]

Essential features in this class of compounds included the presence of a protonatable nitrogen atom which is thought to be significant for uptake and accumulation in the acidic food vacuole of the parasite, the β-amino alcohol moiety (a known antimalarial pharmacophore) and an aromatic group which may be important for hydrophobicity. The latter is important in achieving a fine balance with the hydrophilicity of a compound in order for it to be able to cross the parasitic cell membrane.

ii) the second group of compounds, exemplified by **3.6** incorporated the 4-amino-7-chloroquinoline group, a known antimalarial pharmacophore and a privileged substructure,[7] such as the biaryl unit, on the other terminal. Our efforts to probe this class of β-amino alcohol antimalarials coincided with the work of Harrison *et al*,[12] who established the *in vitro* antimalarial activity of propranalol, a drug used in the treatment of angina. Propranalol **3.7** (Fig. 3.4), a β-amino alcohol, and other drugs of similar structure belong to a class of compounds termed β-blockers. In their study, Harrison and co-workers showed that a racemic mixture of **3.7** was able to prevent *P. falciparum* infection of erythrocytes by up to 87%. They suggested that propranalol worked by inhibiting signal transduction proteins.



3.7

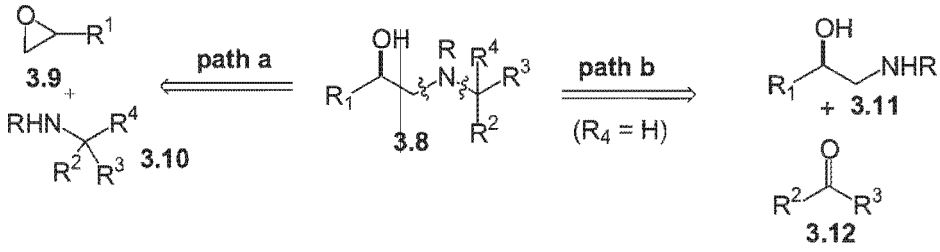
Figure 3.4: Chemical structure of propranalol **3.7**

A survey of the literature indicated that such an approach of combining these chemical entities in the context of malaria had not been previously reported. On this basis, we initiated a project to synthesise exploratory libraries of compounds incorporating the β-amino alcohol moiety and other appropriate functionalities primarily for biological screening against different strains of *P. falciparum*. At this juncture, it must be emphasized that the ease and simplicity of chemical synthesis is an important consideration for diseases like malaria that are prevalent mainly in poor Third World countries. This fact was taken into consideration of our target compounds for the PhD thesis.

3.2. Chemical Synthesis

3.2.1 Retrosynthesis of Tertiary *β*-Amino Alcohols

The synthesis of β-amino alcohols can be achieved by considering the two well-known disconnections in Scheme 3.1: **path a**, involving the alkylation of amines and, **path b**, reductive amination of aldehydes/ketones with primary and secondary amines.



Scheme 3.1: Synthetic routes to *β*-amino alcohols

The reductive amination route (**path b**) suffers from the limitation of not providing access to neopentyl amines (i.e. $R_4 \neq H$), while **path a** affords a greater opportunity for structural diversity due to the increased number of commercially available amines as well as chiral epoxides. The ease with which various appropriately substituted epoxides can be generated from the commercially available alkylating reagent epichlorohydrin (**3.13**, Fig 3.5) and appropriate nucleophiles encouraged us to pursue this path towards *β*-amino alcohols. Other methods for the synthesis of optically active β-amino alcohols have been documented in the literature and include addition of organometallics to *α*-amino aldehydes,[13] nitroaldol reaction,[14] reduction of *α*-amino ketones[15] hydroboration of enamines[16] and electrophile-promoted cyclization of allylic and homoallylic alcohols that bear a nucleophilic nitrogen tethered through an alcoholic oxygen.[17]

3.3. Synthesis of Tertiary *β*-Amino Alcohols

3.3.1 *N*-Alkylation of Phthalimide

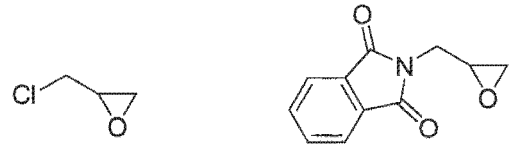


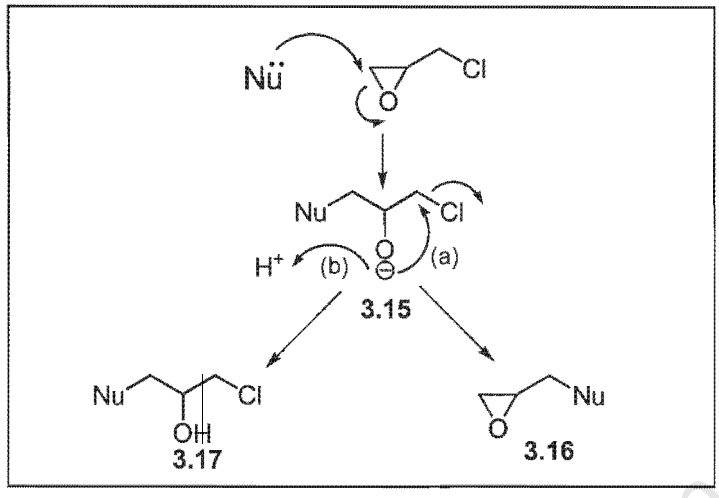
Figure 3.5: Chemical structure of epichlorohydrin **3.13** and key epoxide **3.14**

The initial design of the β-amino alcohols was such as to incorporate a nitrogen atom on one terminal that could subsequently be used as a diversity handle (Fig 3.3, section 3.1, p. 42). Such an approach would then require protection of the N-atom in order to prevent undesirable side reactions from taking place. The Gabriel synthesis[18] of primary amines became an immediate choice as the starting point for the synthesis of the envisaged *β*-amino alcohols. Thus, the first step towards the synthesis of our exploratory library was to synthesise the epoxide **3.14** from epichlorohydrin.

The reactivity of **3.13** is discussed below before a description of the synthesis of **3.14** is outlined.

3.3.1.1 Reactivity of Epichlorohydrin

Epichlorohydrin (**3.13**) is an alkylating reagent that has been used to synthesise epoxides using appropriate nucleophiles *via* addition-elimination reactions.[19] The outcome of the reaction, which at first sight seems to be a nucleophilic substitution reaction, is governed by the reaction conditions that are used during synthesis. Epichlorohydrin has two primary carbon atoms at which nucleophilic attack can be directed, the one bearing the chlorine atom as well as the methylene carbon of the epoxide ring. Given that the epoxide ring is highly strained, nucleophilic attack preferentially occurs at the primary carbon atom of the epoxide ring, leading to the tetrahedral alkoxide anion **3.15**, Scheme 3.2. Hydrogen bonding in polar protic solvents also favours attack at this position as the C-O bond is weakened due to the partial positive charge that the O atom develops upon H-boding.



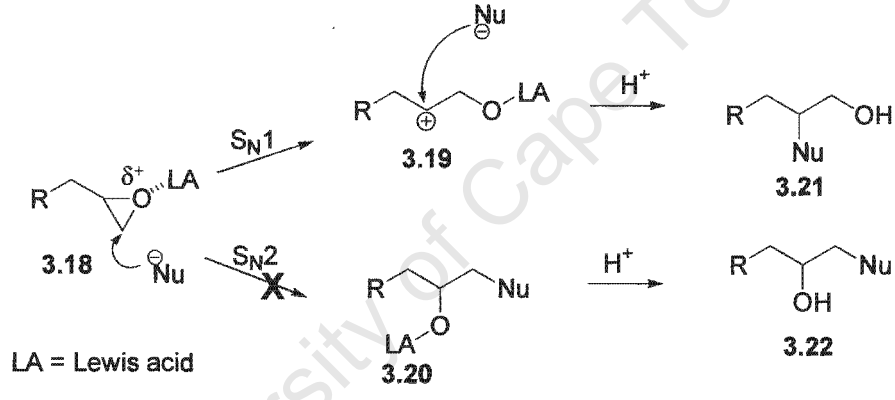
Scheme 3.2: Mechanism of epoxide-opening reaction under different conditions

The fate of the resulting tetrahedral intermediate **3.15** is twofold, either (i) in the absence of a proton source (such as in polar aprotic solvents), the alkoxide anion attacks the chloride-bearing carbon atom expelling the chloride anion (intramolecular concerted displacement of the chloride ion), thus generating the epoxide **3.16**, or, (ii) in the presence of a proton source (such as in polar protic solvents), the alkoxide anion becomes protonated resulting in the chloroalcohol **3.17**.

It must be appreciated that although nucleophilic attack of the epoxide can occur at either positions of the epoxide ring, it instead occurs regioselectively at the less hindered position. The observed regioselectivity is governed purely by steric factors, the attack preferentially occurring at the less substituted carbon.

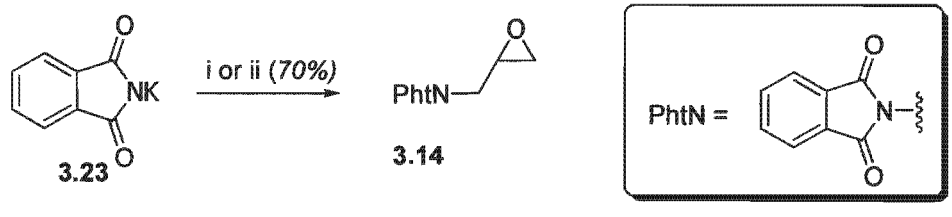
Under appropriate conditions, the regioselectivity of the epoxide-opening reaction can be reversed so that the incoming nucleophile is directed to attack the more hindered position, giving rise to terminal alcohols for monosubstituted epoxides. This reversal of regioselectivity can be achieved by adding to the reaction mixture a Lewis or Brønsted acid. In this case the addition of the acid leads to the species **3.18** in which the oxygen is

complexed to the acidic centre thus creating partial positive charges at the two carbons attached to the oxygen, Scheme 3.3. The result is that the complexation leads to weakening of the two bonds and, either cleavage of one of the two bonds may occur in the process to form carbocation **3.19** (S_N1) or the nucleophile (Nu) may attack the least substituted carbon (more stable TS) to give **3.20**. Cleavage to the more substituted carbon thus leads to the formation of the more stable secondary carbocation **3.19**. Attack of the amine then occurs on **3.19** to give the terminal alcohol **3.21** upon work up. Under these conditions, nucleophilic attack at the less substituted C (S_N2) to give **3.22** *via* **3.20** does not occur (appreciably).



Scheme 3.3: Mechanism of reversal of regioselectivity in epichlorohydrin

We were however not interested in the terminal amino alcohols of the type **3.21** and so the above route was not pursued.



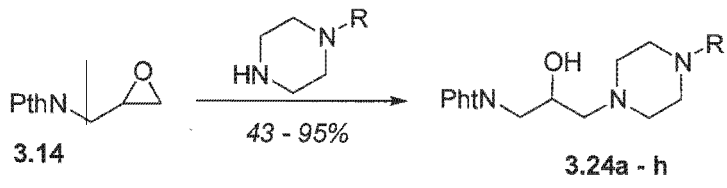
Scheme 3.4: *Reagents and conditions* i) Epichhlorohydrin, TBAB, DMF, K_2CO_3, rt, 8 h or reflux, 2 h; ii) 160 °C, 8 h

An earlier report by Landini and Rolla had indicated that the use of polar aprotic solvents such as DMF greatly accelerates the rate of formation of epoxides of type **3.14**.[20] Accordingly, potassium phthalimide **3.23** was treated with 2 eq of epichlorohydrin, catalytic *tert*-butyl ammonium bromide (TBAB) and 1.5 eq of K_2CO_3 in anhydrous DMF at room temperature (Scheme 3.4) and, after 8 h of reaction no traces of product could be seen from the reaction mixture as evidenced by TLC. The temperature was slowly elevated to between 95 and 100 °C at which the reaction was allowed to proceed for 2 h and immediately afterwards allowed to cool to room temperature. Subsequent analysis of the reaction mixture by ^{1}H NMR upon aqueous work up still revealed no evidence of the formation of the product.

In the second and alternative approach, a slurry of epichlorohydrin and potassium phthalimide **3.23** in a 1:6 ratio was heated at 160 °C for 8 h in the neat to afford **3.14** in 70% yield upon work up.

The proposed structure of the isolate was confirmed by ^{1}H NMR in which the chemical shift values of the diastereotopic protons next to the N-atom of the aromatic ring appeared more downfield as a pair of double doublets at δ 3.97 and 3.80 ppm in contrast to a doublet at δ 3.55 ppm for the same protons in epichlorohydrin. Further proof of the molecular formula was provided by the high resolution mass spectrum which confirmed the molecular ion peak as the base peak at 203 amu.

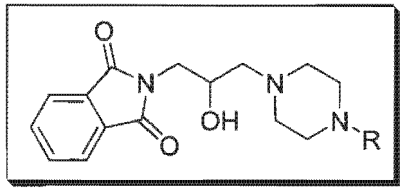
With **3.14** in hand the stage was set for the parallel synthesis of the first β-amino alcohol library. Initially, a number of commercially available piperazinyl amines **3.24a – j** were selected for use in the parallel synthesis. Scheme 3.5 depicts the synthetic approach employed.



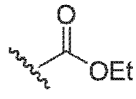
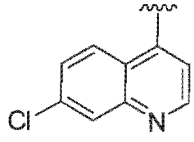
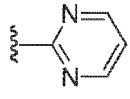
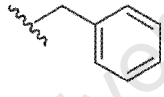
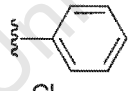
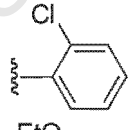
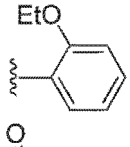
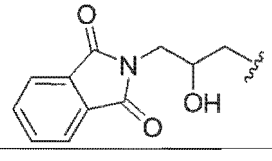
Scheme 3.5: *Reagents and conditions* i) MeOH, 63 °C, 4 – 6 h

The syntheses of **3.24a – j** were easily achieved in operationally simple and straightforward one-step reactions wherein mixtures of the epoxide **3.14** and amines were simply heated in anhydrous MeOH at 63 °C in a parallel array format to afford the products in high yields. All amines were used in slight excess (1.3-excess-fold with respect to epoxide), except for piperazine which was used as the limiting reagent. This led to the *bis*-substituted β-amino alcohol **3.24j**. The amines employed as well as the yields are shown in Table 3.1. Purification was achieved by means of conventional gravity or preparative layer chromatography.

Table 3.1: Amines, reaction times and isolated yields of compounds **3.24a – j**



General Structure of amino alcohols

Entry	R	Product	Time (h)	Yield (%)
1		**3.24a**	3	41
2		**3.24b**	4	93
3	**-Bu^t**	**3.24c**	5	93
4	**-CH$_3$**	**3.24d**	6	68
5		**3.24e**	6	65
6		**3.24f**	6	71
7		**3.24g**	4	83
8		**3.24h**	4	70
9		**3.24i**	6	53
10		**3.24j**	6	51

All the ^{1}H NMR spectra of the resultant β-amino alcohols displayed a common diagnostic multiplet around δ 4 ppm that was assigned to the methine proton bearing the hydroxyl group. It was expected that the two pairs of diastereotopic protons on either side of the stereogenic centre would appear as separate doublets of doublets. Indeed these protons were revealed as the expected doublets of doublets each integrating for a single H in some cases, but in other cases the same protons were revealed as either a doublet integrating for 2 Hs or a multiplet integrating for 2 Hs owing to poor resolution or overlapping of the peaks. The same observation was also made in the case of the methylene protons on the piperazine ring which appeared as either triplets or multiplets. A typical ^{1}H NMR spectrum is shown in Fig 3.6 for compound **3.24g**.

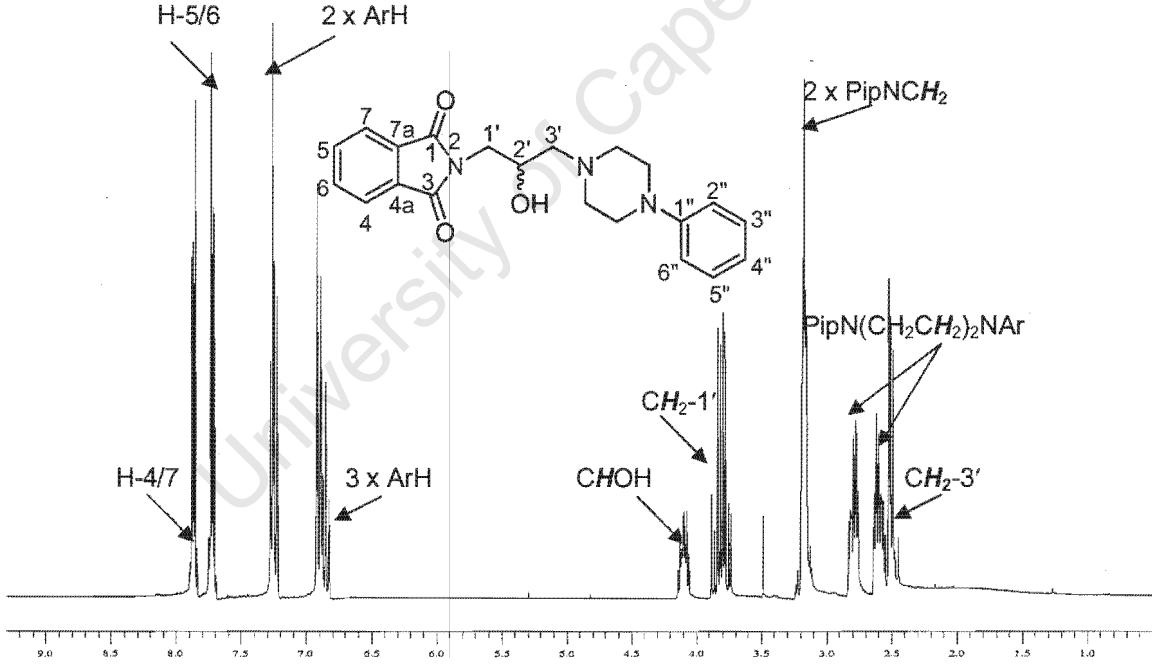


Figure 3.6: ^{1}H NMR spectrum of **3.24g** in $CDCl_3$

The ^{13}C NMR spectra that were obtained were also in consistency with the proposed structures. Figure 3.7 shows the ^{13}C NMR of the same compound **3.24g**.

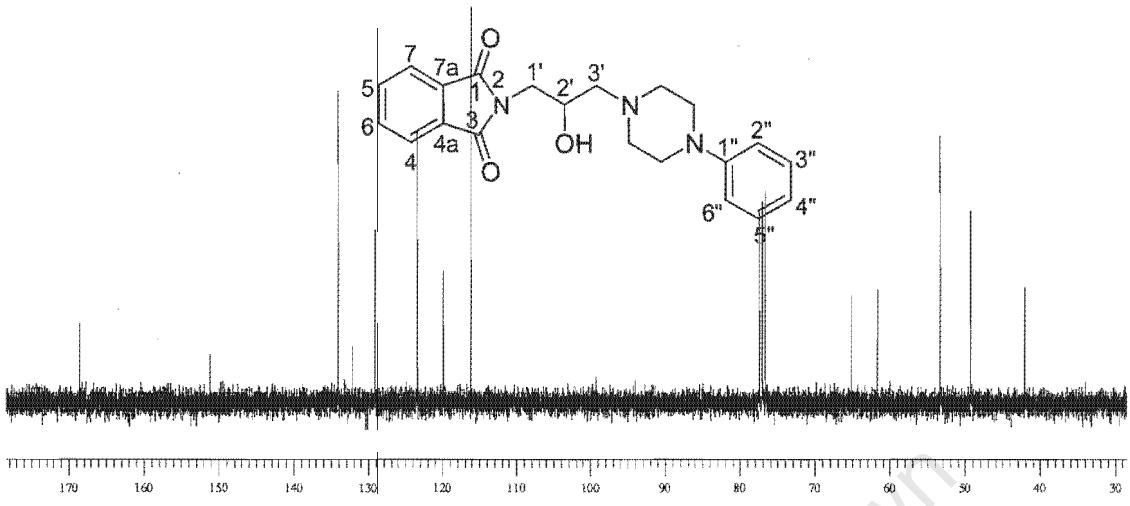


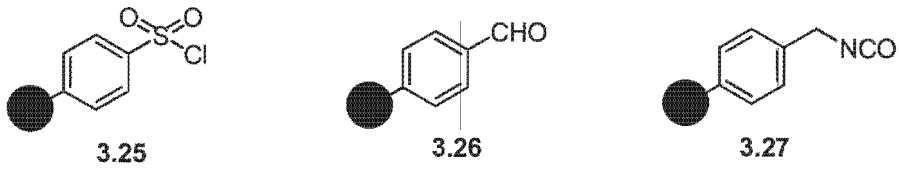
Figure 3.7: ^{13}C NMR spectrum of **3.24g** in $CDCl_3$

In combination with HSQC and DEPT spectra, and the ^{13}C NMR spectrum shown above for **3.24g**, it was possible to assign intuitively the peaks to the different carbon atoms in the molecule.

3.4 Polymer-Assisted Solution Phase Synthesis (PASP)

3.4.1 Purification of β-Amino Alcohols using PASP Synthesis

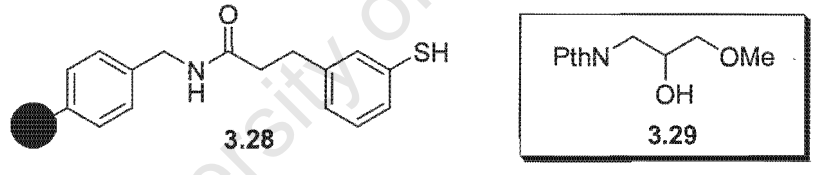
The parallel solution-phase synthesis adopted for the synthesis of compounds **3.24a – j** is ideal for small libraries but would prove laborious for the synthesis of larger libraries. We thus sought an alternative method that would permit us to carry out convenient purification and isolation of our target compounds *via* simple filtration and washing. In this regard two options were available to us: solid-phase organic synthesis (SPOS)[21] or polymer-assisted solution-phase synthesis (PASP).[22] While SPOS introduces two extra steps in a synthetic scheme during immobilisation of reagents onto, and cleavage from, the solid support, PASP offers a less tedious and economical alternative.



3.25 3.26 3.27

Figure 3.8: Electrophilic polymer-supported scavengers: PS-*p*-toluene sulfonylchloride, PSTsCl (**3.25**), PS-aldehyde (**3.26**) and PS-isocyanate (**3.27**)

Purification of our compound library could be achieved by using a scavenger for either starting amine or epoxide. In the case of the former, an electrophilic polymer-supported reagent such as PSTsCl **3.25** (23), PSCHO **3.26**[23,24] or PSNCO **3.27**[23,25] (Fig. 3.8) would be ideal. Although PSCHO would be compatible with the solvent of choice (MeOH), it does not react with secondary amines and thus excess of amine would not be scavenged.



3.28 3.29

Figure 3.9: Nucleophilic polymer-supported scavenger: PS-thiophenol (**3.28**) and *β*-methoxy alcohol **3.29**

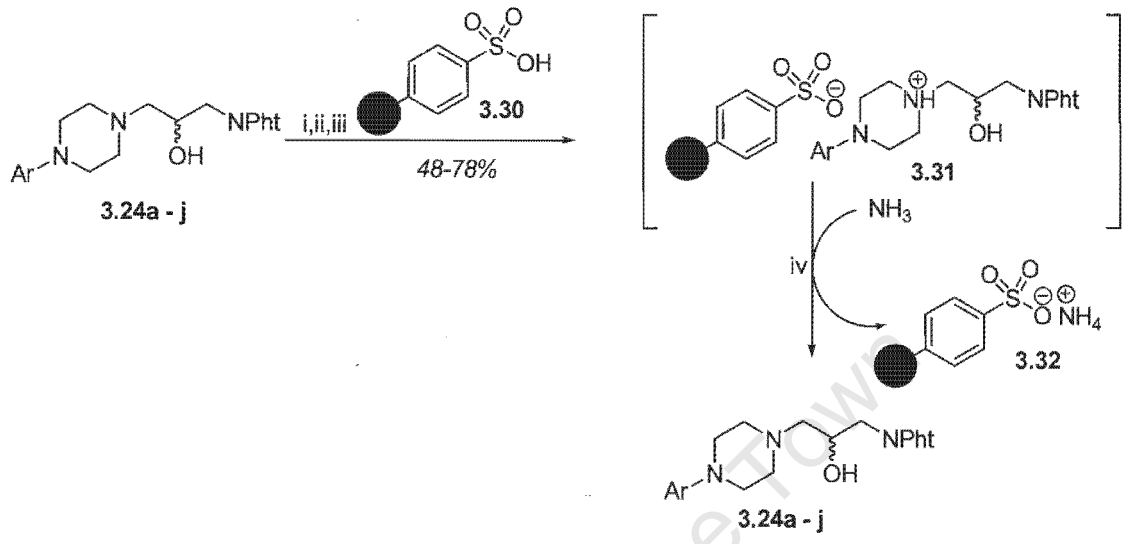
In the case of excess of epoxide, a polymer-supported nucleophile such as polymer-supported thiophenol **3.28** (PS-SH, Fig. 3.9)[26] could suffice. However, such an approach may not be able to rid the reaction mixtures of any non-reacting by-products. In our case, if the epoxide is used in excess under the reaction conditions (high temperature and MeOH), reaction between the solvent and the epoxide as a side reaction would give rise to the β-methoxy alcohol (**3.29**), Fig. 3.9. Hence, the electrophilic scavengers would not be able to discriminate between desired products and the side products. This is likely

to reduce the purity of the library should such an approach be taken. In any case, other compatible solvents such as THF and DMF could suffice.

An alternative approach would involve a "catch and release"[27] principle, in which a polymer-bound reagent selectively binds a compound *via* an ionic interaction, removing it from solution. Resin washing and subsequent "release" from the resin with appropriate reagents should then furnish the products in good yield and purity. The choice of such a reagent is crucial to obtaining high purity products. Ideally, it should be able to discriminate between starting materials, unwanted by-products and desired products. In other words, targeted functional group(s) on which to exploit the methodology must only be inherent in the desirable compounds.

Using a readily available polymer-supported reagent as an isolation and purification tool, compounds **3.24a – j** were regenerated in a parallel array format. Macroporous polymer-supported toluene-sulfonic acid resin (MP-TsOH) **3.30**[28] was found to give excellent results in achieving the dual purpose of isolation and purification. The use of MP-TsOH is based on the "catch and release" principle, which exploits the basic nature of the piperazine nitrogen in the product. The use of an excess of epoxide to drive the reaction to completion leads to the isolation of pure products from the reaction mixtures without possible contamination from either starting materials, as the excess epoxide (or **3.29**) cannot be scavenged by the MP-TsOH due to its non-basic nature while the amine, although basic, is completely consumed in the reaction. The probable events that lead to the isolation of target compounds are depicted in Scheme 3.6. Ionic interaction between **3.30** and β-the amino alcohol leads to the resin-bound salt **3.31**, which is retained on the resin support on washing the beads with solvent. Sequential rinsing of the resins with the stronger base ammonia (NH_3) attains base interchange so that the

target compounds are released into solution while the NH_3 forms the resin-bound salt **3.32**.



Scheme 3.6: *Reagents and conditions* i) **3.30**, MeOH, rt, 1 h; ii) Filter, wash; iii) 3% NH_3/MeOH; iv) Filter, wash

The resynthesized compounds were obtained in modest to high yields (Table 3.2).

Table 3.2: Amino alcohols from polymer-assisted solution-phase synthesis

Product	Rxn time (h)	Yield (%)	HPLC Purity (%)
3.24a	3	67	93
3.24b	4	78	99
3.24c	5	48	96
3.24d	6	60	87
3.24e	7	66	91
3.24f	6	45	92
3.24g	6	64	99
3.24h	4	62	98
3.24i	6	73	97

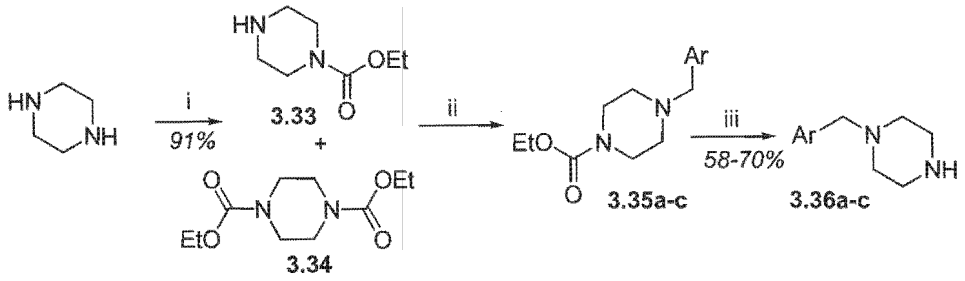
HPLC Conditions: Flow rate = 1.5 ml/min; UV 220 and 254 nm; 30:70 CH_3CN: 25 mM Phosphate buffer

With the exception of compounds **3.24a** and **3.24i**, the yields were lower than those obtained from solution-phase synthesis. Despite the lower yields, however, the methodology developed here is advantageous in being convenient, avoiding repeated column chromatography thus making purification much easier; also the rate of turnover is higher within a given time frame. Lastly, it is economical in that it avoids the use of large volumes of solvents to purify compounds and is environmentally benign.

3.4.2 Validation of PASP Methodology

To establish the generality of the methodology described above, second generation compounds based on benzylpiperazine were designed in consistency with the most biologically active compound **3.24f** from a preliminary biological screen. In preparing a library of tertiary amines, Siegel *et al* used cation exchange chromatography for the purification and isolation of their compounds.[29] We reasoned that the use of a similar approach in which the ionic reagent would be bound on a resin would yield similar results.

These second generation compounds required the synthesis of substituted benzylpiperazine amines, which we envisaged would be accessible *via* reductive amination of selected aromatic aldehydes. The syntheses were achieved by way of Scheme 3.7.

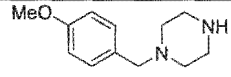
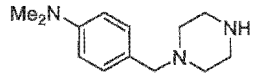
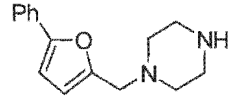


Scheme 3.7: *Reagents and conditions* i) EtOCOCl, H_2O, pH~ 2 – 3, 2 h; ii) ArCHO, $(AcO)_3BH$, rt, 3 – 6 h; iii) LiOH, EtOH (aq), reflux, 2 h

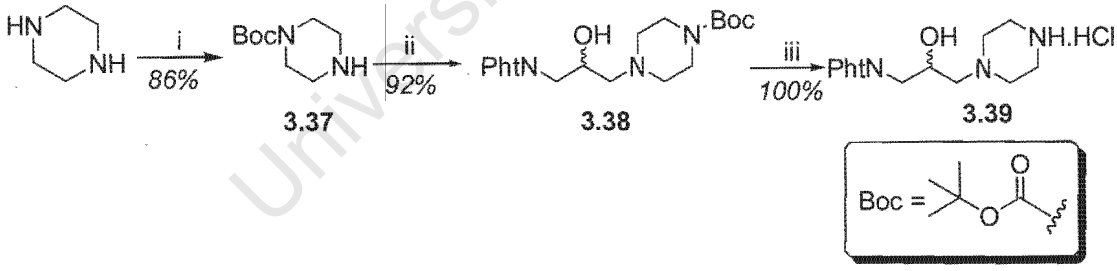
The first step towards the synthesis of the *N*-substituted benzylpiperazines was the mono-protection of piperazine as the carbamate **3.33** by treatment with 1.1 eq of ethylchloroformate (EtOCOCl) in acidified H_2O. An isolated yield of 91% was obtained for **3.33** alongside an unappreciable amount of the *bis*-acylated piperazine **3.34** (<3%). On monitoring the reaction progress, compound **3.33** could be distinguished easily from both piperazine and **3.34** on TLC plate in that it had an R_f value that was intermediate between those of piperazine and **3.34**. Condensation of **3.33** with appropriate aldehydes (ArCHO) in DCE at ambient temperature and *in situ* reduction of the resultant iminium ions with the mild reducing reagent $NaBH(OAc)_3$ afforded *N*-protected piperazine amines **3.35a – c**.[30] The mild nature of the reducing agent stems from the combined negative inductive effects of the three attached acetyl groups whose effect is to reduce the nucleophilicity of the hydride anion.

Saponification of the *N*-protected amines **3.35a – c** with KOH in refluxing aqueous EtOH afforded the free amines **3.36a – c**, Table 3.3.

Table 3.3: Isolated yields of amines *via* reductive amination

Amine	Structure	Yield
3.36a		65
3.36b		58
3.36c		70

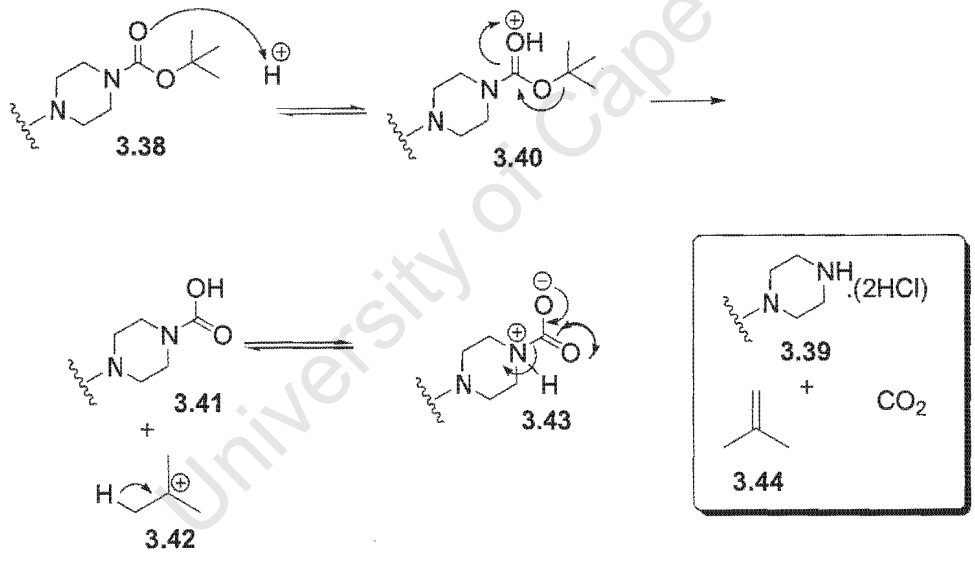
However, this method of generating benzylpiperazines did not prove to be satisfactory for other aldehydes used subsequently; in some cases only starting aldehydes and amines were recovered. In these cases, an alternative method was pursued in which boc-protected piperazine **3.37**, prepared by treatment of excess piperazine with boc-anhydride Boc_2O,[31] was instead reacted with key epoxide **3.14** in MeOH, affording the *N*-boc-protected β-amino alcohol **3.38** as a key intermediate (Scheme 3.8).



Scheme 3.8: *Reagents and conditions* i) Boc_2O, DCM, rt, 18 h; ii) **3.14**, MeOH, 63 °C, 4 h; iii) HCl/MeOH, 2 h, rt

Removal of the boc-protecting group from **3.38** was achieved by treatment with a solution of 5% HCl in MeOH[32] for 2 h to afford the HCl salt **3.39**. The rapid removal of the boc-protecting group can be appreciated by considering the by-products of the

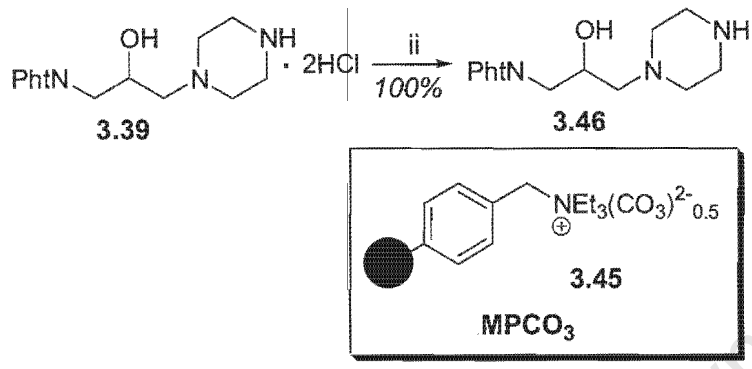
reaction in the mechanism depicted in Scheme 3.9. Protonation of the carbonyl of the carbamate **3.38** leads to the intermediate **3.40**, which gives rise to **3.41** and the *tert*-butyl cation **3.42** upon cleavage of the C-O bond. The intermediate **3.41** is in equilibrium with **3.43**; decarboxylation from **3.43** and loss of a proton from **3.42** leads to the HCl salt of the amine **3.39**, and isobutylene **3.44** respectively, and CO_2. The irreversible reactions involving the loss of the two gaseous by-products (isobutylene **3.44** and CO_2) drives the reaction in the forward direction; the thermodynamic stability (or the entropy) of the gaseous products relative to starting materials is greatly increased so that the forward reaction occurs very rapidly.



Scheme 3.9: Mechanism of removal of the boc-protecting group

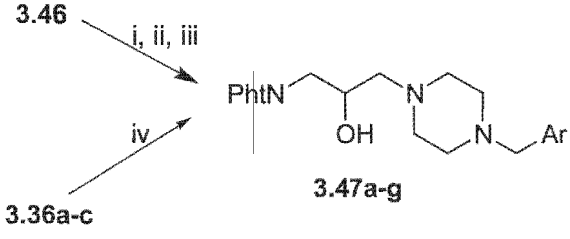
Basification of the resultant HCl salt **3.39** with $MPCO_3$ **3.45**[33] in MeOH cleanly afforded the free base **3.46** in quantitative yield. The basification process occurs in what could be seen as a "catch and release" manner, wherein the acid component of the salt (HCl) is

transferred to the more basic $MPCO_3$. In this case though, the desired compound remains in solution as opposed to being resin-bound.



Scheme 3.10: *Reagents and conditions* i) **3.45**, MeOH, rt, 2 h

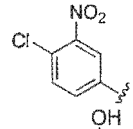
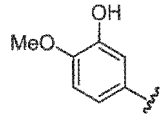
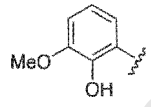
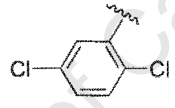
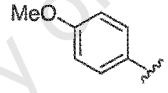
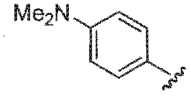
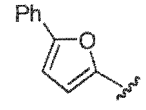
Accordingly, **3.46** was treated with an array of aldehydes (excess) and a catalytic amount of AcOH in MeOH at room temperature, followed by *in situ* reduction of the intermediate iminium ions with $NaCNBH_3$,[34] Scheme 3.11. After dilution of the reaction mixtures with MeOH, resin-bound MP-TsOH (**3.30**) was added to ensure scavenging of the tertiary amine products (**3.47a – g**) from solution. Upon filtration and washing of the resins, the products were released into MeOH by the addition of anhydrous 3% NH_3/MeOH. Amines **3.36a – c** were reacted with **3.14** and purified accordingly.



Scheme 3.11: *Reagents and conditions* i) ArCHO (1.0 eq), AcOH, MeOH, $NaCNBH_3$ (1.3 eq), rt, 4 – 6 h; ii) MP-TsOH, 4 h, filter; iii) 3% NH_3/MeOH, filter; iv) **3.14**, MeOH, 63 °C, 4 h

Table 3.4 shows the isolated yields of compounds **3.47a – d** obtained form the method described above, and **3.78e – f** for the two different methods.

Table 3.4: Isolated yields of second generation β-amino alcohols **3.47a – g**

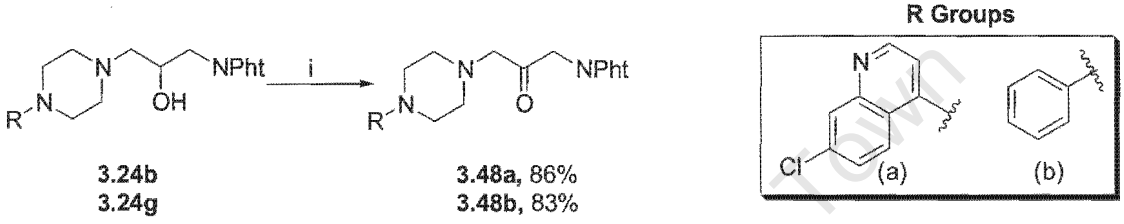
Entry	Compound	Ar Group	Yield (%)
1	**3.47a**		63
2	**3.47b**		63
3	**3.47c**		52
4	**3.47d**		51
5	**3.47e**		61 (55)*
6	**3.47f**		59 (50)
7	**3.47g**		50 (50)

(*Yields in parentheses indicate those achieved *via* PASP)

The above approach offers the obvious advantage of providing access to high purity β-amino alcohols in a parallel array format whilst avoiding the tedious work involved in chromatography; it is also economical in the sense that only a single and readily available commercial reagent is required for the purification of large numbers of compounds.

3.5 Further Elaboration of Tertiary *β*-Amino Alcohols

It was decided that the β-amino alcohols **3.24a – i** be converted to other classes of compounds containing other known antimalarial pharmacophores (potential cysteine protease inhibitor amino ketones). As such two compounds were randomly selected for oxidation to their corresponding amino ketones prior to removal of the phthalimide protecting group by way of Scheme 3.12.1

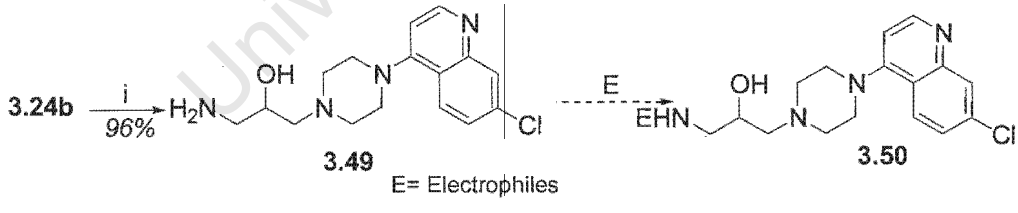


3.24b
3.24g

3.48a, 86%
3.48b, 83%

Scheme 3.12: *Reagents and conditions* i) SO_3.Pyr, Et_3N, DMSO, DCM, rt, 24 – 26 h

Employing the Parikh-Doering oxidation method,[35] a modification of the method originally developed by Pfitzner and Moffat,[36] **3.24b** and **3.24g** were treated with a SO_3.-pyridine complex/Et_3N/DMSO cocktail in DCM to afford the amino ketones **3.48a** and **3.48b** in yields of 86 and 83% respectively.



Scheme 3.13: *Reagents and conditions* i) $NH_2NH_2.H_2O$, EtOH, reflux, 30 min

It was then decided that the phthalimide-protecting group be removed with a view to introducing chemical diversity *via* **3.49**. Removal of the protecting group in **3.24b** was thus achieved by refluxing its solution in EtOH in the presence of hydrazine monohydrate.[37] Reaction of **3.49** with benzaldehyde, benzylsulfonyl isocyanate,

benzylisocyanate and benzylsulfonyl chloride at three different temeperaturs (0, 25 and 65 °C) would then provide access to different classes of 1,3-diamino alcohols, sulfonyl ureas, sulfonamides and sulfonamides (**3.50**) respectively. Oxidation of these intermediates would then lead to potential novel potential 1,3-diamino ketone cysteine protease inhibitors. However, all the reactions that were attempted gave complex mixtures that were not easy to purify. It is possible that the observed results may have been a result of a competing reaction of the free OH group of the amino alcohols, although in a report by Gutcait *et al*, β-amino alcohols similar to **3.49** reacted with electrophiles with a free hydroxyl group.[38] Unfortunately the reasons for these results could not be established as aspects of this project were not pursued any further.

3.6 β-Amino Alcohols Incorporating Privileged Structures

3.6.1 Introduction: Privileged Structures

The term "privileged structures" was first used by Evans *et al* in 1988 after noting the ability of 1,4-benzodiazepine-2-ones to bind various receptors. Appropriately, the term privileged structure was defined as "a single molecular framework able to provide ligands for diverse receptors".[39] Today the term has been used to describe ligands that bind multiple, unrelated proteinacious enzymes and receptors with high affinity.[40]

An inspection of the structural diversity clearly reveals that most of the privileged substructures known to date are natural products or derivatives of these. Such a finding suggests that the binding abilities of these substructures to multiple targets are a result of evolutionary selection over time. It is also possible that the binding may result from the biosynthetic processes used in their preparation. Since the biosynthesis of natural products would involve intermediates that bind to proteins to catalyze their assembly,

preferential selection for physiochemical features that would favour protein binding would occur.

Despite being non natural products, biaryls, indoles, benzimidazoles and arylpiperazines have the ability to selectively bind diverse receptors just like natural products. This is in view of the ability of aromatic substituents in drugs to bind to proteins *via* aromatic and hydrophobic interactions.[41,42] Favourable interactions also result from interaction of aromatic substituents with polar substituents and positively charged groups.[41] It is this versatility of aromatic units in binding that makes the biaryl unit a favourable and common unit in drugs.

We deliberately limited our exploratory libraries only to bicyclic privileged substructures since these are known to provide molecular rigidity and good bioavailability. Indeed, studies have revealed that the number of rotatable bonds in a drug candidate is a major contributor to good oral bioavailability.[43]

3.6.1.1 Rationale For Drug Design

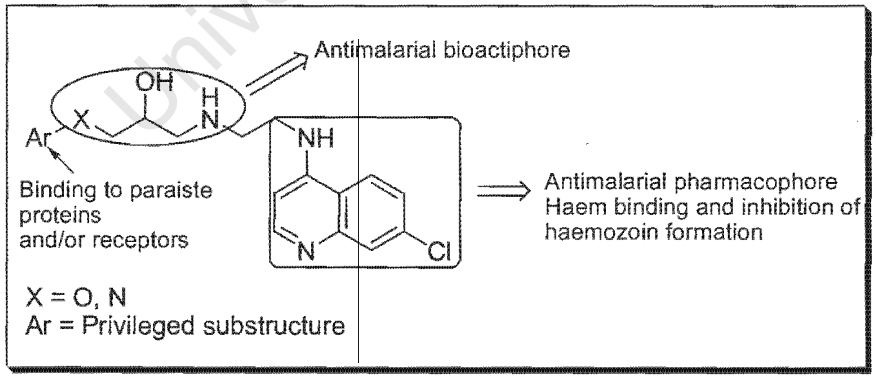


Figure 3.10: Rationale for design of target compounds

In brief, the rationale for the design of the target compounds was based on the summary illustrated in Fig. 3.10 as such: presence of two antimalarial pharmacophores, namely the 4-amino-7-chloroquinoline and *β*-amino alcohol subunits, should provide maximal antimalarial activities in the new compounds. We also reasoned that the appendage of the privileged substructures to the former pharmacophores would improve the activities of the new compounds *via* interactions with parasitic receptors and/or proteins.

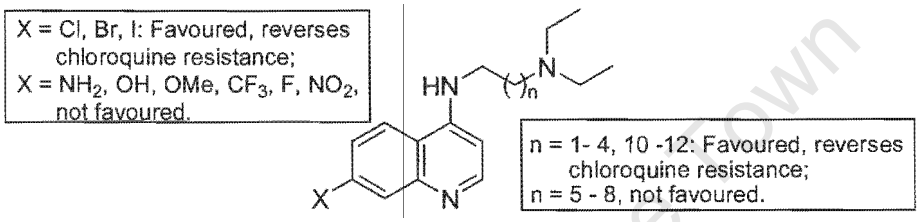


Figure 3.11: Summary of 4-aminoquinolines based on chloroquine

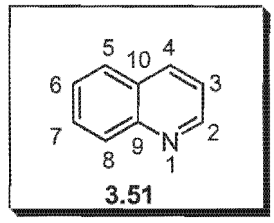
As earlier indicated (section 1.6.1, pp. 16-17), modifications to the chloroquine side chain have led to the identification of various classes of compounds that are active against both chloroquine-resistant and chloroquine-sensitive strains of the malaria parasite. Studies by De *et al* established that the number of carbons between the two nitrogens in the diamino side chain of chloroquine derivatives exemplified by Fig. 3.11 is a major determinant of antiplasmodial activity against chloroquine-resistant *P. falciparum*.[44] The size of the carbon spacer between the nitrogens correlated to antiplasmodial activity in the chloroquine-sensitive Haiti 135 and chloroquine-resistant Indochina strain thus: aminoquinolines with short (2 – 4 carbons) and long (10 – 12) chains were more active against chloroquine-sensitive and resistant strains than those with intermediate chains (5 – 8 carbons). Additionally, compounds with both 7-bromo- and 7-iodo-substitutions were more active than the 7-chloro-substituted compounds,

though not significantly so. Independent studies on similar compounds by Kaschula *et al* on β-haematin inhibition established that association of β-haematin depends on the nature of the substituent at the C-7 position of quinoline, although this does not directly translate into good antiplasmodial activity.[45] Accordingly, they concluded that strongly electron-donating groups at C-7 inhibit β-haematin the most, although moderately electron-withdrawing groups such as Cl, Br and I exhibit better antiplasmodial activities. Inhibition of β-haematin formation, though, was found to correlate with good antiplasmodial activity, with the antiplasmodial IC_{50} having a linear dependence on inhibition of β-haematin formation.

Such a variation in the side chain in which the 4-amino-7-chloroquinoline and a privileged substructure moiety are coupled to the β-amino alcohol group, we envisaged, could lead us to compounds with broad antiprotozoal activities. The presence of the privileged substructure should also introduce hydrophobicity and rigidity within the molecular framework. The latter property is significant in reducing the number of rotatable bonds which has implications on both the stability (and/or bioavailability) and probable side effects as the number of interactions with various targets and/or enzymes and receptors reduce. Also, by varying the number of the methylene spacers in the 4-aminoquinoline amine side chains we hoped to get an insight into the SAR across the different substitution patterns.

Before discussing the specific privileged substructures selected for use in this part of the project, the reactivity of quinoline **3.51** is described in the next section. The quinoline unit (Fig. 3.11) is the starting point for building the target compounds of this section.

3.6.1.2 General Reactivity of Quinoline



3.51

Figure 3.12: Chemical Structure of quinoline

Considering that quinoline (Fig. 3.12) is a fused benzo[b]pyridine heterocycle, its reactivity can be expected to parallel that of the two rings from which it is derived, namely pyridine and benzene. Indeed, quinoline behaves in the same manner that benzene behaves towards nucleophiles, and as pyridine does towards electrophiles. In other words, electrophilic reactions in quinoline occur on the benzene ring, whilst nucleophillic reactions occur on the pyridine ring. The presence of the N-atom renders quinoline π-electron-deficient in two ways, *viz.*, by the mesomeric and inductive effects (Fig. 3.13), in which case the effects are more pronounced at the C-2 and C-4 positions, which are in close proximity to the ring N-atom. Reactivity at these positions is further enhanced by the presence of electron- withdrawing groups (e.g. halogens).

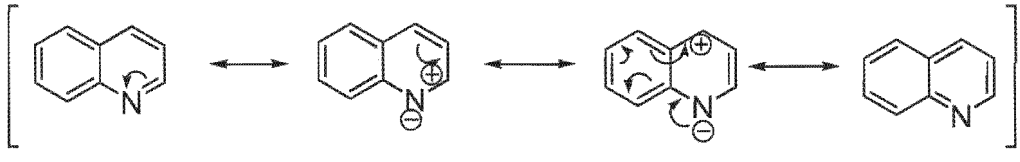


Figure 3.13: Canonical structures for the inductive and mesomeric effects of N-atom on aromatic ring

Compared to pyridine, quinoline is more reactive towards electrophiles and this is attributable to the stabilisation of the reaction intermediate by the presence of the second aromatic ring.

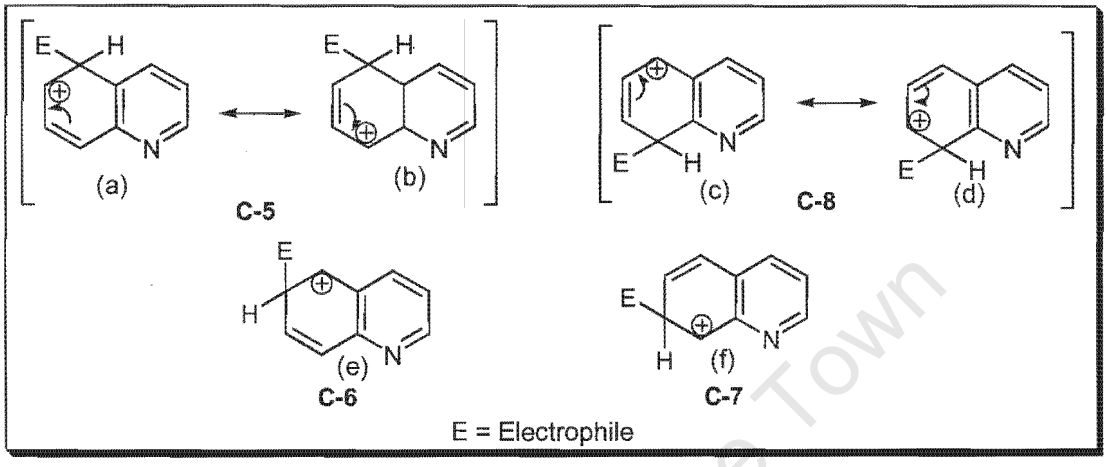
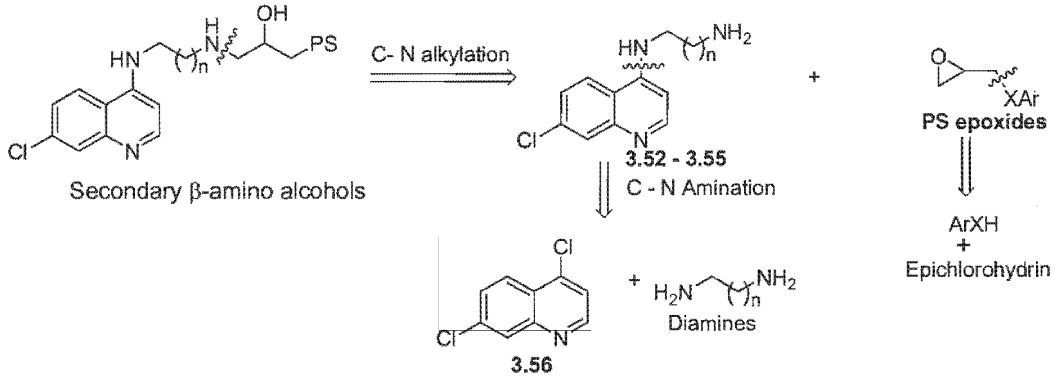


Figure 3.14: Mesomeric effects in quinoline depicting sites of attack by electrophiles

Thus electrophilic substitution (e.g. nitration) in quinoline occurs predominantly at the C-5 and C-8 positions, in which the intermediates are stabilized by resonance as can be seen above, Fig 3.14.

3.6.2 Retrosynthetic Analysis of Secondary β-Amino Alcohols



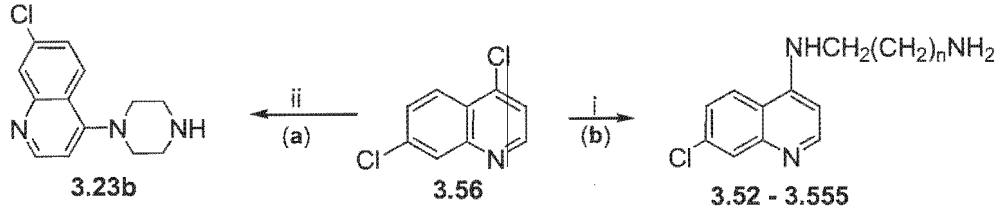
Scheme 3.14: Retrosynthesis of secondary β-amino alcohols

The retrosynthesis of secondary β-amino alcohols is shown in Scheme 3.14. A C-N disconnection leads to the aminoquinoline amines **3.52 – 3.55** and the privileged structure-containing epoxides. Further analysis of the primary amines **3.52 – 3.55** leads to commercially available 4,7-dichloroquinoline **3.56** and diamines, while further elaboration of the epoxides leads to epichlorohydrin and the privileged substructures (ArXH).

3.6.3 Synthesis of 4-Aminoquinoline Diamines

The 1-(4′-amino-7′-chloroquinoline)-diamines were prepared following a literature procedure described by De *et al*, Scheme 3.15.[46] In all cases the statistical effect was exploited to obtain the desired monosubstituted amines by using large excesses of the appropriate amines. In this approach no less than 4 equivalents of the amine were required to suppress formation of the terminally disubstituted amines.

The starting amine required for the synthesis of the amine for the 5-carbon spacer was not available at the time of synthesis. Excepting the synthesis of **3.23b**, all reactions were performed in the neat, while synthesis of the former was achieved by way of path (**a**) employing *N*-methylpyrrolidinone (NMP) as solvent, in the presence of Et_3N and a catalytic amount of K_2CO_3.[47]

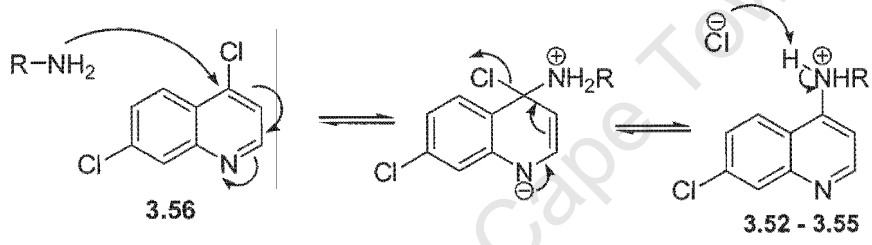


3.23b **3.56** **3.52 - 3.555**

Scheme 3.15: *Reagents and conditions* i) n-Alkyl diamines, 80 °C, 1 h, then 135 °C, 3 h; ii) Piperazine, (5 eq), Et_3N, K_2CO_3, NMP, 4 h

3.6.3.1 Mechanistic Comments

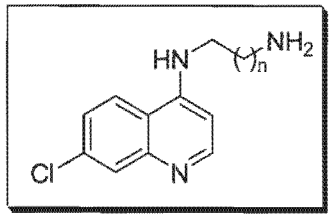
As stated earlier, the presence of electronegative groups in the C-2 and C-4 positions of quinoline renders these positions particularly susceptible to nucleophilic attack. The ability of the quinoline nitrogen to act as an electron 'sink', and the closer proximity relative to the Cl on C-7 allows the incoming nucleophile to be directed at the C-4 position. The resonance stabilisation that occurs, as well as the closer proximity to the N-atom are the major driving forces for the observed regioselectivity.



Scheme 3.16: Mechanism of formation of 1-(7'-chloro-4'-amino)-diamines

Synthesis of compound **3.53** has been reportedly achieved in low yield[48] but, more recently, Madrid *et al*[49] reported an improved yield in which solid-phase extraction was employed to enhance the yield. In our hands, the synthesis of **3.53** could be achieved in quantitative yield by working up the reaction mixture and extracting into EtOAc while still hot. The isolated yields of the amines **3.52 – 3.55** and **3.23b** are tabulated in Table 3.5 below.

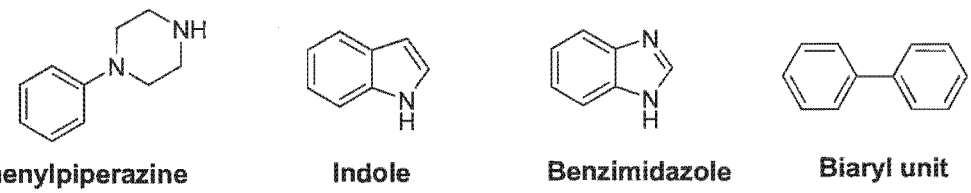
Table 3.5: Isolated yields/melting points of 1-(7′-chloro-4′-amino)-diamines



Compound	n	Yield (%)	M.p., (lit mp.)
3.52	1	100	137 – 138 °C, (137 – 139 °C)[50]
3.53	2	100	125 – 127 °C, (125 – 127 °C)[50]
3.54	3	100	45 – 47 °C, (43 – 47 °C)[50]
3.55	5	96	136 – 138 °C, (136 – 138 °C)[50]
3.23b	–	86	113 – 115 °C, (113 – 115 °C)[51]

3.6.4 Synthesis of Epoxides Incorporating Privileged Substructures

Despite the plethora of privileged structures that are known and accessible to date, either by synthesis or from commercial sources, we decided to limit our choices for exploratory studies to unsubstituted phenylpiperazine, indole, benzimidazole and biphenyl systems (Fig. 3.15) towards the synthesis of an exploratory series of *β*-amino alcohols. We reasoned that in order for us to make any meaningful future SAR studies worthwhile endeavours, unsubstituted privileged substructures would be an ideal starting point. Identification of biologically active compounds from the different classes would then allow us to pursue a particular series for SAR studies. Each of the envisaged target compounds required the synthesis of epoxides from commercially available starting materials and the syntheses are outlined below.



Phenylpiperazine **Indole** **Benzimidazole** **Biaryl unit**

Figure 3.15: Structures of phenyl piperazine, indole, benzimidazole and biphenyl unit

3.6.4.1 Synthesis of Indole-Derived Epoxide

i) The indole substructure is probably the most significant scaffold in drug discovery[52] possessing a variety of therapeutic uses. Frequently being found in natural products and derivatives thereof, the indole ring is also found in a variety of compounds including but not limited to tryptophan **3.57**, a naturally-occurring amino acid, serotonin **3.58**, a key CNS neurotransmitter that regulates smooth muscle function in the cardiovascular and gastrointestinal systems, factor Xa inhibitors and antiinflammatories[53] such as indomethacin **3.59** (Fig. 3.16) .

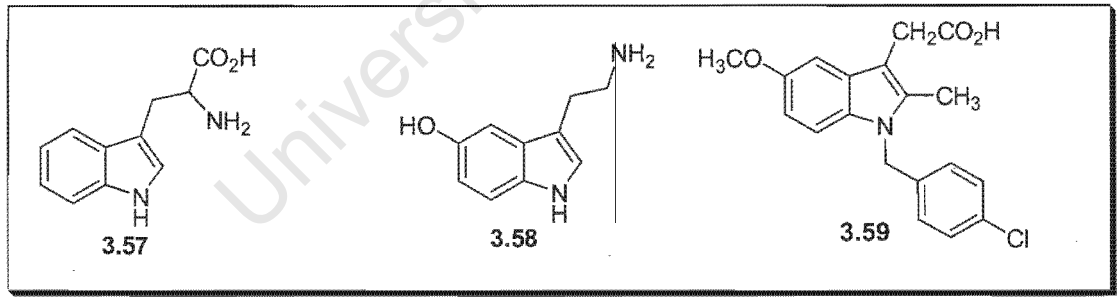
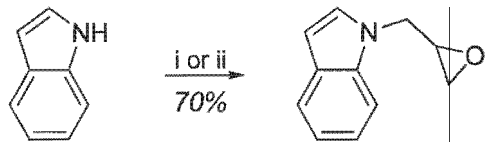


Figure 3.16: Structures of tryptophan (**3.57**), serotonin (**3.58**) and indomethacin (**3.59**)

Clearly, indole is an established privileged substructure that renders itself as a scaffold upon which drug discovery may be based.

The indole epoxide **3.60** was synthesized by way of Scheme 3.17. Mild conditions for the *N*-alkylation of indole were envisaged in this reaction given the ease with which the reactions are known to proceed.[54]



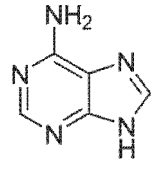
Indole **3.60**

Scheme 3.17: *Reagents and conditions* i) KF-Al_2O_3, epichlorohydrin, CH_3CN, rt, 22 h, 72% or ii) NaH, epichlorohydrin, DMF, 0 °C then rt, 18 h, 70%

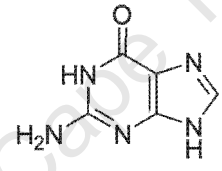
Thus K_2CO_3, a weak base that is known to deprotonate indole was elected as the first choice. However, when the synthesis of **3.60** was attempted using K_2CO_3 as base and either DCM or acetonitrile as the reaction medium, only the indole starting material could be retrieved after 24 h of reaction at room temperature or after refluxing. The use of potassium fluoride bound on a solid support (aluminium oxide), KF-Al_2O_3 has been exploited extensively to carry out efficient alkylation reactions on the nitrogen atom.[55,56] The advantages of using this base cannot be overemphasized; its compatibility with a range of solvents and ease of removal after reaction by means of simple filtration gives it an edge over most bases, making it more amenable to the synthesis of combinatorial libraries as it precludes work up protocols. In this regard KF-Al_2O_3 became an immediate choice to effect the deprotonation. Thus, an immediate switch was made from K_2CO_3 to KF-Al_2O_3 and, as anticipated, the alkylation step proceeded at ambient temperature to afford the expected product in high yield (72%). Similarly, use of NaH in DMF[57] afforded the desired epoxide in 70% yield. Interestingly, and in contrast with observations made with other synthesized epoxides, the indole-derived epoxide had a lower R_f value as seen on TLC plate than the nonalkylated indole.

3.6.4.2 Synthesis of Benzimidazole-Derived Epoxide

The structural similarity between benzimidazole and indole makes it just as an important privileged substructural motif and as such sees extensive use in medicinal chemistry. The therapeutic window in which benzimidazole derivatives find use is wide and includes applications in anticancer, antiulcer, fungicidal, antihelminthic and antiviral to mention but a few. Its close resemblance to the purine framework found in DNA (**3.61** and **3.62**, Fig. 3.17) makes it an attractive template in the search for antimalarials as *P. falciparum* lacks a *de novo* pathway to synthesize these purine bases[58] and has to rely on the salvage pathway.



3.61



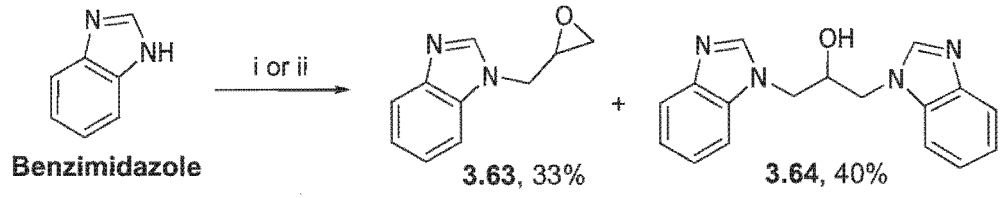
3.62

Figure 3.17: Structures of purine bases present in DNA: adenine (**3.61**) and guanine (**3.62**)

Therefore, the benzimidazole motif presents itself as an attractive scaffold for the discovery of new drug candidates.

It was immediately apparent that the synthesis of the desired benzimidazole-derived epoxide **3.63** from benzimidazole could not be achieved in the absence of a base. Firstly, the aniline nitrogen in itself is not sufficiently nucleophilic due to donation of its lone pair into the aromatic ring. Secondly, it was perceived that in the absence of a base, formation of the chloroalcohol alluded to earlier (section 3.3.1.1, p. 47) would be a competing, if not predominant, reaction should the reaction be conducted at high temperature. This made the inclusion of a base in the reaction particularly crucial. Thus, benzimidazole was treated with NaH in anhydrous DMF at 0 °C and subsequently

elevated to room temperature. We opted for the much stronger base considering that the aniline proton is not very acidic and therefore deprotonation would require the use of a strong base prior to the alkylation. The approach used is depicted in Scheme 3.18.



Benzimidazole **3.63**, 33% **3.64**, 40%

Scheme 3.18: *Reagents and conditions* i) NaH, epichlorohydrin (5 eq), DMF, 0 °C – rt, 24 h, or ii) epichlorohydrin (5 eq), KF-Al_2O_3, CH_3CN, 18 h.

Interestingly, the reaction proceeded to form three products at room temperature as was indicative of the TLC. Subsequent purification of the reaction mixture indeed led to three isolable products. Characterization was attempted on all three isolates, but only two of the three could be unambiguously identified as **3.63** and **3.64**. The third fraction gave a complicated ^{1}H NMR spectrum that could not be assigned easily. The proposed structure of the unanticipated compound **3.64** was apparent from the ^{1}H NMR (Fig. 3.18), which was suggestive of a symmetrical compound. Except for the methine proton on the OH-bearing carbon, all protons integrated for twice their expected number; the high resolution mass spectrum also confirmed the molecular formula of compound **3.64** ($C_{27}H_{16}N_4O$, M^+ = 292.13208).

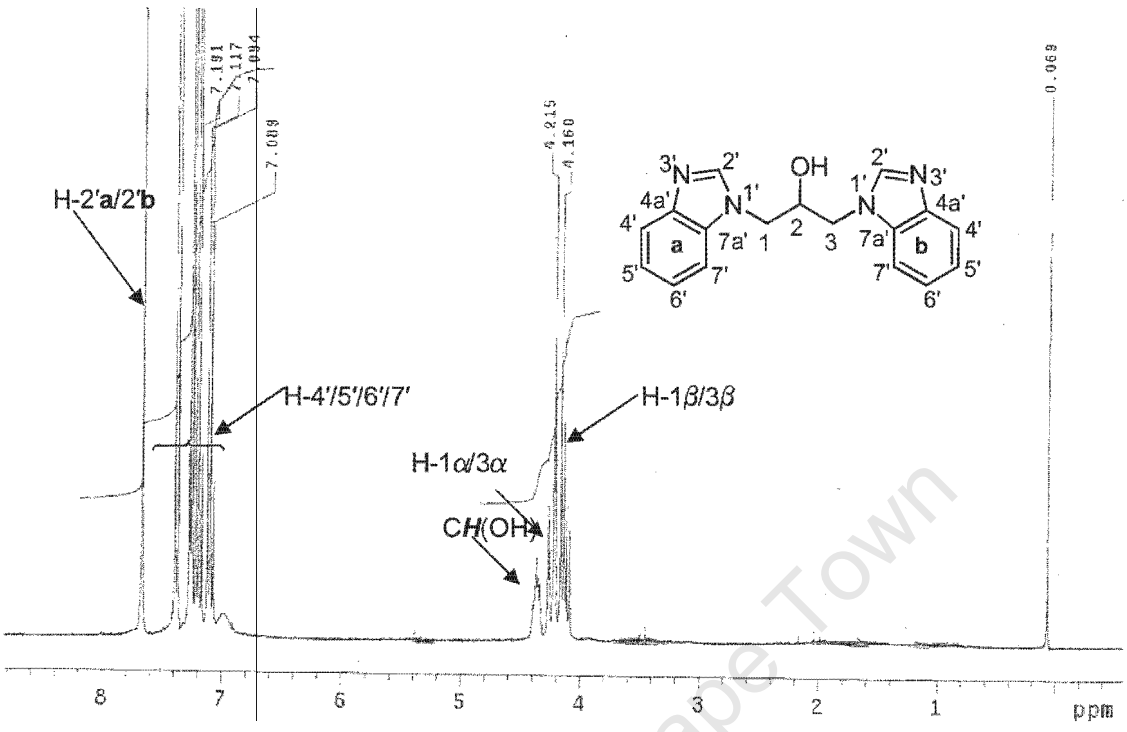


Figure 3.18: ^{1}H NMR of **3.64** in $CDCl_3$

To further explore this unexpected outcome, we decided to investigate different reaction conditions in order to note any differences in the product distribution.

In the first attempt, a 1.5-excess-fold equivalent of KF-Al_2O_3 relative to benzimidazole was employed, while 3 equivalents of epichlorohydrin were added in order to drive the reaction to completion. Acetonitrile was initially chosen as reaction solvent due to its compatibility with the base and its ease of removal at the end of the reaction. When the reaction was conducted in CH_3CN with KF-Al_2O_3 as the base the same quality of product distribution was obtained. Reproducible results were obtained with Et_3N as the base in the absence of solvent between 0 and 30 °C. Similarly when the solvent was switched to THF the same set of results were obtained in terms of quality. The only differences were

in the actual isolated yields of the products. A summary of the conditions employed and the results obtained appears in Table 3.6.

Table 3.6: Isolated yields of **3.63** under varying conditions

Entry	Base	Solvent	Temp. (° C)	Rxn Time (h)	Yield (%)
1	NaH	DMF	25	26	33
2	NaH	THF	25	24	40
3	KF-Al_2O_3	THF	25	18	46
4	NaH	CH_3CN	25	22	42
5	KF-Al_2O_3	CH_3CN	25	18	40
6	Et_3N	THF	0 – 30	48	0*
7	Et_3N	None	25	48	39

* Only starting material was recovered

Although the initial yield with NaH in DMF was lower (entry 1), it could be improved by allowing the mixture of NaH and benzimidazole to stir between 0 and 25 °C for 2 h prior to addition of the alkylating reagent. Similar results were obtained with KF-Al_2O_3, but in this case the yields were slightly improved when the number of equivalents of the base was at least 2-fold. Practically, the use of the excess of KF-Al_2O_3 was advantageous because of its ease of removal after reaction, otherwise there was no improvement in the isolated yield of the desired epoxide **3.63**.

The outcome of the reaction, *vis-à-vis* **3.64**, may be explained by considering the canonical structures of the anion of benzimidazole shown in Fig 3.19.

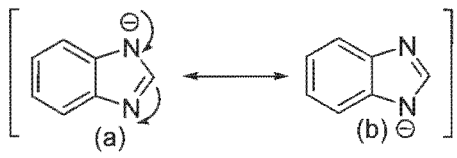
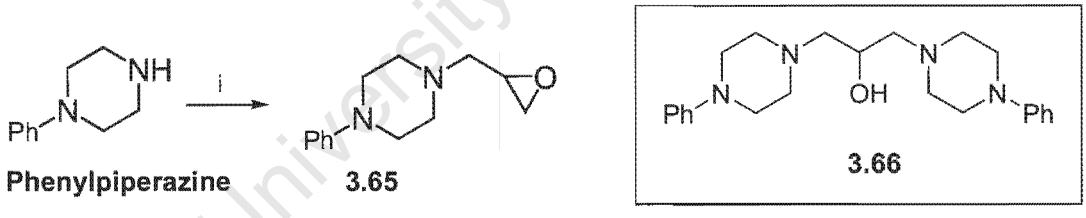


Figure 3.19: Canonical structures of benzimidazole anion

Despite the presence of the aromatic ring into which electrons may be delocalized, the negative charge is more localized on either nitrogen atom as opposed to being delocalized into the aromatic ring as in the case of indole where a second nitrogen atom is absent. Because of this delocalization onto the two nitrogens, the anion reacts with the epoxide leading to unexpected product **3.64**.

3.6.4.3 Synthesis of Phenylpiperazine-Derived Epoxide

As earlier mentioned, the arylpiperazine moiety is one of the most occurring frameworks present in a number of drug candidates (section 3.1, p. 42).



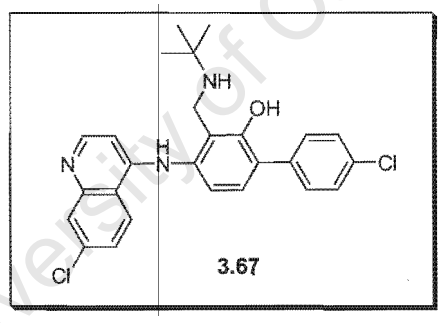
Scheme 3.19: *Reagents and conditions* i) KF-Al_2O_3, CH_3CN, rt, 24 h

Prior to the synthesis of the phenylpiperazine epoxide **3.65**, we were concerned that the potential reactivity of the secondary amine phenylpiperazine (or its anion) towards **3.65** would result in the *bis*-alkylated amino alcohol **3.66** (Scheme 3.19), although the use of a large excess of the alkylating reagent could seemingly overcome this hurdle. This realization thus made the choice of base particularly crucial. However, under the reaction conditions employed (room temperature-CH_3CN) when the alkylation was

attempted with KF-Al_2O_3 in CH_3CN, the product could be isolated in near quantitative yield after a 24 h period of reaction with no trace of **3.66**. The same reaction when carried out with NaH did not go to completion after 3 days and only gave **3.65** in 68% yield.

3.6.4.4 Synthesis of Biphenyl-derived Epoxides

The biaryl framework remains one of the most popular privileged substructures that are known to date, reportedly being present in over 4% of the drugs on the market.[7] Classes of compounds containing this scaffold have found use as antiinfective, antifungal, antiarythmic, analgesic, antitumor, and antihypertensive agents.[59] In terms of antiinfectives, the biphenyl framework is found in the antimalarial agent tebuquine **3.67**, Fig. 3.20.[60]

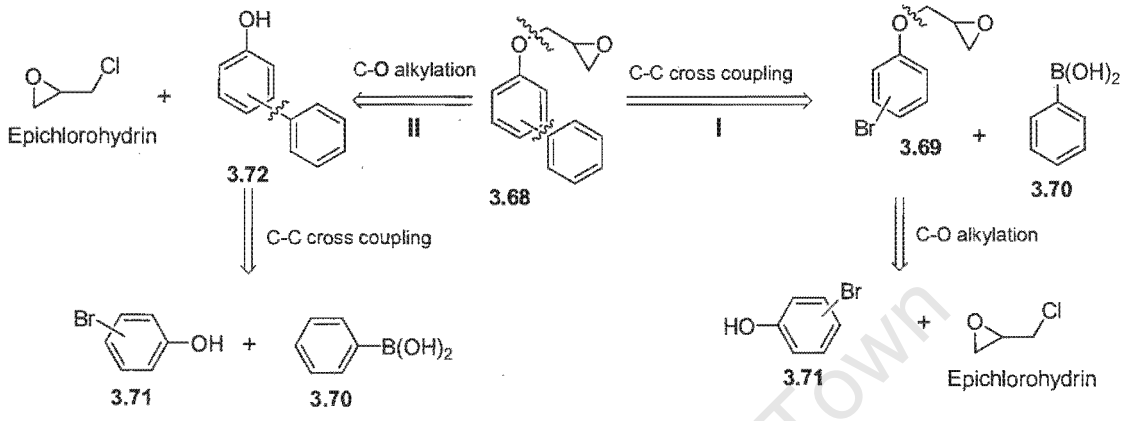


3.67

Figure 3.20: Tebuquine **3.67**

Various methods exist for the synthesis of biaryls, including the Ullmann reaction[61] for the synthesis of symmetrical biaryls and the Stille,[62] Negishi,[63] and Suzuki[64] reactions, as well as the use of organosilicates for the creation of both symmetrical and unsymmetrical biaryls. The wide range of reaction conditions and functional groups inherent in the starting materials that the Suzuki reaction tolerates makes it a famous approach for the generation of biaryls.

Generation of the biphenyl fragments of the envisaged β-amino alcohols could be achieved by considering the retrosynthetic analysis shown in Scheme 3.20.



Scheme 3.20: Retrosynthesis of biphenyl epoxides

On the one hand, retrosynthesis of the biphenyl epoxide **3.68** leads to the epoxide of the bromophenol **3.69** and phenylboronic acid **3.70**, *via* path **I**. Further analysis of **3.69** leads to bromophenol **3.71** and epichlorohydrin as the starting materials. On the other hand, the alternative approach involves reversal of sequences so that, retrosynthesis of the biphenyl epoxide **3.68** (path **II**) leads to the biphenyl alcohol **3.72** and epichlorohydrin, the former being obtainable from phenylboronic acid **3.70** and the bromophenol **3.71**. Although either synthetic approach was deemed feasible, path **II** was adopted. Before delving into the synthesis of **3.72**, a brief account of the Suzuki reaction used to couple the aryl units is given below.

3.6.5 The Suzuki Reaction

The coupling of aryl halides and boronic acids has come to be known as the Suzuki reaction and, since its first report has developed into one of the most important reactions in organic chemistry.[64] The reaction is mediated by catalytic Pd(0) in the presence of a

base, usually at high temperature. Sources of catalytically active palladium of Pd(0) such as $Pd(PPh_3)_4$ which are electron-rich and nucleophilic and thus prone to oxidation are used. Mechanistically, it is similar to the Heck coupling of a vinyl halide and an aryl halide[65] in having three principal steps, Fig. 3.21.

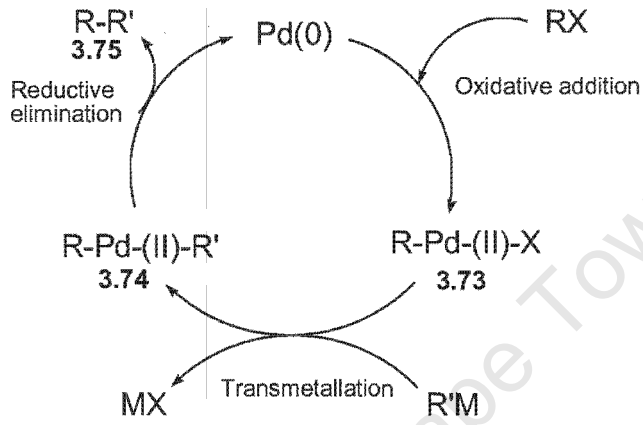
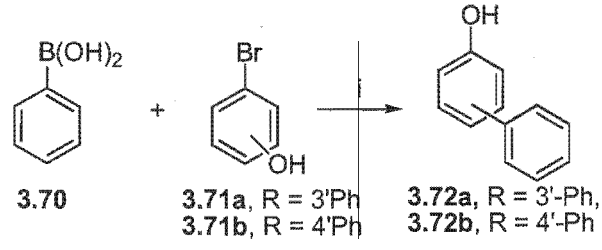


Figure 3.21: Catalytic cycle of the Suzuki reaction

i) Oxidative Addition: This step can be seen as the starting point of the catalytic cycle. The term is used because Pd(0) is formally oxidized to Pd(II) by the addition of RX (aryl halide) to the metal. Addition of the halide RX leads to the complex **3.73**. Because the halide substrate is reduced in the process, electron-deficient halides are in general more reactive than their electron-rich counterparts. Since reductive elimination occurs rapidly at temperatures of –20 °C or higher, the halide cannot have β-hydrogens. Therefore, the reaction is restricted to aryl and vinyl substrates.

ii) Transmetallation: refers to the transfer of the R group in RX to Pd for the halide (X) to generate the diarylpalladium complex **3.74** and the metal halide (MX). Invariably, transmetallation is the rate-limiting step in cross-coupling reactions, occurring with retention of stereochemistry in the organic group.

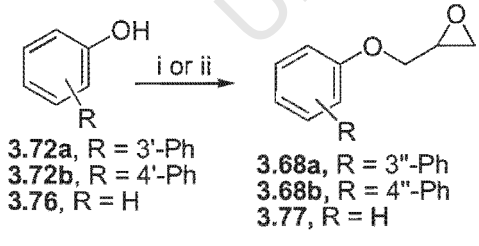
iiii) Reductive Elimination: refers to the rearrangement of the *trans* diarylpalladium (II) complex to the *cis* complex to produce the coupled product **3.75** and regenerating the Pd(0) catalyst. Since reductive elimination from the diarylpalladium complex is faster than *β*-elimination, β-hydrogens in the R group of the boronic acid may be tolerated.



Scheme 3.21: *Reagents and conditions* i) $Pd(PPh_3)_4$, K_2CO_3 (aq), toluene-*i*PrOH, reflux, 14 h

Cross-coupling of **3.70** and 3- or 4-bromophenol **3.71a** or **3.71b** respectively, was achieved by refluxing the reactants in an aqueous toluene/isopropanol solution in the presence of K_2CO_3 and catalytic $Pd(PPh_3)_4$ (tetrakis triphenylphosphine palladium), as a source of Pd(0) for 14 h, Scheme 3.21.[66] The desired biphenols **3.72a** and **3.72b** were obtainable in yields of 73 and 70% for the 3- and 4-substituted positions respectively, following chromatographic purification.

Table 3.7: Isolated yields of epoxides



R	Product	Yield (%)
3′-Ph	**3.68a**	70
4′-Ph	**3.68b**	73
H	**3.81**	95

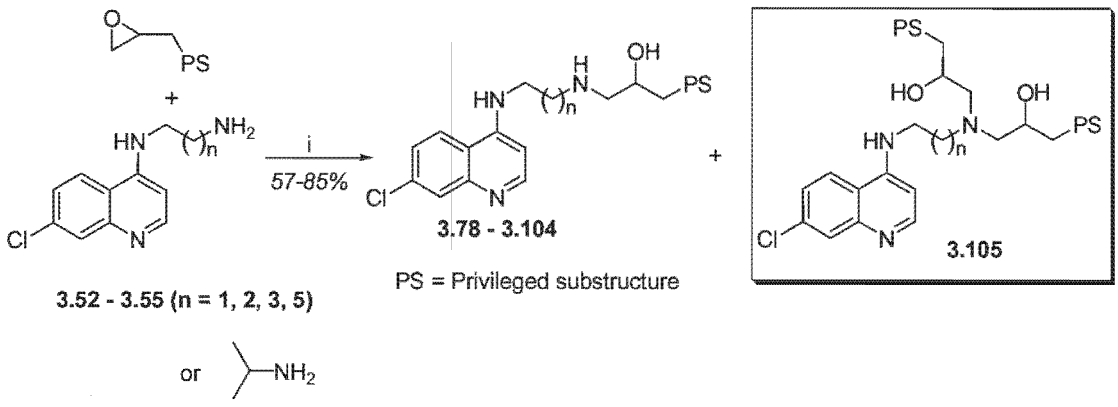
Scheme 3.22: *Reagents and conditions* i) NaH, DMF, 0 °C – rt or ii) KF-Al_2O_3, CH_3CN, rt, 48 h

Alkylation of the biphenyl alcohols was achieved with KF-Al_2O_3 in CH_3CN to afford the 3″ and 4″-substitued biphenyl epoxides **3.68a** and **3.68b** respectively in good yield.

Similarly, phenol **3.76** was alkylated with epichlorohydrin and KF-Al_2O_3 to afford the epoxide **3.77** in 95% yield (Scheme 3.22). However, in the case of the latter higher temperatures were required to effect the alkylation. Yields for the three epoxides are alongside Scheme 3.22, Table 3.7.

3.7 Synthesis of Secondary β-Amino Alcohols

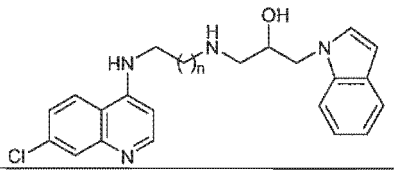
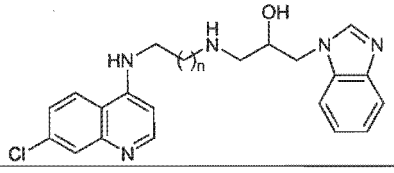
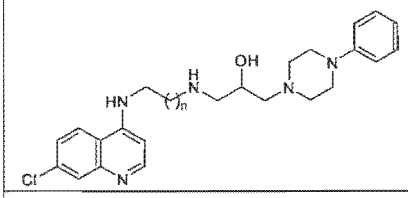
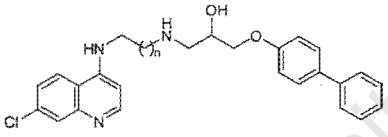
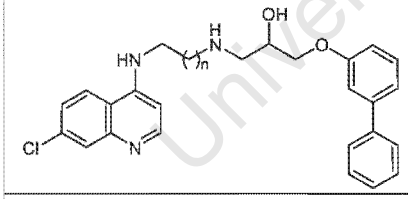
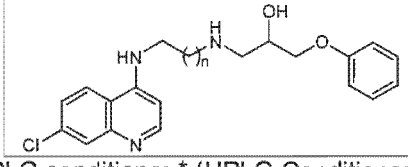
Scheme 3.23 represents the synthesis of amino alcohols **3.78 – 3.105**. The alkylation of primary amines with epoxides is known to be problematic,[67] usually requiring high temperatures and excess of primary amine. Oftentimes, under the reaction conditions the initial product of the reaction which is a secondary amine, reacts with the starting epoxide to yield the *bis*-alkylated tertiary amino alcohol such as **3.105**. Purification of the reaction mixture by conventional chromatography then becomes a labour intensive and time consuming undertaking. In order to avoid this, several reagents have been developed that reduce the nucleophilicity of the nitrogen atom for both conventional solution-phase and combinatorial approaches.[27,67] These reagents, such as trimethylsilyl acetamide, react with the primary amine to afford an amide which is less reactive towards the epoxide. However, at high temperatures and prolonged reaction times the amide nitrogen is able to react with the epoxide without the danger of over-alkylation. The approach precludes the use of excess of amine, allowing the use of stoichiometric amounts of reagents. Cleavage of the protecting group cleanly affords the secondary β-amino alcohols.



Scheme 3.23: *Reagents and conditions* i) MeOH, 65 °C, 8 h

Despite the foreseeable problems, the secondary β-amino alcohols could be synthesized in solution without the need to pre-treat the primary amines with any one of developed reagents for this purpose. A 1.3-fold excess of the amines was sufficient to drive the reactions to completion and only the secondary *β*-amino alcohols were isolated as the major products (Scheme 3.23). Where the reaction mixtures gave other by products, the yields of side products were negligible and characterization of these isolates was not attempted. Purification was easily achieved by means of chromatography on silica gel. Characteristically, all products had R_f values that were much lower than either starting epoxides or by-products, usually barely running above the baseline in 10 – 15% MeOH in DCM.

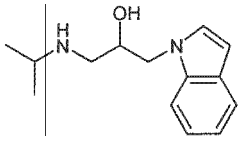
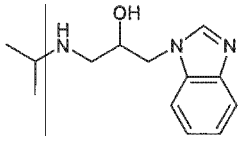
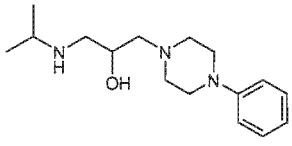
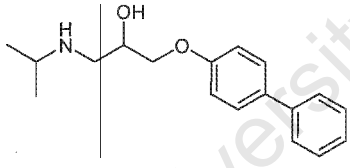
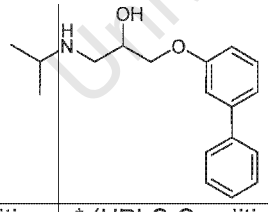
Table 3.8: Products, isolated yields and purities of β-amino alcohols **3.82 – 3.104**

General Product Structure	Product	n	Yield (%)	HPLC Purity (%)[a]
	**3.78**	1	71	99
	3.79	2	65	94
	3.80	3	59	94
	3.81	5	74	97
	**3.82**	1	67	91
	3.83	2	64	94
	3.84	3	74	95
	3.85	5	66	98
	**3.86**	1	66	99
	3.87	2	60	98
	3.88	3	71	90
	3.89	5	63	97
	**3.90**	1	66	95
	3.91	2	72	90
	3.92	3	69	92
	3.93	5	60	95
	**3.94**	1	57	99
	3.95	2	76	99
	3.96	3	67	96
	3.97	5	66	92
	**3.98**	1	85	98
	3.99	2	78	94

[a] HPLC conditions: * (HPLC Conditions: $CH_3CN:H_2O$) 60:40, 25 mM Phosphate buffer (pH~7), flow rate = 1.5 ml/min)

Tables 3.8 and 3.9 show the isolated yields and purities of the compounds. On average, the yields were high, and the purities excellent.

Table 3.9 Products, isolated yields and purities of β-amino alcohols **3.100 – 3.105**

Compound Structure	Product	Yield (%)	Purity (%)[a]
	**3.100**	84	99
	**3.101**	81	94
	**3.102**	77	97
	**3.103**	67	98
	**3.104**	79	95

[a] HPLC conditions: * (HPLC Conditions: $CH_3CN:H_2O$) 60:40, 25 mM Phosphate buffer (pH~7), flow rate = 1.5 ml/min)

Characteristic IR bands included the broad peak around 3500 cm^{-1} and ~3300 cm^{-1} due to OH and NH stretching respectively. All compounds depicted the presence of the other functional groups with ArH and C=C stretches appearing around ~3000, and ~1650.

All ^{1}H NMR spectra that were obtained were in agreement with the proposed structures. The most notable differences in the spectra among the different classes of secondary β-amino alcohols included the multiplicities and relative positions of the methylene protons adjacent to the OH-bearing carbon. As before, the multiplicities of these protons varied between multiplets, double doublets and doublets. A typical ^{1}H NMR spectrum obtained for compound **3.80** is shown in Fig. 3.22.

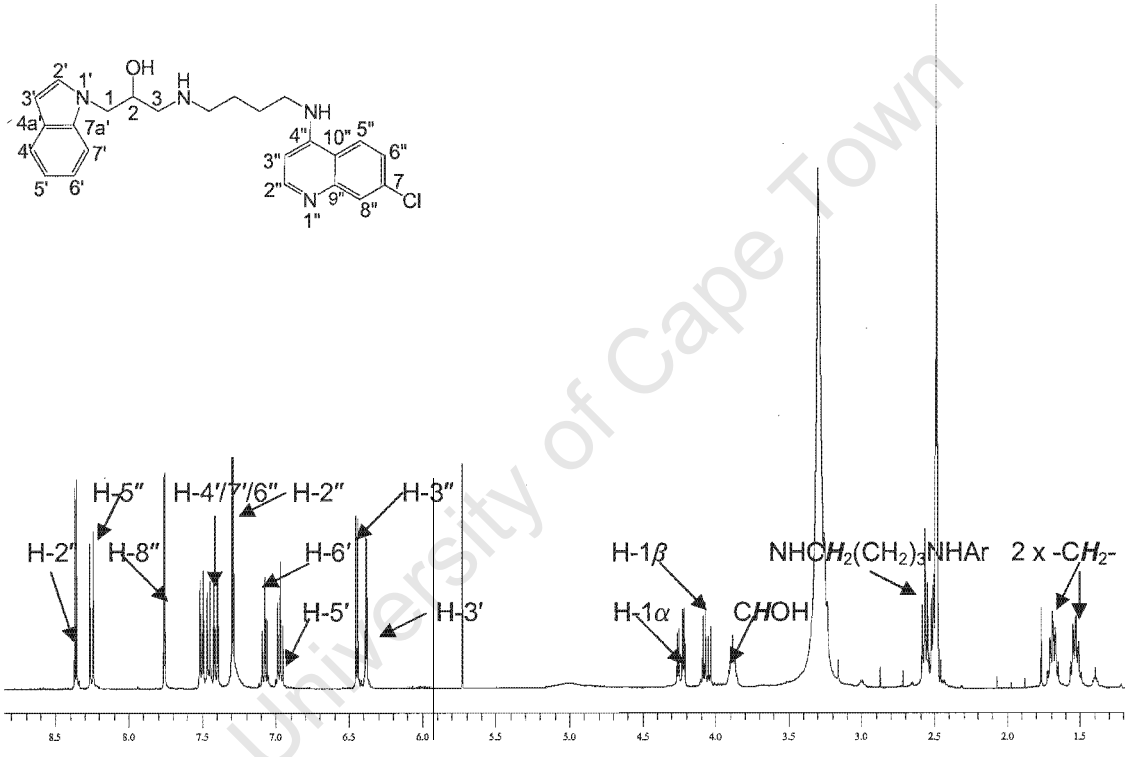


Figure 3.22: ^{1}H NMR spectrum of **3.80** in DMSO-d_6

The ^{13}C NMR data for all the β-amino alcohols were also found to be consistent with the proposed structures. In the cases of those β-amino alcohols arising from privileged substructures, the only differences lay in the number of aliphatic carbons within each series. As the chain length was increased, an extra sp^3 carbon peak in the alkyl region

was evidenced in respective ^{13}C NMR spectra. A representative ^{13}C NMR spectrum obtained for **3.78** is shown in Fig. 3.24.

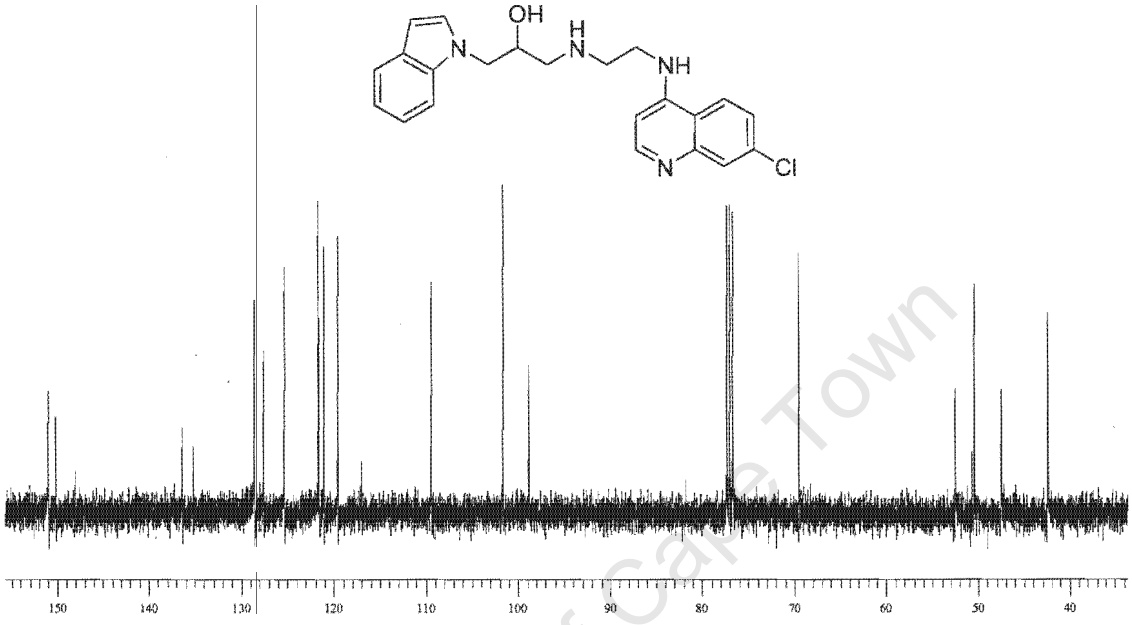


Figure 3.23: ^{13}C NMR of **3.78** in $CDCl_3$

Low-resolution fast atomic bombardment mass spectroscopy was principally used to determine the presence of the parent molecular ion. All compounds showed the molecular ion peak to differing degrees. The purities of the compounds were determined by means of HPLC, eluting with a 1:1 mixture of CH_3CN and a 25 mM phosphate buffer solution. All compounds were deemed pure, with purity averaging 95% (Table 3.9), for biological evaluation.

3.8 Biological Results and Discussion

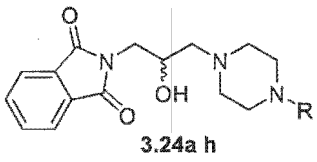
The biological activities of the synthetic *β*-amino alcohols **3.24a – j** were determined in the laboratories of Professor P. Grellier, of the Museum National d'Histoire Naturelle in Paris, France, while the antiplasmodial activities of **3.78 – 3.104** were determined in the laboratories of Professor P. J Smith (UCT Department of Pharmacology). The synthesized target compounds were principally generated in order to determine their abilities to inhibit plasmodial growth by determining the IC_{50} and ED_{50} values, terms that are used interchangeably throughout. The lower the IC_{50} or ED_{50} value of a compound, the greater is its efficacy. However, the more important property that a good drug should exhibit is that of selectivity. A non-selective drug with low IC_{50} or ED_{50} values is no good if it has poor selectivity between host and parasite. In other words, a good drug must be selectively cytotoxic to the parasite and not the host. All experimental details pertaining to biological tests are given in the experimental section.

3.8.1 Results

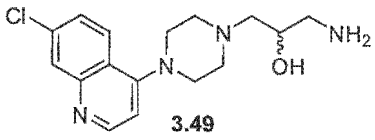
3.8.1.1 *In Vitro* Testing of Tertiary β-Amino Alcohols 3.24a – h

Compounds **3.24a – h** and **3.49** (Table 3.10) were screened for their antiplasmodial activity against the chloroquine-resistant K1 strain of *P. falciparum*. As can be seen from the table, none of the compounds exhibited any significant antiplasmodial activity. The most active compound was **3.49** (IC_{50} = 0.33 μM), in which the phthalimide-protecting group had been removed. All the other compounds were over several hundred-fold less efficacious than chloroquine.

Table 3.10: Intrinsic antiplasmodial activities of compounds

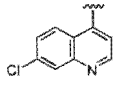
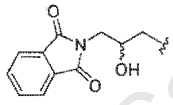
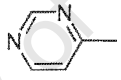
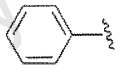
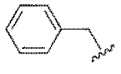
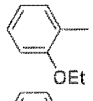


3.24a h



3.49

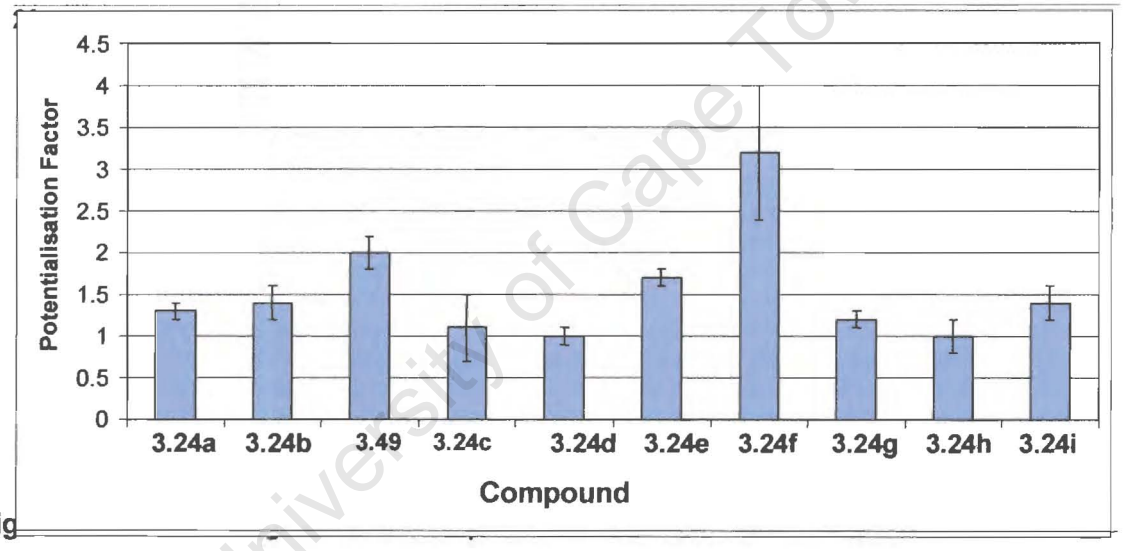
General structure of β-Amino Alcohols

Compound	R	K1 IC_{50} (μM)
Chloroquine	–	0.11 ± 0.0024
3.24a	–COOEt	>100
3.24b		18.7
3.24j		>100
3.24d	$–CH_3$	>100
3.24e		54.8
3.24g		>100
3.24f		28.7
3.24i		>100
3.24h		>100
3.49	see above	0.33

The compounds' activities were too poor to warrant further investigation as potential antiplasmodial agents. However, the most active compound, the 4-aminoquinoline-bearing β-amino alcohol **3.49** (IC_{50} = 0.33 μM) had the phthalyl protecting group removed, and contained the 4-amino-7-chloroquinoline subunit, a well established

antimalarial pharmacophore. At worst, it was still three times less active than chloroquine in the chloroquine-resistant K1 strain.

On close examination, we reasoned that compounds **3.24a – h** possessed properties inherent in chloroquine-resistance reversal agents. These features include weak intrinsic antiplasmodial activity, the presence of hydrophobic moieties[68] that give lipid solubility, two planar aromatic rings, a cationic charge and a tertiary nitrogen atom. Thus the compounds were re-evaluated for their ability to reverse resistance to, or potentiate the action of chloroquine in the same strain, the results of which are shown in Figure 3.24.



Fig

Figure 3.24: Chloroquine potentialisation effects of compounds **3.24a – h**

The potentialisation factor, in the context of chloroquine resistance, is defined as the ability of a drug to reverse the resistance of *Plasmodium* species to chloroquine. In this assay, the compounds were fed to the parasite in combination with chloroquine and the IC_{50} values re-determined. Compounds with potentialisation factors well above 1 were judged to possess inherent chloroquine-resistance reversal properties. Thus, compound **3.24f** was found to be the most active chloroquine-resistance reversal agent, causing a

3-fold increase in parasite sensitivity to chloroquine. The chloroquine-resistance reversal activity was followed by **3.49**, **3.24e**, and **3.24i** in this order. Compounds **3.24a**, **3.24b**, **3.24c**, **3.24d**, **3.24g**, and **3.24h** with potentiation factors close to 1 were judged to be acting synergistically with chloroquine.

Due to reasons beyond our control, the second generation β-amino alcohols **3.47a – g** whose rationale of design was based on the most active chloroquine-potentiating compound **3.24f** were not evaluated for either antiplasmodial activities or chloroquine-reversal abilities and so no biological data are presented.

3.8.1.2 *In Vitro* Testing of 4-Aminoquinoline-containing Secondary β-Amino Alcohols

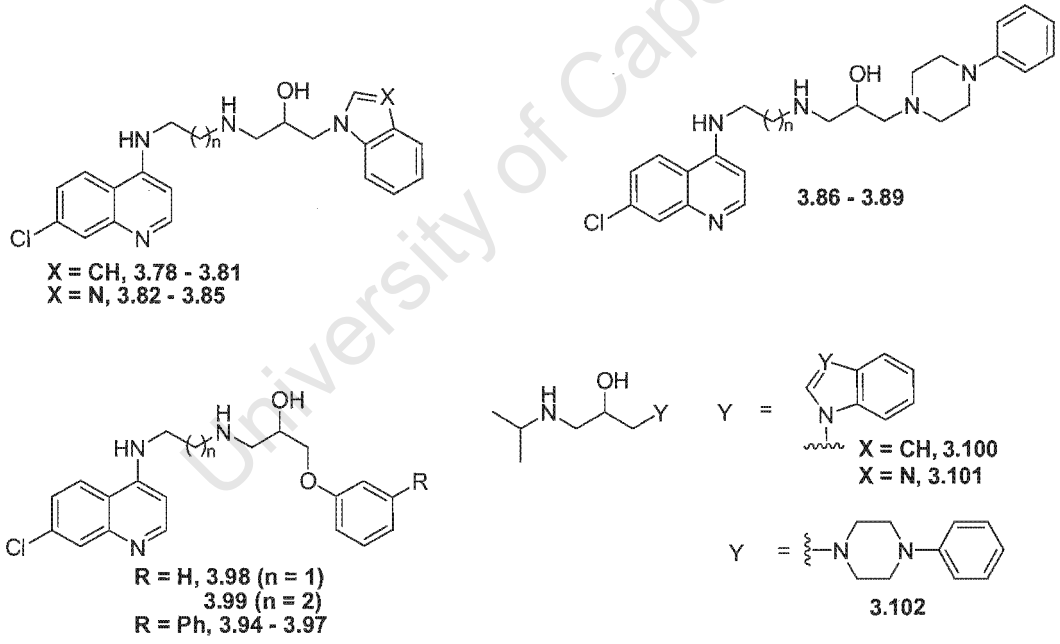


Figure 3.25: General Structures of β-amino alcohols **3.78 – 3.102**

Compounds **3.78 – 3.102** were screened against the chloroquine-sensitive D10 strain and the percent viabilities of the parasite cultures in the presence of the inhibitors determined. The percentage viability is a quick but reliable measure of the inhibitory

activity of a compound that was in this case determined at three different concentrations of inhibitor. Compounds that significantly inhibited plasmodial growth at the lowest concentration (0.0625 μM) by 60% or more were tested further to determine their respective IC_{50}s.

Table 3.11: Antiplasmodial activities of amines **3.52 – 3.55** and β-amino alcohols **3.78 – 3.102**

	Concentration		
	% Parasite Survival		
Compound	**0.250 μg/ml**	**0.125 μg/ml**	**0.625 μg/ml**
Chloroquine	33.2	37.4	75.7
3.52	44	50	54
3.53	61	74	94
3.54	82	99	96
3.55	20	23	77
3.78	55	60	69
3.79	63	58	74
3.80	69	73	90
3.81	34	43	46
3.100	>100	>100	>100
3.82	22	29	41
3.83	29	38	55
3.84	42	51	65
3.85	40	49	88
3.98	56	70	72
3.99	74	79	87
3.90	34	68	78
3.91	27	43	66
3.92	52	61	76
3.93	71.6	75.8	84.4
3.94	53	97	87
3.95	35.9	44.2	43.5
3.96	46	47	76
3.97	39	41	44
3.89	20	60	84
3.102	3	22	77

The results in Table 3.11 show that the four 4-aminoquinoline amines **3.52 – 3.55**, as well as the majority of the compounds were by and large devoid of antiplasmodial activity as revealed by the high parasite viabilities at the lowest concentration tested. Nonetheless, three compounds exhibited good activities at 0.0625 μM, namely **3.86**, **3.87** and **3.88** (% viability data not shown). Both **3.86** and **3.87** were twice as active in the D10 strain as chloroquine (chloroquine IC_{50} = 0.093 μM), both inhibiting parasite development with IC_{50}s of 0.053 μM, while, the potency of **3.88** (IC_{50} = 0.21 μM) was half that of chloroquine (Table 3.12).

General structure of the most potent β-amino alcohols

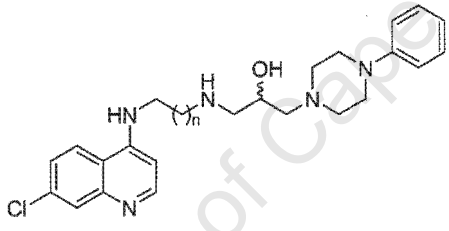


Table 3.12: Antiplasmodial activities of β-amino alcohols **3.86 – 3.88**

Compound	n	IC_{50} (μg/ml)	IC_{50} (μM)
Chloroquine	–	0.0305	0.096
3.86	1	0.0232	0.053
3.87	2	0.024	0.053
3.88	3	0.096	0.21

3.8.2 Discussion:

Considering that the compounds bear the 4-aminoquinoline moiety, it is possible that they may be acting by a mechanism similar to that for chloroquine, namely, the inhibition of haemozoin formation.[69] The result of this inhibition, then, would be the accumulation of haem to levels that are parasiticidal. Possibly, the compounds may be acting on the major parasite proteases (plasmepsins); indeed β-amino alcohols have been shown to

inhibit malarial plasmepsins.[5] The phenylpiperazine subunit is a privileged substructure, therefore it has the ability to bind a range of proteins and receptors. Among the privileged substructures used, it is tempting to speculate that the superior activities observed with the β-amino alcohols containing the phenylpiperazine unit are better ligands for binding their targets, whatever the mechanism of action of these compounds may be. However, to delineate meaningful SARs, more compounds in which the phenyl ring is omitted or replaced by other groups would need to be synthesized. Since all the compounds were tested as their racemates, it is possible that the observed low antiplasmodial activities associated with the inactive compounds were a result of opposing activities of respective enantiomers. Therefore, before these compounds are dismissed as being devoid of antiplasmodial activity, it would be interesting to note the antiplasmodial activities of their individual enantiomers.

3.8.3 *In Vivo* Testing of Citrate Salt of Compound 3.86: Results and Discussion

Table 3.13: *In Vivo* Antiplasmodial activity of Tricitrate salt β-amino alcohol **3.86**

No. of days/ injection	% Parasitaemia		
	Control	Exp 1	Exp 2
1	0	0	0
2	0.65	0.64	0.34
4	1.6	0.72	0.75
5	7.2	0.58	0.46
7	19	0.75	0.90
8	20.5	0.53	3.4
11	34.2	0.59	0.9
13	***	0.62	0.74
14	***	0.70	0.57
15	***	0.67	0.54

The parasiticidal compound **3.86** was insoluble in water and so it was decided that its solubility be improved by synthesizing a water-soluble salt. Thus, the *in vivo* activity was determined using the tricitrate salt of **3.86**, which was soluble in water at >5 mg/ml. The two independent tests in Table 3.13 reveal that the results against *P. berghei*-infected mice were reproducible within limits of experimental error. As can be seen, the mean parasitaemia in the control experiment showed a progressive increase, rising from 0.65 on day 2 to 20.5 on day 8 post infection. At this point, the control mice started dying with only 2/5 surviving, while in the case of the parasite-infected mice treated with the citrate salt, the parasitaemia was suppressed and was kept constant up to 15 days. At this point in time, the mice treated with the tricitrate salt of **3.86** showed a 5/5 health rating and the parasite suppression expressed as a percentage was determined to be 97.4%. That the compound was able to suppress a rise in parasitaemia suggests that it was acting at the blood stage of malaria, consistent with the mode of action of 7-chloro-4-aminoquinolines.[69,70] The long survival time of the compound-treated rodents indicates that the compound is not cytotoxic towards the mice. This observation of an absence of observable toxicity in mice is encouraging considering the cytotoxicity data (highest therapeutic index of the series) obtained for the same compound against the human KB cell line (section 3.8.4, Table 3.14, p. 99). The observed *in vivo* activity of **3.86** suggests that this compound is (orally) absorbed and may have a favourable pharmacokinetic profile. This is noteworthy.

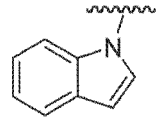
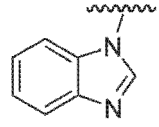
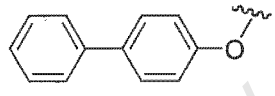
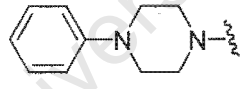
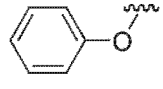
3.8.4 *In Vitro* Activities of Secondary β-Amino Alcohols against *T. brucei*: Results

The abilities of select amino alcohols to inhibit the activity of cultured *T. b. brucei* parasites were determined at 4 different concentrations prior to determining actual

ED_{50}s. With the exception of the 6 compounds **3.82 – 3.85**, **3.100** and **3.101**, all compounds nearly inhibited parasite growth completely at the lowest compound concentration tested (1 μg/ml). The encouraging inhibitory activities of these compounds warranted further analysis of the compounds in the parasite cultures, the results of which are presented in Table 3.14. Data for the standard control drugs pentamidine (*T. brucei*) and Podophyllotoxin (cytotoxicity) is included for comparative purposes.

As is apparent from the table, most compounds were active in the low to mid-micromolar range. Despite the promising results for these new compounds, however, some of the compounds (**3.79**, **3.80**, **3.81**, **3.95**, **3.89** and **3.98**), exhibited unfavourable cytotoxicities towards the human KB cell line used. The immediate implication of these high cytotoxicities is that the compounds make unfavourable leads for further development. A few other compounds displayed very low and desirable ED_{50}s against the parasite, and were only cytotoxic to the human cells at very high concentrations, implying high therapeutic indices. The most notable compounds in this regard are **3.86** and **3.99** both of which have selective parasite inhibitions of over 700. Other noteworthy compounds in these series include **3.78**, **3.87** and **3.102**.

Table 3.14: *In vitro* activities of 4-aminoquinoline *β*-amino alcohols against *T. brucei* (see p. 93, Fig. 3.25 for general structures).

Compound	R	n	ED_{50} μg/ml	ED_{50} μM	Cyt. ED_{50} μg/ml	Cyt. ED_{50} μM	TI*
Pentamidine	–	–	0.00007	0.0002	–	–	
Podophyllotoxin	–	–	–	–	0.00024	–	–
3.78		**1**	**0.022**	**0.056**	**5**	**12.7**	**227**
3.79		2	0.26	0.63	0.7	1.7	3
3.80		3	0.17	0.37	0.83	1.8	5
3.81		5	0.16	0.36	1.0	2.2	6
3.82		1	3.9	9.76	24.0	60.5	6
3.83		2	3.0	7.27	6.9	16.8	2
3.85		3	6.7	15.7	6.7	15.8	1
3.85		5	3.6	8.0	5.4	11.9	1
3.94		1	0.03	0.060	3.0	6.7	112
3.95		2	0.26	0.53	0.3	0.7	1
3.96		3	0.012	0.025	2.0	4.2	168
3.97		5	0.022	0.045	1.8	3.6	80
3.86		**1**	**0.0076**	**0.017**	**5.9**	**13.4**	**788**
3.87		**2**	**0.013**	**0.029**	**4.0**	**8.8**	**303**
3.89		5	0.17	0.34	1.0	2.0	6
3.98		1	0.48	1.29	1.1	4.1	3
3.99		2	**0.028**	**0.072**	**19.9**	**51.7**	**718**
3.100	–	–	4.52	19.4	196.0	841.0	43
3.101	–	–	0.96	4.1	30.7	131.0	32
3.102	–	–	**0.0112**	**0.040**	**5.2**	**18.7**	**466**

*TI, Therapeutic Index = *T. brucei* ED_{50}/Mammalian cells ED_{50}.

3.9 Conclusions and Recommendations for Future Work

The results obtained show that the most active antiplasmodial compounds are also potential antitrypanosomal agents. This is especially seen with the two compounds **3.86** and **3.87**, both of which are highly efficacious against *P. falciparum* and *T. brucei* cultures, and yet lack toxicity towards the human KB cell line.

As the compounds were synthesized, isolated and tested in racemic form, the primary suggestion would be to isolate the most active compounds into their respective enantiomers and have these tested again in the same strain. Secondly, and more importantly, before the inactive compounds are dismissed all compounds (including the most active) need to be tested against a range of malaria parasites with varying degrees of resistance towards chloroquine.

The secondary amino position and phenyl ring of **3.86** and **3.87** serve as diversity points for the establishment of meaningful SAR studies. Thus, using these as diversity handles in the lateral side chain, further compounds may be synthesized in the hope of generating even more potent compounds than those obtained herein.

As for the *in vivo testing*, one suggestion would be to retest the compounds in the mouse models and monitor recrudescence (or short term relapse) after a longer period than the 15 days in the initial tests. The compound may also have to be tested for its curative ability in pre-infected (sick) mice for observation of parasite clearance.

In terms of antitrypanosomal activity, **3.79**, **3.86**, **3.87**, **3.99** and **3.101** warrant further investigation (e.g. *in vivo*) in view of their high favourable therapeutic indices. Overall mechanistic studies with respect to targets of the most active compounds need to be performed. For *P. falciparum* testing of **3.86** for binding to haem and inhibition of haemozoin formation as well as testing against the *P. falciparum* aspartic proteases (plasmepsins) are obvious starting points. In order to establish the possible targets for

antitrypanosomal compounds, a good starting point is the antioxidative enzyme trypanothione reductase (TryR).[71] Trypanothione reductase is a validated target for drugs which disrupt the natural redox defence systems in the parasitic protozoa *Trypanosoma* and *Leishmnia*. Both chloroquine and quinacrine (mepacrine) as well as derivatives are known inhibitors of trypanothione reductase.[72,73]

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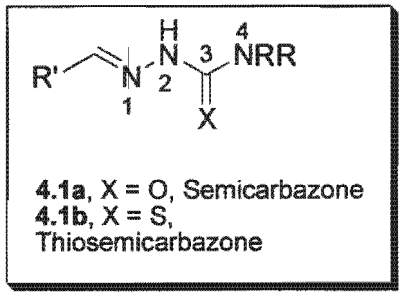
CHAPTER 4

THIOSEMICARBAZONES AND SEMICARBAZONES AS POTENTIAL ANTIPROTOZOAL CYSTEINE PROTEASE INHIBITORS

4.1. Introduction: Background to Thiosemicarbazones and Semicarbazones

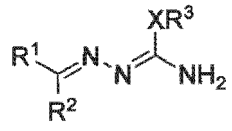
The following chapter describes the design, synthesis and biological evaluation of a series of thiosemicarbazones and semicarbazones, and provides rationales behind the syntheses. A background on the chemistry and biological applications is given, followed by rationales for the syntheses of the different classes of thiosemicarbazones and semicarbazones. The biological results from *in vitro* studies are presented, preceded by the chemistry employed in synthesizing the compounds. As before, the compounds were designed and synthesized for cross screening against malaria parasites and other (principally trypanosomal) parasites, and the associated enzymes falcipain-2 and rhodesain, (as well as cruzain, for which data are given in the Appendix). Only biological data corresponding to *P. falciparum*, falcipain-2, *T. brucei* and rhodesain are presented in this chapter.



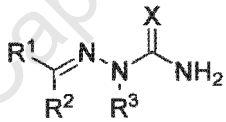
4.1a, X = O, Semicarbazone
4.1b, X = S,
Thiosemicarbazone

Figure 4.1: General Structure of thiosemicarbazones and semicarbazones

Thiosemicarbazones and semicarbazones have the general structure shown in Fig. 4.1. Oftentimes, derivatives that have substituents on the X atom, such as **4.2** or on the 'hydrazinic' nitrogen, as in **4.3** (Fig. 4.2) are usually included in this class.



4.2



4.3

Figure 4.2: Other structures of thiosemicarbazones and semicarbazones

Thiosemicarbazones and semicarbazones have emerged as important targets in the search for new chemotherapeutic agents, presenting themselves as potentially important pharmacological agents in the areas of antiprotozoal,[1] anticonvulsant,[2] antibacterial,[3] antiviral,[4] and anticancer research.[5] They are grouped together with a class of Schiff bases that are chelators of metals, particularly Fe. The idea of using Fe chelators in the discovery of new antimalarial agents stems from the central role that this metal plays in the proliferation of the parasite as well as the arrest of parasite growth both *in vitro* and in *vivo*.[6,7] It must be stressed here that chelation of iron in itself may not be able to achieve a well-defined role in the chemotherapy of malaria until new agents are

designed that specifically possess antimalarial properties. The ability of antimalarial metal ion chelators has led to some suggested modes of action of these compounds.[8,9]

4.1.1 Possible Modes of Action of Thiosemicarbazones and Semicarbazones

As chelators of iron, thiosemicarbazone and semicarbazone antimalarials can be categorized into three major groups depending on the predominant mechanism of inhibition of parasite growth. Two of these categories depend on an interaction with iron in order for them to exert their antimalarial activities, while the third one depends on an interaction with cysteine proteases.

4.1.1.1 Withholding Iron from Plasmodial Metabolic Pathways

One possibility *via* which Fe chelators are able to arrest plasmodial growth is by sequestration of the iron that is necessary for plasmodial replication rather than directly affecting the parasite, or sequestering other essential trace elements. This mechanism is supported by experimental data for Fe(III) chelators which reveal that the ability of these compounds to inhibit plasmodial growth is completely abrogated when incubation mixtures of these compounds are saturated with Fe.[10] The ultimate stagnation of growth observed in the parasite is a result of either the inhibition of one or more metalloenzymes that are critical to survival, e.g. ribonucleotide reductase which is involved in the biosynthesis of DNA, or the deprivation of the much needed Fe for growth and replication.

4.1.1.2 Forming Toxic Complexes with Iron

A second plausible mode of action of the thiosemicarbazones and semicarbazones is that based on the formation of toxic complexes. Compounds that form toxic complexes

with Fe are essentially Fe(II) chelators. Rather than withholding iron, it appears that these compounds form a complex with Fe extracellularly, which then enters the parasitized erythrocyte resulting in a rapidly lethal free radical-mediated intracellular reaction.[11]

4.1.1.3 Inhibition of Cysteine Proteases

The chemical structure of thiosemicarbazones presents two electrophilic sites which may serve as sites for nucleophilic attack by the thiolate anion of cysteine proteases (Fig. 4.3). One of the implications of this is that by attacking either of these positions thiosemicarbazones can reversibly inhibit malaria cysteine proteases which are known to be important for parasite survival.[12] The metal complexes alluded to earlier (section 4.1.1.2) can also inhibit cysteine proteases since the thiolate anion can bind to the metal.

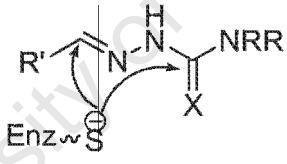


Figure 4.3: Potential sites of attack by cysteine proteases

The rich chemistry with which sulfur is endowed, namely nucleophilicity, ability to form stable metal chelates and ability to form radicals *via* redox chemistry, makes its inclusion in the design of thiosemicarbazones highly critical. Coupled with the properties discussed, we became interested in thiosemicarbazones and semicarbazones due to their nonpeptidic nature (suggesting better bioavailability), small size and the economic cost of production for the generation of new potential antiparasitic agents, with emphasis being laid largely on malaria.

4.1.2 Characteristics that Affect the Antimalarial Activities of Iron Chelators

It is essential that for Fe chelators to be effective, the compound must be able to gain entry into the parasitic compartment of the infected erythrocyte since this is the most likely residence of the Fe to be withheld. Thus, it should possess physical properties that allow it to penetrate the lipid membrane well; additionally it should have a high affinity for Fe and preferably selectively bind Fe(III) rather than Fe(II).[11] These properties are detailed in the following sections.

4.1.2.1 Affinity for Iron

As a prerequisite for Fe chelation and thence antimalarial activity, a high affinity for Fe is of utmost significance.[13] This activity can be achieved by incorporating into the compound chemical groups that coordinate with Fe. Among several groups of compounds and/or atoms that can chelate Fe, the thiosemicarbazones have 2 – 3 groups (N and S atoms) that can coordinate with Fe.

4.1.2.2 Hydrophilic/Hydrophobic Balance

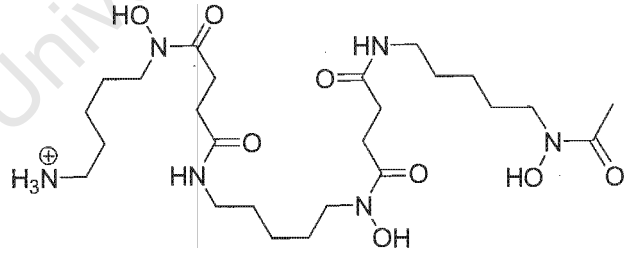
A balance of the hydrophilic/hydrophobic properties of a compound is an important factor in the movement of a compound across a lipid-containing membrane for it to enter a cell. Thus, the usefulness of an Fe chelator as an antimalarial agent can be determined by its relative hydrophobic/hydrophilic balance or lipophilicity.[14] Indeed, experimental evidence attesting to a direct positive correlation between the lipophilicity of an Fe chelator and its antiplasmodial inhibitory activity has been established.[15]

4.1.2.3 Selectivity for Fe over other Cations

The significance of selectivity for Fe over other metal ions is twofold: (i) the limited ability of the malaria parasite to recover from Fe deprivation in comparison to the human host makes Fe a reasonable target relative to other essential metals;[16] (ii) nonselective metal chelators have the ability to remove other essential biometals (e.g. Ca, Zn, Mg) from the human host.

4.1.2.4 Selectivity for Fe(III) over Fe(II)

It seems that chelators that bind Fe(III) have more potential than those that bind Fe(II). Desferrioxamine-B, DFO (**4.4**, Fig. 4.4) is a widely used chemotherapeutic agent that works by chelating Fe in patients suffering from iron overload and is also an active antiplasmodial agent *in vitro*.[17] In experiments conducted on amino derivatives of DFO, it was found that these derivatives had substantially reduced ability to bind Fe(II) but still retained their ability to chelate Fe(III).[17] Unlike the parent unmodified DFO, these compounds retained their inhibitory effects on cultured *P. falciparum* parasites while virtually not inhibiting the growth of mammalian cells.



4.4

Figure 4.4: Desferrioxamine-B

4.1.2.5 Number of Coordination sites

Since the Fe^{3+} ion has six coordination sites, it would be expected that hexadentate chelators would form the most stable complexes. Penta- and quadridentate inhibitors

would leave one or two of the coordination sites unbound, potentially exposing these to partake in toxic reactions that could damage host tissues. On the other hand, tri-and bidentate chelators could fully occupy the coordination sites of Fe^{3+} by forming 2:1 or 3:1 complexes respectively. The two classes of thiosemicarbazones and semicarbazones that we targeted had either 2 or 3 (Fig. 4.5) such coordination sites inherent in their structures. Fig. 4.5 illustrates the formation of a tridentate chelate with the three participating groups in our envisaged thiosemicarbazones.

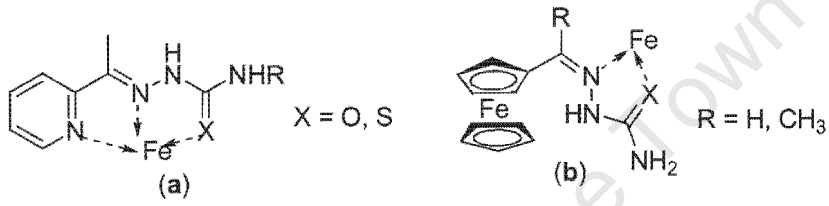
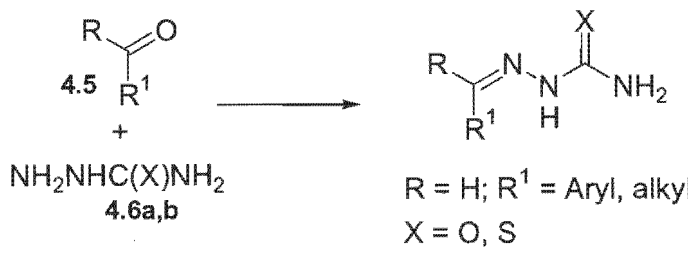


Figure 4.5: Possible (a) tridentate and (b) bidentate Fe chelates of thiosemicarbazones and semicarbazones

Unfortunately, at very low concentrations of the tri- and bidentate chelators, partial dissociation from Fe may occur thereby leaving the now free sites to participate in toxic reactions.

4.2. Chemical Synthesis

4.2.1. General Synthesis of Thiosemicarbazones and Semicarbazones



Scheme 4.1: General synthesis of thiosemicarbazones and semicarbazones

By and large, thiosemicarbazones and semicarbazones are prepared by the condensation of the appropriate thiosemicarbazide **4.6a** (X = S) or semicarbazide **4.6b** (X = O) and a carbonyl compound (aldehyde or ketone) **4.5**. Compared to imines, thiosemicarbazones and semicarbazones are more stable because the adjacent electronegative nitrogen can participate in delocalization of the imine double bond as shown in Fig. 4.6.

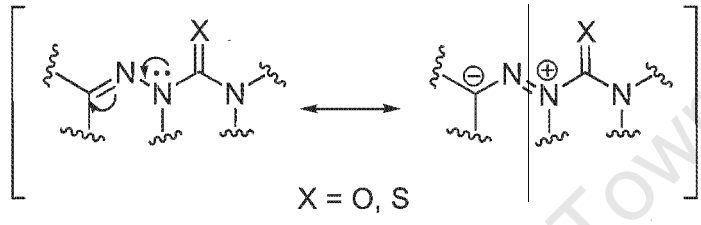


Figure 4.6: Resonance structures of semicarbazones and thiosemicarbazones

Delocalization decreases the $\delta+$ charge on the carbon atom of the thiosemicarbazone and semicarbazone imine double bond and thus raises the energy of the LUMO, making it less susceptible to nucleophilic attack. One of the implications of this stability is that thiosemicarbazones and semicarbazones are more stable to hydrolysis and thus they would be expected to be more bioavailable than their imine counterparts.

4.2.2 Synthesis of 2-Pyridyl Thiosemicarbazones

4.2.2.1 Rationale of Drug Design

The *in vivo* antimalarial activities of 2-pyridyl thiosemicarbazones were reported in the literature more than 25 years ago by Klayman and co-workers who conducted their studies on *P. berghei*-infected mice.[18] In the initial studies they found that N^4 monosubstituted thiosemicarbazones were found to be curative at doses below 200 mg/kg/day. Subsequent SAR studies revealed that the incorporation of N^4 into a medium-sized ring system improved activity.[19] The position of the nitrogen atom in the

ring was also found to be central to the antimalarial activities of the compounds. Thus, when the position of the nitrogen was switched to afford either the 3 or 4-pyridyl thiosemicarbazones, the antimalarial activities were lost. The more active compounds in the series typically had ring sizes of 4 – 9 and 13 carbons and the optimal size was established as being resident in the six-membered rings. It is noteworthy that the targets of these compounds in *P. falciparum* were unknown. In summary, they established the following features as being important for antimalarial activity (Fig. 4.7):

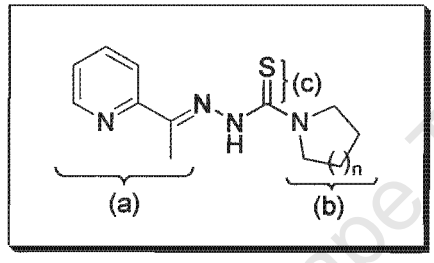


Figure 4.7: Important molecular features for antimalarial activity in 2-acetylpyridine thiosemicarbazones

(a) presence of the 2-pyridylethylidene moiety,

(b) cyclic structures at N^4 (certain other bulky noncyclic substituents are tolerated), and,

(c) the presence of the thiocarbonyl (C=S) group as opposed to the carbonyl (C=O) group.

Our thought process for the design of the target compounds is summarized in Fig 4.8 and is discussed below.

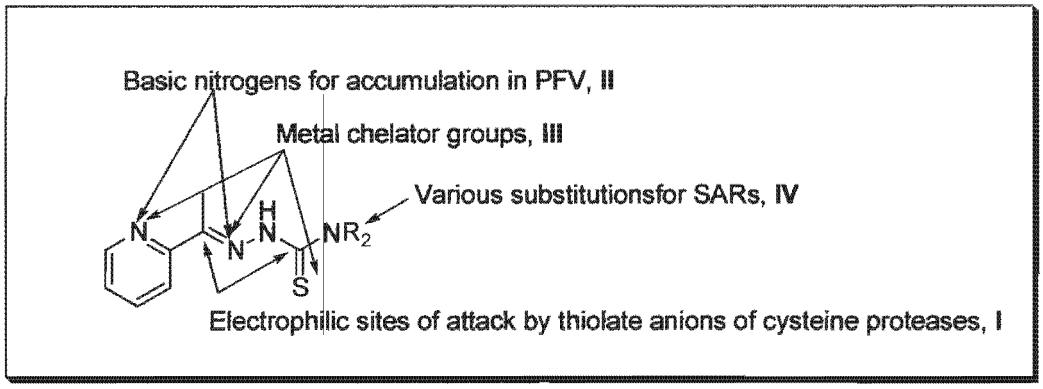


Figure 4.8: Rationale for design of 2-pyridyl thiosemicarbazones

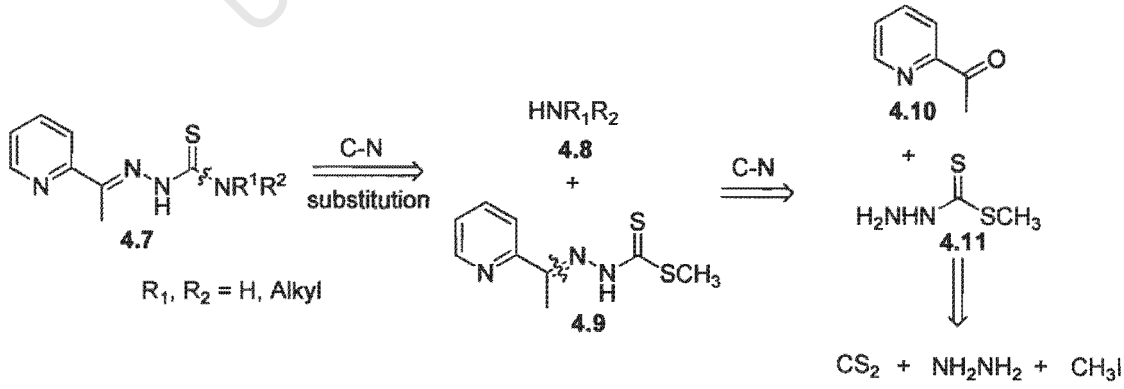
I) presence of electrophilic sites that may present themselves as sites of attack by the nucleophilic thiolate anions of cysteine proteases;

II) presence of two basic nitrogen atoms which may be significant for accumulation in the acidic parasitic food vacuole *via* pH trapping; additionally the presence of the 2-pyridyl N is essential for antimalarial activity;

III) presence of electron-donating groups which may be important for metal selectivity and affinity in terms of metal chelation; and

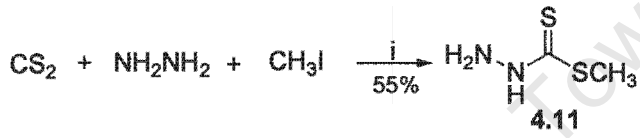
IV) variations in the N^4 substituents which may be important for specificity.

4.2.3 Retrosynthetic Analysis



Scheme 4.2: Retrosynthetic analysis of 2-pyridyl thiosemicarbazones

The synthesis of 2-pyridyl thiosemicarbazones was envisaged according to the retrosynthetic analysis depicted in Scheme 4.2. Accordingly, C-N disconnection in **4.7** should lead to amines **4.8** and the key 2-pyridylthiosemicarbazone thioester scaffold **4.9**, whose further analysis leads to commercially available 2-acetylpyridine **4.10** and the thiosemicarbazide thioester **4.11**. The latter is easily obtainable from CS_2 (carbon disulfide), NH_2NH_2 (hydrazine) and CH_3I (methyl iodide), all of which were accessible from commercial sources.



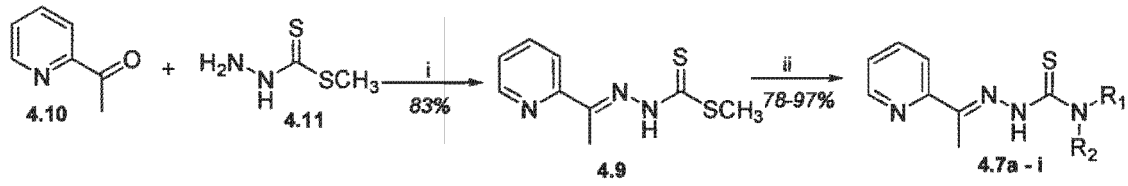
4.11

Scheme 4.3: *Reagents and conditions* i) KOH, rt, 3 h, *i*PrOH

The thiosemicarbazide thioester **4.11**, characterized by an offensive odour, was accessed by way of the method described by Klayman *et al*,[19] Scheme 4.3. Sequentially reacting CS_2 with NH_2NH_2 in an aqueous KOH-*i*PrOH solution and CH_3I afforded **4.11** in 55% yield following recrystallisation from DCM. Because of the insoluble nature of **4.11**, both ^1H NMR and ^{13}C NMR experiments were run in deuterated DMSO and both could only reveal singlets for the methyl protons and the thiocarbonyl group respectively. The presence of the molecular ion at 123 amu in the low resolution mass spectrum was useful in confirming the proposed molecular weight of **4.11**.

Having synthesized **4.11**, the subsequent step was to generate an exploratory library of thiosemicarbazones *via* scaffold **4.9** (synthesized from 2-acetylpyridine and **4.11**), Scheme 4.4. As alluded to earlier, the synthesis of thiosemicarbazones is a simple one-step condensation of aldehyde/ketone and thiosemicarbazide in a polar solvent such as

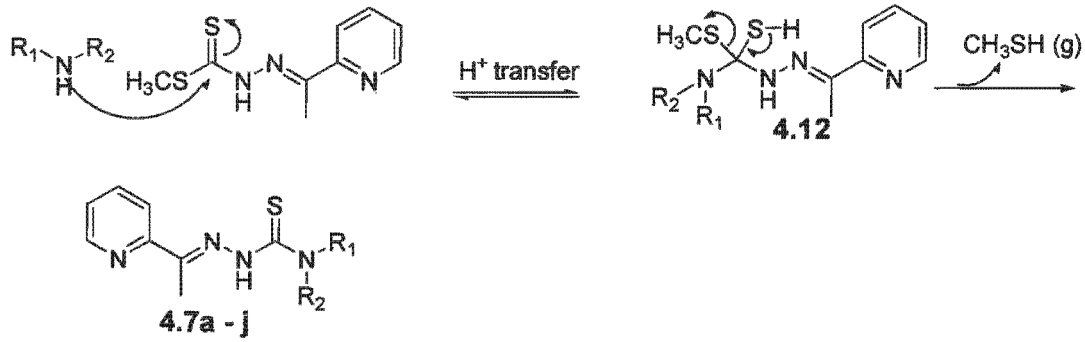
MeOH or EtOH usually requiring high temperatures and prolonged reaction times to go to completion.



Scheme 4.4: *Reagents and conditions* i) MeOH, reflux, 20 h; ii) **R_1R_2NH**, MeOH, reflux, 20 h

A series of commercially available amines (R_1R_2NH, not shown) and the synthetic 4-aminoquinoline amines **3.23b**, **3.52** and **3.53** were selected as the initial set of compounds. Using a parallel solution-phase approach, the amines were individually condensed with scaffold **4.9** in a 1:1 molar ratio in refluxing methanol. The reactions were allowed to reflux overnight (20 h) until the evolution of the foul-smelling methane thiol gas had ceased. The reactions were characterized by a tendency to deposit the products as brightly-coloured insoluble precipitates which were isolated by means of filtration. All but two products (**4.7a** and **4.7b**) precipitated upon cooling while **4.7a** and **4.7b** only crystallized upon removal of solvent.

4.2.3.1 Mechanistic Details

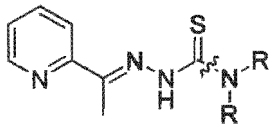
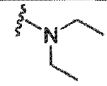
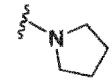
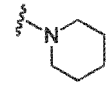
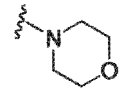
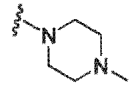
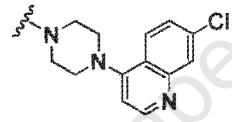
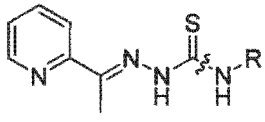
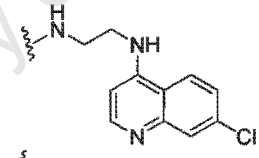
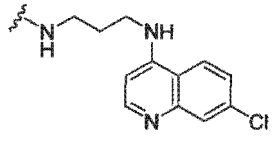


Scheme 4.5: Mechanism of substitution

In terms of mechanism, the events that lead to the N^4-substituted thiosemicarbazones **4.7a – i** are shown in Scheme 4.5. Nucleophilic attack of the amine on the electrophilic thiocarbonyl carbon of the thioester **4.9** leads to the tetrahedral intermediate **4.12** that collapses to release methane thiol gas and thiosemicarbazones **4.7a – i**. The last step is a non-reversible reaction whose driving force is the increased entropy of resultant products.

As can be seen from Table 4.1, the isolated yields of the initial thiosemicarbazones were good to excellent, and only those thiosemicarbazones that were synthesized from secondary amines formed *E,Z* isomers while those from primary amines resulted in single isomers. The structures and isolated yields of the resultant thiosemicarbazones are depicted in Table 4.1.

Table 4.1: Isolated yields and ratios of isomers of thiosemicarbazones **4.7a – i**

General structure	Compound	Substituent	Yield (%)	Ratio of isomers
	**4.7a**		96	1:1
	4.7b		97	4:7
	4.7c		95	2:3
	4.7d		93	1:1
	4.7e		92	1:1
	4.7f		92	2:1
	**4.7g**		97	1
	4.7h		78	1
	4.7i		94	1

Data emanating from studies on thiosemicarbazones and semicarbazones suggest that in their free unsubstituted forms in solid state, the C=N-NH-CX-NH_2 (X = O, S) backbone is usually almost planar, with the X (O, S) atom being *trans* to the azomethine N atom (E configuration, **I** in Fig. 4.9). Among the factors that may contribute to this preferred configuration is that the *trans* arrangement places the amine and azomethine nitrogen atoms in relative positions that are suitable for intramolecular hydrogen bonding.[20] Also,

thiosemicarbazones in which N^4 is fully substituted crystallize with the S atom *cis* to the azomethine N (Z configuration, **II**).

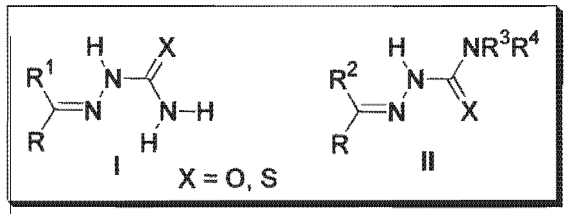
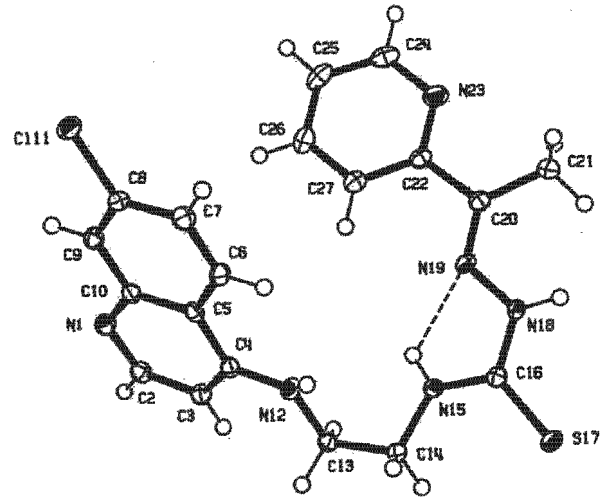


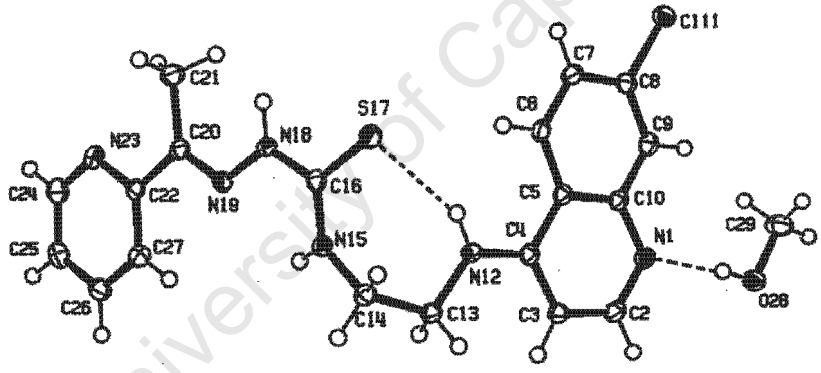
Figure 4.9: Conformations of thiosemicarbazones and semicarbazones

In contrast to this, all the fully substituted N^4 thiosemicarbazones **4.7a – f** crystallized to afford mixtures of *E/Z* isomers. This was clearly evident from their respective ^{1}H NMR spectra that were obtained. In the monosubstituted thiosemicarbazones **4.7g – i**, the products precipitated to yield single isomers which, according to the crystal structures obtained for **4.7h** in MeOH and EtOH (Fig. 4.10 respectively) selectively formed the *trans* product.

In all reaction mixtures the isomers could not be distinguished by means of TLC plate as they had similar R_f values when visualized under ultraviolet light.



(a) 4.7h (*trans*)



(b) 4.7h.MeOH (*trans*)

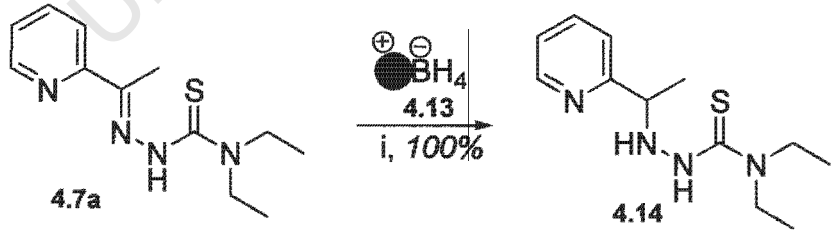
Figure 4.10: X-ray crystal structures of **4.7h** (**a**) and **4.7h** incorporating a molecule of MeOH (**b**)

Crystal structures of **4.7h** (crystallized from EtOH) and **4.7h·MeOH** (a solvated form of **4.7h** crystallized from MeOH) were determined. Although the crystal structures of these two compounds revealed different molecular conformations as well as different hydrogen bonding schemes, the configuration was *trans* with respect to the thionyl (C=S) and the azomethine groups.

The conformation of **4.7h** can be described as being almost helical (Fig. 4.10(a)). The only torsion angle that deviates significantly from 0/180° is N12-C13-C14-N15 with a value of 58.5(2)°. The *gauche* conformation around C13-C14 thus results in the overall molecular conformation appearing to be helical. The intramolecular hydrogen bond between N19 and the hydrogen atom attached to N15 stabilizes this conformation.

The conformation of **4.7h·MeOH** can be described as V-shaped (Figure 4.10(b)). The principal moieties are planar. However, torsion angles N12-C13-C14-N15 and C13-C14-N15-C16 have magnitudes of 58.8(2)° and 104.3(2)° respectively, giving rise to the V-shape. The intramolecular hydrogen bond between the S atom and the hydrogen attached to N12 stabilizes this conformation. The crystal structures of **4.7h** and **4.7·MeOH** are stabilized by intermolecular hydrogen bonds. In the solvate, the MeOH molecule acts as both a hydrogen donor and acceptor, linking the host molecules.

The variations at the N^4 position of the thiosemicarbazones were meant to establish SAR within this class of compounds. Another way in which further SAR could be established would be reduction of the imine double bond and, accordingly one of the compounds **4.7a** was reduced by way of Scheme 4.6.



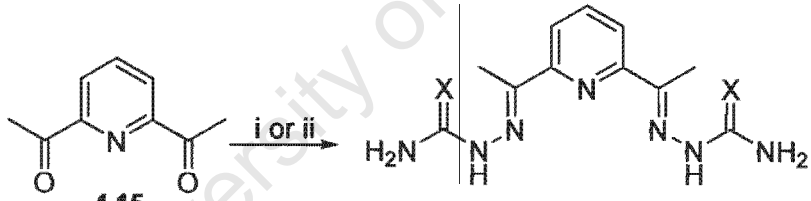
Scheme 4.6: *Reagents and conditions* i) PS-borohydride resin **4.13**, MeOH, 48 h

Thus, treating a methanolic solution of **4.7a** with polymer-supported borohydride **4.13** cleanly afforded the product **4.14** in quantitative yield after filtration and resin washing.

Two new diagnostic peaks in the ^{1}H NMR spectrum of **4.21**, a quartet at δ 3.55 ppm and a doublet at δ 1.45 confirmed the conversion of **4.7a** into **4.14**.

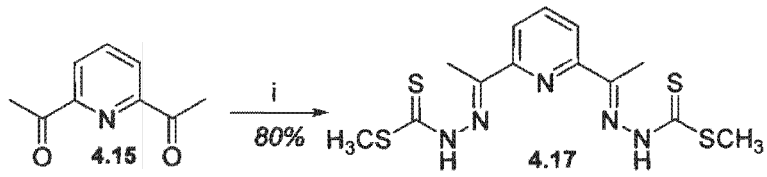
4.2.4 Synthesis of 2,6-Diacetylpyridyl Thiosemicarbazones and Semicarbazones

N^4-unsubstituted *bis*-thiosemicarbazones and *bis*-semicarbazones were also envisaged for the purpose of extending SAR studies. Thus, the requisite *bis*-thiosemicarbazone and *bis*-semicarbazone **4.16a** and **4.16b** were obtained by the treatment of commercially available 2,6-diacetylpyridine **4.15** with either thiosemicarbazide or semicarbazide. In the case of semicarbazones **4.16b** basification of the HCl salt of semicarbazide was necessary prior to addition of the ketone component and this was easily achieved by stirring a suspension of semicarbazide hydrochloride and NaOAc (1 eq) in EtOH for at least 30 min.



Scheme 4.7: *Reagents and conditions* i) $NH_2NHC(O)NH_2$.HCl, NaOAc, EtOH, rt, 24 h, or ii) $NH_2NHC(S)NH_2$, MeOH, reflux, 24 h

Simply refluxing the reaction mixtures in MeOH deposited the products as characteristic yellow crystalline compounds which could be easily isolated by simple filtration. However, in the case of semicarbazone **4.16b** it was necessary to wash the reaction mixture after consumption of starting materials with water in order to get rid of the inorganic by-products. Yields were excellent in all cases.

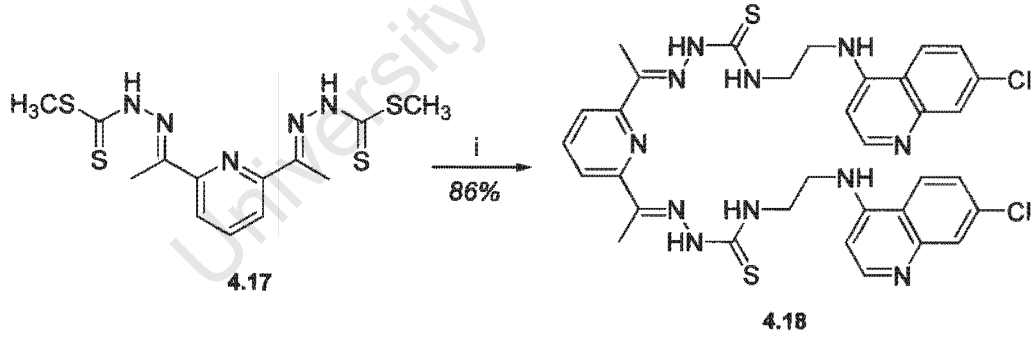


Scheme 4.8: *Reagents and conditions* i) $H_2NNHC(S)SCH_3$, MeOH, reflux, 24 h

To further extend SAR studies within this series of compounds, it was decided the symmetrical *bis*-thiosemicarbazone **4.17** be converted to the N^4,N^4-*bis*-substituted thiosemicarbazone **4.21**.

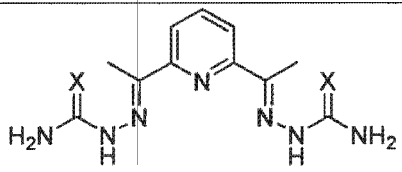
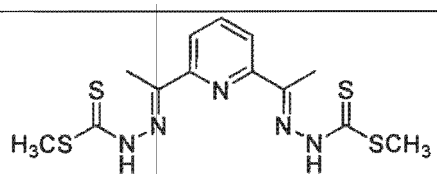
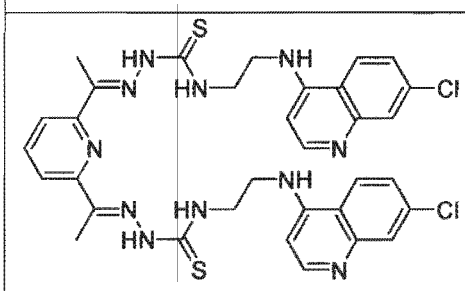
Therefore, a solution of 2,6-diacetylpyridine **4.15** and thiosemicarbazide thioester **4.11** was refluxed in MeOH (Scheme 4.8) and, after 3 h the product was deposited as a yellow precipitate which could easily be isolated by gravity filtration to afford the desired compound **4.17**.

Sequentially treating **4.17** with 2 eq of **3.52** furnished to the desired target **4.18** in excellent yield (86%).



Scheme 4.9: *Reagents and conditions* i) **3.52** (2 eq), MeOH, reflux, 18 h

Table 4.2: Yields of thiosemicarbazones and semicarbazones **4.19 – 4.20**

Structure	Compound	Yield (%)
	**4.16a** (X = O)	97
	4.16b (X = S)	79
	**4.17**	80
	**4.18**	86

The ^{1}H NMR spectral data of both **4.17** and **4.18** were suggestive of symmetrical compounds as were the ^{13}C NMR spectra. In both **4.17** and **4.18**, all quinoline ring protons integrated for 2 Hs. High resolution mass spectrometry was helpful in confirming the molecular formula of the proposed structure of **4.7h** with the molecular ion being observed at *m/z* 717.17399 ($C_{33}H_{33}Cl_2N_{11}S_2$).

Fig. 4.11 depicts the ^{1}H NMR spectrum of **4.7h**, which was typical for the group of compounds.

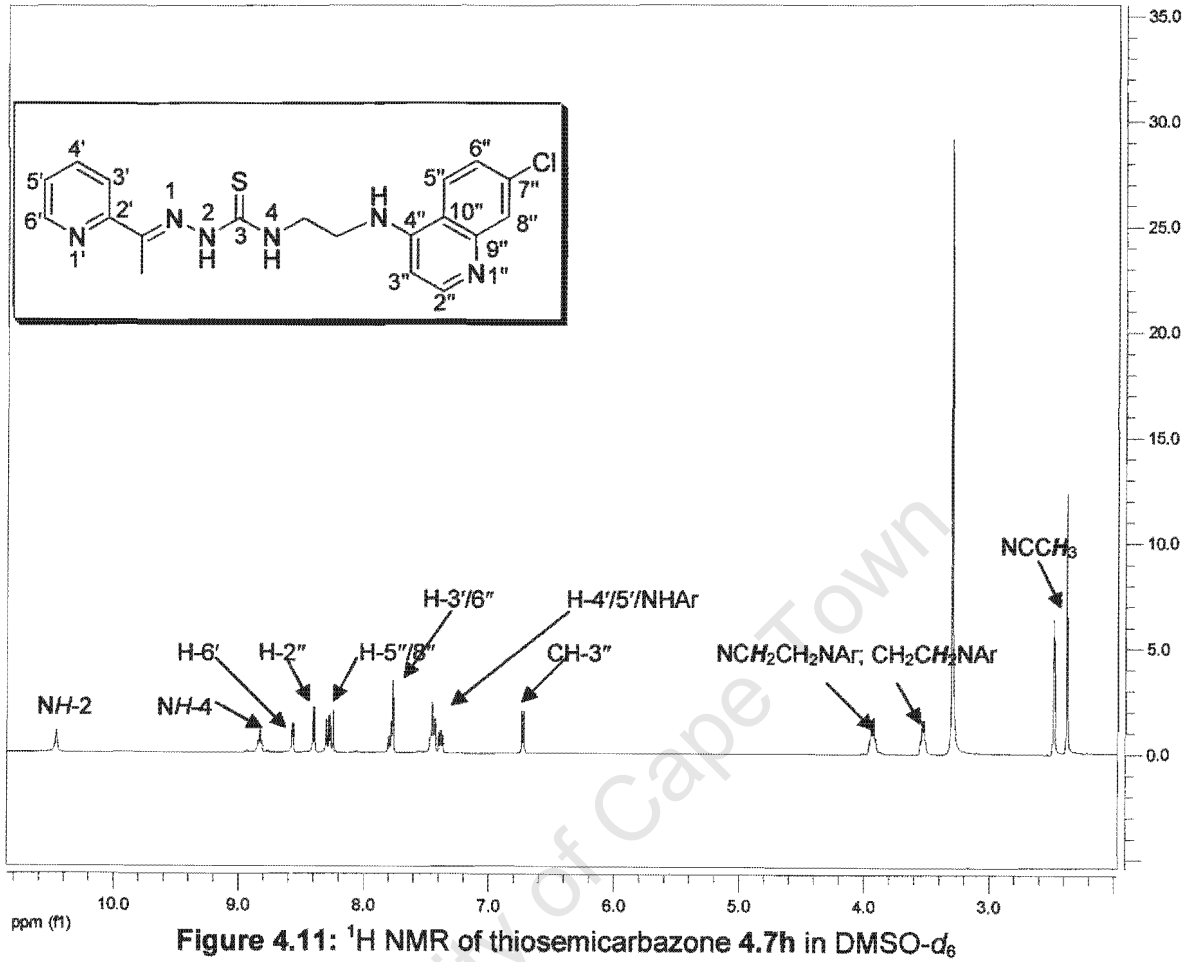
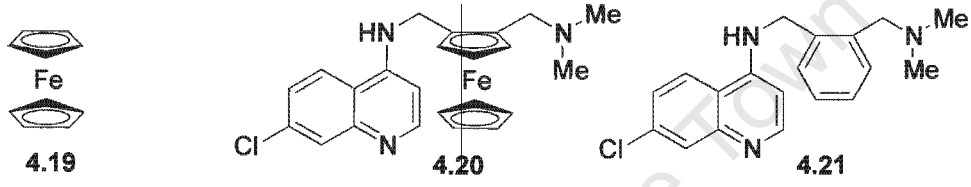


Figure 4.11: ^{1}H NMR of thiosemicarbazone **4.7h** in DMSO-d_6

4.2.5 Synthesis of Thiosemicarbazones and Semicarbazones Containing Ferrocene

4.2.5.1 Rationale for Drug Design

One of the major successes to overcome chloroquine resistance in the malarial parasite was demonstrated by Brocard *et al* in 1997, who carried out slight modifications to the chloroquine core structure[21] by incorporating ferrocene **4.19** (Fig. 4.12), in the side chain.



4.19 **4.20** **4.21**

Figure 4.12: Chemical structures of ferrocene **4.19**, ferroquine **4.20** and phenylequine **4.21**

The initial design of these compounds was based on what was termed the 'bait and hook' hypothesis in which it was assumed that ferrocene would be a source of Fe for the malaria parasite. Appreciating that the parasite's survival within the host is dependent on the presence of free iron, they postulated that given the parasite's avidity for Fe, the resistance associated with chloroquine might be effectively removed by the addition of Fe to a chloroquine molecule. Therefore, they incorporated the ferrocene (dicyclopentadienyl iron) nucleus **4.19** into the chloroquine structure and generated a series of compounds, among which was **4.20** (now commonly called ferroquine), that was as active *in vitro* in all the chloroquine-sensitive strains as the parent drug chloroquine but more active than chloroquine in the chloroquine-resistant strains against which it was tested. While the specific role of ferrocene has not been clearly established it has been demonstrated that ferroquine forms stable complexes with haematin in

solution ($\Delta G°$ = –0.95 kcal/mol).[22] It is thus possible that the mechanism of action of ferroquine is in part similar to that of chloroquine, probably involving haematin as the drug target and inhibition of haemozoin formation.[22] It remains to be established whether ferroquine is oxidized to the ferricinium ion (Fe^{3+}) or not. If indeed this were the case, it would then be possible that the ferrocene present as Fe^{2+} could catalyze a Fenton-like reaction in which the hydroxyl radical may be generated (Scheme 4.10).

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^{\bullet} + HO^{-}$$

Scheme 4.10: Fenton reaction

However, the involvement of ferrocene itself in the Fenton reaction seems extremely unlikely as it is an 18 electron complex and so is coordinatively saturated and cannot accept any new ligands precluding direct interaction between the hydrogen and peroxide oxygen atoms and the iron atom during a catalytic cycle.

In a similar vein, Beagley *et al* used the chloroquine modification strategy and, by incorporating a phenyl ring between the side chain nitrogens they synthesized compound **4.21** (phenylequine).[23] In the *in vitro* assays **4.21** was found to be more active than chloroquine in both chloroquine-resistant and chloroquine-sensitive strains. These independent findings suggest that the presence of ferrocenyl or phenyl units in chloroquine helps achieve an optimal hydrophobic/hydrophilic balance which may be significant for crossing the parasite membrane and for hydrophobic interactions with key targets.

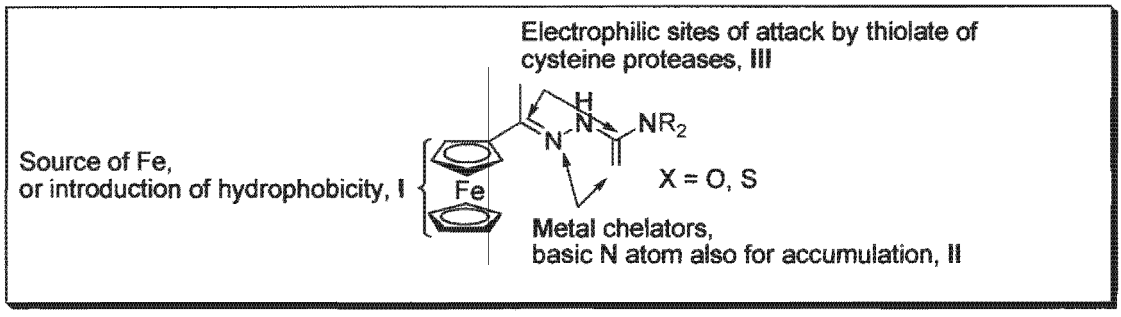


Figure 4.13: Rationale for design of ferrocene-containing thiosemicarbazones and semicarbazones

In designing the new series of thiosemicarbazones and semicarbazones (Fig. 4.13), we appended the ferrocene unit into the thiosemicarbazone and semicarbazone frameworks while retaining two of the features discussed for 2-pyridyl thiosemicarbazones *viz.* the presence of metal-chelating sites (**II**) and electrophilic sites of attack (**III**) (section 4.1.1.3). It is worthy to note that although this approach led to a reduction in the number of metal coordination sites by one unit, in theory the new compounds would retain their ability to form a 3:1 complex with Fe(III) so that all coordination sites are occupied.

4.2.6 Chemical Synthesis

The ferrocene aldehyde **4.22**, *bis*-aldehyde **4.24** and the ketone **4.23** and *bis*-ketone **4.25** (Fig. 4.14) employed here and in subsequent sections were donated to us by the laboratories of Prof. J. Brocard of the 'Sciences et Technologies de Lille', in Lille, France.

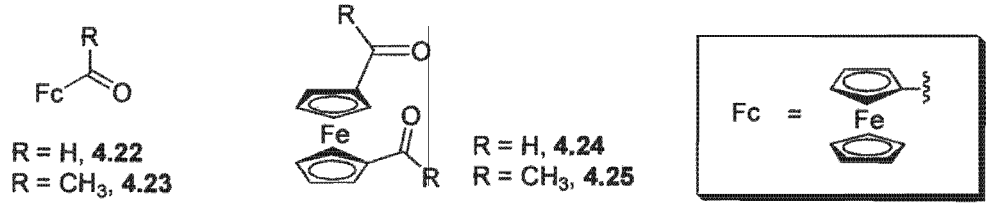
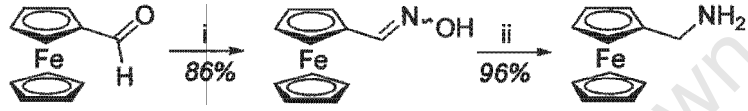


Figure 4.14: Structures of ferrocene and *bis*-ferrocene aldehydes and ketones

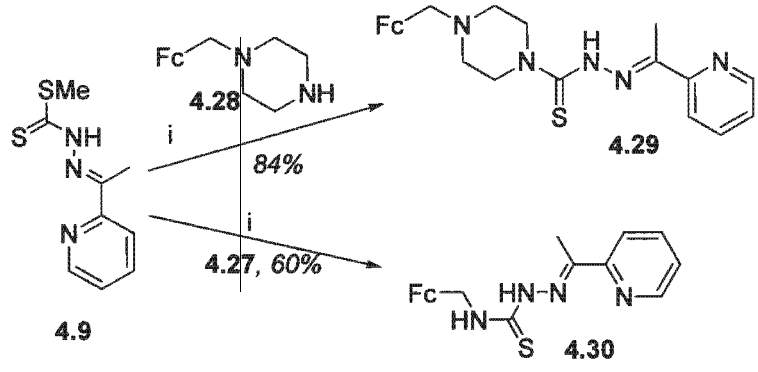
As a starting point, the synthesis of 2-pyridyl thiosemicarbazones was continued, this time incorporating the ferrocene unit in the amine component. Two such amines, a primary ferrocenemethyl amine **4.27** (Scheme 4.11) and the secondary ferrocenemethyl piperazine amine **4.28** (Scheme 4.12) were required to synthesize the desired thiosemicarbazones. While the latter amine was donated along with the aldehydes and ketones, the former was synthesized in our laboratories by way of Scheme 4.11.[21]



4.22 **4.26** **4.27**

Scheme 4.11: *Reagents and* conditions i) $NH_2OH.HCl$, NaOH (aq), EtOH, reflux, 6 h; ii) $LiAlH_4$, THF, reflux, 2 h

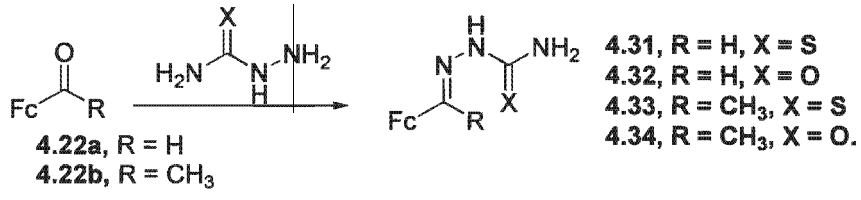
Hydroxylamine hydrochloride ($NH_2OH.HCl$) was basified by the addition of aqueous NaOH (2 eq) to its suspension in EtOH. In itself, the hydrochloride salt was insoluble in absolute EtOH, as such the addition of an aqueous solution of NaOH was necessary to achieve complete dissolution. After basification of the solution, the free base was condensed with ferrocenecarboxaldehyde **4.22** to afford the oxime **4.26** in good yield (86%), Scheme 4.11. The isolate was revealed as a mixture of two geometric isomers (^1H NMR) in a 1:1 ratio and no effort was made to separate the two. Sequential reduction of the oxime **4.26** was achieved by refluxing in anhydrous THF in the presence of $LiAlH_4$ to afford the free amine **4.27** in 96% yield after purification by column chromatography.



Scheme 4.12: *Reagents and conditions* i) **4.27** or **4.28**, MeOH, reflux, 12 h

Having synthesized **4.27**, attention was now directed towards synthesizing the two ferrocene thiosemicarbazones **4.29** and **4.30**, Scheme 4.12. Thus, treating solutions of the amines **4.27** and **4.28** in MeOH with 2-pyridylthiosemicarbazone thioester **4.9** for 12 h yielded the desired thiosemicarbazones **4.29** and **4.30** in high yields (76% and 60%) respectively. The modest yield of **4.30** was as a result of the difficulty encountered during purification by either recrystallisation (as with other thiosemicarbazones) or column chromatography. The latter was a result of the large number of fractions that were eluting from the column. The crude mixture had to be run three times on the column (silica gel) in order to obtain **4.30** of high purity.

Monosubstituted aldehyde **4.22** and ketone **4.22b** were converted to the corresponding thiosemicarbazones **4.31** and **4.33** and semicarbazones **4.32** and **4.34** by treatment with either thiosemicarbazide or semicarbazide hydrochloride respectively (Scheme 4.13).



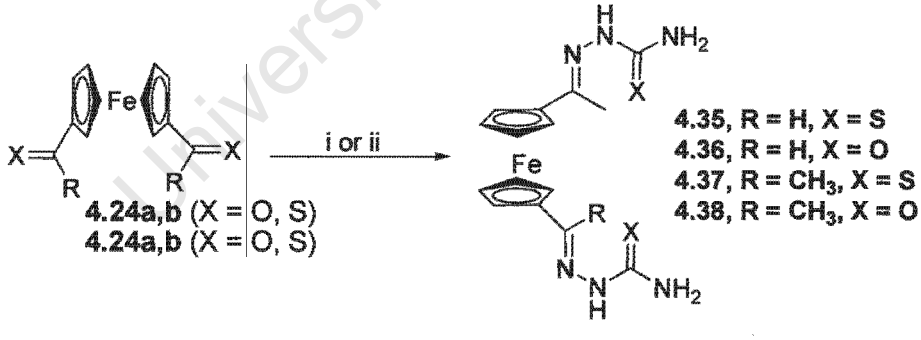
Scheme 4.13: *Reagents and conditions* i) EtOH, rt, o/n or ii) EtOH, reflux, o/n

Yields were excellent in all cases as can be seen from Table 4.3, ranging from 88 – 100%.

Table 4.3: Yields of ferrocene thiosemicarbazones and semicarbazones **4.31 – 4.34**

Product	X	*R*	Yield (%)
4.31	S	H	93
4.32	O	H	93
4.33	S	CH_3	88
4.34	O	CH_3	100

As before, basification of the HCl salt was necessary prior to addition of the carbonyl compound and this was achieved as previously described (NaOAc). We found that conducting the reactions with semicarbazide hydrochloride at room temperature yielded better results in terms of yields and quality of the products compared to refluxing the reaction mixtures as in the case of thiosemicarbazide. With prolonged heating, the semicarbazones deposited an insoluble black tar alongside the desired products, while at room temperature only the desired products were deposited.



Scheme 4.14: *Reagents and conditions* i) EtOH, rt, o/n, or ii) EtOH, reflux, 12 – 24 h

We then wished to compare the effects of *bis*-substitution on the ferrocene subunit on the biological activities. Thereby the *bis*-thiosemicarbazones and *bis*-semicarbazones **4.35 – 4.38** were generated from the *bis*-ferrocene aldehyde **4.24** and ketones **4.24** by

condensation with thiosemicarbazide or semicarbazide hydrochloride/NaOAc mixture (Scheme 4.14). By following a similar protocol mentioned for compounds **4.31** and **4.34** the new *bis*-substituted compounds **4.35 – 4.38** were synthesized in excellent yields (Table 4.4).

Table 4.4 Yields of ferrocene *bis*-thiosemicarbazones and semicarbazones **4.35 – 4.38**

Product	X	*R*	Yield (%)
4.35	S	H	95
4.36	O	H	100
4.37	S	CH_3	100
4.38	O	CH_3	100

All compounds were fully characterized by means of IR, ^{1}H NMR, ^{13}C NMR and mass spectrometry. The most common peaks in this group of compounds were the NH stretches of the thioamides in the 3400 cm^{-1} region, aromatic CH, C=N stretches, stretches and C=S stretches in the 3000, 1550 and 1435 cm^{-1} regions respectively.

All compounds gave satisfactory NMR data (^{1}H and ^{13}C) consistent with the proposed structures. Notably, the aromatic protons on the quinoline ring were better resolved than those on the pyridine ring. Thus, it was possible to assign unambiguously the identity of these protons based on the observed coupling constants.

In order to determine the purity of the target compounds, melting points and combustion analysis (microanalysis) were employed, the latter was being used to confirm the atomic composition of the suggested molecular formulae.

4.3 Biological Results and Discussion

4.3.1 Biological Evaluation of Thiosemicarbazones and Semicarbazones

The synthetic thiosemicarbazones and semicarbazones were evaluated in three different *in vitro* assays. In the first of these, the compounds were assayed against the chloroquine-resistant W2 *P. falciparum* strain, using chloroquine as the control drug. In the second assay, the compounds were evaluated for their abilities to inhibit the activity of the plasmodial cysteine protease falcipain-2. All antiplasmodial and enzyme inhibition tests were conducted at the Department of Medicine, San Francisco General Hospital, University of California at San Francisco (UCSF), in collaboration with Professor P. J. Rosenthal. The antitrypanosomal activities of the compounds against *T. brucei* parasite cultures were determined at the London School of Hygiene and Tropical Medicine, University of London in collaboration with Dr. V. Yardley.

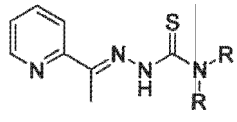
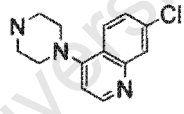
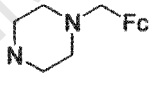
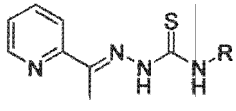
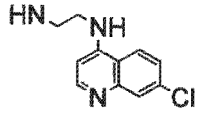
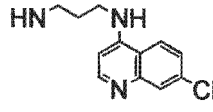
4.3.2 Results

4.3.2.1 *In Vitro* Antiplasmodial Activity of Thiosemicarbazones and Semicarbazones

The antiplasmodial activities of compounds **4.7a – i**, **4.29** and **4.30** are tabulated in Table 4.5. The data shown in this table reveal that compounds synthesized from secondary amines were generally more active than those generated from primary amines in the *in vitro* antiplasmodial assay, with only the chloroquine-type compound **4.7f** deviating from this observation. Compounds **4.7a – d** and **4.14** showed greater antiplasmodial activities in the resistant W2 strain (IC_{50} ranging from 0.008 to 0.21 μM) than the reference drug chloroquine (IC_{50} = 0.24 μM). The activity of **4.7b** shows a 3-fold increase in efficacy when the *N*-4 substituent of **4.7a** is cyclized into a five membered-ring. However, further expansion of this ring into a six-membered ring **4.7c** caused a 3–fold reduction in activity. Interestingly, when the methylene (CH_2) group in **4.7c** was

replaced by O as in compound **4.7d**, the antiplasmodial activity was enhanced twofold. On the other hand, substitution of the O atom with the *N*-methyl group as in compound **4.7e**, had no apparent effect on the antiplasmodial activity of **4.7d**. Compounds **4.7a – d** have been previously synthesized and their antiplasmodial activities against a chloroquine-sensitive Smith strain have appeared in the literature.[24] All were very active against the strain tested, with IC_{50}s ranging from 16 – 56 nM.

Table 4.5: Antiplasmodial and falcipain-2 inhibitory activities of 2-pyridyl compounds

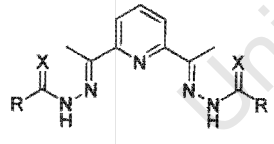
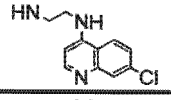
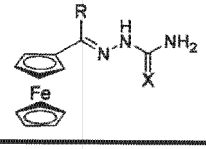
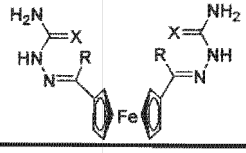
General Structure	–NRR	Compound	IC_{50} (μM) W2 *P. falciparum*	Rec. F–2*
	–	**Chloroquine**	0.24	–
		**4.7a**	0.025	>20
		**4.7b**	0.008	>20
		**4.7c**	0.028	>20
		**4.7d**	0.013	>20
		**4.7e**	0.013	>20
		**4.7f**	0.212	>20
		**4.29**	0.025	19.2
		**4.7g**	0.260	>20
		**4.7h**	0.212	>20
		**4.7i**	0.190	>20
		**4.30**	0.016	16.7
	–	**4.14**	0.032	>20

*Rec. F-2 = Recombinant Falcipain-2;

Compound **4.7g** and the 4-aminoquinoline-containing compounds **4.7f**, **4.7h** and **4.7i** on the other hand showed comparable efficacy to chloroquine (Table 4.5). Both ferrocene-containing compounds **4.29** and **4.30** showed significant antiplasmodial activities against the W2 strain with respective IC_{50}s of 0.025 μM and 0.016 μM. Generally, none of the compounds in Table 4.5 was able to significantly inhibit falcipain-2 *in vitro* at the highest concentration tested (20 μM), with only the ferrocenyl compounds **4.29** and **4.30** being able to inhibit the enzyme below 20 μM with IC_{50}s of 19.2 and 16.7 μM. All other compounds had IC_{50} values greater than 20 μM.

Of note, the structurally related compounds **4.7a** and **4.14** showed comparable inhibitory activities against whole parasite isolates, and both did not inhibit the free malarial enzyme falcipain-2.

Table 4.6: Antiplasmodial and falcipain-2 activities of compounds **4.16 – 4.38**

Compound General Structure	X	R	Compound	IC_{50} (μM) W2 *P. falciparum*	Rec. F–2*
Chloroquine	–	–	–	0.224	–
	**S**	NH_2	**4.16a**	0.14	>20
	O	NH_2	**4.16b**	6.52	>20
	S	SCH_3	**4.17**	1.68 (3.26)	2.13
	S		**4.18**	0.10 (0.21)	0.18
	**S**	H	**4.31**	>10	2.79
	O	H	**4.32**	>10	>20
	S	CH_3	**4.33**	>10	0.58
	O	CH_3	**4.34**	>10	6.72
	**S**	H	**4.35**	>10	0.82
	O	H	**4.36**	>10	>20
	S	CH_3	**4.37**	>10	>10
	O	CH_3	**4.38**	>10	>10

*Rec. F-2 = Recombinant Falcipain-2;

Among the four 2,6-pyridyl *bis*-thiosemicarbazones **4.16a**, **4.16b**, **4.17** and **4.18** (Table 4.6), the most active compound against both parasite cultures and falcipain-2 was **4.18** (IC_{50} = 0.10 and 0.18 μM respectively), which was twice as active in the chloroquine-resistant W2 *P. falciparum* strain as chloroquine. Although both *bis*-thiosemicarbazone **4.16a** and *bis*-semicarbazone **4.16b** inhibited parasite development, the former was 47 times more efficacious than the latter. However, neither compound showed inhibitory activity against recombinant falcipain-2 (IC_{50} > 20 μM for both compounds).

The intermediate *bis*-thiosemicarbazone thioester **4.17** showed an improvement in activity against falcipain-2. Although it was less active than **4.16a** against W2 parasite cultures in two independent tests, it was able to inhibit the activity of falcipain-2 with a much lower IC_{50} (2.13 μM) than that of **4.18** (IC_{50} >20 μM).

Bis-substitution of the thiomethyl (SCH_3) groups in **4.17** with the 4-aminoquinoline amine **3.52** resulted in **4.18**, which exhibited improved efficacy against W2 parasites by a factor of 17, resulting in comparable activity to that of **4.16a**. The antiplasmodial activity of the new *bis*-substituted derivative **4.18** improved to twice that of the monosubstituted 2′-pyridyl thiosemicarbazone **4.7h** and was also much higher against the enzyme (IC_{50} = 0.18 μM).

All the ferrocene containing thiosemicarbazones, semicarbazones and their *bis*-substituted counterparts did not show significant inhibitory activities against whole (W2 *P. falciparum*) parasites. Despite this lack of activity against parasite cultures, however, the ferrocenyl thiosemicarbazones **4.31**, **4.33 – 4.35** displayed significant activities against falcipain-2, with the most active compound **4.33** having an IC_{50} of 0.58 μM. Against whole parasite cultures, it seems that neither mono- nor *bis*-substitution favours antiplasmodial activity in the ferrocenyl compounds in this *P. falciparum* strain. Comparing the structurally related compounds **4.16a** and **4.35**, as well as **4.16b** and

4.36, it seems that the 2-pyridyl-containing compounds are more active against whole parasite cultures. Against falcipain-2 though, the ferrocenyl *bis*-thiosemicarbazone **4.35** possesses superior activity to its 2,6-pyridyl counterpart. Lastly, all thiosemicarbazones were more active than their semicarbazone counterparts, lending further support to the SARs established by Klayman *et al*[19] that substitution of O for S in these compounds causes a loss of antiplasmodial activity.

4.3.3 Discussion

The superior activities (and equipotencies in some cases) of 2-pyridyl thiosemicarbazones and semicarbazones against cultured W2 parasites were not accompanied by corresponding activities against the malarial cysteine protease falcipain-2. These data suggest that the antiplasmodial activities were not entirely a result of inhibition of falcipain-2. Although falcipain-2 does not appear to be the principal target for these compounds, it is possible that they may be acting through the inhibition of another cysteine protease, since there are many other cysteine proteases in *P. falciparum*. This possibility is supported by the abilities of **4.29** and **4.30** to bind and inhibit (albeit weakly) falcipain-2. Alternatively, the compounds may be acting *via* a protease-independent mechanism as has been concluded for tridentate metal-chelating 2-pyridyl thiosemicarbazones.[25] Since all the compounds are potential metal chelators, they may be acting in one of three ways discussed below:

i) The compounds may be acting by chelation and withholding of essential metals in *P. falciparum* such as Fe. This would then result in the malfunctioning of Fe-dependent enzymes such as ribonucleotide reductase. Tridentate chelating thiosemicarbazones have been shown to inhibit ribonucleotide reductase, an enzyme

essential for DNA synthesis.[26,27] Tridentate chelating thiosemicarbazones have also been proposed to act by inhibiting dihydrofolate reductase.[28,29]

ii) They may be acting by formation of toxic complexes. There is evidence that copper complexes of thiosemicarbazones produce significant oxidative stress by binding endogenous reducing agents such as glutathione.[30]

iii) They may be inhibiting cysteine proteases either directly or indirectly as metal-interactive cysteine protease inhibitors.[8] Thiosemicarbazone inhibitors possess two potential sites of attack by nucleophilic thiolate anions of parasitic cysteine proteases. In attempting to establish the mode of action of a series of thiosemicarbazone antitrypanosomal agents based on modeling and experimental results, Du *et al* proposed that the thiocarbonyl group of thiosemicarbazones is the site of attack by the thiolate anion (of the enzyme) after noting that the compounds were time-dependent inhibitors of cruzain.[31] Figure 4.15 below illustrates inhibition of a cysteine protease *via* the formation of a reversible tetrahedral adduct:

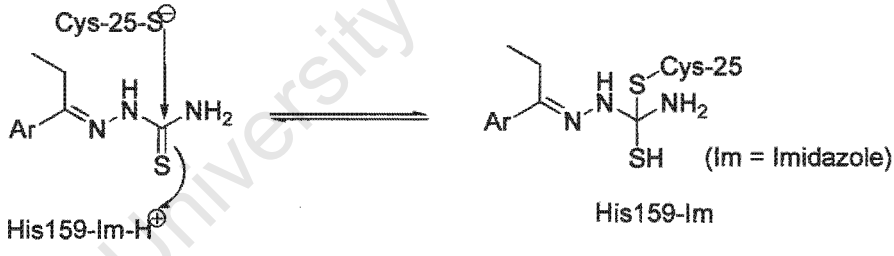


Figure 4.15: Mechanism-based inhibition of a cysteine protease by thiosemicarbazones

Metals alone have the ability to inhibit cysteine protease reactions. Thus, the thiosemicarbazone-metal complex alluded to above may subvert other unknown endogenous mechanisms that protect protease reactions against the action of inhibitory metals. Thiosemicarbazones may indeed be acting as metal-interactive cysteine protease inhibitors.[8a] This feature of the ability of metal chelator-metal ion complexes to inhibit cysteine proteases is best illustrated by metal chelating biguanides such as

metformin (not shown). In one such experiment, the activity of falcipain-2 was measured in three different conditions. In the presence metformin, no inhibition of enzyme was observed, whilst in the presence of 20 μM Fe^{3+} ions, the activity of the enzyme was reduced by 24%. In the presence of 20 μM of the metformin- Fe^{3+} complex, however, the activity of falcipain-2 was reduced by 80%.

The enhanced antiplasmodial activities of **4.7h**, **4.7i** and **4.18** may be attributed to the presence of the 4-amino-7-chloroquinoline subunit, which may result in binding to haem thereby preventing biomineralization of haem into inert haemozoin.[32] The consequence, then, would be an accumulation of haem which kills the parasite. The low IC_{50} value observed for **4.18** against falcipain-2 also suggests that the compound may be exerting its antiplasmodial activity by directly inhibiting the enzyme. The improvement in activity of the *bis*-substituted compound also suggests that the 4-aminoquinoline subunit may be directly binding to falcipain-2 resulting in loss of enzyme activity.

At the highest concentrations tested (10 μM), the ferrocene compounds **4.31 – 4.37** did not exhibit antiplasmodial activity, although 4 out of 8 compounds displayed activity against falcipain-2. The absence of activity against whole parasites may be a result of poor transport across the parasitic cell membrane and hence low accumulation within the parasite. It may also be possible that the ferrocenyl subunit does not play a similar role to that played by the 2-pyridyl subunit in inhibiting parasite development. The lack of antiplasmodial activities of the ferrocene compounds lends further support to the significance of the 2-pyridyl unit for expressing antiplasmodial activity in thiosemicarbazones. This might be a case of tridentate chelation in the 2-pyridyl system being more crucial compared to bidentate chelation in the ferrocene system.

A commonly used phenomenon in medicinal chemistry and drug discovery is "Lipinski's rule of 5' for oral drugs, which is a set of rules that predicts the physicochemical properties of drugs.[33] It predicts that the permeability of a drug across cell membranes is favoured if a drug (candidate) obeys at least three of four principles: i) the CLogP, which is a measure of the partition coefficient of a drug in a water/octanol solution is less than or equal to 5, ii) the number of hydrogen bond-donors is less than or equal to 5, iii) the number of hydrogen bond-acceptors is less than or equal to 10, and iv) the molecular weight is less than or equal to 500. A compound that adheres to 3 of the four properties is said to obey Lipinski's rule. Table 4.10 shows these physical properties in relation to 'Lipinski's rule of 5'. CLogP values were estimated using ChemDraw® v.8.03 and, although the programme does not give precise values, it gives comparative results in terms of lipophilicity for a compound series.

Table 4.10: Physical properties of some thiosemicarbazones compatible with reasonable pharmacokinetics and bioavailability

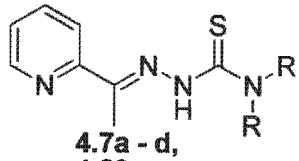
Compound	Mol. Wt	H-Bond Donors	H-Bond Acceptors	ClogP	Criteria Met
Rule	<500	<5	<10	<5	–
4.7g	279	2	4	1.76	4
4.7h	398	3	4	4.4	4
4.7i	412	3	4	4.8	4
4.17	371	2	5	3.2	4

The physical (Lipinski's rule of 5') properties of the most active compounds identified from the different series, presented in Table 4.10, show that all the compounds are fully compatible with Lipinski's rule, i.e. the compounds possess physical properties that would favour permeability across cell membranes. If the compounds are exerting their antiplasmodial activities in the food vacuole of the parasite, then lipophilicity will play a significant role in compound accumulation. The CLogP values of the most active

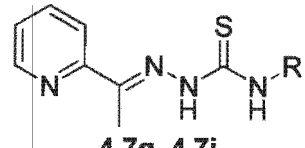
compounds (Table 4.10) show that they are indeed within limits and therefore favourable. As stated earlier (section 4.1.2.2, p. 110), an effective antiplasmodial Fe chelator should have the ability to cross the parasitic membrane and have selectivity for Fe(III) over Fe(II) and other endogenous metals.

4.3.4 *In Vitro* Assays against *T. brucei*

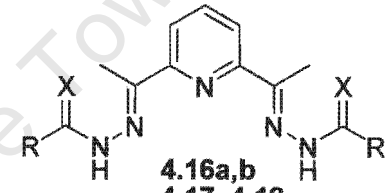
Table 4.7: *In vitro T. brucei* activities of Thiosemicarbazones and Semicarbazones (for substituents at R and X, see Table 4.5 and 4.6, pp. 135 and 136 respectively).



4.7a - d, 4.29,



4.7g, 4.7i, 4.30



4.16a,b 4.17, 4.18

General structures of thiosemicarbazones and semicarbazones evaluated against *T. brucei*

Compound	ED_{50} µg/ml	ED_{50} µM	Cyt.[a] ED_{50} µg/ml	Cyt. ED_{50} µM	Ther.* Index
Pentamidine	0.00007	0.0002	–	–	–
Podophyllotoxin			0.00024		
4.7a	0.038	0.15	0.04	0.16	1
4.7c	**0.0015**	**0.0057**	**0.3**	**1.15**	**201**
4.7d	0.27	1.0	1.8	6.82	7
4.7g	**0.0014**	**0.0050**	**13.5**	**48.4**	**9677**
4.7i	**0.019**	**0.046**	**10**	**24.3**	**528**
4.16a	–	–	>300	>970	–
4.16b	0.55	1.98	>300	>1079	>545
4.29	1.33	2.89	0.57	1.24	0
4.30	0.054	0.14	3.08	7.85	56
4.17	**0.0009**	**0.0024**	**18.2**	**49.1**	**20458**
4.18	0.018	0.025	2.9	4.04	159

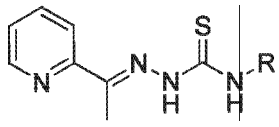
[a]Cyt. = Cytotoxicity; *Ther. Index = Therapeutic Index.

A few select compounds were evaluated against parasite cultures of *T. brucei*, the causative agent of African sleeping sickness or African trypanosomiasis. The results for these assays, presented in Table 4.7, reveal that a few compounds showed good promise as antitrypanosomal agents. Compounds **4.7c** and **4.18** were very active with ED_{50} values in the low micromolar range (0.006 and 0.026 μM respectively), with quite low toxicities towards the human KB cell line used in these experiments. Both compounds exhibited therapeutic indices over 100. Compound **4.7i**, with an ED_{50} value of 0.046 μM was 528 times more cytotoxic to the parasite than to the human KB cell line. On the other hand, compounds **4.7g** (ED_{50} = 0.005 μM) and **4.17** (ED_{50} = 0.0024 μM) showed even higher activities and lower cytotoxicities towards the mammalian cells. The former was ~9600 times more cytotoxic to *T. brucei* than the mammalian cells, while the latter was ~20 000 times more cytotoxic to the parasite. The most active compound **4.17** was still 13 times less efficacious than pentamidine (ED_{50} = 0.0002 μM), the standard treatment drug for Chagas' disease. Nevertheless **4.17** represents a promising series of antitrypanosomal thiosemicarbazones.

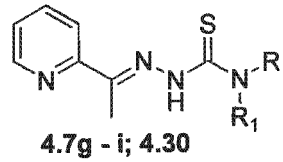
4.3.4.1 *In vitro* Activities Against Rhodesain

The activity of trypanosomal cysteine protease rhodesain was determined in the presence of compounds at 10 μM concentration. IC_{50} values were determined only for those compounds that were able to reduce the observed enzyme activity to 50% or below. Only a few select compounds were assayed in these tests, the results of which are presented in Table 4.8.

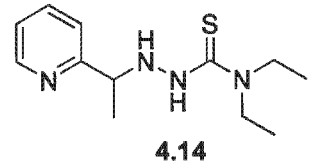
Table 4.8: *In Vitro* activities of thiosemicarbazones against Rhodesain



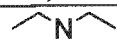
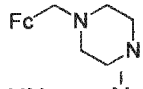
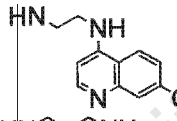
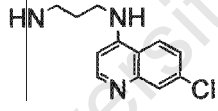
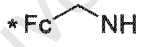
4.7a - e, 4.29



4.7g - i; 4.30



4.14

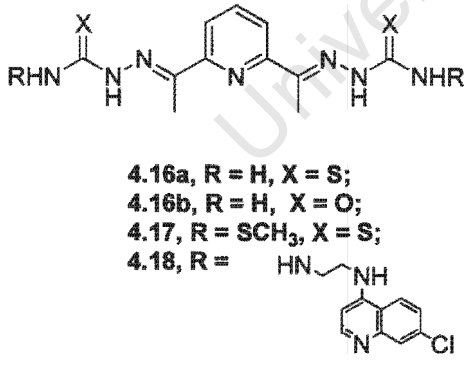
Compound	NRR_1 Group	Rhodesain % Residual activity at 10μM	Rhodesain IC_{50} (μM)
4.7a		22	4.0
4.7b		0	0.11
4.7c		37	4.0
4.7d		104	nd
4.7e		1	0.70
4.29		~100	nd
4.7g		6	1.8
4.7h		5	0.42
4.7i		67	nd
4.30		~100	nd
4.14	See Above	83	nd

*Fc = Ferrocene

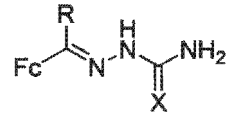
It can be seen from Table 4.8 that of the compounds tested, half were able to inhibit the activity of rhodesain at a concentration of 10 μM. Whereas **4.7a** and **4.7c** could not inhibit the activity of the malarial cysteine protease falcipain-2, they exhibited significant inhibitory activities towards the closely related cysteine protease rhodesain. Compound **4.7b** was the most active among the compounds tested; it completely inhibited the activity of rhodesain at the concentration tested with an IC_{50} value of 0.11 μM. On the

other hand, **4.7c** reduced the activity of rhodesain to 37% with an IC_{50} of 4.0 μM. The variabilities of these results compared with those obtained for falcipain-2 for the same compounds suggest differences in the structures of the active sites between the two enzymes. Other notable compounds in this regard were **4.7g** and **4.7h** which inhibited rhodesain with respective IC_{50}s of 1.8 and 0.42 μM respectively. Interestingly, despite the significant reduction in the activity of rhodesain caused by **4.7h**, its analogue **4.7i** showed much reduced activity (33%) against the same enzyme at the same concentration. Besides being very active against whole *T. brucei* parasite cultures and free enzyme rhodesain, compounds **4.7c** and **4.7g** were both quite nontoxic to the mammalian KB cell line used in the determination of cytotoxicity. The correlation between antiparasitic and enzyme inhibitory activity suggests that these compounds may be exerting their antitrypanosomal activity by direct inhibition of rhodesain.

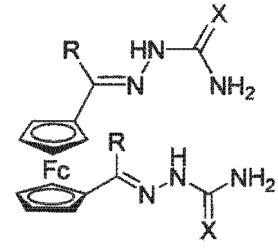
As was the case with falcipain-2, the two ferrocene-containing thiosemicarbazones **4.29** and **4.30** did not significantly inhibit the activities of either enzyme at the concentration tested (10 μM).



4.16a, R = H, X = S;
4.16b, R = H, X = O;
4.17, R = SCH_3, X = S;
4.18, R =



4.31, R = H, X = S;
4.32, R = H, X = O;
4.33, R = CH_3, X = S;
4.34, R = CH_3, X = O;



4.35, X = S, R = H;
4.36, X = O, R = H;
4.37, X = S, R =CH_3;
4.38, X = O, R = CH_3

Figure 4.16: General structures of thiosemicarbazones and semicarbazones

The chemical structures of the 2,6-pyridyl *bis*-thiosemicarbazones and ferrocene-containing thiosemicarbazones and semicarbazones that were assayed for their inhibitory activities against rhodesain are shown in Fig. 4.16, and the results in Table 4.9.

Table 4.9: *In vitro* activities of *bis*-(thio)semicarbazones and Fc–containing (thio)semicarbazones against Rhodesain

	Rhodesain	
	% Residual activity	
Compound	**at 10μM**	**IC_{50} (μM)**
4.17	3	0.3
4.18	0	0.4
4.16a	22	5.5
4.16b	29	4.0
4.31	~100	nd
4.32	~100	nd
4.33	20	4.2
4.34	~100	nd
4.35	8	1.8
4.36	~100	nd
4.37	58	10
4.38	~100	nd

Among the compounds tested, only 6 caused a greater than 50% reduction in enzyme activity at 10 μM, *viz.* **4.17**, **4.18**, **4.16a**, **4.16b**, **4.33** and **4.35** (Table 4.9). The activities of **4.17** and **4.18** were comparable, inhibiting enzyme activity with IC_{50} values of 0.3 and 0.4 μM respectively. Also, the activities of the related *bis*-substituted compounds **4.16a** (IC_{50} = 5.5 μM) and **4.16b** (IC_{50} = 4.0 μM) were comparable.

The ferrocenyl thiosemicarbazone compound **4.30** was completely inactive against rhodesain at the concentration tested (10 μM). The introduction of a methyl substituent into this compound **4.33** enhanced activity against the enzyme (**4.33**, IC_{50} = 4.2 μM). Whereas *bis*-substitution of the ferrocene compound **4.31** to **4.35** enhanced the efficacy

against the enzyme (IC_{50} rhodesain = 1.8 μM), there seemed to be no measurable effect on the activity of the *bis*-substituted compound **4.36** compared to monosubstituted compound **4.32**. Introduction of the two methyl substituents into the side chains of **4.35** caused a modest loss of activity in the enzyme (**4.37**); a similar change in the semicarbazone **4.36** did not yield any change in the activity of **4.38** upon introduction of the two methyl substituents. The new compound was inactive against both enzymes at 10 μM.

4.4 Conclusions and Recommendations for Future Work

The simplicity of the chemistry employed during synthesis, coupled with the commercial availability and economic cost of reagents makes the thiosemicarbazones attractive lead compounds in the search for new antiparasitic (antiplasmodial and antitrypanosomal) agents. More importantly, thiosemicarbazones possess sought-after properties in drug discovery and development, namely: i) generally low host cell cytotoxicity as seen here with several compounds; ii) physical properties that are known to be compatible with desirable pharmacokinetics i.e. low molecular weight, favourable CLogP values, favourable hydrogen-bonding and accepting capabilities; iii) high potency, with IC_{50} values in the low nanomolar range; iv) nonpeptidic nature, a property which is likely to impart reasonable bioavailability. We have synthesized modest libraries of thiosemicarbazones and semicarbazones and demonstrated their dual potential as both antiplasmodial and antitrypanosomal agents.

In terms of further development, more 2'pyridyl thiosemicarbazones bearing different substitutions at the *N*-4 position may need to be synthesized in order to obtain meaningful SARs. Given the high safety ratios associated with compounds **4.7c**, **4.7g**,

4.7h and **4.17**, they may be taken as lead compounds and further studies and development made on these. As *in vitro* activity does not always translate into *in vivo* activity, a suggestion would be that the compounds be tested in animal models to note if indeed they would be capable of curing malaria in parasite-infected animal models. Since the compounds possess at least two basic nitrogens, the aqueous solubilities of these compounds may be improved by making different salt derivatives with a view to enhancing absorption under physiological conditions which are aqueous in nature. Also, given the elusive mechanism of action of this class of compounds, it would indeed be a good idea to employ chemical proteomics to identify the targets with which they interact.[34,35] This strategy requires synthesis of suitable chemical probes that covalently modify a target enzyme in such a way that it can be subsequently identified and/purified. Lastly, in view of the fact that resistance to antiplasmodial agents is often strain-specific, it would be of significance to test the compounds discussed in various other strains of *P. falciparum* with varying degrees of resistance.

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CHAPTER 5

4-AMINOQUINOLINE-CONTAINING COMPOUNDS *VIA* MULTI-COMPONENT REACTIONS

5.1 Introduction

This chapter describes the synthesis of novel 4-aminoquinoline-based compounds *via* multicomponent reactions as potential antimalarial agents, with some restriction to the Ugi multicomponent reaction and its variants. Schematic and brief descriptions of rationales are provided for the different classes of compounds. All compounds were synthesized to determine any inherent antiplasmodial activities against whole *Plasmodium* parasites, falcipain and trypanosomes. It was hoped that the structural diversities resulting from variations of the inputs in the multicomponent reactions should provide an insight into structure activity relationships (SARs) within each particular classes of compounds.

5.1.1 Multicomponent Reactions (MCRs)

Multicomponent reactions (MCRs) are reactions in which more than two starting materials, all present together in one reaction vessel combine to form a final product that is derived from all the starting materials.[1-3] The starting materials do not react simultaneously in a single step, but rather in a series of ordered steps. Multicomponent reactions can be basically grouped into three separate classes,[2b] Table 5.1.

Table 5.1: The basic types of multicomponent reactions

MCR Type	General reaction scheme
I	$A + B \rightleftharpoons C \rightleftharpoons \ldots\ldots O \rightleftharpoons P$
II	$A + B \rightleftharpoons C \rightleftharpoons D \ldots\ldots O \rightarrow P$
III	$A \rightarrow B + C \rightarrow D \rightarrow \ldots\ldots O \rightarrow P$

Multicomponent reactions in which the starting materials, intermediates and products are in a constant equilibrium belong to type I. In this case yields between 0 and 100% are possible as different states of equilibrium can prevail. As such, most products occur as mixtures with the starting materials and/or intermediates.

Type II multicomponent reactions encompass those whose elementary reactions are equilibria and irreversible partial reactions, and whose last reaction step is irreversible. Such reactions are advantageous since the total equilibrium is shifted in the direction of the products by the irreversible step. Lastly, type III multicomponent reactions are sequences of irreversible elementary reactions that rarely occur in preparative synthetic chemistry. These reactions include biochemical reactions in the living world, many of whose irreversiblities arise from either thermodynamic circumstances or due to a combination of endothermal and exothermal reactions.

The variability of MCRs is seen from the large numbers of compounds that can be synthesized from a few starting materials. Compared to conventional multistep organic syntheses, MCRs are advantageous owing to their greater atom efficiency and accessibility to large numbers of compounds and complex molecules. The wide structural diversity and simplicity of their one-pot procedures make them amenable to combinatorial synthesis. Several MCRs exist to date, e.g. the Mannich reaction,[4] Strecker amino acid synthesis, Biginelli's dihydropyrimidine synthesis,[5] Hantsch's dihydropyridine synthesis,[6] but a large number and important classes of these belong to

isocyanide multicomponent reactions (IMCRs).[2] Since the majority of these reactions depend on the reactivity of isocyanides, a brief account of the syntheses and reactivities of isocyanides will be presented here.

5.1.1.1 Structure of Isocyanides

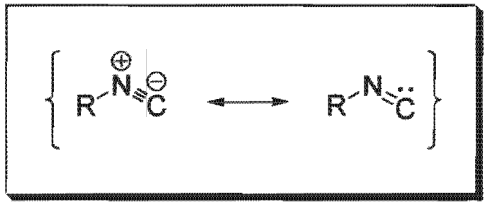
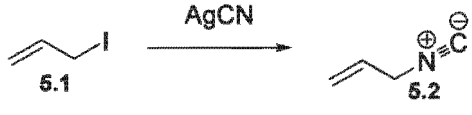


Figure 5.1: Resonance structures of isocyanides

The structure of isocyanides (Fig. 5.1) was proposed by Lindemann and Wiegrebe[7] long after the class of compounds was identified as discrete compounds. In analogy to the structure of carbon monoxide and in accordance with the octet rule, the authors proposed the structure shown in Fig. 5.1. Isocyanides are structurally related to carbenes and are the only stable organic compounds with a divalent carbon atom.

5.1.1.2 Synthesis of Isocyanides

5.1.1.2.1 From allyl iodide and silver cyanide (AgCN)



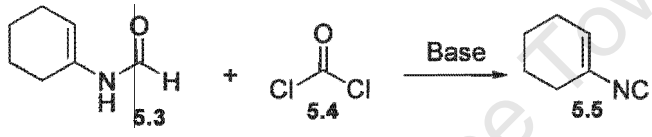
Scheme 5.1: Lieke's synthesis of isocyanide

Isocyanides were first synthesized by Lieke in 1859[7] when he attempted to synthesize allyl nitrile from allyl iodide **5.1** and AgCN (Scheme 5.1). In lieu of the anticipated nitrile,

he obtained the isocyanide **5.2** in reasonable yield. On attempting to transform the presumed nitrile into the corresponding carboxylic acid (crotonic acid) by acid hydrolysis, he obtained only formic acid. However, he discontinued the studies on this anomalous hydrolysis reaction because of 'continuing complaints in the neighbourhood about the vile odour.'

5.1.1.2.2 Dehydration of *N*-Monosubstituted Formamides

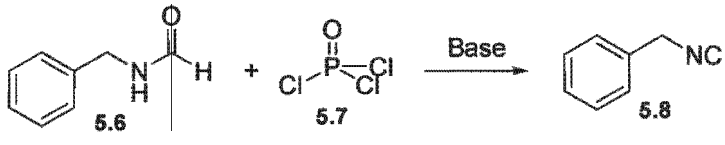
(i) Phosgene Method



Scheme 5.2: Phosgene-mediated synthesis of isocyanides

Many years of work on the synthesis of isocyanides followed after the first attempt by Lieke.[2,7] To date, dozens of methods for the synthesis of isocyanides have been documented, although most isocyanides are generated by the dehydration of *N*-formamides with phosgene or its surrogates such as di- and triphosgene and appropriate bases (Scheme 5.2), owing to the associated low costs, high yields and ease of synthesis.

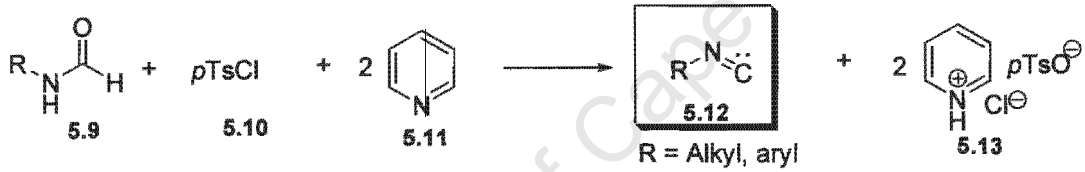
(ii) Phosphorous Oxychloride Method



Scheme 5.3: $POCl_3$-mediated synthesis of isocyanides

As an alternative to using phosgene to dehydrate formamides, $POCl_3$ **5.7** usually in combination with a tertiary amine base or a hindered secondary amine[7,8] has become the more general method of accessing isocyanides (Scheme 5.3). In fact, Zhu and coworkers have recently developed a mild dehydration method based on $POCl_3$ that preserves chirality at the α-carbon in chiral formamides by using an excess of Et_3N in a solvent (usually DCM) at very low temperatures (–20 to –25 °C).[9] An example of the use of $POCl_3$ is illustrated in Scheme 5.3 for the synthesis of benzyl isocyanide **5.8** *via* dehydration of benzylformamide **5.6**.

5.1.1.2.3 Arylsulfonyl Chlorides



Scheme 5.4: Arylsulfonylchloride-mediated synthesis of isocyanides

Corey and Hertler found that by treating *N*-monosubstituted formamides **5.9** with *p*-TsCl **5.10** and pyridine **5.11**, a dehydration reaction occurred leading to the corresponding isocyanide **5.12**[10] and the pyridinium salt **5.13**, Scheme 5.4. The generality of the method was established by treating three diverse formamides from the acyclic, alicyclic and aromatic groups. Yields for the then new method ranged from 50 – 93%. Benzenesulfonyl chloride has also been documented to dehydrate formamides to corresponding isocyanides.[1]

Other dehydrating agents that have been used to effect dehydration of formamides include $SOCl_2$[1] and PPh_3.[11]

5.1.1.3 Reactivity of Isocyanides

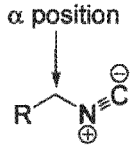


Figure 5.2: Illustration of the α-acidic position of isocyanides

The chemistry of isocyanides has been described as being characterized by three properties, namely the easy formation of radicals, the α-acidity and the α-addition (Fig. 5.2). The α-acidity of isocyanides is greatly increased by the presence in the α-position of electron-withdrawing groups such as nitriles, esters and sulfonyl groups. Schöllkopf has used α-metalated starting materials for the synthesis of α,β-unsaturated isocyanides and amino acids[12] by exploiting the reactivity of the α-carbon. Isocyanides are easily hydrolyzed by protic acids to formamides and, as such, acidic conditions should be avoided in reactions involving isocyanides. However, the most significant property of isocyanides is their ability to undergo reaction with both electrophiles and nucleophiles at the same isocyanide carbon, a property that is only shared by carbon monoxide and carbenes. This unique dual reactivity is in contrast with most other functional groups in organic chemistry which react at different centres, more so with their nitrile isomers.

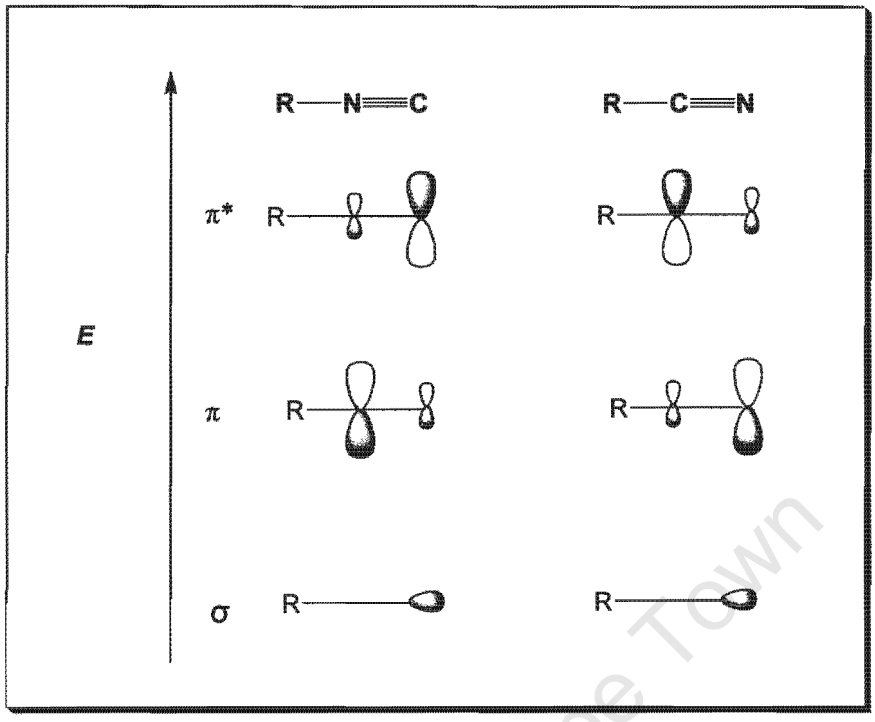


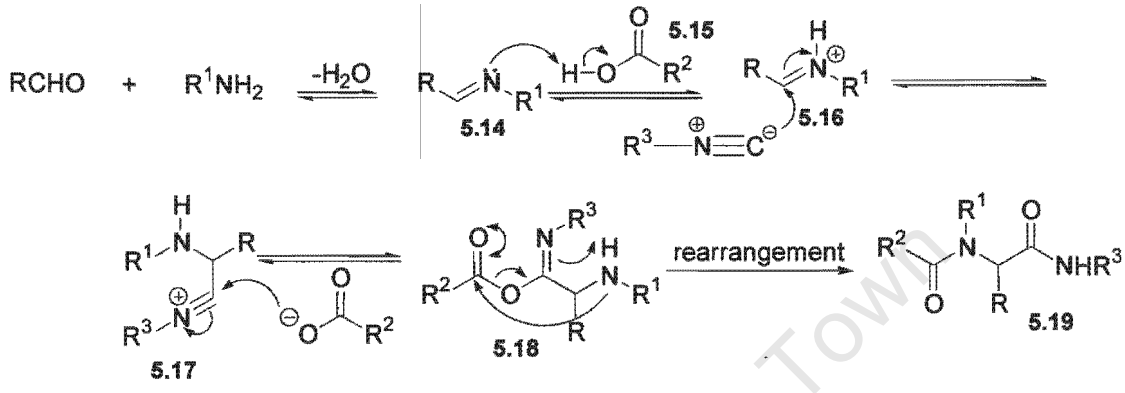
Figure 5.3: Qualitative comparison of the frontier orbitals of isocyanides and nitriles[2b]

The dual reactivity of isocyanides can be explained in terms of molecular orbital theory, Fig. 5.3.[2b] The explanation is that the orbital coefficients of the isocyanides at the carbon atom in the π^* orbital is higher, leading to nucleophilic attack at the carbon atom. Electrophiles react with the σ orbital of the HOMO-1 and therefore also at the carbon atom. On the other hand, nitriles are attacked by nucleophiles at the carbon atom since it has the higher π^* orbital coefficient and, by electrophiles at the nitrogen atom where the σ orbital coefficient is higher.

5.1.2 The Ugi 4 Component Condensation Reaction

The first report of the Ugi 4-component condensation (4CC) reaction appeared in the literature in 1959[13] and, within a few weeks following the publication most of the multicomponent condensations known today were discovered. In its classic form, the Ugi

reaction is a 4CC of aldehyde (RCHO), amine (R_1NH_2), carboxylic acid (R_2CO_2H) and isocyanide (R_3NC) to afford an α-acylamino amide. The reaction and mechanistic events that lead to the formation of the Ugi adducts are depicted in Scheme 5.5.



Scheme 5.5: Mechanism of the Ugi 4CC

In the first step of the reaction, the aldehyde RCHO (or ketone) and the amine R_1NH_2 condense to form the imine **5.14** *via* a hydroxy aminal (not shown). Protonation of the nitrogen atom of the imine by the carboxylic acid **5.15** then generates the activated iminium ion **5.16** in which the electrophilicity of the C=N bond is increased. The electrophilic iminium ion **5.16** and the nucleophilic carboxylate anion (R_3COO^-) then add to the carbon atom of the isocyanide R_3NC to generate an α-adduct **5.18** analogous to an acid anhydride *via* **5.17**. Intramolecular acyl transfer and subsequent rearrangement of the resultant hydroxyimine-amide then results in the formation of the stable Ugi adduct **5.19**. With the exception of the rearrangement step, which lies exclusively on the product side, all steps in the Ugi reaction are equilibria. The driving force for the irreversible reaction is oxidation of the isocyanide C^{II} atom to the amide C^{IV} atom.

From the reactivity series of the Ugi 4CC reaction as a function of solvent and concentration, it has been established that the reaction is mainly influenced by inductive

and, to a lesser extent steric effects. In fact, the concentration of the reactants has been found to be much more important than the properties of the solvent in which the reaction is conducted.[14] Other generalizations about the Ugi 4CC valid for solution-phase reactions that have been established are: use of low molecular weight alcohols, such as MeOH, EtOH or trifluoroethanol[15] as solvents in the reaction; other polar aprotic solvents such as DMF, THF or dioxane have been used and have been described as being advantageous. Additionally, the reaction can be performed in biphasic aqueous media. Indeed, the rate-accelerating effect of aqueous media in the Ugi reaction was disclosed recently.[16] Reactant concentrations of between 0.5 and 2 molar give optimal yields; precondensation of amine and aldehyde or ketone also favours the reaction. Finally, the addition of Lewis acids can be advantageous, understandably so given the reaction mechanism *via* which the reaction is believed to proceed.

5.2 Rationale for Drug Design

Our long term interest in MCRs initially involved the Ugi reaction to generate libraries of novel quinoline-containing α-acylamino amides in our quest for new antimalarial agents. The simplicity and attractiveness of MCRs in rapid exploration of SAR was a major consideration. While isocyanide-based MCRs have found application in other disease areas, to our knowledge, prior to the commencement of our work, there were no reports of the application of these reactions to antiparasitic drug discovery and SAR studies.

The initial exploratory target α-acylamino amides were designed based on the classic Ugi 4CC reaction of amine, aldehyde, carboxylic acid and isocyanide. The compounds were designed in such a way as to incorporate the 4-amino-7-chloroquinoline subunit into the amine input, Fig. 5.4.

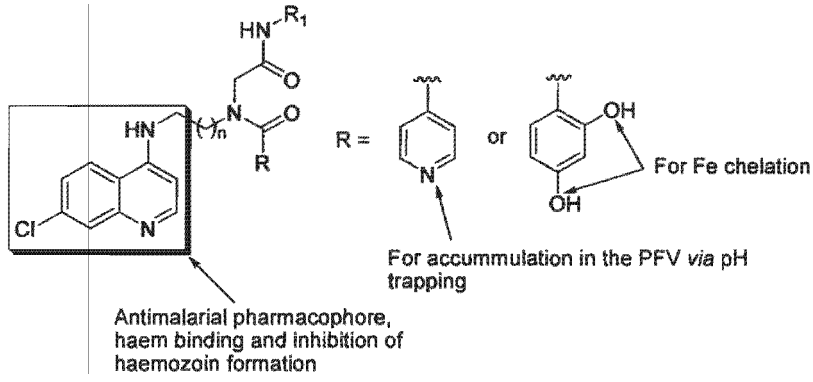
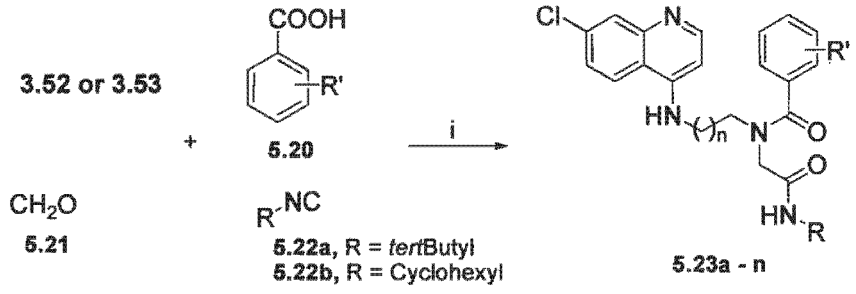


Figure 5.4: Rationale of design of 4-aminoquinoline-based α-acylamino amides

The basis of the design was that the 4-aminoquinoline substructure is present as the pharmacophore in a number of established antimalarial drugs such as chloroquine, mefloquine, quinine etc, where its presence is known to be significant in inhibiting haemozoin formation.[17,18,19] The initial choice of hydroxyl-containing carboxylic acids was made on the basis of the ability of the hydroxy functionality to chelate iron,[20] while we reasoned that the presence of a nitrogen atom in the carboxylic acid would present a second protonatable site within the molecular framework which would aid in compound accumulation within the acidic parasitic food vacuole *via* pH trapping, as well as improve the aqueous solubility of the compounds *via* salt formation. The use of formaldehyde as the aldehyde input was governed by our desire to obtain low molecular-weight compounds,[21] while the choice of isocyanides was initially limited by their commercial availability.

5.3 Synthesis of 4-Aminoquinoline-containing α-Acylamino Amides



Scheme 5.6: *Reagents and conditions* i) MeOH, rt, 36 – 48 h

With the exception of the 4-amino-7-chloroquinoline amines **3.52** and **3.53** whose synthesis has been described, all the inputs required for the desired Ugi 4CC reaction were obtained from commercial sources. The desired compounds were generated by means of parallel solution-phase synthesis according to Scheme 5.6. Thus, condensation of amines **3.52** or **3.53**, paraformaldehyde, aromatic carboxylic acids **5.20a – c** and isocyanides **5.22a** and **5.22b** in MeOH at room temperature afforded the target compounds **5.23a – n**. Simply removing the solvent under reduced pressure and subsequently subjecting the crude products to thin layer chromatography afforded the Ugi adducts (Fig. 5.5). The yields of the compounds were generally high, ranging from 63 to 77%.

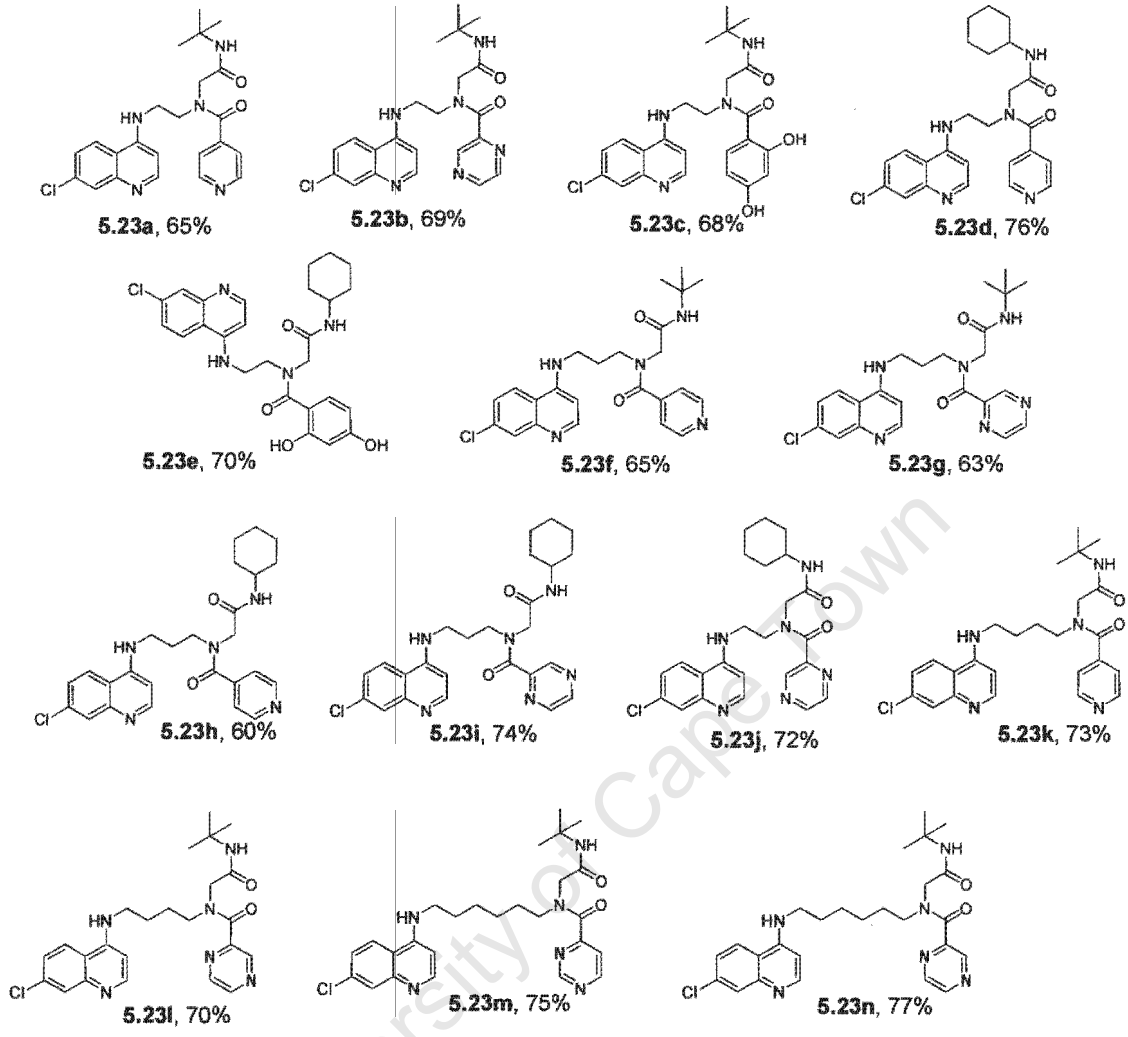


Figure 5.5: Structures of initial Ugi adduct targets **5.23a – n**

Infrared spectroscopy was used insofar as possible, to confirm the presence of certain functional groups in the proposed structures. The IR spectra depicted the bands identifiable with stretches due to amide NH (~3500 cm^{-1}), aromatic CH (~3000 cm^{-1}), carbonyl and aromatic C=C (~1700 cm^{-1} and 1550 cm^{-1} respectively).

Characterization of all the new compounds was done by means of ^{1}H NMR and ^{13}C NMR and mass spectrometry.

Both the ^{1}H NMRs and ^{13}C NMR spactra of the resultant α-acylamino amides showed duplication of individual peaks in the spectra, an observation that was attributed to slow interconversion between the *cis* and *trans* isomers about the tertiary amide bond.[22] The phenomenon of interconversion is depicted in the $^{1}HNMR$ spectra of a crude sample of **5.23a** (Fig. 5.6), in which it can be observed that all peaks at different chemical shift values are duplicated in the spectrum. However, by running variable temperature (VT) NMR for the compounds between 60 and 90 °C, near coalescence and peak broadening of the duplicating peaks was observed at the higher temperature.

High-resolution mass spectrometry was employed in determining the presence of the parent molecular ion. The compounds were characterized by the presence of the $(M^{+} + H)^{+}$ and M^{+} peaks.

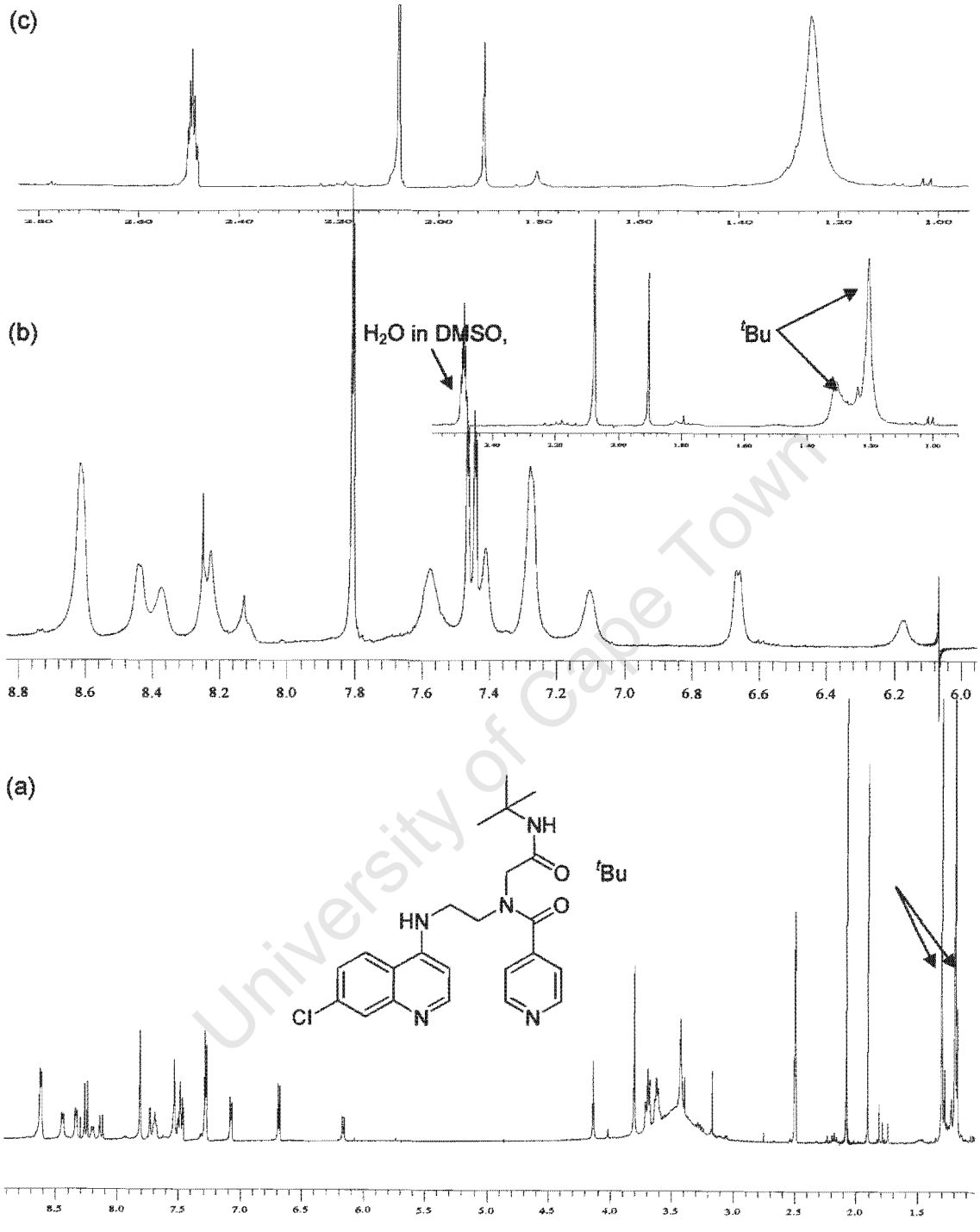


Figure 5.6: ^{1}H NMR of **5.23a** at (a) 30 °C, (b) 60 °C (aromatic region) and, (c) 90 °C (only ^{t}Bu group) in DMSO-d_6

5.4 Synthesis of Heterocycles *via* Ugi 3-Component 4-Centre (3C/4C) Reactions

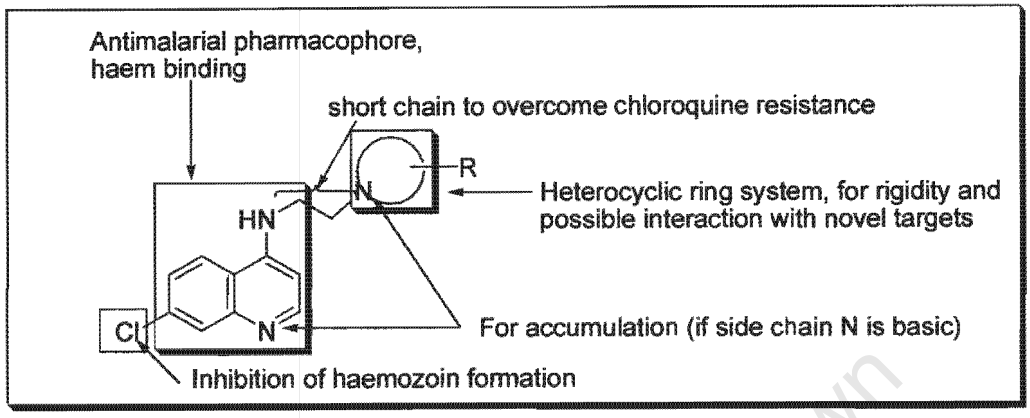


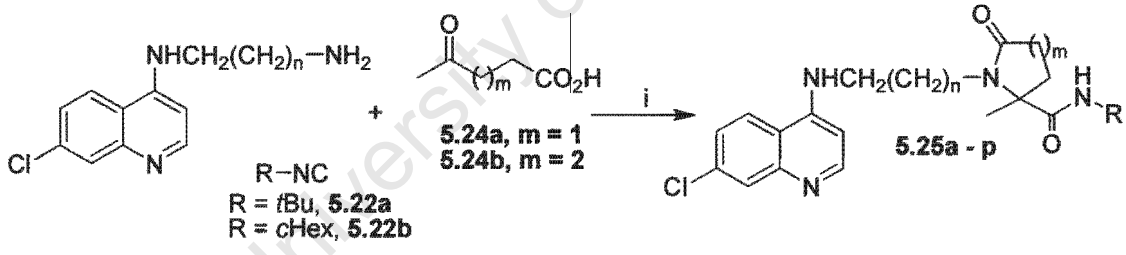
Figure 5.7: Rationale for design of 4-aminoquinoline compounds containing heterocyclic side chains

The classical Ugi reaction used in the synthesis of α-acylamino amides, despite its synthetic potential, is limited by giving rise to products that are flexible and peptidic in nature, often being described as being 'non-drug-like' and therefore making them less attractive targets in the search for new drugs. Importantly though, several novel intramolecular variations and secondary transformations of the Ugi 4CC that produce more constrained, biologically relevant heterocyclic compounds including tetrazoles,[23] diketopiperazines,[24] benzodiazepines[25] and morpholines[26] have been developed. In one variant of the Ugi 4CC, termed the Ugi 3-component 4-centre (3C/4C) reaction, two of the functional groups that take part in the reaction are tethered onto one starting material, leading to heterocyclic compounds. This strategy has been used to synthesize β-, and δ- and γ-lactams.[27,28] We thus became interested in the synthesis of heterocyclic compounds *via* multicomponent reactions built around the 7-chloro-4-aminoquinoline unit, a known antimalarial pharmacophore. The rationale behind the synthesis is summarized in Fig. 5.7.

5.4.1 Synthesis of 4-Aminoquinoline γ– and δ–Lactams

As mentioned before, several variations of the classical Ugi 4CC reaction that lead to heterocycles have been described in the literature.[3,23-26,29] While many choices of heterocycles were available to us, we decided to restrict our synthetic efforts towards generating modest exploratory libraries of compounds in which the 4-amino-7-chloroquinoline subunit was incorporated into the amine. As a starting point, we chose to synthesize γ- and δ-lactams, in which one of the amide bonds is incorporated into a heterocyclic ring. Indeed, different groups have used the Ugi reaction to synthesize β -lactams as potential inhibitors of the cysteine proteases[27] by incorporating an amide bond into the 4-membered heterocyclic ring system. Similarly, γ- and δ-lactams have been synthesized on solid support using the modified Ugi reaction.

Synthesis of an exploratory library of γ- and δ-lactams was achieved by way of Scheme 5.7.

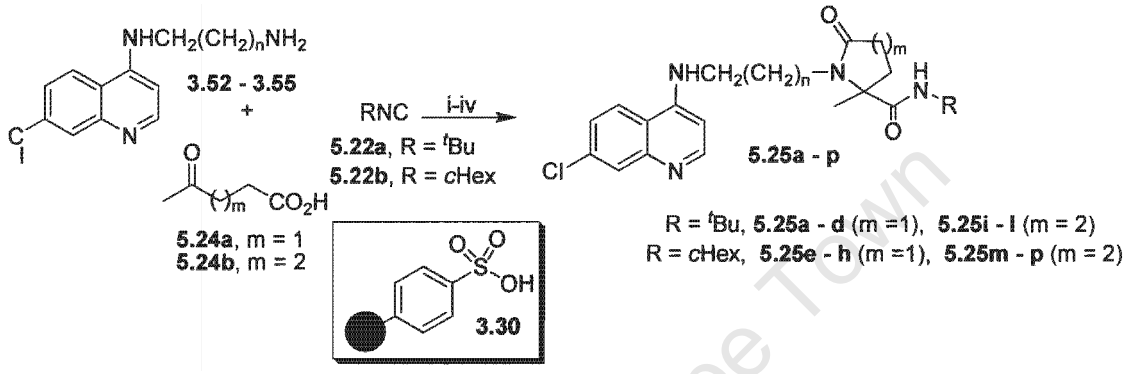


Scheme 5.7: *Reagents and conditions* i) MeOH, rt, 18 – 30 h

The synthesis was a one-pot reaction using a slight excess of amines (1.2 eq) **3.52 – 3.55**, ketoacid (levulinic acid **5.24a** or 4-acetylbutyric acid **5.24b**, 1.0 eq) and isocyanides **5.22a** and **5.22b** (1.0 eq) in MeOH at room temperature (Scheme 5.7). We found that the reactions in which cyclohexylisocyanide was used as the isocyanide input took longer to go to completion as compared to those where *tert*-butyl isocyanide was used.

5.4.1.1 PASP Synthesis of γ– and δ–Lactams

As before (in the synthesis of a β-amino alcohol library, section 3.4.1, p. 53), we sought a methodology that could allow us to prepare a library of γ- and δ-lactams of satisfactory purity while eliminating any chromatographic techniques.



Scheme 5.8: *Reagents and conditions* i) MeOH, rt, 12 - 18 h; ii) MPTsOH **3.30**, 1 h; iii) Filter, wash; iv) 3% NH_3/MeOH, filter, wash

We reasoned that the basicity (albeit weak) of the quinoline nitrogen could be exploited for the capture of the products followed by purification by washing and subsequent release in this case. The quinoline nitrogen is rendered basic due to the resonance or mesomeric release mediated by the 4-amino group, Fig. 5.8.

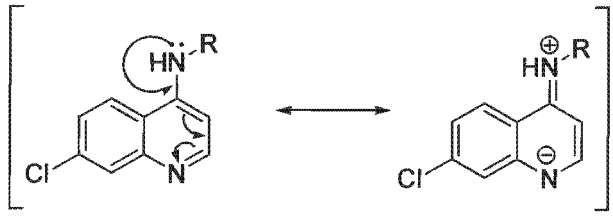


Figure 5.8: Increased basicity of quinoline nitrogen due to mesomeric effects

The reactions were performed in parallel using an excess of isocyanides (**5.22a** and **5.22b**) and ketoacids (**5.24a** and **5.24b**) while the diamines **3.52 – 3.55** were used as the limiting reagents. The ratio of reactants was carefully determined in such a way as to minimize the quantity of amines used in the reactions.

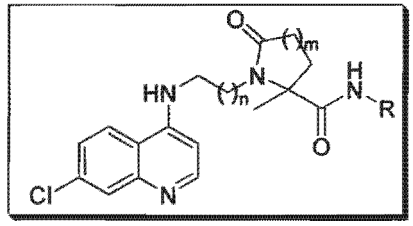
Since the presence of the quinoline nitrogen renders the lactam products basic and, since the "catch and release" concept relies on the formation of an ionic pair between a basic and an acidic compound, use of excess of the diamines would ultimately be a source of impurities in the final products. The predictable inability of MPTsOH **3.30** to discriminate between starting amines **3.52 – 3.55** and products **5.25a – p** in solution thus dictated the use of the former as the limiting reagents.

Again, we found the acidic resin-bound macroporous *para*-toluene sulfonic acid **3.30** (MP-TsOH) to be an effective tool for achieving the dual purpose of purifying and isolating our target lactams. Since the starting amines from which the lactams derive their basicity are completely consumed in the reactions, the addition of **3.30** at the end of reaction should achieve selective capture of the target compounds, leaving behind excess of ketoacid and isocyanide in solution, which can then be removed by simple filtration and resin washing with the reaction solvent.

Therefore, after the reactions were deemed complete as indicated by TLC, MP-TsOH was added to the reaction mixtures to scavenge the products from solution. After 1 h following addition of MP-TsOH, all reactions revealed complete scavenging of the products from solution (TLC) and were thus filtered and washed repeatedly to remove excess of isocyanides (**5.22a** and **5.22b**) and ketoacids (**5.24a** and **5.24b**). Release of the products was achieved by agitating the resins in anhydrous methanolic ammonia for 30 min. Yields were generally high to excellent and were comparable with those from traditional solution-phase synthesis. More interestingly, the purities of compounds **5.25a**

– **p** were high enough to permit their direct use in biological assays (Table 5.2). For biological evaluation purposes, however, compounds purified using conventional chromatography were used.

Table 5.2: Isolated yields and purities of lactams **5.25a – p**



General structure of lactams **5.25a – p**

Entry	Compound	m	R	Yield[a] (%)	Yield[b] (%)	HPLC Purity (%)
1	**5.25a**	1	^{t}Bu	57	60	91
2	**5.25b**	1	"	84	79	92
3	**5.25c**	1	"	77	73	90
4	**5.25d**	1	"	75	69	94
5	**5.25e**	1	*c*Hex[d]	84	75	88
6	**5.25f**	1	"	67	70	91
7	**5.25g**	1	"	70	68	95
8	**5.25h**	1	"	84	77	94
9	**5.25i**	2	^{t}Bu	65	75	91
10	**5.25j**	2	"	76	71	96
11	**5.25k**	2	"	75	69	87
12	**5.25l**	2	"	70	74	93
13	**5.25m**	2	*c*Hex	76	66	95
14	**5.25n**	2	"	80	72	94
15	**5.25o**	2	"	78	74	91
16	**5.25p**	2	"	79	72	93

[a] Yields from solution-phase synthesis; [b] Yields from polymer-assisted solution-phase synthesis; [c] RP-HPLC Purity was determined as area under a curve, UV 220 and 254 nm); [d] *c*Hex = cyclohexyl.

Using IR, the presence of the NH groups was confirmed by peaks around 3300 cm^{-1}, while the presence of the C=O groups were confirmed by strong peaks in the near 1700 cm^{-1} region. All the new lactams were characterized by means of their ^{1}H NMR and ^{13}C NMR spectroscopy and all gave spectra that were in agreement with their proposed structures. The proposed molecular formulae were confirmed by means of high resolution mass spectrometry and all compounds gave the expected molecular formulae and molecular weights.

5.5 Synthesis of 4-Aminoquinoline-containing 2-Imidazolines

5.5.1 Background

The 2-imidazoline scaffold (Fig. 5.9) is widespread in a number of natural products and derivatives thereof, and is a useful intermediate for the synthesis of biologically active compounds.[30] Derivatives of imidazolines exhibit a wide variety of biological activities including potent antihypercholesterolemic,[31] antidiabetic[32] and antihypertensive.[33] Our interest in the 2-imidazolines stemmed from previous work on the synthesis of α-acylamino amides described previously (section 5.3, p. 162). Indeed imidazolines have been used as amide bond replacements[34] to stabilize peptidic compounds. The amidine that is present in the imidazoline ring has similar resonance structures to those present in the parent amide bond and as such mimics the features of the amide bond. Similarities between the two include presence of two heteroatoms, similar configuration of the double bond, similar hydrogen bonding possibilities and similar steric properties, Fig. 5.9. The greater basicity of the amidine functionality compared with that of the amide provides stability towards hydrolysis under physiological conditions that are likely to prevail in biological systems.[35] Despite the great biological potential of 2-imidazolines, they have not been previously investigated as antimalarial compounds, or indeed as antiparasitic compounds in general.

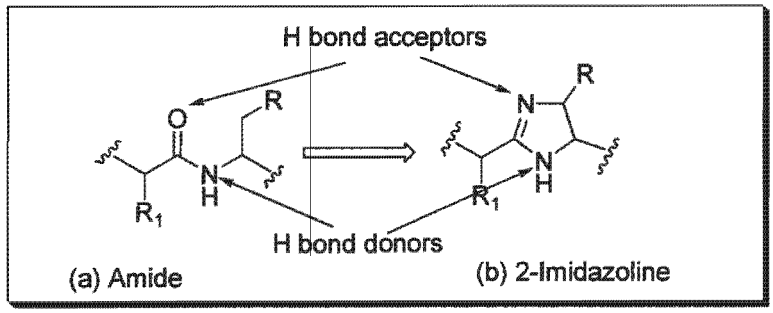


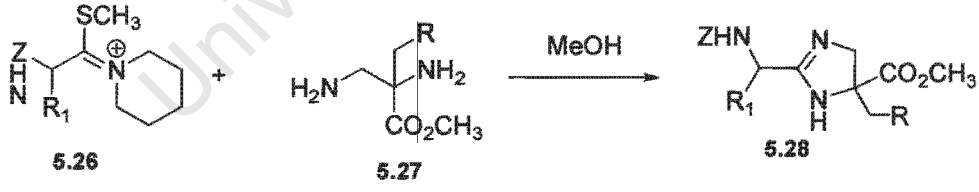
Figure 5.9: Structure of amide (a) and 2-imidazoline (b) showing similar H bond donors and heteroatomic H bond acceptors

5.5.2 General Synthetic Methods of 2-Imidazolines

Several methods for the synthesis of 2-imidazolines have appeared in the literature, a few of which are discussed below.

5.5.2.1 From 1,2-Diamines

The most commonly reported synthesis of 2-imidazolines involves ring-closure of 1,2-diamines (or their derivatives) and carboxylic acids and derivatives of these.[36] Other synthetic approaches based on 1,2-diamines through which 2-imidazoline rings may be formed have appeared in the literature.[37]



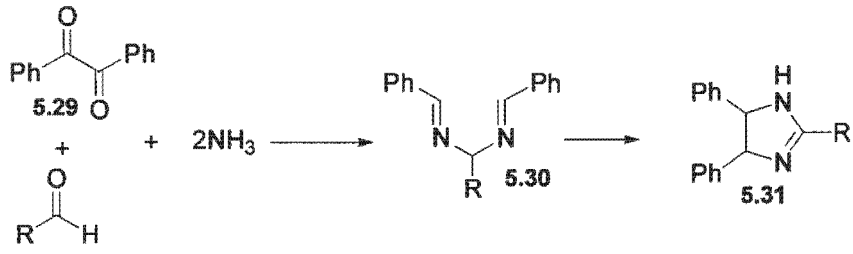
5.26 **5.27** **5.28**

Scheme 5.9: Gilbert's synthesis of 2-Imidazolines

The method shown in Scheme 5.9, developed by Gilbert *et al* involves the use of the *in situ* generated S-methylthioimidate salt **5.26** and immediate condensation with the 1,2-diamino compound **5.27** in MeOH to afford the target 2-imidazolines **5.28** in yields ranging from 42 to 91%.[35] Other methods in this category include reaction of 1,2-

diamines (and their derivatives) with carboxylic acids, esters, amino ethers, nitriles and amidines and guanidines.[36]

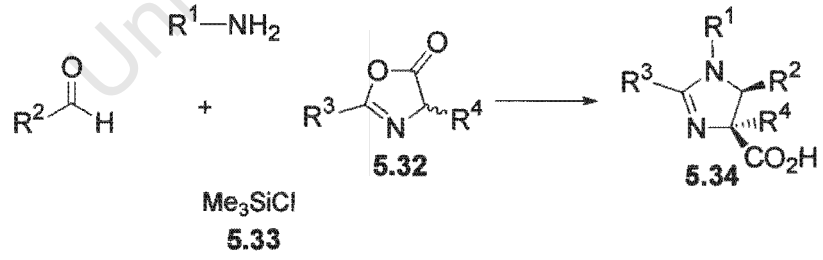
5.5.2.2 From Carbonyl-containing Compounds and Ammonia



Scheme 5.10: Synthesis of 2-Imidazolines

The reaction of benzil **5.29**, aromatic aldehydes (RCHO) and ammonia yields the imine **5.30**, which on heating may cyclise to give the 2,4,5-triaryl-2-imidazoline **5.31**,[38] Scheme 5.10. Other aromatic aldehydes have also been used in place of benzil in a similar fashion to produce 2,4,5-triaryl-2-imidazolines.

5.5.2.3 From Multicomponent Reactions: Teppe's Synthesis of 2-Imidazolines



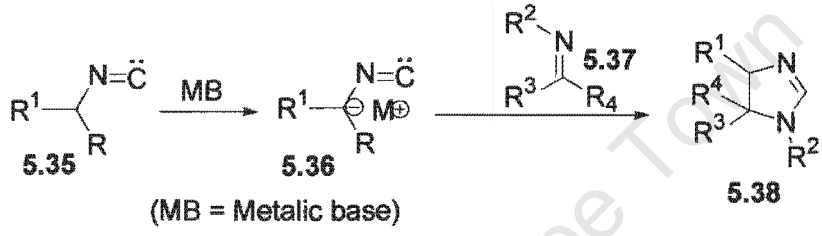
Scheme 5.11: *Reagents and conditions* i) DCM, 40 °C, 51 – 78%

This method, described by Teppe and coworkers, was probably the first multicomponent reaction towards the synthesis of 2-imidazolines,[39] Scheme 5.11. The reaction has four

components, namely an aldehyde R^2CHO (or ketone), an amine R_1NH_2, an oxazolone **5.32** and trimethylchlorosilane (TMSCl) **5.33**, and is highly diastereoselective in affording the *trans* diastereomer **5.34** (with respect to R^2 and R^4). In this case the reactions were done in DCM at 40 °C, affording the 2-imidazolines in good to high yields.

5.5.2.4 Isocyanide-Based Syntheses of 2-Imidazolines

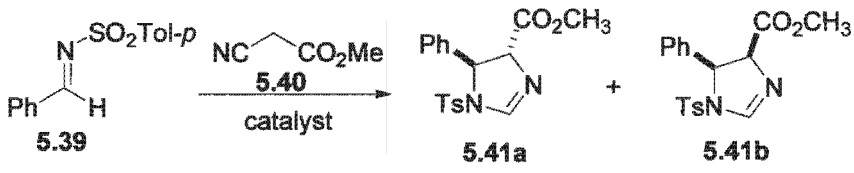
5.5.2.4.1 Schollköpf's Synthesis



Scheme 5.12: *Reagents and conditions* i) THF, –60 °C 51 – 78%

One of the earliest methods for the synthesis of the 2-imidazoline-ring system, developed by Schollköpf *et al*,[40] uses an isocyanide as one of the inputs. In this reaction an isocyanide **5.35** possessing an active α-hydrogen atom is deprotonated with base such as n-butyllithium, 1,8-diazabicyclo-[5.4.0]undec-7-ene (DBU), Et_3N, NaOH etc. without the need for isolation of the α-metalated isocyanide intermediate anion **5.36**, Scheme 5.12. The intermediate **5.36** has an ambivalent nature; it contains, on the one hand, a nucleophilic centre that can add to polar multiple bonds and, on the other hand an electrophilic centre at the isocyanide carbon that allows cyclization of intermediates to form heterocycles. Addition of the preformed imine **5.37** to a solution of **5.36** results in the formation of the 2-imidazoline **5.38**.

5.5.2.4.2 Hayashi's Synthesis

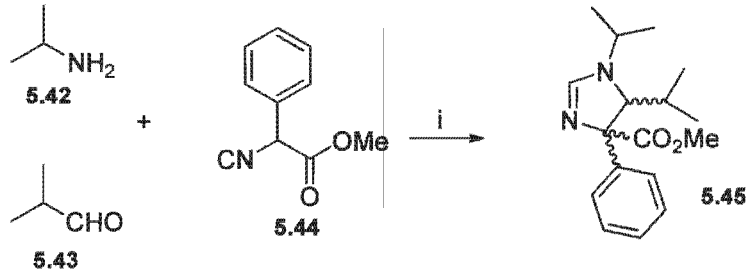


Scheme 5.13: *Reagents and conditions* i) DCM, reflux 89 – 99%

Hayashi and coworkers reported a synthesis of 2-imidazolines based on sulfonylimines **5.39** and isocyanoacetate **5.40** in the presence of a metallic catalyst to afford the diastereomeric 2-imidazolines **5.41a** and **5.41b** in excellent yields (>95%), Scheme 5.13[41] In trying to optimize the conditions, the diastereoselectivity of the reaction was improved from 1:1 *cis:trans* using a Rh catalyst to as high as 1:19 in favour of the *cis* compound with a Au catalyst. However, Hayashi's method was only applicable to sulfonylimines of the type **5.39**; when other imine inputs were used the expected 2-imidazolines did not form.

5.5.2.4.3 Orru's Synthesis

Recently, Orru *et al* reported a one-pot synthesis of 2-imidazolines that precludes the addition of base to effect anionisation.[42] Thus, the 4,4,5 trisubstituted 2-imidazolines **5.45** are obtained *via* a 3-component condensation of isopropylamine **5.42**, isobutyraldehyde **5.43** (aldehyde or ketone) and isocyanoacetate **5.44** under very mild conditions, Scheme 5.14.

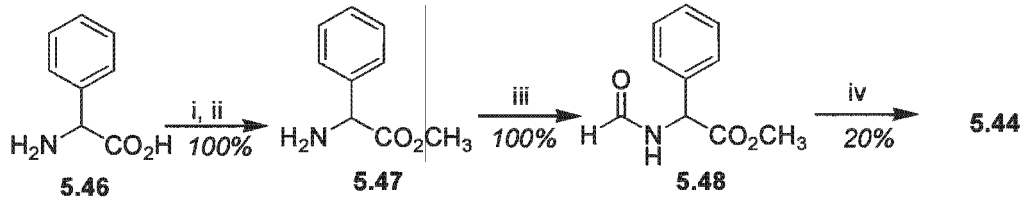


Scheme 5.14: *Reagents and conditions*: i) MeOH (or THF or DCM), rt, 18 h

In this reaction, it is thought that the slight excess of amine that is employed is sufficient to serve as the deprotonating base. The limitation to this approach is that on a semi-synthetic scale, it is only applicable to isocyano acetates that contain a stabilizing group such as the phenyl ring in the α-position. On the other hand, because the approach is amenable to combinatorial and parallel synthesis, it is advantageous in that conditions can be easily manipulated to allow for novel purification strategies that would ease the amount of effort generally required for the synthesis and purification of large compound libraries. Although this method was developed based on the concept originally developed by Schollköpf, its translation into a smooth multicomponent reaction makes it a more favourable approach in combinatorial synthesis. The ease of introduction of diversity in a single step also renders it a very elegant approach.

5.5.3 Chemical Synthesis of 4-Aminoquinoline 2-Imidazolines

One of our primary aims was to generate quickly exploratory libraries *via* known multi-component reactions. Of the various methods described in the literature we found the combinatorial approach developed by Orru and others to be the more appealing approach for our parallel array format synthesis. The following section describes the synthetic steps towards the desired 4-aminoquinoline-containing 2-imidazolines.



Scheme 5.15: *Reagents and conditions* i) $SOCl_2$, MeOH, 0 °C – rt, 2 h; ii) K_2CO_3, MeOH; iii) HCOOH/Ac_2O, DCM, reflux, 1 h; iv) Et_3N, $POCl_3$, –23 °C – 0 °C, 2 h

The isocyanoacetate **5.44** required for the multicomponent reaction in combination with select aldehydes and 4-aminoquinoline diamines **3.52 – 3.54**, was realized by way of Scheme 5.15. Commercially available racemic phenylglycine **5.46** was esterified *via* the acid chloride (not shown here) using $SOCl_2$/MeOH at ambient temperature.[43] Besides its shorter reaction time, the esterification reaction employing $SOCl_2$/MeOH required milder conditions than the use of HCl/MeOH and was thus the more preferable approach.

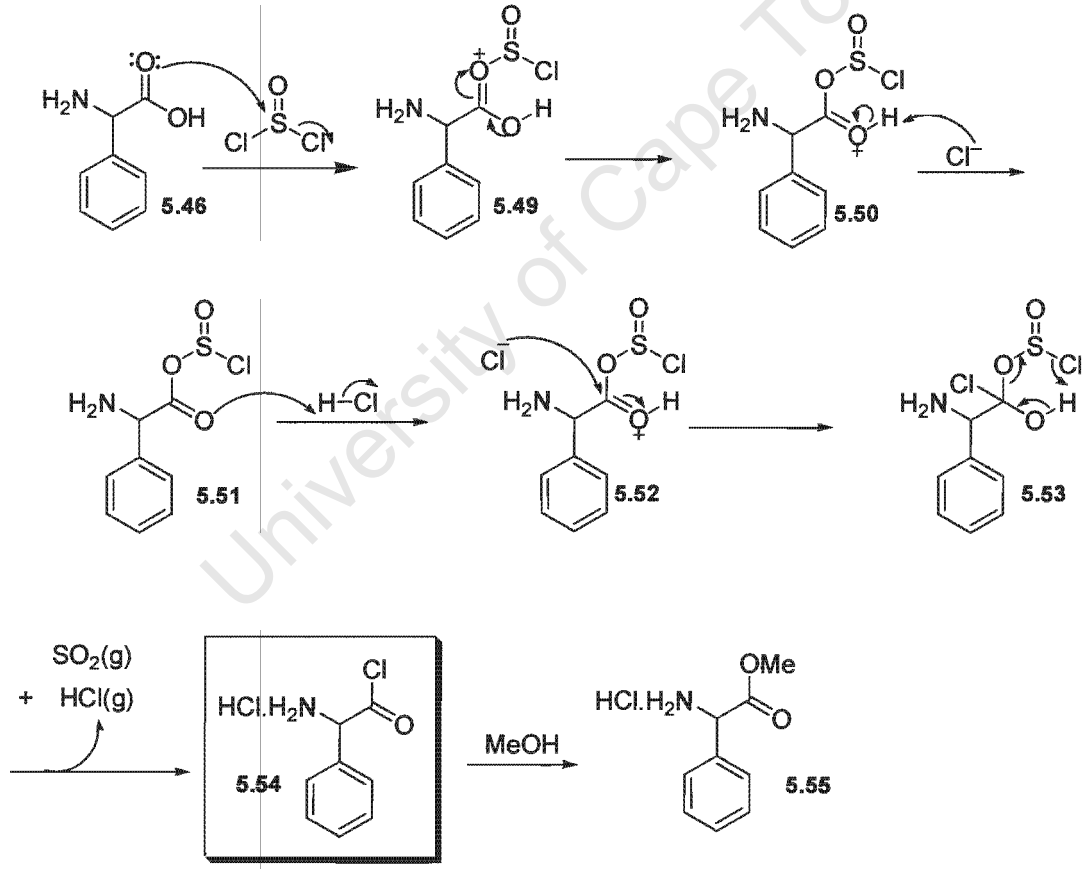
The product was obtained by continuous evaporation of the reaction mixture with DCM, quantitatively yielding the HCl salt of **5.47** as a while solid. Basification of the salt was achieved by simply stirring its methanolic solution in the presence of $NaHCO_3$. Subsequent filtration of the inorganics, followed by drying and evaporation afforded the free base of the ester **5.47** in quantitative yield. The appearance of a singlet at δ 3.8 ppm in the ^{1}H NMR spectrum corresponding to the methyl group confirmed the esterification of the amino acid. A corresponding peak in the ^{13}C NMR spectrum for the methyl carbon was also observed at ~δ 53 ppm.

The free base was then formylated by the method described by Orru *et al.*[42] Thus, a suspension of the amino ester **5.47** was refluxed in DCM in the presence of the preformed mixed anhydride of Ac_2O and HCOOH. The free base of the ester was not completely soluble in DCM but slowly went into solution upon addition of the mixed anhydride and subsequent refluxing. The resultant formamide was isolated by repeated

dilution of the mixture with water and continued evaporation without the need to perform any extractive processes. Using this method of isolation, the formamide **5.48** was obtained in quantitative yield and with high purity.

The diagnostic peak for the formamide proton appeared at δ 8.05 ppm, indicating that the free amino group of **5.47** had been converted to the formamide **5.48**. Both the ^{13}C NMR and IR spectra revealed the presence of a second carbonyl group around δ 170 ppm and 1700 cm^{-1} respectively.

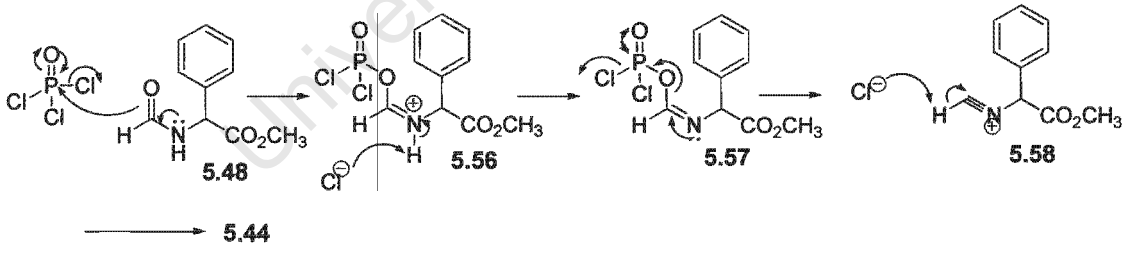
In terms of mechanism, the sequence of events that leads to the methyl lester **5.57** is shown in Scheme 5.16.



Scheme 5.16: Mechanism of esterification of L-phenylalanine

Thionyl chloride ($SOCl_2$) is electrophilic at the S atom and is consequently attacked by the carboxyl group of the amino acid **5.46** to give the unstable intermediate **5.51** *via* **5.49** and **5.50** after loss of HCl. Protonation of **5.51** by HCl gives rise to the highly electrophilic intermediate **5.52**, which is then attacked by the weakly nucleophilic Cl anion to give the tetrahedral intermediate **5.53**. Collapse of **5.53** in an irreversible reaction releases SO_2 (g) and HCl (g) as well the salt of the acid chloride **5.54**, which is in turn attacked by the MeOH to afford the hydrochloride methylester **5.55**.

Finally, dehydration of the formamide **5.48** to the isocyanide **5.44** was achieved by treatment of the former with $POCl_3$ in anhydrous DCM in the presence of 5 equivalents of Et_3N. Mechanistically, dehydration of the formamide is believed to proceed *via* the sequence of events depicted in Scheme 5.17. S_N2 attack on the P atom by the amide oxygen generates the intermediate **5.56**, followed by deprotonation to give **5.57**. The C-O bond is cleaved next with assistance from the lone pair of the N atom leading to the nitrilium ion **5.58**, followed by deprotonation by the Cl anion to give the isocyanide **5.44**.

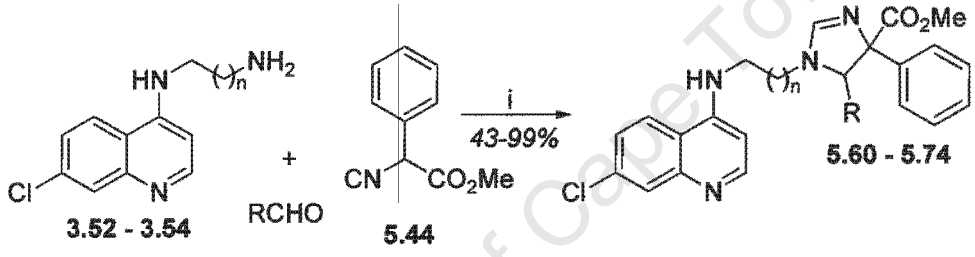


Scheme 5.17: Mechanism of $POCl_3$–mediated dehydration of formamide

The isocyanoacetamide **5.44** could only be realised in 20% yield after column chromatography on SiO_2 gel despite the yield of the crude product having been much higher. Orru and coworkers reported that whilst attempting to synthesize **5.44**, they

observed "some" decomposition of the isocyanide on the column during the purification step.[42] In our hands the decomposition happened to have occurred extensively on either acidic SiO_2 or Et_3N-deactivated SiO_2 gel. Similar observations were made when the stationary phase was changed to basic Al_2O_3.

Having synthesized **5.44**, it was decided to attempt the multicomponent reaction to generate a modest exploratory library of the 4-aminoquinoline-containing 2-imidazolines. Deliberately, we limited ourselves to the use of amines **3.52 – 3.54** and randomly chose five aldehydes (RCHO) for use in and carried out the multicomponent reactions as described, Scheme 5.18.



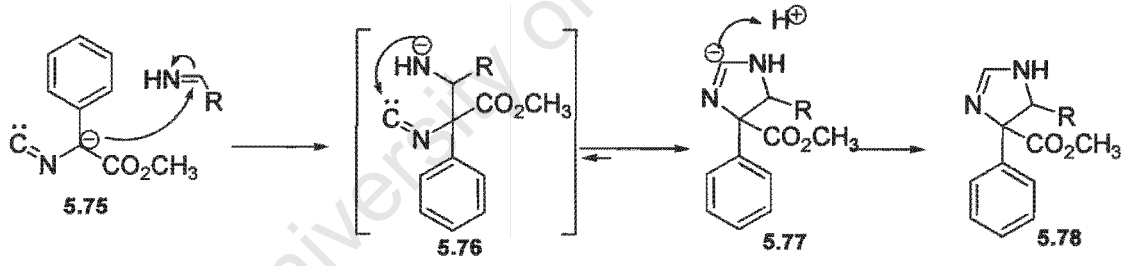
Scheme 5.18: Reagents *and conditions* i) MeOH, 45 °C, 2 h

The multicomponent reaction to 2-imidazolines was previously optimized as reported by Orru *et al* and accordingly, MeOH was found to be one of the better solvents for the reaction. Thus, the synthesis of the 2-imidazolines was conducted in MeOH. Orru and coworkers conducted their synthesis at room temperature in the presence of a drying agent (Na_2SO_4) to remove the water produced during the condensation of amine and aldehyde. Perhaps the low temperature at which the reactions were performed was responsible for the prolonged reaction times that were seen with these reactions (up to 18 h).

In our laboratories we found that conducting the reactions at elevated temperatures greatly accelerated the forward reactions towards formation of the 2-imidazolines. However, elevated temperatures may have lowered the diastereoselectivity of our

reactions. Typically, all reactions were allowed to proceed for 20 minutes prior to addition of phenylisocyanoacetate **5.44** and were deemed complete within a space of 2 h (TLC) after addition of **5.44**. A temperature of 45 °C was established to be optimal, although higher temperatures were also tolerated. The preclusion of a drying agent from the reaction mixtures was found to have no effect on the isolated yields of the products. The elevated temperatures may have lowered the diastereoselectivities of our reactions. The average diastereoselectivity reported by Orru *et al* was 1:3 *syn* vs. *anti* diastereomer, whereas in our cases the diastereoselectivites were as high as 1:5. According to Orru *et al*, formation of the *anti* diastereomer is favoured over the *syn* diastereomer irrespective of the solvent employed. Based on these findings therefore, we think that the major products isolated from the reactions were the *anti* diastereomers.

5.5.3.1 Mechanistic Comments

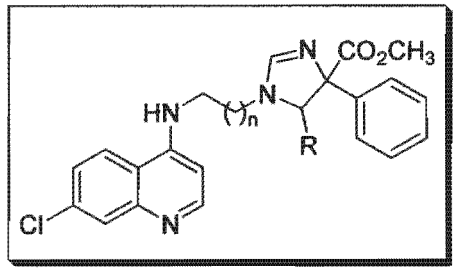


Scheme 5.19: General mechanism of formation of the 2-imidazoline ring

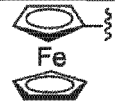
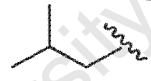
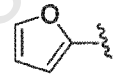
It is postulated that the traces of the starting amine present in the reaction mixture act as the base and anionise the isocyanide **5.44** to give the anion **5.75** (Scheme 5.19), although, given that the intermediate imine is basic it is also possible that it can anionise the isocyanide. Nucleophilic attack on the imine carbon by **5.75** leads to the intermediate **5.76** (Scheme 5.19), whose equilibrium is believed to lie far to the right to **5.77**. Finally, protonation of **5.88** results in the 2-imidazoline **5.78**.

Because the reactions proceed *via* a flat imine intermediate, the use of substituted aldehydes (other than paraformaldehyde) or ketones results in the formation of diastereomers. With the exception of the reactions using the paraformaldehyde as the aldehyde input, all other reactions gave inseparable mixtures of diastereomers with similar R_f values (TLC). The diastereomeric mixtures were only revealed on examination of their respective ^{1}H NMR spectra. The diastereomeric ratio in a mixture (dr, only calculated after column chromatography) was determined as the ratio of the integration of a proton in one diastereomer to the same proton in the other diastereomer from the respective ^{1}H NMR spectra. The non-crystalline nature of the products made it difficult to separate the diastereomers by selective crystallization, so that all products were simply isolated as diastereomeric mixtures. Purification was achieved by means of column chromatography on SiO_2 gel by eluting with MeOH/DCM 0 – 10% with yields ranging from 43 to 99% (Table 5.3).

Table 5.3: Isolated yields and structures of 4-aminoquinoline 2-imidazolines **5.70 – 5.85**



General structure of 2-imidazolines **5.60 – 5.75**

Compound	n	R	Yield (%)	*dr*	HPLC Purity (%)
5.60	1	**H**	84	-	92
5.63	2		78	-	95
5.66	3		67	-	94
5.69	1	**CH_3-**	82	1:3	96
5.72	2		55	1:4	96
5.61	3		43	1:3	95
5.64	1		76	1:3	98
5.67	2		77	1:4	92
5.70	3		74	1:3	97
5.73	1		86	1:3	95
5.62	2		56	1:5	99
5.65	3		52	1:3	96
5.68	1		92	1:4	93
5.71	2		65	1:5	94
5.74	3		99	1:4	90

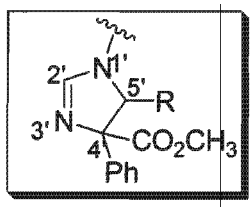
HPLC Conditions: Flow rate = 1.5 ml/min; UV 220 and 254 nm; 30:70 CH_3CN: 25 mM Phosphate buffer

All the synthetic 4-amino-7-chloroquinoline 2-imidazolines gave IR spectra that indicated characteristic functional groups in their structures.

The ^1H NMR data obtained for the new compounds **5.60 – 5.74** were consistent with the proposed structures. The most notable differences among the spectra were the relative positions of the H-5′ proton of the 2-imidazoline ring and the corresponding multiplicities.

In the unsubstituted derivatives **5.60**, **5.61** and **5.63** the two protons appeared as AB doublets at ~δ 4.4 ppm and 3.6 ppm with coupling constants of 9.6 Hz, whilst the chemical shifts and multiplicities in the substituted derivatives were dependent on the nature of the R group at this position. For instance in **5.69**, **5.72** and **5.61** the H-5′ proton appeared as a quartet at ~δ 4.5/4.0 ppm for the two diastereomers, whereas the same proton appeared as a singlet at ~δ 5.4/4.8 ppm in the ferrocene-substituted compounds **5.64**, **5.67** and **5.70**. A summary of the different chemical shift values, multiplicities and coupling constants for H-5′ are shown in Table 5.3.

Table 5.4: Chemical shift values, multiplicities and coupling constants for H-5′



Compound	R	~δ ppm	Multiplicity	*J* value (Hz)
5.60	H	4.4/3.3	d/d	9.6
5.69	CH_3	4.5/4.0	q/q	6.6
5.64	Fc	5.4/4.8	s/s	–
5.73	Fufuryl	6.0/5.7	s/s	–
5.68	^{i}Bu	3.5/3.4	m/m	–

Figure 5.10 depicts the ^{1}H NMR spectrum of **5.62**. The aromatic region of the spectrum has been expanded to highlight the differences in chemical shifts between the two diastereomers (inset).

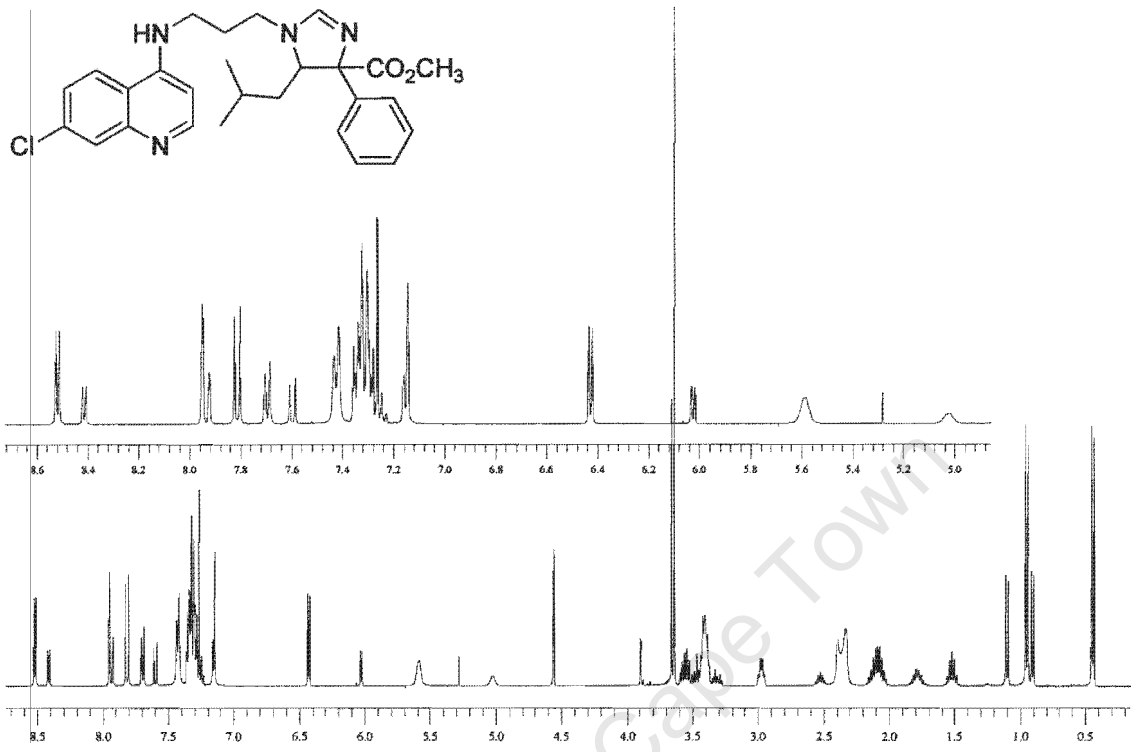
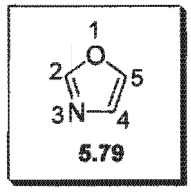


Figure 5.10: ^{1}H NMR spectrum of **5.71** in $CDCl_3$

The ^{13}C NMR data of all the compounds were consistent with the expected values for the different types of carbon atoms. The accurate masses of the parent ions were determined by means of high resolution mass spectrometry and, within acceptable limits of error all compounds gave molecular formulae that were consistent with the proposed structures. Additionally, the isotopic distributions were checked to confirm that they were in agreement with calculated values.

5.6 4-Aminoquinoline-containing 2,4,5-Trisubstituted Oxazoles

The oxazole ring is a 5-membered aromatic heterocycle that contains oxygen and nitrogen (**5.80**, Fig. 5.11). Oxazoles represent an interesting class of heterocycles existing as subunits of biologically active naturally occurring compounds,[44] while those arising from chemical syntheses exhibit favourable pharmacophore profiles in drug discovery programmes.[45] A number of oxazoles isolated from natural sources have shown a wide range of biological activities including antifungal,[46] antiviral,[47] antitumoral, antibiotic[48] and anti-HIV.[49]



5.79

Figure 5.11: Structure of oxazole

While the oxazole ring is present as a core substructure in many biologically active compounds, its potential as an antimalarial has not been pursued. We thus became interested in the oxazole substructure as a bioactiphore, which we envisaged by hybridizing with the 4-amino-7-chloroquinoline subunit could yield novel compounds with interesting antiplasmodial properties. We also intended to verify our hypothesis that the novel compounds with antiplasmodial activities can also be effectively used as antitrypanosomal agents.

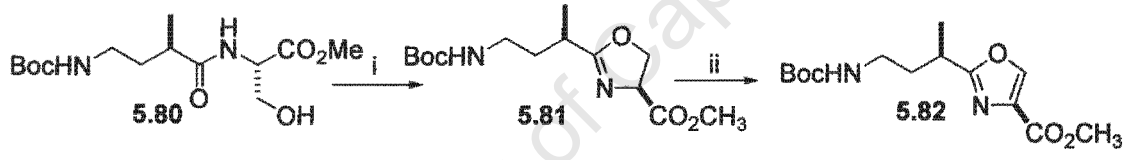
Consequently, we sought an efficient and cost effective approach of hybridizing the 4-aminoquinoline moiety and the oxazole ring system. While the literature abounds with many synthetic ways to assemble the oxazole unit,[45,50] only a few general methods have been developed to date. Therefore a mild and selective formation of the oxazole ring in the presence of other sensitive functional groups remains one of the key challenges of

its synthesis. Several oxazole formation methodologies have been developed to meet these challenges, a few of which are highlighted below.

5.6.1 Synthetic Methods of Oxazoles

5.6.1.1 Evans' Synthesis of Oxazole

Several of the methods appearing in the literature have relied on the use of serine to generate the oxazole ring.[45] One such synthesis is that reported by Evans and coworkers, who assembled the oxazole unit during their synthesis of caliculyin A, a natural product isolated from the marine sponge *Discodermia calyx*, according to Scheme 5.20.[51]

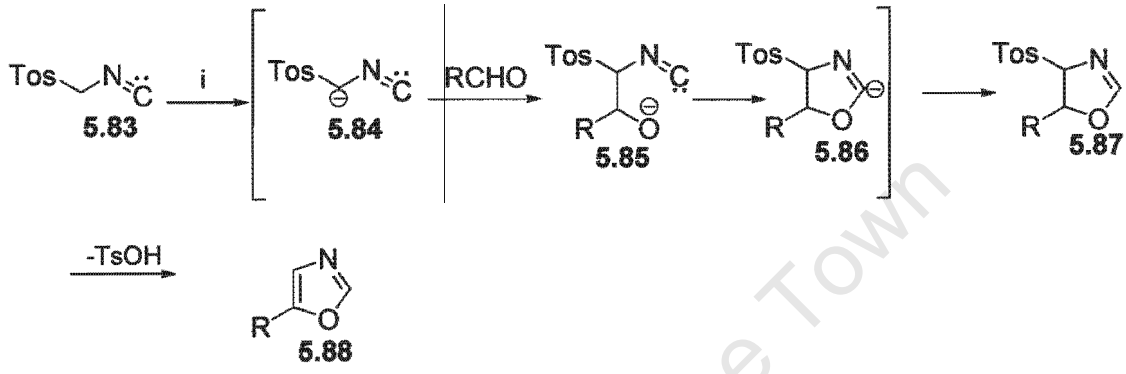


Scheme 5.20: *Reagents and conditions* i) $SOCl_2$, pyridine; ii) nickel peroxide, or a) Boc_2O, DMAP, CH_3CN, b) KHMDS, PhSeCl, c) H_2O_2

The scheme shows only the key steps used during the generation of the oxazole subunit. The first key step towards assemblage of the oxazole ring in this multistep synthesis involved the cyclodehydration of **5.80** using $SOCl_2$ in pyridine to afford the oxazoline **5.81**. The second and final oxazole-generating step involved dehydrogenation of **5.81** to the oxazole **5.82** by treatment with either nickel peroxide or α-selenation followed by oxidative elimination.

5.6.1.2 van Leusens' Synthesis

One of the simplest and most efficient methods for generating the oxazole unit was that developed by van Leusen and co-workers, employing an aldehyde (RCHO) and tosylmethyisocyanide **5.83** (TosMIC) in refluxing MeOH in the presence of K_2CO_3[52] Scheme 5.21.

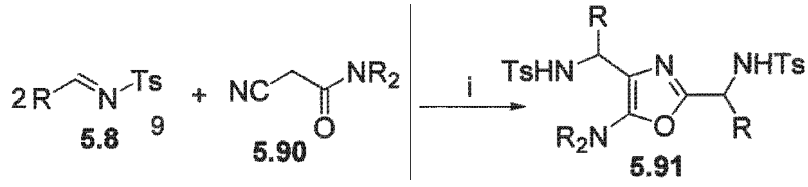


Scheme 5.21: *Reagents and conditions* i) K_2CO_3, MeOH, reflux, 2 h

The reaction is believed to proceed *via* intermediates **5.84 – 5.86**, then oxazolidine **5.87**, which upon a base-catalyzed elimination of the tosyl group affords the oxazole **5.88**. They found that acid chlorides and acid anhydrides participated well in the reaction to afford the oxazole ring under the same reaction conditions. This reaction gave the 2,4 disubstituted oxazoles only.

5.6.1.3 Lin's Synthesis

Lin and co-workers found that the oxazole ring could be formed under milder conditions of room temperature and absence of base to afford the 2,4,5-trisubstituted oxazole **5.92** in a double addition reaction,[53] Scheme 5.22.

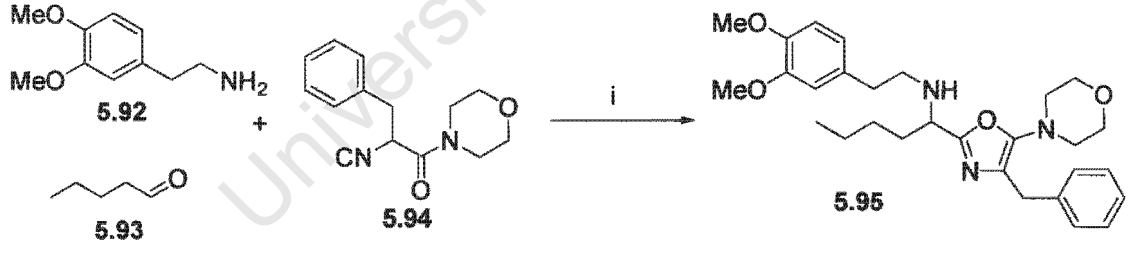


Scheme 5.22: *Reagents and conditions* i) DCM, rt

While attempting to synthesize the imidazoline ring in a control experiment, they found that by replacing the isocyanoacetate **5.40** (section 5.3.2.4.2) with isocyano acetamide **5.90** and precluding base, the reaction proceeded to give the 2,4,5 trisubstituted oxazole **5.91** in very high yields.

5.6.1.4 Zhu's Synthesis

Recently, Zhu *et al* have disclosed an elegant one-pot synthesis of the 2,4,5-trisubstituted aminooxazole ring *via* the isocyanide multicomponent reaction[54] which relies on amines (e.g. **5.92**), aldehyde **5.93** (or ketone) and amino acid-derived isocyanoacetamides such as **5.94**, Scheme 5.24.



Scheme 5.23: *Reagents and conditions* i) MeOH, 55 °C, 4 h

Compared to other approaches, the method described by Zhu *et al* is superior in terms of ease of introduction of diversity, given the large pool of naturally-occurring amino acids and the large number of amines that are available from commercial sources. In terms of combinatorial library design the multicomponent synthesis of 2,4,5-trisubstituted oxazole is amenable to solid-phase synthesis as well as automation.

Thus, we became interested in this multicomponent reaction approach towards synthesizing new hybrids containing the oxazole ring and the 4-amino-7-chloroquinoline subunit.

5.6.2 Rationale for Drug Design

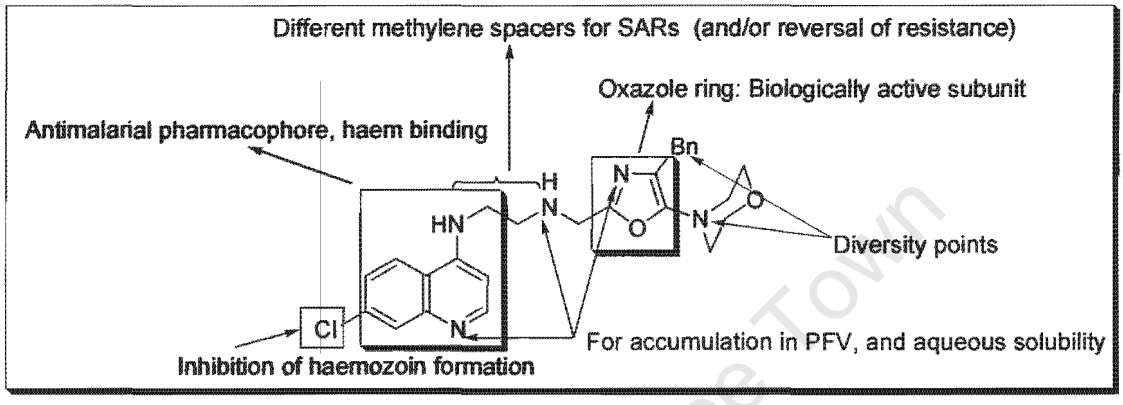


Figure 5.12: Rationale for design of target compounds

Apart from introducing the oxazole bioactiphore, we hoped that the presence of the heterocyclic side chain in the new structures would help stabilize the compounds towards degradation under physiological conditions.

The rationale upon which the design of this class of compounds was based (Fig. 5.12) is summarized below:

i) Presence of the 4-amino-7-chloroquinoline antimalarial pharmacophore[17]

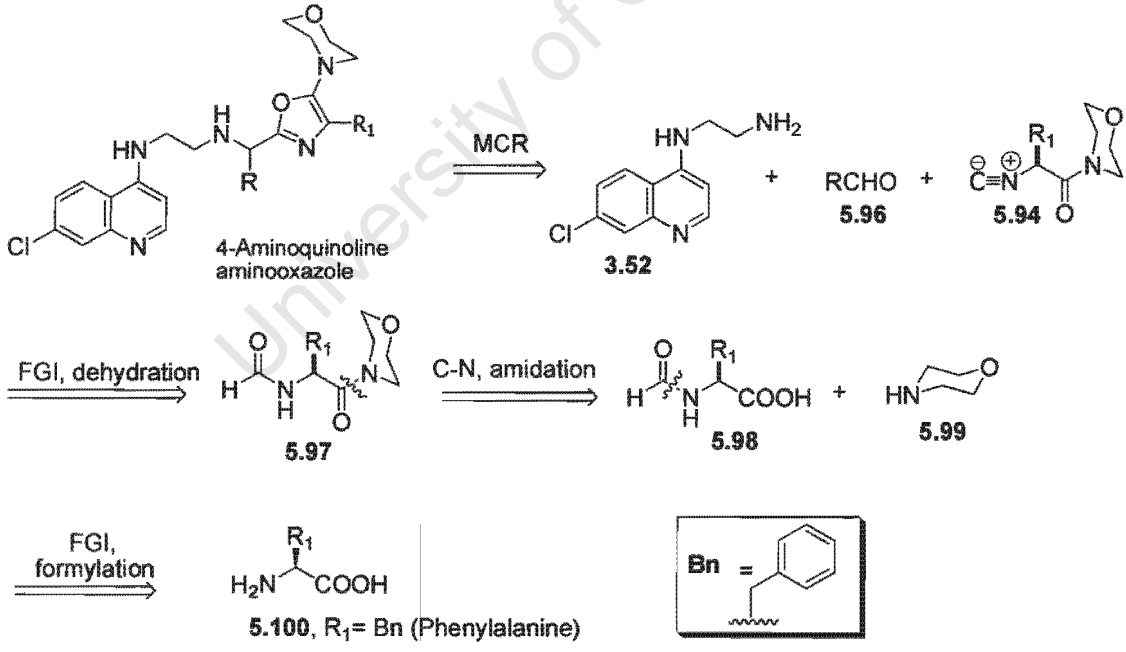
ii) Variation of the size of the alkyl chain in the aminoquinoline side chain, which may help circumvent resistance to chloroquine, and variable lipophilicity;

iii) Presence of the oxazole heterocyclic system, a bioactiphore found as a significant subunit in many natural products. Moreover, the presence of the secondary N in the side chain could be used as a diversity handle to introduce further chemical diversity in these series of compounds.

iv) Presence of basic sites that should aid in the accumulation of the compounds *via* pH trapping in the acidic food vacuole of the parasite, as well as improve aqueous solubility of the compounds *via* salt formation. Also, by increasing the number of H-bond donors and acceptors, these nitrogens may improve the aqueous solubility of these compounds.

5.6.2.1 Retrosynthesis of 4-Aminoquinoline-containing Oxazole

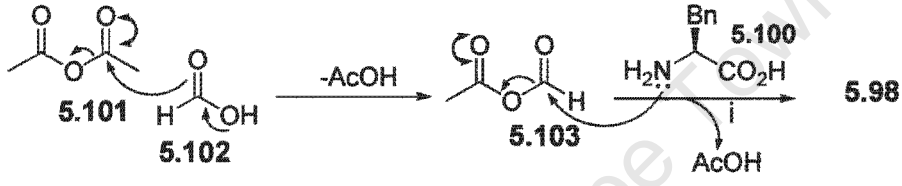
Scheme 5.24 shows the retrosynthesis of our envisaged target 4-aminoquinoline 2,4,5-trisubstituted amino oxazoles. Multicomponent condensation of **3.52**, aldehydes **5.96** and isocyanoacetamide **5.94** should lead to the desired target aminooxazoles. Analysis of isocyanoacetamide **5.94** *via* functional group interconversion (FGI) leads to the formamide **5.97**, which in turn leads to the formamide **5.98** and morpholine **5.99**. The latter, **5.98**, is easily obtained from L-phenylalanine **5.100** and an Ac_2O/HCOOH acid mixture.



Scheme 5.24: Retrosynthetic analysis of 4-aminoquinoline 2,4,5-trisubstituted aminooxazole

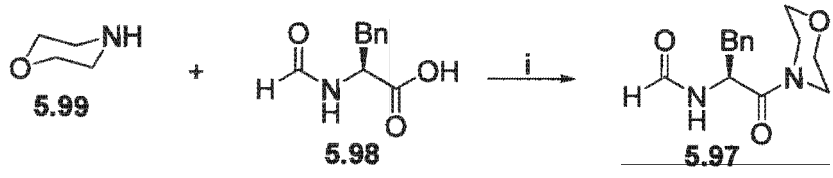
5.6.2.2 Synthesis of Isocyanoacetamide 5.94

The first step leading to the synthesis of the isocyanoacetamide **5.94** required for the multicomponent synthesis of oxazoles of the new 4-aminoquinoline aminooxazoles is depicted in Scheme 5.25. Formylation of L-phenylalanine was achieved by treating the amino acid with the mixed anhydride of formic acid and acetic anhydride at subzero temperature and subsequent elevation to ambient temperature.[55] Alternatively, **5.98** could be obtained in reproducible yield (80%) by refluxing the same reaction mixture over a 2 h period.



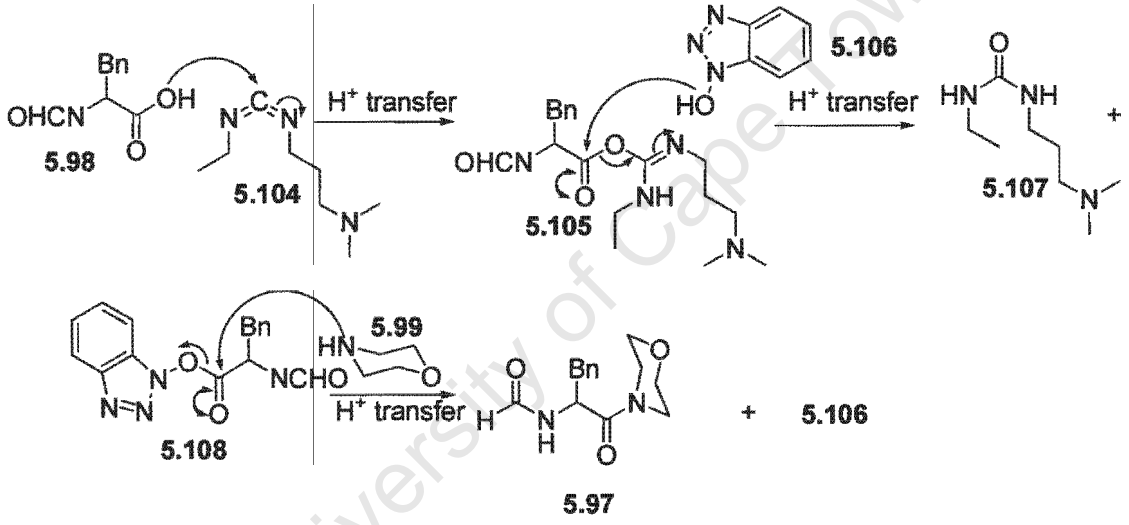
Scheme 5.25: *Reagents and conditions* i) $HCOOH/Ac_2O$, 0 – 25 °C, 26 h, 78%, or reflux, 2 h, 80%

Formic acid **5.102** in itself is not reactive enough to formylate the amino acid and so a more reactive species is required to mediate the conversion to the formamide **5.98**. Activation of formic acid **5.102** is thus achieved by treatment with acetic anhydride **5.101** to give the mixed anhydride **5.103** which is more reactive than formic acid. The reaction leading to the formation of the mixed anhydride is highly exothermic and thus external cooling of the reaction vessel is usually required. The mixed anhydride **5.103** in turn reacts with L-phenylanine **5.100** to furnish the formamide **5.98**.



Scheme 5.26: *Reagents and conditions* i) a) EDC.HCl, HOBt, DCM, rt, 90%, or, b) CDI, DCM, 100%

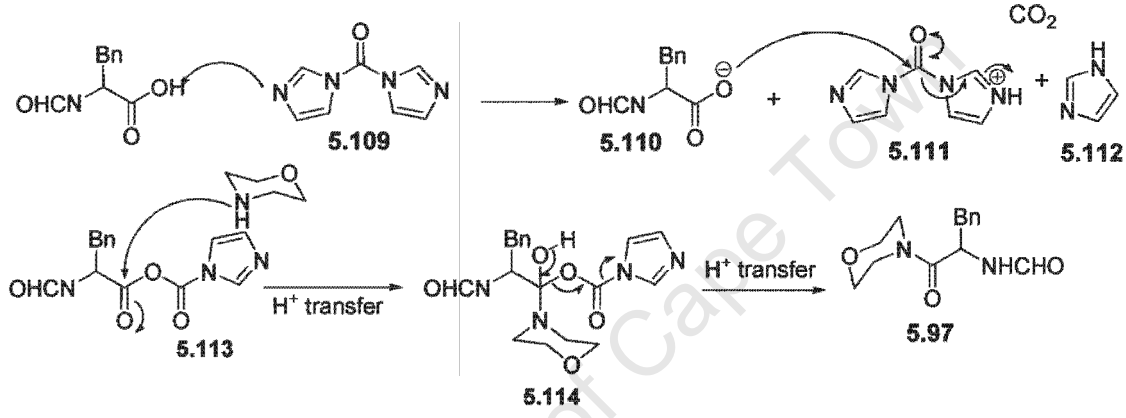
Having generated the formamide **5.98**, the next step was to couple its carboxyl group and the selected amine morpholine **5.99** in an amidation reaction. Use of *N,N*-dimethylaminopropyl carbodiimide (EDC)/hydroxybenzotriazole hydrate (HOBt)-mediated coupling between amines and carboxylic acids is a mild reaction that occurs in high yield[58] and accordingly was our first choice among the various known methods for peptide coupling, Scheme 5.26. The use of HOBt in this coupling reaction has the dual purpose of preserving chirality at the α-carbon as well as speeding up the amidation reaction. The mechanism for the coupling reaction is depicted in Scheme 5.27.



Scheme 5.27: Mechanism of EDC/HOBt-mediated peptide coupling

Reaction of the carboxyl group of **5.98** and EDC **5.104** leads to formation of the activated ester **5.105** which is then intercepted by HOBt **5.106** to form the urea derivative **5.107** and the new ester intermediate **5.108** and. In the absence of HOBt the ester **5.105** can be attacked by an amine, but this causes racemization at the α-carbon. In this case, however, **5.108** does not racemize mainly because the reaction is accelerated by the addition of HOBt. Attack by the amine nucleophile then occurs on the HOBt-ester **5.108** to give the nonracemic amide **5.97** and HOBt.

In the second approach, amide bond formation between the formamide **5.98** and morpholine **5.99** was mediated by carbonyldiimidazole (CDI), **5.109**.[57] The reaction conditions for this method are just as mild, the coupling being achievable at room temperature in anhydrous DCM. A 1.0 eq molar ratio of **5.109** and **5.98** was used in the reaction and the coupled product **5.97** was obtained in quantitative yield. The mechanistic events that lead to the formation of the amide are worthy of mention and are depicted in Scheme 5.28.



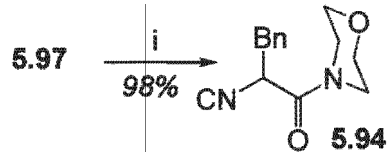
Scheme 5.28: Mechanism of CDI-mediated coupling between formamide **5.98** (MC128) and morpholine **5.99**

Carbonyldiimidazole (CDI) **5.109** deprotonates the carboxyl group of the formamide to generate the more nucleophilic carboxylate anion **5.110** and the more electrophilic imidazolium cation **5.111**. Nucleophilic attack on the imidazolium cation by the carboxylate anion then generates imidazole **5.112** and the corresponding activated ester intermediate **5.113**. The amine (morpholine) then comes in and attacks the activated ester **5.113** to generate the tetrahedral intermediate **5.113** that immediately collapses in an irreversible reaction liberating CO_2 and a second molecule of imidazole. The irreversible nature of the last step is inherent in the increased summation of the entropy of the products formed. That the CDI-mediated coupling reaction is feasible at a very high rate with the weakly nucleophilic carboxylate anion would suggest that other weak

nucleophiles could take part in the reaction. Indeed when H_2O, a weak nucleophile, is present in the reaction mixture it attacks a molecule of CDI in a similar fashion, immediately decomposing it to imidazole and CO_2. Thus, it was essential to ensure that both the solvent and the substrates were completely dried to avoid CDI decomposition into imidazole and CO_2.

Although this coupling method is less superior to that employing EDC/HOBt in that it induces epimerization at the chiral centre in the starting material,[58] subsequently, it was found to be more efficient in terms of both yield and time. Firstly, whereas coupling with EDC/HOBt required a reaction time of 12 h, the CDI-mediated reaction was complete within an hour. Secondly, isolation of the amide could be achieved simply by removing the solvent under reduced pressure and purifying the residue by means of flash chromatography. Although the imidazole by-product could be removed by aqueous work up, we found that the yield was significantly reduced when this was done. In any case the CDI-mediated epimerization is of little consequence as the stereogenic centre is destroyed anyway in the final target oxazole product.

Finally, conversion of **5.97** to the corresponding isocyanoacetamide **5.94** was achieved by dehydration as earlier described ($POCl_3$), affording the desired product in excellent yield (98%), (Scheme 5.29).

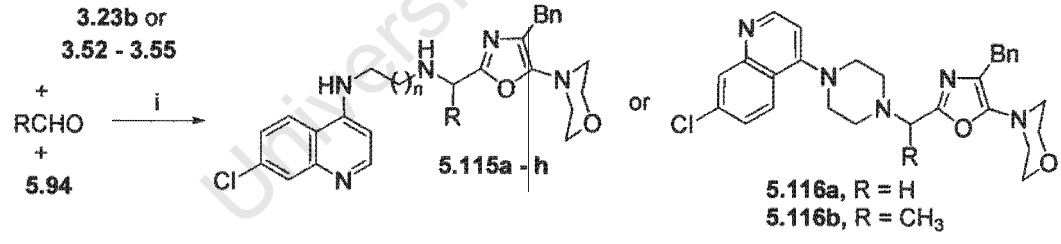


Scheme 5.29: *Reagents and conditions* i) Et_3N, $POCl_3$, CH_2Cl_2, 18 h

As in the previous case, conversion of the formamide group in **5.97** to the isocyano group was confirmed by both IR and ^{1}H NMR spectroscopy. In the former case loss of a peak around ~1680 cm^{-1} was accompanied by the appearance of a sharp peak in the IR spectrum at ~2150 due to C-N stretching of the isocyanide group, while in the ^{1}H NMR spectrum disappearance of the singlet at δ ~8.0 ppm due to the formamide proton was clear evidence of a successful conversion.

5.6.2.3 Chemical Synthesis of 4-Aminoquinoline-containing 2,4,5-Trisubstituted Aminooxazoles

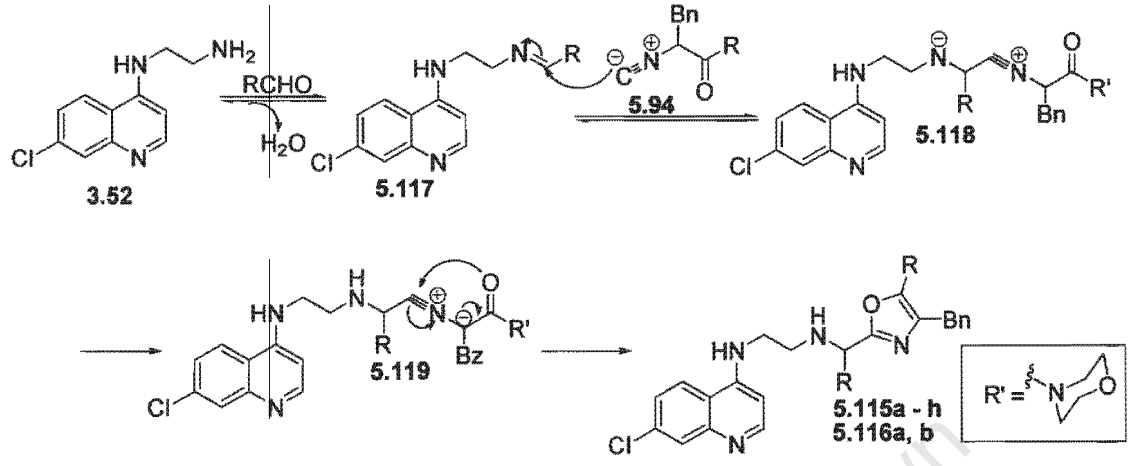
With the isocyanoacetamide **5.94** in place, efforts were now directed towards generation of an exploratory library of aminoquinoline-containing 2,4,5-trisubstituted aminooxazoles. The key amines **3.52 – 3.54** and **3.23b** were treated in parallel with either formaldehyde or CH_3CHO at 55 °C for 30 min to initiate formation of imines (Scheme 5.30). As before, the initial choice formaldehyde and CH_3CHO as the aldehyde inputs was governed by our desire to obtain low molecular weight compounds.



Scheme 5.30: *Reagents and conditions* i) MeOH, 55 °C, 1.5 h, then 60 °C

An hour after addition of isocyanoacetamide **5.94**, the temperature of the Carousel Reaction Station® on which the parallel synthesis was performed was elevated to 60 °C as the formation of the oxazoles was observed to be sluggish at the lower temperature (TLC).

5.6.2.3.1 Mechanistic Details

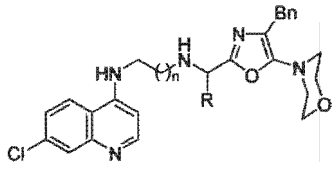
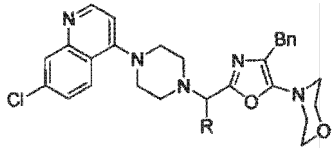


Scheme 5.31: Mechanism of formation of 2,4,5-trisubstituted aminooxazole

The plausible mechanism leading to the 2,4,5-trisubstituted oxazole ring is depicted in Scheme 5.31. Condensation of amine **3.52** and aldehyde (RCHO) furnishes the imine **5.117** with concomitant release of H_2O. Reaction of **5.117** with **5.94** yields the intermediate nitrilium **5.118**. Upon tautomerisation of **5.118** to **5.119**, which may be catalyzed by excess amine, cyclization occurs irreversibly to give the aminooxazoles **5.115** and **5.116**.

Yields of the 4-aminoquinoline-containing 2,4,5-trisubstituted aminooxazoles were generally good to excellent, the lowest yields being obtained for **3.55** with the two different aldehydes (Table 5.5).

Table 5.5: Isolated yields and purities of aminooxazoles **5.115a – j**

General Oxazole Structure	R	Product	n	Yield	*Purity
	H	**5.15a**	1	70	93
	"	**5.15b**	2	68	95
	"	**5.15c**	3	62	94
	"	**5.15d**	5	39	91
	CH_3	**5.15e**	1	68	96
	"	**5.15f**	2	51	97
	"	**5.15g**	3	67	96
	"	**5.15h**	5	43	94
	H	**5.16a**	–	93	99
	CH_3	**5.16b**	–	87	98

*HPLC Purity, conditions: Flow rate: 1.0 ml/min, UV 220 and 254 nm, 30% CH_3CN:70% 25 mM Phosphate buffer

Consistent with findings reported by Zhu *et al*, the two cases in which the secondary amine **3.23b** was employed gave the corresponding aminooxazoles in higher yields in comparison to primary amines; the purities of the reaction mixtures were also higher as evidenced by HPLC.[54] All compounds were purified by means of column chromatography on SiO_2 gel eluting with 2 – 10% MeOH:DCM. The isolated yields and HPLC purities of the aminooxazoles obtained from the parallel synthesis are shown in Table 5.5.

The presence of the NH group was confirmed by the appearance of weak, broad peaks near 3400 cm^{-1} in the respective IR spectra. These peaks were absent in the two aminooxazole spectra that resulted from the piperazine amine **3.23b**. Other peaks that could be unambiguously assigned to other functional groups and their corresponding absorbances included stretching vibrations due to aromatic C-H (~3020 cm^{-1}), aliphatic

C-H (~ 2970 cm^{-1}), aromatic C=C (1611 cm^{-1}), C=N (1582 cm^{-1}) and C-O (1223 cm^{-1}) groups.

All the new 4-amino-7-chloroquinoline-derived 2,4,5-trisubstituted aminooxazoles gave ^1H NMR spectra that were consistent with their proposed structures. Figure 5.13 shows the ^1H NMR spectrum of compound **5.15a** that is representative of this class of compounds. The spectra resulting from the two aldehyde inputs were by and large similar, save for the differences in the multiplicities of the H atoms next to the oxazole ring. When *para*-formaldehyde was used the methylene protons appeared as a singlet whereas, in the case of acetaldehyde a quartet resulted due to coupling with the methyl group.

Similarly, all compounds gave ^{13}C NMR spectral data that were consistent with the proposed structures. Two dimensional (2D) ^{13}C NMR (DEPT, COSY and HSQC) in combination with ^1H NMR spectroscopy were used to differentiate the carbons as being primary, secondary, tertiary or quaternary.

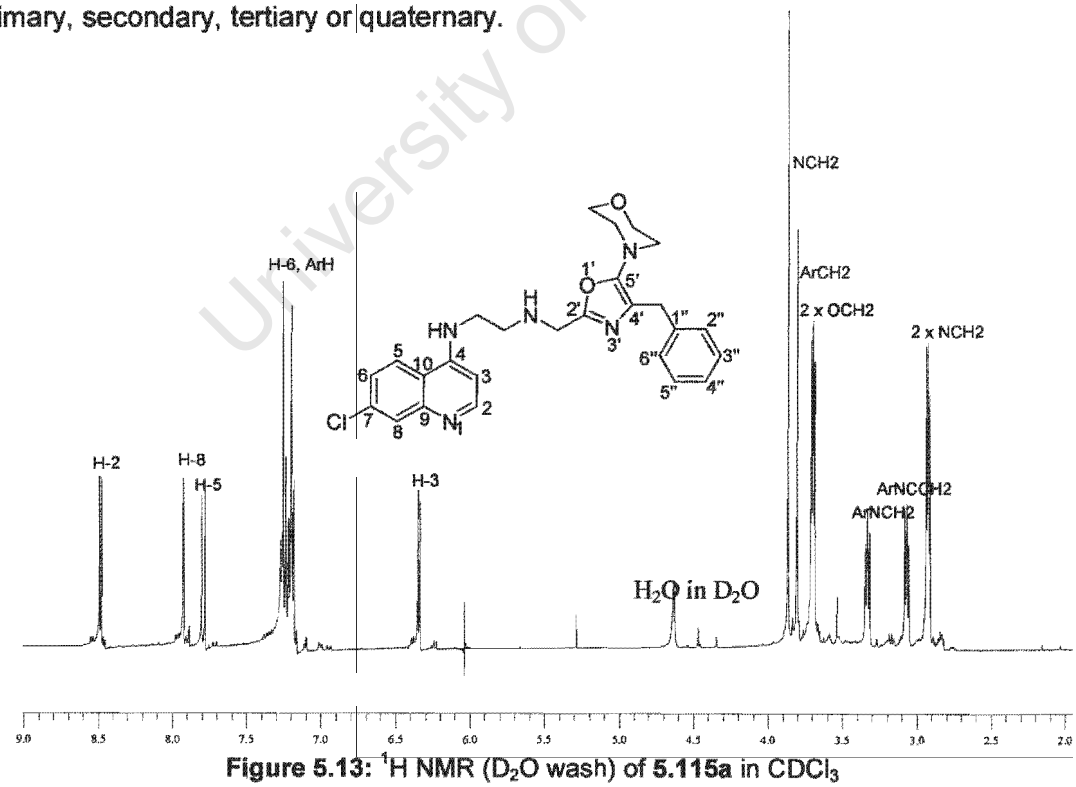


Figure 5.13: ^1H NMR (D_2O wash) of **5.115a** in $CDCl_3$

The accurate masses and molecular formulae of the proposed structures were determined by means of electron impact high resolution mass spectrometry and all compounds gave satisfactory results as per calculations. Finally, HPLC was used to determine the purities of the compounds for assay purposes.

5.7 Biological Results and Discussion

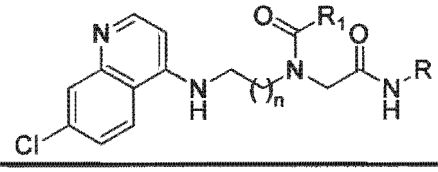
The biological activities of the compounds synthesized in this part of the project were determined at different laboratory facilities run by project collaborators. All antiplasmodial activities against the chloroquine-sensitive D10 *P. falciparum* strain were determined at the Division of Pharmacology, in the laboratories of Professor PJ Smith, (UCT), while those against the chloroquine-sensitive (3D7) *P. falciparum* and chloroquine-resistant (K1) *P. falciparum* strains, as well as *T. brucei* antitrypanosomal and cytotoxicity assays were conducted in the laboratories of Dr. V Yardley (LSHTM). Chloroquine-resistant W2 antiplasmodial and falcipain-2 inhibitory activities were conducted in the laboratories of Professor PJ Rosenthal (UCSF). Finally, antiplasmodial activities against the chloroquine-resistant FCM29 *P. falciparum* strain were determined in the laboratories of Professor P. Rassanaivo Madagascar at IMRA in Madagascar.

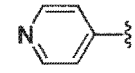
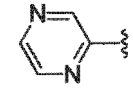
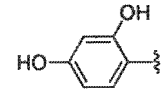
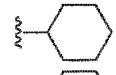
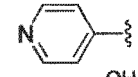
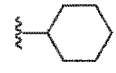
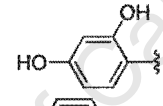
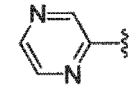
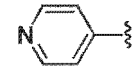
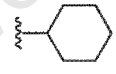
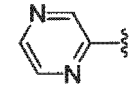
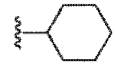
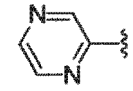
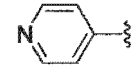
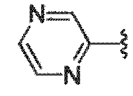
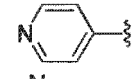
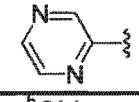
5.7.1 *In Vitro* Antiplasmodial Activities of 4-Aminoquinoline α-Acylamino Amides

The α-acylamino amides **5.23a – o** derived *via* the Ugi MCR were assayed against three different strains of *P. falciparum* at two different laboratories facilities. The results for the independent tests are presented in Table 5.6.

Table 5.6: Antiplasmodial activities of α-acylamino amides **5.23a – n**

General structure:



Compound	n	R	R_1	IC_{50} (μM) D10[a]	K1[b]	W2[b]
Chloroquine	–	–	–	0.037	0.57	0.24
5.23a	1			1.064	1.10	0.99
5.23b	1			0.712	1.13	1.19
5.23c	1			5.65	5.79	4.15
5.23d	1			0.54	0.53	0.62
5.23e	1			1.67	3.83	5.94
5.23f	2			>20	18.15	3.81
5.23g	2			1.38	2.30	1.65
5.23h	2			0.24	0.073	1.57
5.23i	2			0.24	0.52	0.88
5.23j	1			0.79	1.35	>10
5.23k	3			nd	nd	0.26
5.23l	3			nd	nd	0.22
5.23m	5			nd	nd	0.13
5.23n	5			nd	nd	0.17

[a]Chloroquine-sensitive; [b]Chloroquine-resistant

The compounds displayed moderate to significant antiplasmodial activities in all three strains of the malaria parasite with activity averaging in the single micromolar range. In the absence of data on D10 and K1 strains for **5.23k – n** compounds, **5.23i** was generally the most active against all three strains. Compounds **5.23k – n** were either as active as chloroquine (IC_{50} = 0.24 μM) or more active (though not significantly so) in the W2 strain, **5.23n**, the most active having an IC_{50} of 0.13 μM. Compound **5.23h** on the other hand exhibited very high activity in the D10 and K1 strains (0.24 and 0.073 μM), but was not as active in W2 (IC_{50} = 1.57 μM). **5.23d**, (IC_{50} = 0.62 μM) was nearly as active in W2 as in the other two strains (D10 IC_{50} = 0.52 μM and K1 IC_{50} = 0.53 μM).

In terms of the isocyanide input (R), compounds with the cyclohexyl ring were generally more active than those with *tert*-butyl group as seen with **5.23a** vs. **5.23d** (all 3 strains tested) and **5.23c** vs. **5.23e** (2 out of 3 compounds tested). The results for **5.23b** vs. **5.23j** are not conclusive as only one compound was more active, while the other two were equipotent.

The differently substituted carboxylic acid inputs gave variable results, although in all cases 2,4-dihydroxybenzamide substituted compounds showed the least activities within the different groups (consider **5.23a** vs. **5.23b** vs. **5.23c**; **5.23d** vs. **5.23e** vs. **5.23k**).

5.7.2 Discussion

Despite the generally lower antiplasmodial activities associated with these compounds with respect to chloroquine, the α-acylamino amides **5.23a – k** on average possess favourable resistance indices (Table 5.7). The resistance index is a measure of the cross resistance of a compound between two species of the parasite, determined as the ratio of the IC_{50} in the resistant strain to that in the sensitive strain. Ideally, compounds with low resistance indices or none are more favorable.

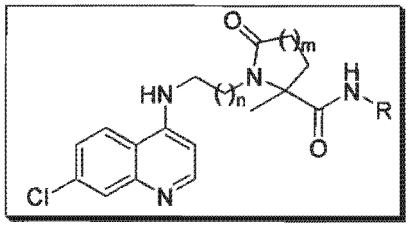
Table 5.7: Resistance Indices of α-Acylamino amides **5.23a – j**

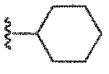
Compound	Resistance Index (K1)	Resistance Index (W2)
Chloroquine	15.5	6.5
5.23a	1	1
5.23b	1.6	1.7
5.23c	1	0.7
5.23d	1	1
5.23e	2.3	3.5
5.23f	nd	nd
5.23g	1.7	1.2
5.23h	0.3	6.6
5.23i	2	3.6
5.23j	nd	nd

Whereas chloroquine has resistance indices of 15.5 and 6.5 in K1 and W2 respectively, compounds **5.23a – j** generally have values far below those of chloroquine. This implies that the compounds have lower cross resistance between the chloroquine-sensitive and chloroquine-resistant strains. The only exception to this observation was compound **5.23h** in W2. In this regard, compounds **5.23a – d** and **5.23g** had the more favourable resistance indices across the different strains.

5.7.3 *In Vitro* Antiplasmodial Activities of 4-Aminoquinoline γ– and δ–Lactams

Table 5.8: *In vitro* Antiplasmodial activities of γ– and δ–lactams **5.25a – p**



Compound	m	n	R	W2 *P. falciparum* IC_{50} (μM)	Rec. F-2* IC_{50} (μM)
Chloroquine	–	–	–	0.24	–
5.25a	1	1		0.67	>10
5.25b	1	2	"	0.98	>10
5.25c	1	3	"	1.11	>10
5.25d	1	5	"	0.18	>10
5.25e	1	1		0.35	>10
5.25f	1	2	"	0.63	>10
5.25g	1	3	"	0.52	>10
5.25h	1	5	"	0.27	>10
5.25i	2	1		0.21	>10
5.25j	2	2	"	0.31	>10
5.25k	2	3	"	0.37	>10
5.25l	2	5	"	0.46	>10
5.25m	2	1		0.87	>10
5.25n	2	2	"	0.83	>10
5.25o	2	3	"	0.87	>10
5.25p	2	5	"	0.096	17.62

*Rec. F-2 = Recombinant Falcipain-2;

Initially all lactams **5.25a – p** were tested for their activities against parasite cultures of the chloroquine-resistant W2 strain of *P. falciparum* (IC_{50} chloroquine = 0.24 μM). It was found that the activities of the compounds were dependent upon the size of the lactam ring for compounds containing the same *N*-alkyl substituent. Thus, compounds containing the six-membered lactam ring were in general more active than those with the five-membered ring (Fig. 5.14). From the modest library, compound **5.25p** with and IC_{50} of 0.096 μM exhibited the best activity, being 2.5 times as potent in this strain as chloroquine.

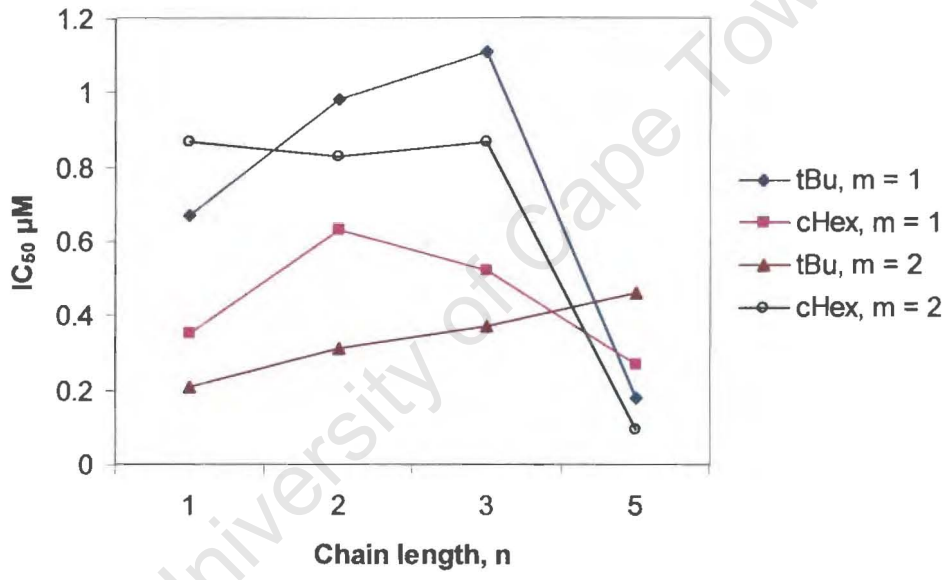


Figure 5.14: Graph correlating methylene spacer length (n) and IC_{50} values in γ- and δ-lactams.

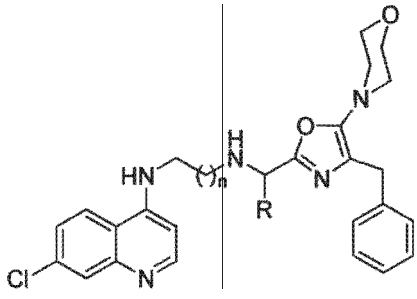
Compounds **5.25d** (IC_{50} = 0.18 μM), **5.25h** (IC_{50} = 0.27 μM) and **5.25i** (IC_{50} = 0.21 μM) exhibited comparable activities to chloroquine, while **5.26e** (IC_{50} = 0.35 μM) **5.26j** (IC_{50} = 0.31 μM) and **5.25k** (0.37 μM) exhibited slightly reduced activities. The remaining compounds on the other hand were less efficacious than the control drug. In terms of methylene spacer length, compounds with the 6-carbon spacer were generally the more

efficacious, while in the case of the five-membered γ-lactams, those containing the cyclohexyl ring turned out to be more potent than those with the *tert*-butyl group. On the contrary, the *tert*-butyl-containing δ-lactams were more efficacious than the cyclohexyl counterparts.

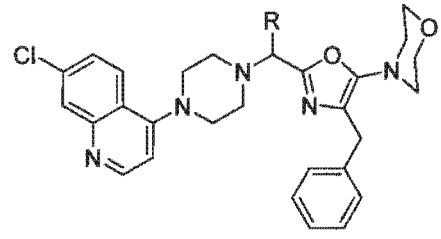
The generally modest activities of these compounds may be due to their overall reduced basicities. Whereas chloroquine accumulation in the acidic food vacuole of the parasite is dependent upon presence of two protonatable nitrogen atoms, the absence of a second protonatable nitrogen is likely to have implications for accumulation and hence antiplasmodial activity. Consequently, these lactams may be accumulating to much lower concentrations than those required to significantly inhibit plasmodial growth. Moreover, studies have revealed that chloroquine adopts a bioactive conformation in which the inter-nitrogen separation (N-(quinoline) – N-(diethyl)) of 0.83 Å[58] is equivalent to that of the central Fe atom and the two oxygens in the carboxylate groups of haem,[59] confirming that ferriprotopophyrin (haem) is the "receptor" target. Such similarity is unlikely to be present in these series of compounds in the absence of a second basic nitrogen in the side chain. Therefore, it may be possible that the modest antiplasmodial activities displayed by these compounds may result from their inability to interact with haem or from not being delivered to the requisite site of action within the acidic food vacuole. However, the reduced basicities of the compounds relative to chloroquine do not fully explain the observed antiplasmodial activities. Some compounds (such as **5.26d**, **5.26h** and **5.26i**) have comparable activities to chloroquine and yet they lack the terminal basic nitrogen, which is more critical for the accumulation in the acidic food vacuole. The varying degrees of activity of the compounds in W2, a chloroquine-resistant strain, support the assertion that *P. falciparum* resistance is compound specific. Indeed, as already mentioned in this thesis (section 1.6.1.1, pp. 16 & 17), modifications

to the side chain of chloroquine have led to derivatives which are able to circumvent the chloroquine resistance mechanism.[60] In any case, testing of these compounds in a sensitive strain would have been more informative. The most notable result from this limited series of compounds is that from **5.26p** (W2 IC_{50} = 0.096 μM, falcipain-2 IC_{50} = 17.7 μM). To our knowledge, this is the first report of an aminoquinoline lactam (albeit weak) inhibitor of falcipain-2. The antiplasmodial activity of **5.26p** may in part be due to the inhibition of falcipain-2, which may be attacking a carbonyl group of one of the amide bonds of this compound (**5.26p**) forming a tetrahedral intermediate like that depicted in Fig. 2.2 (section 2.1.1.2, p. 26).

5.7.4 *In Vitro* Antiplasmodial Activities of 4-Aminoquinoline Aminooxazoles



R = H, 5.115a - d;
R = CH_3.5.115f h.



R = H, 5.116a;
R = CH_3, 5.116b.

Table 5.9: Antiplasmodial Activities against Chloroquine-Sensitive 3D7 Strain

Compound	n	R	3D7 IC_{50}		Cytotoxicity		Ther. Index[a]
			µg/ml	µM	µg/ml	µM	
Chloroquine*	–	–	***0.01***	***0.02***			
5.115a	1	H	0.004	0.0084	40.0	83.8	9979
5.115b	2	"	0.02	0.041	51.8	105.5	2572
5.115c	3	"	0.04	0.079	25.3	50.0	634
5.115d	5	"	0.28	0.15	8.9	16.7	111
5.116a	–	"	0.26	0.52	43.1	85.7	165
5.115e	1	CH_3	0.06	0.12	11.3	23.0	192
5.115f	2	"	0.04	0.079	44.0	87.0	1102
5.115g	3	"	0.002	0.0038	15.5	29.9	7868
5.115h	5	"	0.01	0.018	6.5	11.9	660
5.116b	–	"	0.27	0.14	26.9	52	371

[a]Ther.. Index = Therapeutic Index; *ED50 of the diphosphate salt of chloroquine.

The antiplasmodial activities of compounds **5.115a – h**, **5.116a** and **5.116b** against the chloroquine-sensitive and chloroquine-resistant 3D7 and K1 strains respectively, as well as cytotoxicity were determined in the laboratories of Dr. Vanessa Yardley at the London School of Hygiene and Tropical School (London, UK).

From the results in Table 5.9, compound **5.115g** was the most active in the chloroquine-sensitive 3D7 strain which, with an ED_{50} = 0.0038 µM, was 5 times more efficacious than chloroquine (ED_{50} = 0.02 µM). Also of note was **5.115a** (ED_{50} = 0.0084 µM) which was

also active in the low nanomolar range. On the other hand, **5.225b** exhibited slightly weaker potency at ED_{50} = 0.041 μM than chloroquine although not significantly so, while **5.115c** (ED_{50} = 0.0.079 μM), **5.115d** (ED_{50} = 0.15 μM), **5.115f** (ED_{50} = 0.15 μM) and **5.116a** (ED_{50} = 0.14 μM) exhibited weakened antiplasmodial activities in comparison to chloroquine. Interestingly, the introduction of a methyl substituent into the side chains of compounds **5.115a** and **5.115b** caused reductions in the activities of the corresponding derivatives **5.115e** and **5.115f** respectively. In contrast, the introduction of a methyl substituent into the side chains of **5.115c** and **5.115d** resulted in significant improvements in the efficacies of the resulting compounds **5.115g** and **5.115h**. With ED_{50}s of 0.0038 μM and 0.018 μM respectively, **5.115g** and **5.115h** exhibited the best potencies, showing 21-fold and 8-fold improvements in efficacy against the chloroquine-sensitive strain. Compound **5.115d** (ED_{50} = 0.52 μM), the least active in the sensitive strain, was only active at high nanomolar concentration. All compounds displayed favourable therapeutic indices. However, these results are not quite as remarkable, as the strain used here is susceptible to chloroquine. Ideally, chloroquine-derived compounds displaying high antiplasmodial activity and favorable (minimal) therapeutic indices across chloroquine-resistant parasites are most sought after.

Table 5.10: Antiplasmodial Activities against Chloroquine-Resistant K1 Strain

Compound	n	R	K1 IC_{50} Expt 1 (µg/ml)	Expt 1 (µM)	Expt 2 (µg/ml)	Expt 2 (µM)
Chloroquine	–	–	*0.13*	*0.25*	*0.53*	*1.03*
5.115a	1	H	0.11	0.23	–	–
5.115b	2	"	0.07	0.14	–	–
5.115c	3	"	0.05	0.099	–	–
5.115d	5	"	–	–	0.06	0.11
5.116a	–	"	–	–	***0.18***	***0.36***
5.115e	1	CH_3	–	–	***0.13***	***0.25***
5.115f	2	"	–	–	0.08	0.16
5.115g	3	"	–	–	0.010	0.019
5.115h	5	"	–	–	0.020	0.037
5.116b	–	"	–	–	***0.5***	***0.97***

Although the results from the independent experiments against the chloroquine-resistant K1 strain in Table 5.10 reveal a comparatively higher ED_{50} value for the standard control drug chloroquine, it must be stated that the value (1.03 µM) is within range and that the results for other compounds are comparable as the conditions in which these experiments were conducted were similar.

The results in Table 5.10 reveal that all compounds were more active than chloroquine in the K1 resistant strain. In the first experiment, chloroquine had ED_{50} = 0.25 µM whereas the 2 out of the 3 compounds tested along with the control drug, namely **5.115b** and **5.115c** were both more potent than chloroquine with respective ED_{50}s of 0.23, 0.14 and 0.099 µM. In the second experiment, compounds **5.115d – 5.115h** were several orders of magnitude more active than chloroquine. In fact, **5.115f**, the least active among the three (ED_{50} = 0.16 µM) was 6 times more potent than chloroquine (ED_{50} = 1.66 µM), while **5.115g** (ED_{50} = 0.019 µM) was 54 times more efficacious than chloroquine and exhibited the greatest potency.

The results suggest that the introduction of a methyl substituent in **5.115b** (ED_{50} = 0.14 μM) to yield **5.115f** (ED_{50} = 0.16 μM) had no effect on the antiplasmodial activity, whereas a similar change for **5.115c** (ED_{50} = 0.099 μM) resulted in significantly improved efficacy for compound **5.115g** (ED_{50} = 0.019 μM). A similar increase in potency was seen in the analogous compounds **5.115d** (ED_{50} = 0.11 μM) and **5.115h** (ED_{50} = 0.037 μM). It was also observed that compound **5.115f** with an alicyclic side chain was nearly four times as active as the piperazinyl derivative **5.116b** in the resistant strain.

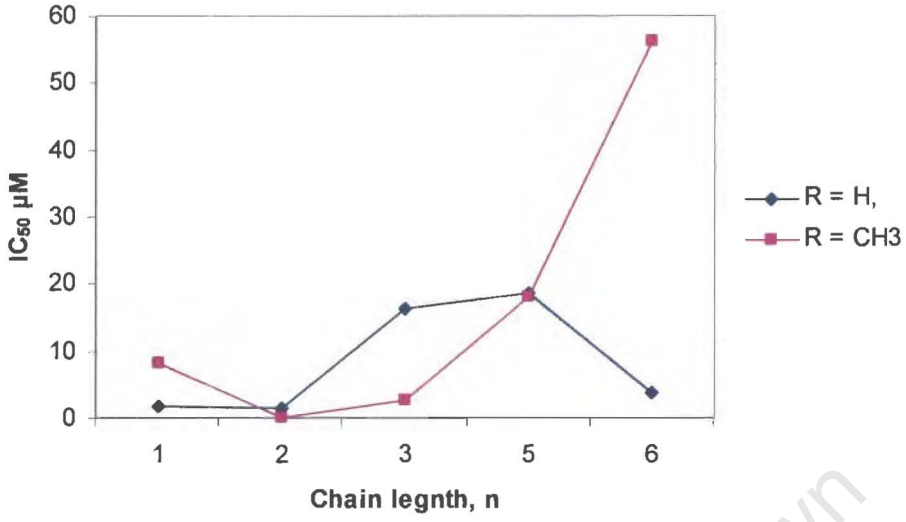
Table 5.11: Antiplasmodial activities against chloroquine-resistant FCM29 strain

Compound	n	R	FCM29 IC_{50} (μg/ml)	FCM29 IC_{50} (μM)	Resistance Index[a]
Chloroquine	–	–	*0.051*	*0.16*	5.1 (0.1)
5.115a	1	H	0.50	1.72	7.5
5.115b	2	"	0.56	1.56	38
5.115c	3	"	4.64	16.43	208
5.115d	5	"	5.94	18.61	124
5.116a	–	"	1.21	3.71	7.1
5.115e	1	CH_3	1.42	8.30	69
5.115f	2	"	0.00156	0.0035	0
5.115g	3	"	0.48	2.68	705
5.115h	5	"	3.83	17.92	996
5.116b	–	"	4.41	56.14	401

[a]Resistance indices calculated based on 3D7 results shown in Table 5.9

From the results shown in Table 5.11, it can be seen that all but one compound **5.115f** IC_{50} = 0.0035 μM (3.5 nM) were less active than chloroquine in the chloroquine-resistant FCM29 strain, with activity ranging from single digit nanomolar range to low midmicromolar range.

Figure 5.15 shows the relationship between the size of the methylene spacer and antiplasmodial activity in this strain.



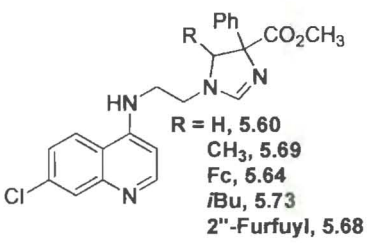
*Represents compounds **5.116a** and **5.116b**

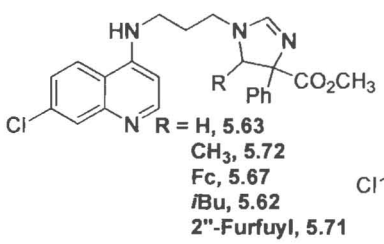
Fig. 5.15: Correlation of chain length n to antiplasmodial activity in the FCM29 strain

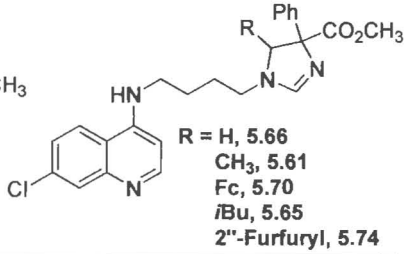
According to Fig. 5.15, the consequence of altering the length of the carbon spacer in the lateral side chain was not uniform in either the unsubstituted or methyl-substituted derivatives. In the unsubstituted compounds **5.115a – d**, the two compounds with the 2 and 3-carbon spacers were the more active, while this activity was lost in the 4 and 5-carbon-spaced compounds. On the contrary, the methyl-substituted compound **5.115e** with the 2-cabon spacer was not as active as the 3-carbon spaced compound **5.115f**. The lowest activity was observed with the piperazinyl derivative **5.115c**.

5.7.5 *In Vitro* Antiplasmodial Activities of 4-Aminoquinoline 2-Imidazolines

Table 5.12: *In Vitro* Antiplasmodial Activities of compounds **5.60 – 5.74**







Compound	3D7 ED_{50}		K1 ED_{50}		Cytotoxicity		Therapeutic Index	
	(μg/ml)	(μM)	(μg/ml)	(μM)	(μg/ml)	(μM)	a	b
Chloroquine	***0.01***	***0.02***	***0.53***	***1.03***				
5.60	0.14	0.34	0.20*	0.49*	239.9	587.8	1722	1200
5.69	0.02	0.047	0.23	0.54	41.8	99.0	2106	183
5.64	0.03	0.051	0.17	0.29	24.4	41.2	808	142
5.73	0.02	0.043	0.07	0.15	37.7	81.2	1889	541
5.68	0.04	0.084	0.18*	0.38*	52.8	111.4	1326	293
5.63	0.04	0.095	0.41*	0.97*	47.7	112.3	1182	116
5.72	0.01	0.023	0.30*	0.69*	22.9	52.5	2283	76
5.67	0.002	0.0033	0.02	0.033	5.1	8.4	2550	255
5.62	0.003	0.0063	0.04	0.084	22.4	46.8	7435	557
5.71	0.002	0.0041	0.19*	0.38*	8.6	17.6	4297	46
5.66	0.001	0.0023	0.20*	0.46*	48.4	111.0	48246	241
5.61	0.01	0.022	–	nd	6.3	14.0	636	–
5.70	0.0003	0.00048	0.01	0.016	1.6	2.6	5375	163
5.65	0.001	0.0020	0.01	0.020	3.0	6.1	3047	305
5.74	0.02	0.04	0.22	0.44	12.5	24.9	622	57

*Chloroquine (diphosphate salt of) ED_{50} was determined as 0.08 μg/ml (0.16 μM); Therapeutic Index **a** = Cytotoxicity/3D7 ED_{50}; Therapeutic Index **b** = Cytotoxicity/K1 ED_{50}.

Table 5.12 shows the results obtained against the chloroquine-sensitive 3D7 and chloroquine-resistant K1 strains; the compounds were tested at the LSHTM (Dr. V. Yardley) as diastereomeric mixtures.

From the results shown in Table 5.12, it is apparent that most compounds indeed show great promise as antiplasmodial agents as most had activities comparable to chloroquine or even better. In two separate independent experiments, the chloroquine

ED_{50} ranged from 0.01μg/ml (0.02 μM) – 0.53 μg/ml (1.03 μM), whereas in a third experiment (compounds marked with asterisk '*'), the ED_{50} of chloroquine was 0.08 μg/ml (0.16 μM). When the compounds were tested against 3D7, it was found that 7 out of 15 compounds possessed greater potencies in comparison to chloroquine (ED_{50} chloroquine = 0.02 μM). With an ED_{50} of 0.34 μM, **5.60** was the least active, exhibiting 17 times less efficacy than chloroquine and was the only compound whose activity did not approach that of the standard drug. As can be seen from Table 5.12, the next least active compound, **5.63** (ED_{50} = 0.095 μM) was 5 times less active than chloroquine. On the other hand, the most potent compound in this assay, **5.70** (ED_{50} = 0.00048 μM), showed a 42-fold improvement in activity over chloroquine in 3D7. In terms of SAR, compounds with the shortest ethylene spacer **5.60**, **5.64**, **5.68**, **5.69** and **5.73** were generally less efficacious than those with the longer carbon spacers (in the chloroquine-sensitive strain); in fact the former were all less active than chloroquine (including **5.63** and **5.74**). Furthermore, compounds possessing a substituent at the 5'-position were generally more active than unsubstituted compounds except for **5.74**; notably, compounds with the ferrocenyl (**5.64**, **5.67** and **5.70**) and isobutyl groups (**5.65**, **5.71** and **5.73**) were the most active among their respective groups. Another general trend that was seen was that compounds with the longer 4-carbon spacer **5.66**, **5.61**, **5.70**, **5.65** and **5.74** were the more active within their respective groups. As can be seen from the table, all compounds show favourable resistance indices, with the most active compound **5.70** being 5375 times more cytotoxic to the parasite than the mammalian KB cells used in the cytotoxicity assays. Even more notable was compound **5.66** whose cytotoxicity towards the parasite over the mammalian cells was over 48 000. Again, these results are not striking in view of the susceptibility of the parasite strain to chloroquine.

Against the chloroquine-resistant K1 strain, the results show that most compounds had activities comparable to those of chloroquine except **5.60**, **5.68**, **5.63**, **5.72**, **5.71** and **5.66**. The remaining compounds all showed greater potencies than chloroquine. Ferrocenic compound **5.70** (ED_{50} = 0.016 μM) and isobutyl compound **5.65** (ED_{50} = 0.02 μM) were again the most potent in the resistant strain within the modest library.

A similar trend in activity in K1 as seen in 3D7 with regard to the size of the carbon spacer between the side chain nitrogens was only observed for compounds **5.64** vs. **5.67** vs. **5.70** and **5.73** vs. **5.62** vs. **5.65**. Among these compounds, **5.70** and **5.65** showed the best activities. These two compounds were >100 and >80 times more active in the chloroquine-resistant strain than the standard drug chloroquine respectively. Despite their lower antiplasmodial activities, compounds **5.69**, **5.64** and **5.73** all exhibited several hundred-fold greater efficacy than chloroquine (IC_{50} = 1.03μM). The therapeutic indices reveal that the compounds were generally nontoxic to the mammalian KB cell line used in the assay. The low toxicities associated with the compounds are manifest in the high therapeutic indices obtained, from which it can be seen that the majority of compounds are over 100 times more cytotoxic to the parasite than the human cell line. Considering the more active ferrocenyl and isobutyl-substituted compounds, it is noted that the former **5.64**, **5.67** and **5.70** are generally more cytotoxic than the latter **5.73**, **5.62** and **5.65**, Table 5.13 below shows the resistance indices associated with these compounds.

Table 5.13: Resistance Indices of compounds **5.60 – 5.74**

Compound	Resistance Index	Compound	Resistance Index
Chloroquine	53.5 (8.0*)	**5.67**	10.0
5.60	1.4	**5.68**	4.5*
5.61	–	**5.69**	11.5
5.62	13.3	**5.70**	33.3
5.63	10.2*	**5.71**	9.3*
5.64	5.7	**5.72**	30.0*
5.65	10.0	**5.73**	3.5
5.66	200*	**5.74**	2.0

The two resistance indices for chloroquine appearing in Table 5.13 are representative of the two different ED_{50} values obtained for the drug in the resistant K1 strain. Resistance indices bearing an asterisk are in comparison with the value for chloroquine given in parenthesis. It is apparent that the majority of compounds possess high resistance indices despite having lower values than those associated with chloroquine. Ideally, a favourable antiplasmodial compound should possess a resistance index that approaches unity, so that it should be active against both the resistant and susceptible strains. In this regard, the most favourable compounds were **5.60** and **5.73**. The four most active compounds against the K1 parasite **5.67**, **5.62**, **5.70** and **5.65** had resistance indices that were lower than that obtained for chloroquine (53.5), with **5.70** having the least favourable index of 33.

5.8 Discussion: Oxazoles and 2-Imidazolines

The improved activities of the novel 4-aminoquinoline 2,4,5-trisubstituted aminooxazoles and 4-aminoquinoline-2-imidazolines may be speculated to be a result of several factors. It is known that the 4-amino-7-chloroquinoline subunit is an antimalarial pharmacophore

that inhibits haem dimerization (biomineralization) into nontoxic haemozoin.[19] Therefore, the primary antiplasmodial activities associated with these compounds may be arising from inhibition of haem biomineralization causing a build up of toxic haem, which may be resulting in parasite death. It is also possible that the increase in basicity caused by the side chain NH and the oxazole N on the one hand and the two nitrogens of the imidazoline ring on the other may be improving the accumulation of the compounds in the acidic food vacuole of the parasite *via* pH trapping. The presence of the heterocycle in the lateral chains may also be resulting in secondary interactions with other targets apart from haem, resulting in the observed antiplasmodial activities. The C=N double bonds of the 2-imidazoline and oxazole rings are also both potential electrophilic sites of attack by the major malarial proteases; therefore these compounds may be acting as mechanism-based inhibitors which when attacked at the C=N bond by the proteases may be ultimately resulting in parasite death.

Considering the ferrocenyl compounds **5.64**, **5.67** and **5.70**, whose activities seem to be superior to those of their counterparts, one may speculate that the Fe in ferrocene present as Fe^{2+} (ferrous iron) may be catalyzing a Fenton-like reaction once inside the intraerythrocytic vacuole, which may be resulting in the generation of hydroxy radicals as follows:

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + HO^- + HO^{\cdot}$$

However, as previously deiscussed (section 4.2.5.1, p128), the ferreocene iron is unlikely to be involved in the Fenton reaction as it is cooridinatively saturated. It may also be possible that the increased lipophilicities of these compounds compared to chloroquine is favouring their transport across the parasitic cell membrane (Table 5.14), causing much increased concentrations within the parasite.

The lipophilicity may also be important in circumventing chloroquine resistance for a number of the described oxazoles and imidazolines found to be more potent than chloroquine against resistant strains. It is known that the chloroquine resistance mechanism is much less effective for certain classes of aminoquinolines, as already mentioned. Within the context of lipophilicity, the chloroquine resistance mechanism does not seem to recognize lipophilic compounds.[61] For example, quinolines such as mefloquine are more lipophilic than 4-aminoquinolines such as chloroquine. It may be suggested that mutations in the *pfcrt* gene, the gene implicated in chloroquine resistance, may not favour or recognize more lipophilic chloroquine-based 4-aminoquinolines such as the oxazoles and imidazolines described in this thesis.

Table 5.14: 'Lipinski's Rule of 5' properties of select 4-aminoquinoline-2-imidazolines

Compound	CLogP	H-Bond Donors	H-Bond Acceptors	Molecular Weight	No. of Criteria Met
Chloroquine	***5.0***	***1***	***2***	***319.2***	***4***
5.60	5.4	1	4	408.1	4
5.62	7.7	1	4	478.2	3
5.64	>7.9	1	4	592.1	2
5.65	7.6	1	4	492.2	3
5.67	>8.1	1	4	606.1	2
5.69	5.9	1	4	422.2	3
5.70	>8.0	1	4	620.2	2
5.73	7.4	1	4	464.2	3

As is apparent from the table, the ferrocenyl compounds are highly lipophilic in comparison to chloroquine. These greater lipophilicities may be influencing the permeabilities of the compounds across the parasite's cell membrane thereby reaching the intended site of action in high concentration, a factor that may be promoted by the presence of the three protonatable nitrogen atoms within the compounds. More

interestingly, all the isobutyl substituted compounds (**5.62**, **5.65** and **5.73**) meet three out of the four criteria of Lipinski's rule of 5, are quite lipophilic and active across both strains. Therefore, it is tempting to suggest that lipophilicity may be playing a significant role in antiplasmodial activity. Lastly, the different substitutions at the R position of the 2-imidazoline ring may be favoring hydrophobic interactions with unknown receptors and/or proteins, which may be leading to malfunction of parasitic functions. This is seen with the more lipophilic ferrocene and isobutyl derivatives.

5.9 *In Vitro* Antitrypanosomal activities of 4-aminoquinoline2-Imidazolines

Table 5.15: *In vitro* activities *T. b. brucei* (see Table 5.12 for structures, p. 213)

Compound	% Inhibition (μg/ml)		ED_{50} μg/ml	ED_{50} μM	Cyt. ED_{50} μg/ml	Cyt. ED_{50} μM	TI*
	3.33	1.11					
Pentamidine	–	–	>0.0001	>0.0003	–	–	–
Podophyllotoxin	–	–	0.002	–	0.00024	–	–
5.60	3.8	1.96	13.94	34.2	239.9	587.8	17
5.69	92.2	27.0	1.83	4.3	41.8	99.0	23
5.64	96.5	63.0	1.19	2.0	24.4	41.2	21
5.73	96.4	90.8	1.12	2.7	37.7	81.2	30
5.68	85.5	5.4	2.25	4.7	52.8	111.4	24
5.63	5.6	0.8	4.88	11.6	47.7	112.3	10
5.72	98.3	56.1	1.40	3.2	22.9	52.5	16
5.67	98.7	97.7	0.43	0.07	5.1	8.4	120
5.62	98.7	98.0	0.61	1.3	22.4	46.8	36
5.71	92.4	64.4	1.81	3.7	8.6	17.6	5
5.66	64.1	2.1	3.93	9.0	48.4	111.0	12
5.61	98.0	88.4	1.06	2.4	6.3	14.0	6
5.70	96.8	97.4	0.43	0.7	1.6	2.6	4
5.65	97.3	97.4	0.38	0.8	3.0	6.1	8
5.74	94.1	49.2	2.48	4.9	12.5	24.9	5

Cyt. = Cytotoxicity; *TI = Therapeutic Index.

The compounds were tested at four different concentrations: 30, 10, 3.3 and 1.1 μg/ml and the ED_{50}s determined afterwards. All compounds completely inhibited parasite growth at the two high concentrations, but on further testing the compounds at 1.1 μg/ml,

only five compounds inhibited parasite growth by at least 90%. Determination of the ED_{50}s identified the ferrocenyl compound **5.70** as the most potent (ED_{50} = 0.070 μM), which besides this encouraging activity remains far less superior to the efficacy of the control drug pentamidine. Encouragingly though, **5.70** exhibits good selectivity of parasite cytotoxicity over the mammalian KB cell line used in this assay as is revealed by its high therapeutic index of 120. Since *T. brucei* exists in the bloodstream as extracellular trypomastigotes, the problems that are usually associated with drug penetration of the macrophage are absent and the compound is more likely to reach its site of action. In this regard **5.70** is a potential valid lead compound.

5.10 Conclusions and Recommendations for Future Work

The multicomponent reaction strategy has been exploited to synthesize new compounds in which diversity is introduced in a single step. In this project, multicomponent reactions were utilized to quickly introduce diversity into the lateral chain of chloroquine derivatives giving rise to a number of different classes of new and novel compounds. Using the MCR strategy, we have synthesized and shown that the different 4-aminoquinoline compounds herein are active both as antiplasmodial and antitrypanosomal agents. The three biologically significant classes of compounds identified in this project were those containing the lactam, oxazole and 2-imidazoline heterocyclic ring systems in the side chains, and each class is discussed separately below:

(a) 4-Aminoquinoline γ- and δ-Lactams

Although only one compound **5.25p** was identified as being more potent than chloroquine in the *P. falciparum* chloroquine-resistant strain (W2), the whole series of compounds may be reinvestigated in view of the absence of a second protonatable nitrogen within the basic molecular framework. As such, new compounds can be

synthesized in which the isocyanide or ketoacid input bears an inherently basic nitrogen atom. Alternatively, the second nitrogen may be introduced *via* post condensation modification of the lactams by known reactions, such as the Mannich reaction. Also, the amide bonds may be stabilized (post multicomponent reaction) by converting them into thioamides by treatment with Lawesson's reagent. It would also be of significance to test the compounds in different strains of the parasite before the compounds can be dismissed as being not favourably active.

(b) **4-Aminoquinoline-2,4,5-Trisubstituted Oxazoles**

The most active compound in this limited series was **5.115f** (IC_{50} = 0.0035 μM) in FCM29 and, although not the most active in the chloroquine-resistant K1 strain (IC_{50} = 0.16 μM), it was 6 times more potent than chloroquine. Based on this compound, it would be interesting to carry out extensive SAR studies in which the aldehyde inputs are varied (and also ketones used) to increase diversity at this position. Secondly, both the amine and amino acid inputs may be altered with a view to increasing the number of isocyanoacetamide inputs in the reactions leading to the new oxazoles. Post multicomponent reactions may also be carried out on this compound (and new relatives thereof) at the secondary nitrogen atom to improve diversity. More importantly, since antiplasmodial activity may reside in one enantiomer and/ or diastereomer, it would be interesting to resolve the racemates and/or diastereomeric mixtures into their respective enantiomers and/or diastereomers and have them tested against a horde of *P. falciparum* strains. It would also be interesting to establish the antimalarial activity of the most active compound(s) *in vivo*.

(c) 4-Aminoquinoline-2-imidazolines

Within the series of the new 4-aminoquinoline-2-imidazolines, four compounds were identified as potential leads (Fig. 5.16). To get a more comprehensive picture of the role of the substituent in the 5′-position of the heterocyclic (2-imidazoline) ring, more compounds could be synthesized which bear different substituents, including those that are disubstituted (from ketones). As with the other compounds, it would be worthwhile to separate the 2-imidazolines into respective enantiomers and diastereomers, or to employ asymmetric synthesis in their synthesis and have these tested again *in vitro*. This should lead to more conclusive data as biological activity may be inherent in only one of two enantiomers and/or diastereomers.

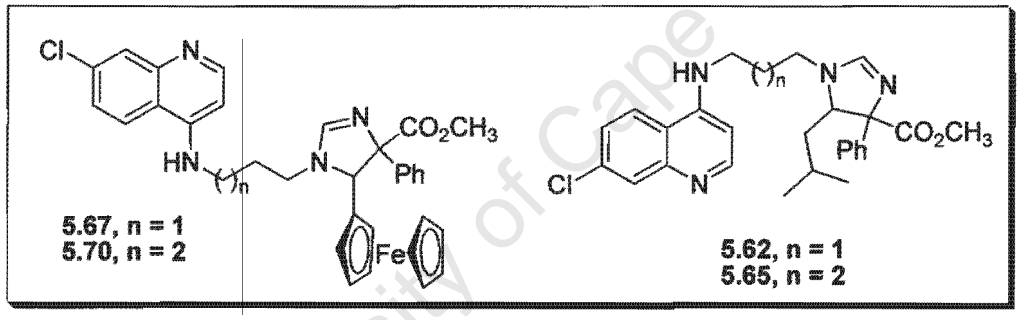


Figure 5.16: Structures of the most active 4-aminoquinoline-2-imidazolines

The compounds may be further explored by removing the methyl ester ($-CO_2CH_3$) group at the 4′-position *via* saponification and subsequent decarboxylation, or alternatively, by introducing diversity at this position through other known chemical transformations of the ester group. As before, it would be interesting to determine the *in vivo* antiplasmodial activities of the most active compounds on both the free bases as well as on the more water-soluble salt derivatives.

Conclusion

Overall, as the adage goes "the most fruitful basis for the discovery of a new drug is to start with an old one", we have successfully employed the 4-amino-7-chloroquinoline moiety to discover new antiplasmodial and antitrypanosomal agents using simple and relatively cheap chemical transformations. Since the 4-amino-7-chloro-quinoline template forms the basis of drug design and synthesis in this and the previous two chapters, the question of potential development of parasite resistance to the new compounds is addressed here. The 4-amino-7-chloroquinoline subunit has come to be accepted as a structural recognition motif for chloroquine resistance in *P. falciparum*, thus the likelihood of the quick development of resistance to the compounds presents a major threat to further development of lead compounds identified here. However, it is hoped that the derivatization of the lateral side chain in these novel compounds will lead to interactions with other unknown targets unlike those associated with chloroquine. In so doing, the compounds would then escape the mechanism by which chloroquine is rendered inactive in these chloroquine-resistant parasites. Whether these new 4-amino-7-chloroquinoline derivatives are acting by different mechanisms to that of chloroquine is an issue that can only be determined by, and is left to further experiment.

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CHAPTER 6

EXPERIMENTAL

All commercially available chemicals were purchased from either Sigma-Aldrich or Merck, South Africa, unless otherwise stated. Reactions were monitored by thin layer chromatography using Merck F_{254} aluminium-backed precoated silica gel plates and were visualized by one of the following: ultraviolet light, iodine vapour, ninhydrin, cerric ammonium nitrate or anisaldehyde. Silica gel chromatography was performed using Merck kieselgel 60: 70-230 mesh for gravity columns and 230-400 mesh for flash chromatography. Melting points were determined on a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Thermo Nicolete FTIR instrument in the 4000 – 600 cm^{-1} range as chloroform or methylene chloride solutions, nujol mulls or as KBr discs. Microanalyses were determined using a Fisons EA 1108 CHNO-S instrument. Mass spectra were recorded on a VG micromass 16F spectrometer operating at 70eV with an accelerating voltage of 4kV and a variable temperature source. Accurate masses were determined using a Kratos limited MS9/50 spectrometer (At Cape Technikon, SA), a VG Micromass 70-SE magnet sector spectrometer (at Kent, UK), VG11-250J Data system, micromass Autospec-TOF double focusing magnetic mass spectrometer (at University of Potchefstroom, SA), VG70-SEQ (FAB, operating at 7kV) and VG70-SEQ (EI, operating at 8kV) instruments (University of the Witswatersrand).

^1H NMR spectra were recorded on a Varian VXR-200 spectrometer at 200 MHz, Varian Mercury spectrometer at 300 MHz or a Varian Unity Spectrometer at 400 MHz. All spectra were recorded in deuterochloroform, deuterodimethylsulfoxide or

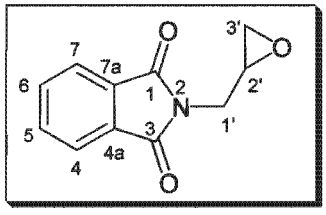
deuteromethanol using tetramethylsilane (TMS) as an internal standard. All chemical shifts are recorded in ppm. **^{1}HNMR coupling constants are rounded off to one decimal place, resulting in exactly the same coupling constants for coupling partners.**

^{13}C NMR spectra were measured on a Varian Mercury spectrometer at 75 MHz or a Varian Unity spectrometer at 100 MHz. **The format used for recording ^{13}C NMR data is that accepted by most international journals (including American Chemical Society journals). In this format chemical shift values are simply listed without specific assignment to carbon atoms.**

HPLC was performed at ambient temperature on a Thermo Separations Products® instrument, fitted with an Xtera® C_{18} 5 μm, 4.6 x 150 mm column and operating on a UV detector. HPLC-grade acetonitrile and ultra filtered water were used as the eluting solvents in the presence of a phosphate buffer.

All solvents were dried by appropriate techniques. Tetrahydrofuran, toluene and diethyl ether were dried over lithium aluminium hydride and subsequently distilled over sodium wire prior to using benzophenone as indicator. Triethylamine was dried over and distilled from calcium hydride and stored over potassium hydroxide pellets. Methylene chloride was dried over phosphorous pentoxide and distilled prior to use. Unless otherwise stated, all other solvents used were anhydrous.

2-Oxiranylmethyl-isoindole-1,3-dione, 3.14



Epichlorohydrin (35 ml, 447 mmol) was added to potassium phthalimide (15.05 g, 81 mmol) in a 100 ml flask equipped with a magnetic stirrer. The mixture was refluxed at 160 °C for 10 h, after which the original pale yellow suspension was gradually transformed into a suspension of deep yellow powder in a yellow liquid. Following consumption of potassium phthalimide (TLC), excess epichlorohydrin was removed under reduced pressure to yield a yellow powder that was dissolved in hot EtOH (100 ml). The inorganic residue was filtered from the hot solution under vacuum and washed with hot EtOH (20 ml). On cooling, the combined EtOH extracts deposited the product as a pale yellow solid. Removal of solvent, followed by recrystallisation from EtOH and concentration on a high vacuum pump afforded the product as a cream white solid (11.82 g, 70%), m.p. 94 – 96 °C, (lit. m.p. 92 – 98 °C);[1] R_f(EtOAc:Hex 2:3) 0.30; δ_H(300 MHz, $CDCl_3$) 7.87 (m, 2H, H-4, H-7), 7.73 (m, 2H, H-5, H-6), 3.97 (dd, 1H, *J* 5.1, 14.4, H-1′α), 3.80 (dd, 1H, *J* 4.8, 14.4, H-1′β), 3.23 (m, 1H, H-2′), 2.82 (dd, 1H, *J* 4.8, 5.1, H-3′α), 2.76 (dd, 1H, *J* 2.7, 5.1, H-3′β); δ_C(75 MHz, $CDCl_3$) 168.0 (2C), 134.0 (2C), 132.0 (2C), 123.5 (2C), 49.1, 46.1, 39.7.

(i) **General Method for the parallel solution-phase synthesis of β-amino alcohols (A)**

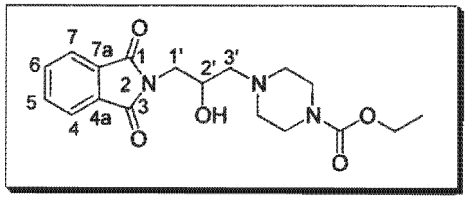
A solution of the epoxide **3.14** (0.50 g, 2.46 mmol) was prepared by dissolving the compound in hot MeOH (5 ml) from which 10 x 500 µl aliquots were obtained and dispensed into 10 labeled vials. Ten (10) different amines (0.318 mmol, 1.3 eq) were added to each of the vials, which were then heated at 63 °C for 4 – 6 h. After consumption of the epoxide (TLC) the reaction mixtures were either filtered (for those

that formed precipitates) and/or evaporated under reduced pressure to yield residues that were purified by chromatography on silica gel (eluent 1 – 5% MeOH/DCM).

(ii) **General Method for the parallel synthesis of amino alcohols *via* the 'Catch and Release' Protocol (B)**

A solution of the epoxide **3.14** (0.50 g, 2.46 mmol) was prepared by dissolving the compound in hot MeOH (5 ml) from which 10 x 500 µl aliquots were obtained and dispensed into 10 labeled vials. Equimolar amounts of 10 different amines (0.246 mmol, 1.0 eq) were added to each of the vials, which were then heated at 63 °C for 3 – 6 h. After consumption of starting materials (TLC), the reaction mixtures were cooled to room temperature, diluted with DCM (5 ml) and MP-TsOH (0.19 g, 0.285 mmol), with a loading capacity of 1.46 mmol/g, added to each vial. The vials were shaken vigorously on an orbital shaker at room temperature for 1 h to scavenge the products from solution, filtered and washed with MeOH to remove any soluble impurities. The resins were finally shaken in a 5% solution of NH_3/MeOH for 1 h, filtered, dried ($MgSO_4$) and concentrated under reduced pressure to yield the products in good yield. Yields for compounds **3.24a – 3.24j** are indicative of those obtained from solution-phase synthesis.

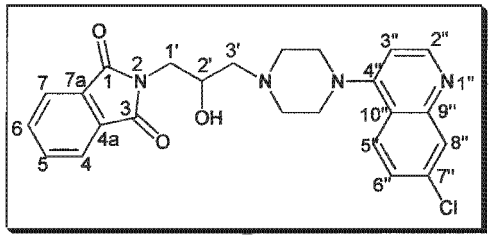
***4-[3-(1,3-Dioxo-1,3-dihydro-isoindole-2-yl)hydroxy-propyl]piperazine-1-carboxylic acid ethyl ester*, 3.24a**



White paste (36 mg, 41%); R_f(Et_2O:Hex) 0.14; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3405 (OH), 3045 (ArCH), 1701 (C=O), 1196 (C-O); δ_H(300 MHz, $CDCl_3$) 7.84 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 4.11 (q, 2H, *J* 6.9, OCH_2CH_3), 4.05 (m, 1H, H-2′), 3.78 (m, 2H, CH_2-1′), 3.42 (m, 4H, 2 x PipNCH_2CO), 2.71 (m, 6H, CH_2-3′, 2 x PipNCH_2), 1.22 (t, 3H, *J* 6.9,

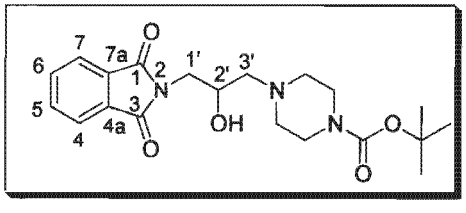
OCH_2CH_3); δ_C(75 MHz, $CDCl_3$) 171.0, 168.7 (2C), 134.0 (2C), 132.2 (2C), 123.5 (2C), 68.8, 65.4, 62.0, 61.5 (2C), 53.2 (2C), 42.2, 14.8; LRMS (EI) *m/z* (19%, M^+); Found: C 60.2, H 6.0, N 11.2, Anal. Calc. C 59.8, H 6.3, N 11.6% for $C_{18}H_{23}N_3O_5$; HPLC Purity: 93%; t_R' = 1.45′.

2-{3-[4-(7-*Chloroquinolin-4-yl*)-*piperazin-yl*]-2-*hydroxy-propyl*}-*isoindole*-1,3-*dione*, 3.24b



Yellow crystals (103 mg, 93%), m.p. 113 – 115 °C; R_f(DCM:MeOH:EtOAc 1:1:1) 0.10; IR ν_{max}(KBr)/cm^{-1} 3128 (OH), 2898 (aliphatic C-H), 1569 (C=N), 1092 (C-O); δ_H(300 MHz, $CDCl_3$) 8.70 (d, 1H, *J* 5.1, H-2″), 8.03 (d, 1H, *J* 2.4, H-8″), 7.95 (d, 1H, *J* 9.3, H-5″), 7.88 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.72 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.20 (dd, 1H, *J* 2.4, 9.3, H-6″), 6.82 (d, 1H, *J* 5.1, H-3″), 4.01 (m, 1H, CH_2-2′), 3.83 (dd, 2H, *J* 5.1, 14.4, CH_2-1′), 3.65 (m, 4H, 2 x PipNCH_2Ar), 3.21 (m, 4H, 2 x PipNCH_2), 2.59 (m, 2H, CH_2-3′); δ_C(75 MHz, $CDCl_3$) 168.6 (2C), 156.7, 150.1, 135.4, 134.1 (2C), 132.0 (2C), 128.9, 126.2 (2C), 125.1, 123.4 (2C), 121.9, 109.0, 65.4, 61.7 (2C), 53.2 (2C), 52.2, 42.1; HRMS *m/z* (FAB) 450.91733 (M^+ $C_{24}H_{23}{}^{35}ClN_4O_3$ requires 450.91742); HPLC Purity: 99%; t_R' = 2.14 min.

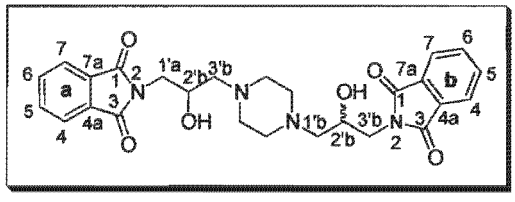
4-[3-(1,3-*Dioxo*-1,3-*dihydro-isoindol-2-yl*)-2-*hydroxy-propyl*]-*piperazine*-1-*carboxylic acid ethyl ester*, 3.24c



Yellow oil (84 mg, 93%); R_f(EtOAc:Hex 3:2) 0.11; IR ν_{max}(KBr)/cm^{-1} 3653 (OH), 3040 (ArCH), 1710 (C=O), 1696 (C=O), 1585 (C=C), 1209 (C-O); δ_H(200 MHz, $CDCl_3$) 7.85 (m, 2H, H-4, H-7), 7.72

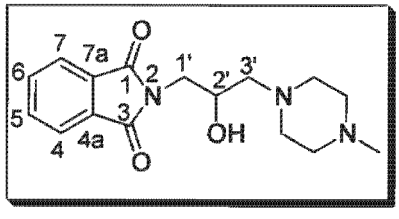
(m, 2H, H-5, H-6), 4.01 (m, 1H, H-2′), 3.77 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.41 (t, 4H, *J* 6.0, 2 x PipNC*H*$_2$Ar), 2.51 (m, 2H, C*H*$_2$-3′), 2.41 (t, 4H, *J* 6.0, 2 x PipNC*H*$_2$), 1.43 (s, 9H, C(C*H*$_3$)$_3$); δ_C(75MHz, $CDCl_3$) 168.4 (2C), 164.7, 134.0 (2C), 132.0 (2C), 123.0 (2C), 79.7, 65.1, 61.7 (2C), 53.1 (2C), 42.0, 28.4, 21.0 (3C); LRMS (EI) *m/z* 389 (39%, M^+ $C_{20}H_{27}N_3O_5$); HPLC Purity: 96%; t_R' = 1.00 min.

1-(3-{4-[3-(2,5-*Dioxopyrridin*-1-yl)-2-*hydroxypropyl*]-*piperazine*-1-*yl*}-2-*hydroxy*-*propyl*)-*isoindole*-2,5-*dione*, 3.24j



White plates (62 mg, 51%), m.p. 197 – 199 °C; R_f(Et$_2$O:Hex 3:7) 0.28; IR ν_{max}(KBr)/cm^{-1} 3010 (ArCH), 2980 (aliphatic C-H), 1689 (C=O), 1208 (C-O); δ_H(300 MHz, $CDCl_3$) 7.86 (m, 4H, H-4a, 4b, 2 x H-7a, H-7b), 7.71 (m, 4H, H-5a, H-5b, H-6a, H-6b), 4.10 (m, 2H, H-2′a, H-2′b), 3.79 (dd, 4H, *J* 5.1, 14.4, C*H*$_2$-1′a, C*H*$_2$-1′b), 2.52 (m, 4H, C*H*$_2$-3′a, C*H*$_2$-3b′), 2.42 (br, 8H, 4 x PipNC*H*$_2$); δ_C(75 MHz) 168.2 (4C), 134.1 (4C), 132.1 (4C), 123.5 (4C), 65.0 (2C), 61.4 (4C), 53.3 (2C), 42.0 (2C); LRMS (EI) m/z 492 (25%, M^+); HPLC Purity: 99%; t_R' = 2.32 min.

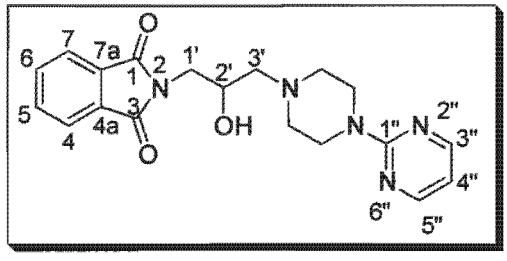
***2*[*2*-Hydroxy-*3*-(*4*-methyl-piperazin-yl)-propyl]-isoindole-*1,3*-dione, 3.24d**



Yellow oil (51 mg, 68%); R_f(MeOH:DCM 1:19) 0.19; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3409 (OH), 3033 (ArCH), 2935 (aliphatic C-H), 1693 (C=O), 1198 (C-O); δ_H(300 MHz, $CDCl_3$) 7.97 (dd, 2H, J 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, J 3.9, 5.7, H-5, H-6), 4.10 (m, 1H, H-2′), 3.75 (dd, 2H, J 5.1, 14.4, CH_2-1′), 2.70 (m, 2H, CH_2-3′), 2.44 (m, 8H, 4 x PipNCH_2), 2.28 (s, 3H, NCH_3); δ_C(75 MHz, $CDCl_3$) 168.5

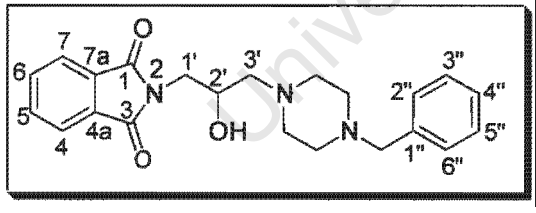
(2C), 134.2 (2C), 132.1 (2C), 123.5 (2C), 65.0, 61.4 (2C), 55.0 (2C), 53.0, 45.8, 42.0; LRMS (EI) m/z 303 (56%, M^+); HPLC Purity: 87%; t_R' = 1.39 min.

2-[2-*Hydroxy*-3-(4-pyrimidin-2-*yl*piperazin-1-*yl*)-*propyl*]-*isoindole*-1,3-*dione*, 3.24e



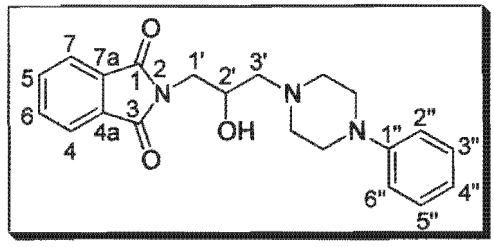
White paste (51 mg, 65%); R_f(Et$_2$O:Hex 3:7) 0.21; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3521 (OH), 3019 (ArC-H), 1691 (C=O), 1556 (C=N); δ_H(300 MHz, $CDCl_3$) 8.14 (d, 2H, *J* 4.8, H-3″, H-5″), 7.89 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.53 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 6.58 (t, 1H, *J* 4.8, H-4″), 4.09 (m, 1H, H-2′), 3.78 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.16 (t, 4H, *J* 6.0, 2 x PipNC*H*$_2$Ar), 2.59 (t, 4H, *J* 6.0, 2 x PipNC*H*$_2$), 2.51 (m, 2H, C*H*$_2$-3′); δ_C(75 MHz, $CDCl_3$) 169.3 (2C), 166.2, 157.1 (2C), 134.3 (2C), 132.2 (2C), 123.4 (2C), 110.8, 70.3, 60.3 (2C), 57.9 (2C), 55.7, 46.9; LRMS (EI) *m/z* 367 (67%, M^+); HPLC Purity: 91%; t_R' = 2.23 min.

2-[3-(4-*Benzyl*-*piperazin*-1-*yl*)-2-*hydroxy*-*propyl*]-*isoindole*-1,3-*dione*, 3.24f



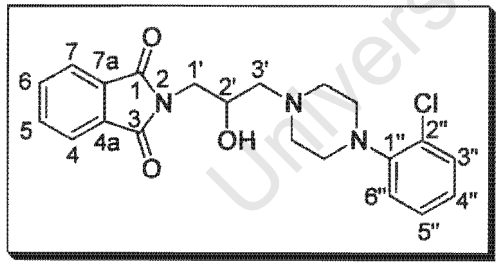
Yellow oil (66 mg, 71%); R_f(Et$_2$O:Hex 3:7) 0.21; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3540 (OH), 3025 ArCH), 1699 (C=O), 1639 (C=C), 1205 (C-O); δ_H(300 MHz, $CDCl_3$) 7.85 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.30 (m, 5H, ArH), 4.1 (m, 1H, H-2′), 3.78 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.48 (s, 2H, ArC*H*$_2$N), 2.63 (m, 2H, C*H*$_2$-3′), 2.43 (m, 8H, 4 x PipNC*H*$_2$); δ_C(75 MHz, $CDCl_3$) 168.0 (2C), 134.0 (2C), 132.0 (2C), 129.2, 129.1 (2C), 128.2 (2C), 127.0, 123.3 (2C), 64.9, 63.4 (2C), 63.0, 61.5, 53.1 (2C), 45.3; LRMS (EI) *m/z* 379 (29%, M^+); HPLC Purity: 92%; t_R' = 2.34 min.

2-[2-*Hydroxy*-3-(4-*phenylpiperazine*-1-*yl*)-*isoindole*-1,3-*dione*, 3.24g



Pale green needles (74 mg, 83%), m.p. 128 – 130 °C; R_f(Et$_2$O:Hex 3:7) 0.13; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3551 (OH), 3029 (ArCH), 1712 (C=O), 1625 (C=C), 1208 (C-O); δ_H(300 MHz, $CDCl_3$) 7.88 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.25 (m, 2H, ArH), 6.88 (m, 3H, ArH), 4.10 (m, 1H, H-2′), 3.79 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.16 (t, 4H, *J* 6.0, 2 x PipNC*H*$_2$Ar), 2.81 (m, 2H, PipNC*H*$_2$), 2.61 (m, 2H, PipNC*H*$_2$), 2.51 (m, 2H, C*H*$_2$-3′); δ_C(75 MHz, $CDCl_3$) 168.5 (2C), 143.0, 134.0 (2C), 132.4 (2C), 129.0 (2C), 123.3 (2C), 119.8 (2C), 116.0, 65.1, 61.6 (2C), 53.3 (2C), 49.2, 42.0; LRMS (EI) *m/z* 365 (32%, M$^+$); Found: C, 69.7, H 6.1; N, 11.5; Anal. Calc. C, 69.4; H, 6.3; N, 11.5; HPLC Purity: 99%; t_R' = 1.99 min.

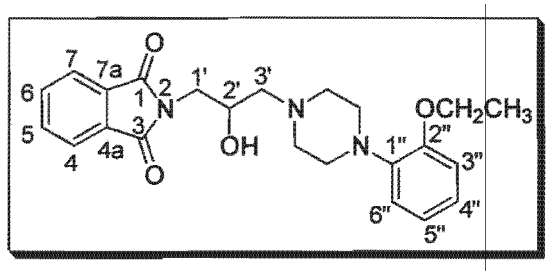
2-{3-[4-(2-*Chloro-phenyl*)-*piperazin-yl*-]-2-*hydroxy-propyl*}-*isoindole*-1,3-*dione*, 3.24h



White crystals (69 mg, 70%), m.p. 165 – 167 °C; R_f(Et$_2$O:Hex 3:7) 0.19; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3551 (OH), 3019 (ArCH), 1718 (C=O), 1633 (C=C), 1218 (C-O); δ_H(300 MHz, $CDCl_3$) 7.88 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.28 (d, 1H, *J* 5.4, H-3″), 7.20 (m, 1H, H-5″), 6.89 – 7.09 (m, 2H, H-4″, H-6″), 4.10 (m, 1H, H-2′), 3.78 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.05 (m, 4H, PipNC*H*$_2$Ar), 2.81 (t, 2H, *J* 6.0, PipNC*H*$_2$), 2.61 (t, 2H, *J* 6.0, PipNC*H*$_2$), 2.51 (m, 2H, C*H*$_2$-3′); δ_C(75 MHz, $CDCl_3$) 168.5 (2C), 149.0, 134.0 (2C), 132.0 (2C), 130.0, 128.8,

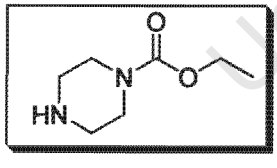
127.6, 126, 123.7, 123.4 (2C), 65.0, 61.4 (2C), 51.3 (2C), 49.3, 42.0; LRMS (EI) *m/z* 299 (76%, M^+); HPLC Purity: 98%; t_R' = 2.79 min.

2-3{-4[(2-*Ethoxyphenyl*)-*piperazine*-1-yl]-2-*hydroxy-propyl*}-*isoindole*-1,3-*dione*, 3.24i



White flakes (53 mg, 53%); R_f (Et_2O:Hex 3:7) 0.27; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3415 (OH), 3019 (ArCH), 2991 (aliphatic C-H), 1703 (C=O), 1633 (C=C), 1215 (C-O); δ_H(300 MHz, $CDCl_3$) 7.88 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 6.88 (m, 4H, ArH), 4.1 (m, 3H, H-2', ArOC*H*$_2$), 3.79 (m, 2H, C*H*$_2$-1'), 3.07 (m, 4H, 2 x PipNC*H*$_2$Ar), 2.82 (m, 2H, PipNC*H*$_2$), 2.62 (m, 2H, PipNC*H*$_2$), 2.51 (m, 2H, C*H*$_2$-3'), 1.43 (t, 3H, *J* 7.2, C*H*$_3$); δ_C(75 MHz, $CDCl_3$) 168.5 (2C), 151.6, 141.2, 134.0 (2C), 142.1, 132.0 (2C), 123.4 (2C), 122.8, 121.0, 118.2, 63.6, 61.7 (2C), 56.4, 53.5 (2C), 50.6, 42.1, 14.9; LREIMS *m/z* 409 (33%, M^+). Found: C 67.5, H 6.3, N 10.1; Anal. Calc.: C 67.5, H 6.6, N 10.3 for $C_{23}H_{27}N_3O_4$; HPLC Purity: 97%; t_R' = 2.65 min.

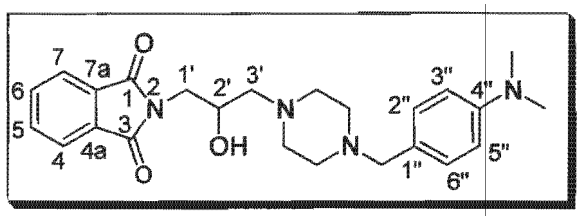
***Ethylpiperazine-1-carboxylate*, 3.33**



Piperazine (2.0 g, 23.2 mmol) was dissolved in H_2O (10 ml) and the pH of the solution adjusted to ~3 by the addition of 2M HCl. EtOCOCl (25.53 mmol, 1.1 eq) was added *via* syringe over 30 min while maintaining the pH between 2 and 3 by the constant addition of 10% NaOH (w/v). The reaction mixture was stirred a further 30 min and then extracted with ether (3 x 20 ml) to remove the 1,4-bis(ethlyoxycarbonyl)piperazine by-product. The aqueous residue was evaporated *in vacuo* and the residue dissolved in cold NaOH (40 % w/v). The mixture was extracted with ether (4 x 20 ml), dried (Na_2SO_4) and concentrated to

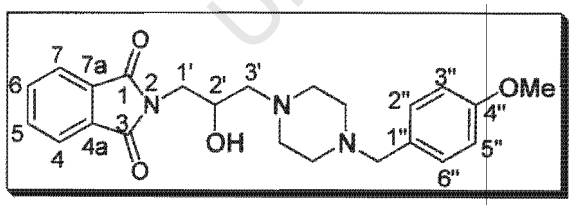
afford the product as a colourless oil (3.34g, 91%); δ_H(200 MHz, $CDCl_3$) 4.11 (q, 2H, *J* 6.0, OCH_2), 3.42 (t, 4H, *J* 5.0, 2 x PipNCH_2), 2.80 (t, 4H, *J* 5.0, 2 x PipNCH_2), 1.34 (s, 3H, OCH_2CH_3); δ_C(75 MHz, $CDCl_3$) 156.6, 61.2, 45.8 (2C), 44.7 (2C), 14.6.

2-(3[4-(4-*Dimethylamino-benzyl*)*piperazin*-1-*yl*]-2-*hydroxy-propyl*}*isoindole*-1,3-*dione*, 3.47f



Yellow oil (55 mg, 59%); R_f(EtOAc:Hex 3:7), 0.15; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3601 (OH), 3025 (ArCH), 2980 (aliphatic CH), 1709 (C=O), 1563 (C=C), 1220 (C-O); δ_H(300 MHz, $CDCl_3$) 7.85 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.15 (d, 2H, *J* 6.0, H-2″, H-6″), 6.69 (d, 2H, *J* 6.0, H-3″, H-5″), 4.1 (m, 1H, H-2′), 3.78 (dd, 2H, *J* 5.1, 14.4, CH_2-1′), 3.4 (s, 2H, ArCH_2N), 2.92 (s, 6H, 2 x NCH_3), 2.65 (m, 2H, CH_2-3′), 2.41 (m, 8H, 4 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 168.0 (2C), 134.0 (2C), 132.0 (2C), 130.2 (2C), 129.8, 127.6 (2C), 123.0 (2C), 113.0, 65.0, 62.5 (2C), 61.5, 53.2 (2C), 52.9, 42.1, 40.7; HRMS (FAB) *m/z* 422.23170 (M^+ $C_{24}H_{30}N_4O_3$ requires 422.23179).

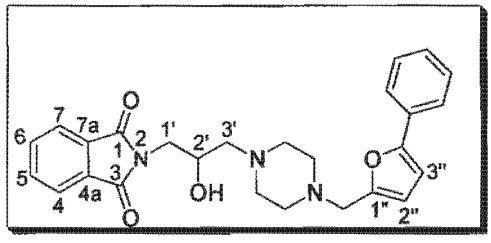
2-(2-*Hydroxy*-3-[4-(4-*methoxy-benzyl*)-*piperazin*-1-*yl*]-*isoindole*-1,3-*dione*, 3.47e



Colourless oil (55 mg, 61%); R_f(EtOAc:Hex 3:7), 0.23; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3609 (OH), 3025 ArCH), 2980 (aliphatic CH), 1653 (C=C), 1220 (C-O); δ_H(300 MHz, $CDCl_3$) 7.93 (dd, 2H, *J* 3.9, 5.7, H-4, H-7), 7.75 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.23 (d, 2H, *J* 5.4, H-2″, H-6″), 6.83 (d, 2H, *J* 5.4, H-3″, H-5″), 4.1 (m, 1H, H-2′), 3.81 (dd, 2H, *J* 5.1, 14.4, CH_2-1′), 3.79 (s, 3H, OCH_3), 3.48 (s, 2H, NCH_2Ar). 2.65

(m, 2H, CH_2-3′), 2.42 (m, 8H, 4 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 168.0 (2C), 133.9, 134.1 (2C), 132.0 (2C), 130.2, 128.6 (2C), 123.6 (2C), 113.5 (2C), 69.3, 64.9, 62.2 (2C), 55.2 (2C), 52.2, 49.3, 42.0; HRMS (FAB) *m/z* 409.47833 (M^+ $C_{23}H_{27}N_3O_4$ requires 409.47818).

2-{2-*Hydroxy*-3-[4-(5-*phenylfuran*-2-*ylmethyl*)-*piperazine*-1-yl}-*isoindole*-1,3-*dione*, 3.47g



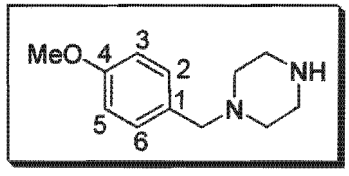
White paste (49 mg, 50%); R_f(Et_2O:Hex 3:2) 0.15; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3609 (OH), 3020 ArCH), 1710 (C=O), 1561 (C=C), 1219 (C-O); δ_H(300 MHz, $CDCl_3$) 7.88 (m H-4, H-7), 7.74 (m, 2H, H-5, H-6), 7.62 (m, 2H, ArH), 7.35 (m, 2H, ArH), 7.21 (m, 1H, ArH), 6.60 (d, 1H, *J* 3.3, H-2″), 6.57 (d, 1H, *J* 3.3, H-3″), 4.10 (m, 1H, H-2′), 3.78 (dd, 2H, *J* 5.1, 14.4, CH_2-1′), 3.60 (s, 2H, ArCH_2N), 2.51 (m, 2H, CH_2-3′), 2.46 (m, 8H, 4 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 168.0 (2C), 152.2, 151.5, 136.7, 134 (2C), 132.0 (2C), 129.0 (2C), 128.5, 127.4 (2C), 123.4 (2C), 110.9, 105.6, 65.3 (2C), 61.2, 56.4, 54.6 (2C), 53.0, 46.9; HRMS (EI) *m/z* 445.19989 (M^+ $C_{26}H_{27}N_3O_4$ requires 445.20016).

General Procedure for the synthesis of piperazinylamines 3.36a – c (C)

A solution of the aldehyde (2.90 mmol, 1.0 eq) in DCE (4.5 ml) was reacted with ethylpiperazine-1-carboxylate **3.33** (3.77 mmol, 1.3 eq) under a nitrogen atmosphere. The reaction mixture was stirred at room temperature for 30 min and $NaBH(OAc)_3$ (3.77 mmol, 1.3 eq) was added at once. After 18 h the reaction mixture was quenched with saturated $NaHCO_3$ (4 ml) and extracted with EtOAc (3 x 15 ml). The combined organic layers were dried and concentrated *in vacuo* to afford residues that were used in the next step without further purification.

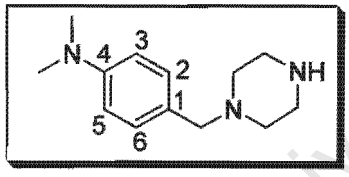
The residues obtained above were dissolved in aquoeus EtOH (15 ml) and refluxed in the presence of KOH (0.21 g, 3.77 mmol) for 24 h. The reaction mixture was cooled to room temperature, evaporated to remove EtOH and extracted with DCM (2 x 10 ml), dried (Na_2SO_4) and concentrated *in vacuo* afforded the crude product that was purified by column chromatography on SiO_2 gel (eluent 10 – 50% MeOH:DCM).

4'-Methoxybenzyl piperazine, 3.36a



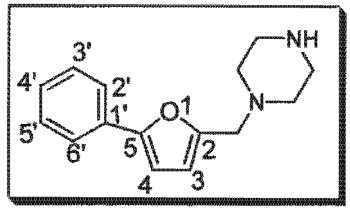
Yellow paste (485 mg, 81%); R_f(NH_3:MeOH 1:49) 0.23; δ_H(400 MHz, $CDCl_3$) 7.22 (d, 2H, *J* 6.0, H-2, H-6), 6.82 (d, 2H, *J* 6.0, H-3, H-5), 3.79 (s, 3H, ArOCH_3), 3.42 (s, 2H, ArCH_2), 2.86 (t, 4H, *J* 6.0, 2 xPipNCH_2Ar), 2.40 (t, 4H, *J* 6.0, 2 x PipNCH_2), 1.89 (br s, 1H, NH); δ_C(75 MHz, $CDCl_3$) 158.0, 130.5 (2C), 130.0 (2C), 113.7, 65.4, 63.2, 54.5 (2C), 53.4 (2C).

4'-*N*, *N*-Dimethylbezylpierazine, 3.36b



Yellow oil (633 mg, 78%); R_f(NH_3:MeOH 1:49) 0.18; δ_H(400 MHz, $CDCl_3$) 7.17 (d, 2H, *J* 8.8, H-2, H-6), 6.69 (d, 2H, *J* 8.8, H-3, H-5), 3.40 (s, 2H, ArCH_2), 2.93 (s, 6H, 2 x CH_3), 2.87 (t, 4H, *J* 6.0, 2 x PipNCH_2Ar), 2.39 (t, 4H, *J* 6.0, 2 x PipNCH_2), 1.78 (br s, 1H, NH); δ_C(75 MHz, $CDCl_3$) 149.9, 130.2 (2C), 125.8, 112.4 (2C), 65.2, 54.1 (2C), 52.9 (2C), 42.7 (2C).

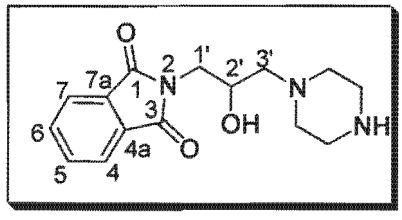
1-(5-Phenyl-furan-2-ylmethyl)piperazine, 3.36c



White hygroscopic solid (640 mg, 70%); R_f(NH_3:MeOH 1:49) 0.30; δ_H(400 MHz, $CDCl_3$) 7.68 (m, 2H, ArH), 7.37 (m, 2H, ArH), 7.27 (m, 1H, ArH), 6.58 (d, 1H, *J* 3.3, H-4),

6.29 (d, 1H, *J* 3.3, H-3), 3.62 (s, 2H, CH_2Ar), 2.92 (t, 4H, *J* 6.0, 2 x PipNCH_2CH_2Ar), 2.50 (t, 4H, *J* 6.0, 2 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 153.1, 150.9, 136.9, 129.7 (2C), 128.6, 127.0 (2C), 113.0, 101.1, 63.3, 56.9 (2C), 53.0 (2C).

2-(2-*Hydroxy*-3-(*piperazin*-1-*yl*)*propyl*)*isoindoline*-1,3-*dione*, 3.46



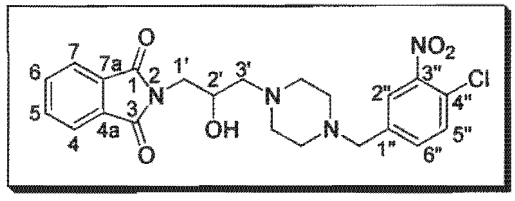
A 5% solution of HCl in MeOH (10 ml) was added at once to a solution of **3.24c** (0.95 g, 2.44 mmol) in MeOH (40 ml) and stirred for 2 h at ambient temperature. The reaction mixture was evaporated to dryness and the resultant HCl salt redissolved in MeOH (30 ml). Resin-bound $MPCO_3$ (2 eq) was added and the reaction mixture shaken at room temperature for 1 h, filtered and washed with MeOH (10 ml). Concentration of the filtrate under reduced pressure afforded the free base as a yellow oil (0.70 mg, 100%); R_f(NH_3:MeOH 1:49) 0.21; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3567 (OH), 3421 (NH), 3030 (ArH), 2987 (CH), 1722 (C=O); δ_H(300 MHz, $CDCl_3$) 7.86 (m, 2H, H-4, H-6), 7.83 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 4.10 (quint, 1H, *J* 6.8, H-2′), 3.76 (d, 2H, *J* 6.0, CH_2-1′), 3.17 (m, 4H, 2 x PipNCH_2Ar), 2.81 (m, 4H, 2 x PipNCH_2), 2.60 (dd, 2H, *J* 7.2, 14.4, CH_2-3′); δ_C(75 MHz, $CDCl_3$) 170.0 (2C), 135.4 (2C), 133.4 (2C), 124.1 (2C), 67.0, 62.1, 51.4 (2C), 44.7 (2C), 43.4; HRMS (FAB) *m/z* 289.14233 (M^+ $C_{15}H_{19}N_3O_3$ requires 289.14262).

General Procedure for the synthesis of β-amino alcohols 3.47a – d, (D)

Amine **3.46** (63 mg, 0.218 mmol) and the appropriate aldehyde (0.272 mmol, 2 eq) were condensed in MeOH (5 ml) at room temperature for 1 h before addition of $NaCNBH_3$ (25 mg, 0.436 mmol). After 12 h MeOH (5 ml) was added followed by MP-TsOH (298 mg, 0.436) and the reaction mixtures were transferred to an orbital shaker where they were agitated for 4 h. The resins were filtered and washed with MeOH before being

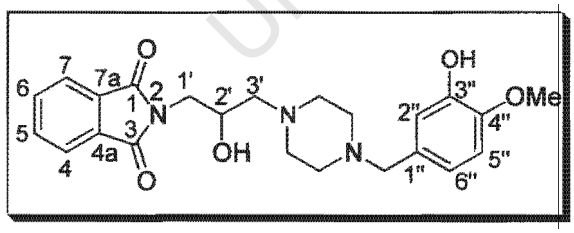
transferred into vials containing anhydrous 3% MeOH/DCM. After shaking for 1 h the resins were filtered, washed with MeOH and the filtrates concentrated *in vacuo* to afford the amino alcohols.

2-{3-[4-(4-*Chloro*-3-*nitro*-*benzyl*)-*piperazin*-1-yl]-2-*hydroxy*-*propyl*}-*isoindole*-1,3-*dione*, 3.47a



White foam (63 mg, 63%); R_f(EtOAc:Hex 2:1), 0.26; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3609 (OH), 1719 (C=O), 1628 (C=C), 1201 (C-O), 687 (Ar C-Cl); δ_H(300 MHz, $CDCl_3$) 7.88-7.82 (m, 3H, H-4, H-7, H-6″), 7.71 (dd, 2H, *J* 3.9, 5.7, H-5, H-6), 7.46 (m, 2H, H-2″, H-5″), 4.1 (m 1H, C*H*-2′), 3.79 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.50 (s, 2H, NC*H*$_2$Ar), 2.51 (m, 2H, C*H*$_2$-3′), 2.45 (m, 8H, 4 x PipNC*H*$_2$); δ_C(75 MHz, $CDCl_3$) 168.9 (2C), 139.3, 134.0 (2C), 133.3 (2C), 132.1 (2C), 131.6, 127.7, 125.5, 123.4 (2C), 65.1, 61.4 (2C), 61.2. 53.1 (2C), 49.6, 42.0; LRMS (EI) *m/z* 458.13696 (M^+ $C_{22}H_{23}{}^{35}ClN_4O_5$ requires 458.13570).

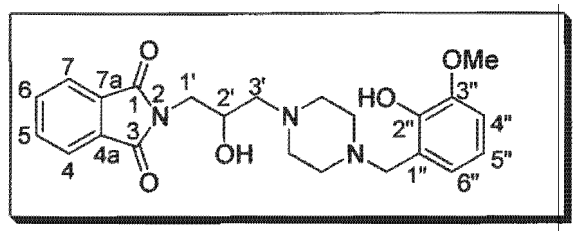
2-{2-*Hydroxy*-3-[4-(3-*hydroxy*-4-*methoxy*-*benzyl*)-*piperazin*-1-*yl*]-*propyl*}-*isoindole*-1,3-*dione*, 3.47b



Yellow oil (48 mg, 52%); R_f(Et_2O:Hex 3:1) 0.19; IR $\nu_{max}(KBr)/cm^{-1}$ 3565 (OH), 3021 (ArCH), 1706 (C=O), 1652 (C=C); δ_H(300 MHz, $CDCl_3$) 7.86 (m, 2H, H-4, H-7), 7.75 (m, 2H, H-5, H-6), 6.72 (dd, 1H, *J* 1.8, 4.8, H-6″), 6.53 (d, 1H, *J* 4.8, H-5″), 6.39 (d, 1H, *J* 1.8, H-2″), 4.1 (m 1H, H-2′), 3.83 (s, 3H, ArOC*H*$_3$), 3.79 (dd, 2H, *J* 5.1, 14.4, C*H*$_2$-1′), 3.54 (s, 2H, NC*H*$_2$Ar), 2.51 (m, 2H, C*H*$_2$-3′), 2.45 (m, 8H, 4 x PipNC*H*$_2$); δ_C(75 MHz, $CDCl_3$) 166.8 (2C), 146.8, 142.6, 132.4 (2C), 132.2 (2C), 130.0, 127.4, 123.2 (2C),

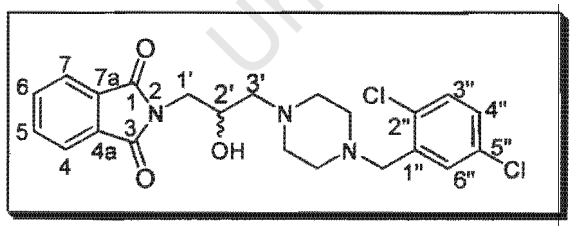
122.0, 117.6, 69.3, 62.1 (2C), 56.9, 56.3, 55.6 (2C), 49.1, 46.3 HRMS (FAB) *m/z* 425.19567 (M^+ $C_{23}H_{27}N_3O_5$ requires 425.19507).

2-{2-*Hydroxy*-3-[-4(2-*hydroxy*-3-*methoxy*-*enzyl*)-*piperazin*-1-yl]-*propyl*}-*isoindole*-1,3-*dione*, 3.47c



White paste (58 mg, 63%); R_f(Hex: EtOAc 2:1) 0.25; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3402 (OH), 2857 (CH), 1691 (C=O), 1208 (C-O); δ_H(300 MHz, $CDCl_3$) 7.85 (m, 2H, H-4, H-7), 7.70 (m, H-5, H-6), 6.72 (t, 1H, *J* 4.8, H-5″), 6.60 (d, 1H, ArH, *J* 4.8, H-6″), 6.52 (d, 1H, *J* 5.1, H-4″), 4.10 (m, 1H, H-2′), 3.86 (s, 3H, OCH_3), 3.78 (m, 2H, CH_2-1′), 3.69 (s, 2H, NCH_2Ar), 2.65 (m, 2H, CH_2-3′), 2.59 (m, 8H, 4 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 168.5 (2C), 148.0, 147.0, 132.5 (2C), 132.0 (2C), 123.4 (2C), 121.2, 120.7, 118.8, 111.2, 65.2, 61.3 (2C), 61.1, 55.9 (2C), 53.0, 52.5, 42.0; HRMS (FAB) *m/z* 425.19524 (M^+ $C_{23}H_{27}N_3O_5$ requires 425.19507).

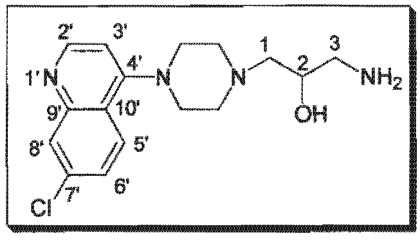
2-{3-[4-(2,5-*Dichloro*-*benzyl*)-*piperazin*-1-*yl*]-2-*hydoxy*-*propyl*}-*isoindole*-1,3-*dione*, 3.47d



White gum (50 mg, 51%); R_f(Et_2O:Hex 3:1) 0.19; IR $\nu_{max}(CH_2Cl_2)/cm^{-1}$ 3601 (OH), 1708 (C=O), 1632 (C=C), 1209 (C-O); δ_H(300 MHz, $CDCl_3$) 7.84 (m, 2H, H-4, H-7), 7.75 (m, H-5, H-6), 7.06 (m, 2H, H-3″, H-6″), 7.16 (dd, 1H, *J* 1.8, 4.2, H-4″), 4.1 (m, 1H, H-2′), 3.79 (m, 2H, CH_2-1′), 3.54 (s, 2H, NCH_2Ar), 2.51 (m, 2H, CH_2-3′), 2.45 (m, 8H, 4 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 168.0 (2C), 138.1, 134.4 (2C), 132.2 (2C), 132.0,

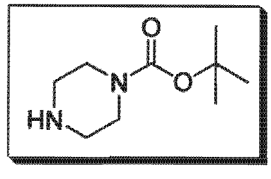
131.4, 130.8, 129.3, 128.8, 123.2 (2C), 65.3, 61.2 (2C), 56.7, 55.8 (2C), 49.3, 46.8; HRMS (FAB) *m/z* 448.34211, (M^+ $C_{22}H_{23}Cl_2N_3O_3$ requires 448.34232).

1-*Amino*-3-[4-(*7-chloro-quinolin-yl*)-*piperazine*-1-*yl*]-*propan*-2-ol, 3.49



Hydrazine monohydrate (0.10 ml, 2.1 mmol) was added to a suspension of **3.24b** (0.3g, 0.68 mmol), in EtOH (10 ml) and refluxed for thirty minutes. The white precipitate that formed was filtered and washed with EtOH. The filtrate was evaporated to dryness and re-dissolved in H_2O, extracted with $CHCl_3$ (2 x 20 ml), washed with saturated ammonium chloride and dried ($MgSO_4$). Concentration *in vacuo* afforded the product as yellow crystals, 0.21 g, 96%, m.p. 101 – 103 °C (from EtOH:H_2O); R_f(25% NH_4OH:MeOH 1:19) 0.19; IR ν_{max}(KBr)/cm^{-1} 3612 (OH), 3221 (NH), 3016 (ArCH), 2987 (CH); δ_H(300 MHz, $CDCl_3$) 8.71 (d, 1H, *J* 5.1, H-2′), 8.03 (d, 1H, *J* 2.1, H-8′), 7.90 (d, 1H, *J* 9.0, H-5′), 7.42 (dd, 1H, *J* 2.1, 9.0, H-6′), 6.83 (d, 1H, *J* 5.1, H-3′), 4.01 (m, 1H, C*H*$_2$-2), 3.25 (m, 4H, 2 x PipNC*H*$_2$), 2.89 (m, 4H, 2 x PipNC*H*$_2$), 2.71 (m, 2H, C*H*$_2$-1), 2.51 (m, 2H, C*H*$_2$-3); δ_C(75 MHz, $CDCl_3$) 156.8, 151.9, 150.2, 134.9, 128.9, 126.2, 125.1, 121.9, 109.0, 68.0, 55.4 (2C), 53.3 (2C), 52.2, 45.5; HRMS (FAB) *m/z* 320.81743 (M^+ $C_{16}H_{21}{}^{35}ClN_4O$ requires 320.81714).

***t*- Butyl piperazine-1-carboxylate, 3.37**



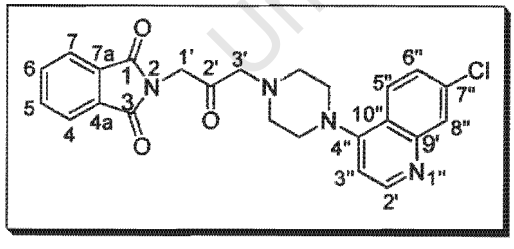
A solution of ditertiarybutyldicarbonate (Boc_2O) (1.80 g, 8.42 mmol) in $CHCl_3$ (25 ml) was added dropwise to a vigorously stirred solution of piperazine (7.1 g, 82.4 mmol) in $CHCl_3$ (35 ml) over 20 minutes. The reaction mixture formed a precipitate that was removed by filtration after 18 h and washed with 2 x 10 ml $CHCl_3$. Removal of solvent under reduced pressure yielded a colourless oil that was dissolved in water (50 ml). The insoluble precipitate that

formed upon addition of water was filtered and the aqueous filtrate extracted into DCM, dried ($MgSO_4$) and evaporated. Concentration under reduced pressure yielded a colourless oil that on standing formed white crystalline needles (1.40 g, 89%), m.p. 43 – 45 °C; R_f(NH$_4$OH:MeOH 1:19) 0.35; δ_H(200 MHz, $CDCl_3$) 3.30 (t, 4H, *J* 5.7, 2 x NCH_2), 2.76 (t, 4H, *J* 5.7, 2 x NCH_2), 2.45 (br s, 1H, N*H*), 1.42 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, $CDCl_3$) 160.3, 72.1, 54.4 (2C), 52.1 (2C), 30.1 (3C).

General Procedure for the oxidation of β-amino alcohols (E)

A solution of the β-amino alcohol (0.66 mmol) in Et_3N (7.52 mmol, 1.0 ml) was mixed with a solution of SO_3.Pyr complex (1.88 mmol) in DMSO:DCM 1:4 (10 ml) and the resultant mixture stirred at ambient temperature for 26 h. After consumption of starting material (TLC), the reaction mixture was partitioned between water and ether, and the organic portion washed with brine (2 x 10 ml), dried ($MgSO_4$) and evaporated to dryness. Recrystallisation from pet ether/water gave the product in good yield.

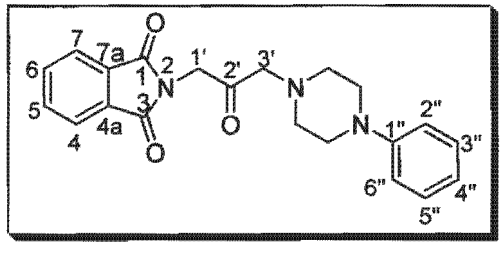
2-{-3-[4-(7-Chloroquinolin-4-yl)-piperazin-1-yl]-2-oxopropyl-isoindole-1,3-dione, 3.48a



(0.25 g, 0.47 mmol) of **3.24b** were used. The product was obtained as yellow crystals (0.21 g, 86%), m.p. 158 – 160 °C; R_f(Et$_2$O:Hex) 0.26; IR ν_{max}(KBr)/cm^{-1} 3030 (Ar CH), 2935 (C-H), 1721 (C=O), 1688(C=O), 1599 (C=C); δ_H(300 MHz, $CDCl_3$) 8.66 (d, 1H, J 5.7, H-2″), 8.11 (d, 1H, J 2.1, H-8″), 7.90 (d, 1H, J 9.0, H-5″), 7.72 (m, 2H, H-4, H-7), 7.72 (m, 2H, H-5, H-6), 7.21 (dd, 1H, J 2.1, 9.0, H-6″), 6.50 (d, 1H, J 5.7, H-3'), 4.70 (s, 2H, CH_2-1'), 3.43 (s, 2H, CH_2-3'), 3.27 (t, 4H, J 6.0, 2 x PipNCH_2), 2.86 (t, 4H, J 6.0, 2 x

PipNCH$_2$); δ_C(75 MHz, $CDCl_3$) 201.1, 167.7 (2C), 156.7, 151.9, 135.0, 134.2, (2C), 132.1 (2C), 129.0, 126.2 (2C), 125.0, 123.5 (2C), 121.9, 109.1, 65.9 (2C), 53.4 (2C), 52.0, 45.8; HRMS (FAB) *m/z* 448.13001 (M^+ $C_{24}H_{21}{}^{35}ClN_4O_4$ requires 448.13022).

2-[2-*Oxo*-3-(4-*phenylpiperazin-1-yl*)-*propyl*]-*isoindole-1,3-dione*, 3.4b

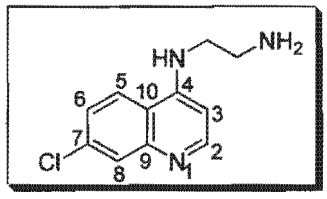


The method used to synthesize **3.48b** was that described in General Procedure (E), using 0.22 (0.37 mmol) of **3.24g**. The product was obtained as light yellow crystals (0.18 g, 83%), m.p. 134 – 136 °C; R_f(Et_2O: Hex) 0.18; IR ν_{max}(KBr)/cm^{-1} 3025 (Ar CH), 2936 (C-H), 1717 (C=O), 1682 (C=O), 1654 (C=C); δ_H(300 MHz, $CDCl_3$) 7.85 (m, 2H, H-4, H-7), 7.72 (m, 2H, H-5, H-6), 7.50 (m, 2H, ArH), 6.63 (m, 3H, ArH), 4.70 (s, 2H, CH_2-1'), 3.43 (s, 2H, CH_2-3'); δ_C(75 MHz, $CDCl_3$) 207.6, 168.0 (2C), 153.1, 134.4 (2C), 132.1 (2C), 129.3, 127.6, 123 (2C), 120.1, 118.1, 116.4, 62.5 (2C), 53.9 (2C) 49.3, 45.5; LRMS (EI) *m/z* 363 (25%, M^+); HPLC Purity: 99%, tR = 2.14 min.

General Synthesis of *N'*-(7-Chloroquinolin-4-yl)-alkyl-1,x-diamines, 3.52 – 3.55 (F)

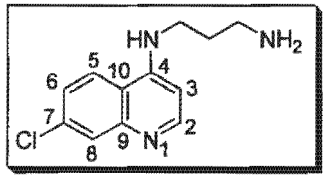
4,7-Dichloroquinoline (1.0 g, 5.05 mmol) was mixed with diamine (3.5 mmol) in a round bottom flask to make a paste that was heated for 1 hour at 80 °C without stirring. The temperature was then raised to 140 °C with stirring. After 12 h the reaction mixture was cooled to room temperature and a 1M solution of NaOH (15 ml) added. The organic product was immediately basified with 10% NaOH (w/v) and extracted with EtOAc (5 x 50 ml), washed with H_2O (2 x 50 ml) and dried ($MgSO_4$). Recrystallisation from EtOAc afforded the amine in excellent yield.

_N'_- (7-Chloroquinolin-4-yl)-ethane-1,2-diamine, 3.52



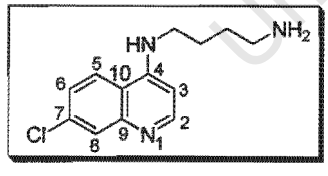
Yellow prisms (1.10 g, 100%); m.p. 115 – 117 °C (lit m.p. 113 – 115 °C);[2] R_f(NH$_3$:MeOH 1:49) 0.20; δ_H(300 MHz, CD_3OD) 8.35 (d, 1H, *J* 5.4, H-2), 8.10 (d, 1H, *J* 9.0, H-5), 7.77 (d, 1H, *J* 2.1, H-8), 7.38 (dd, 1H, *J* 2.1, 9.0, H-6), 6.6 (d, 1H, *J* 5.4, H-3), 3.43 (t, 2H, *J* 6.0, ArNCH_2CH$_2$N), 2.97 (t, 2H, *J* 6.0, ArNCH$_2$CH_2N); δ_C(75 MHz, CD_3OD) 152.9, 150.5, 134.0, 128.9, 126.1, 125.2, 122.0, 117.0, 98.0, 53.6, 46.1.

_N'_- (7-Chloroquinolin-4-yl)-propane-1,3-diamine, 3.53



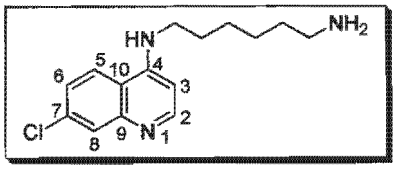
Yellow prisms (1.19 g, 100%), m.p. 124 – 127°C (lit. m.p. 124 – 127 °C);[2] R_f(NH$_3$:MeOH 1:49) 0.21; δ_H(300 MHz, CD_3OD) 8.33 (d, 1H, *J* 5.4, H-2), 8.12 (d, 1H, *J* 9.0, H-5), 7.75 (d, 1H, *J* 2.1, H-8), 7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 6.62 (d, 1H, *J* 5.4, H-3), 3.44 (t, 2H, *J* 6.0, ArNCH_2CH$_2$N), 2.98 (t, 2H, *J* 6.0, ArNCH$_2$ CH$_2$CH_2N), 1.89 (m, 2H, ArNCH$_2$CH_2CH$_2$N); δ_C(75 MHz, CD_3OD) 152.9, 150.5, 134.0, 128.9, 126.1, 125.2, 122.2, 117.0, 98.8, 43.6, 41.4, 30.1.

_N'_- (7-Chloroquinolin-4-yl)-butane-1,2-diamine, 3.54



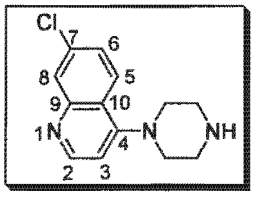
Yellow prisms (1.26 g, 100%), m.p. 45 – 47 °C; R_f(NH$_3$:MeOH 1:49) 0.22; δ_H(300 MHz, CD_3OD) 8.48 (d, 1H, *J* 5.4, H-2), 7.94 (d, 1H, *J* 9.0, H-5), 7.69 (d, 1H, *J* 2.1, H-8), 7.27 (dd, 1H, *J* 2.1, 9.0, H-6), 6.33 (d, 1H, *J* 5.4, H-3), 3.39 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_3$N), 3.04 (t, 2H, *J* 6.0, ArN(CH$_2$)$_3$CH_2N), 1.89 (m, 2H, ArNCH$_2$CH_2(CH$_2$)$_2$N), 1.62 (m, 2H, ArN(CH$_2$)$_2$CH_2CH$_2$NH$_2$); δ_C(75 MHz, CD_3OD) 152.9, 150.5, 134.0, 128.9, 124.9, 126.6, 122.0, 117.0, 98.8, 43.6, 41.4, 30.1, 28.9.

***N'*- (7-Chloroquinolin-4-yl)-hexane-1,2-diamine, 3.55**



Yellow needles (1.40 g, 96%), m.p. 136 – 138 °C; R_f(NH_3:MeOH 1:49) 0.22; δ_H(300 MHz, CD_3OD) 8.39 (d, 1H, J 5.4, H-2), 8.18 (d, 1H, J 9.0, H-5), 7.79 (d, 1H, J 2.1, H-8), 7.44 (dd, 1H, J 2.1, 9.0, H-6), 6.68 (d, 1H, J 5.4, H-3), 3.36 (t, 2H, J 6.0, ArNCH_2(CH_2)$_3$N), 2.67 (t, 2H, J 6.0, NH_2CH_2) 1.77 (m, 2H, $ArNCH_2CH_2$(CH_2)$_2$N), 1.51 – 1.40 (m, 6H, $ArNCH_2$(CH_2)$_3CH_2$N; δ_C(75 MHz, CD_3OD) 152.9, 150.5, 134.0, 128.9, 126.5, 124.9, 122.0, 117.0, 98.8, 42.6, 41.1, 31.8, 28.2, 26.7, 26.5.

7-Chloro-4-(piperazin-1-yl)quinoline



A mixture of piperazine (5.44 g, 63 mmol), K_2CO_3 (0.52g, 3.78 mmol), Et_3N and 4,7-dichloroquinoline (2.50g, 12.62 mmol) in 18 ml of *N*-methylpyrrolidinone (NMP) was refluxed under a nitrogen atmosphere for 4 h. The reaction mixture was cooled to room temperature and diluted with DCM (200 ml), washed with brine (3 x 100 ml) and purified by chromatography on silica gel (eluent MeOH: DCM 1: 9) to afford the free base as cream white crystals (2.74 g, 88%), m.p. 113 – 115 °C (lit m.p. 113 – 115 °C);[3] R_f(MeOH:DCM 1:9) 0.10; δ_H(300 MHz, $CDCl_3$) 8.71 (d, 1H, *J* 5.1, H-2), 8.03 (d, 1H, *J* 2.1, H-8), 7.95 (d, 1H, *J* 9.0, H-5), 7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 6.83 (d, 1H, *J* 5.1, H-3), 3.18 (m, 8H, 4 x PipNCH_2), 1.68 (br s, 1H, NH).

General Procedure for the synthesis of epoxides a – d (G)

Method (a)

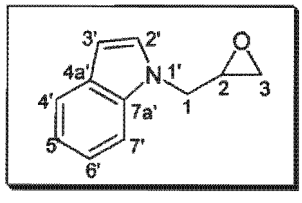
A mixture of the amine (1 eq) and KF-Al_2O_3 in CH_3CN were stirred at ambient temperature for 10 min before addition of epichlorohydrin (3 eq) *via* syringe. After stirring

at room temperature for 18 – 22 h the reaction mixture was filtered and evaporated to dryness. Purification by column chromatography (SiO_2, EtOAc:Hex 1:9) afforded the desired epoxides in moderate to good yields.

Method (b)

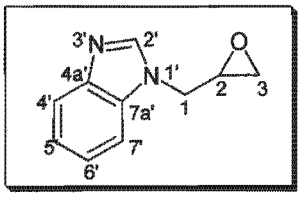
Epichlorohydrin (3 eq) was added to an ice-cold solution of NaH (1.5 eq) and the appropriate amine/phenol (1.0 eq) in DMF (20 ml) at 0 °C under an atmosphere of nitrogen. The suspension was allowed to warm to room temperature and stirred for 22 h. Excess epichlorohydrin was removed *in vacuo* and the residue diluted with H_2O, extracted with EtOAc, dried ($MgSO_4$) and evaporated. The residue was purified by column chromatography on SiO_2 eluting with 10 – 30% EtOAc in hexanes.

1-(Oxiran-2-ylmethyl)-1*H*-indole, 3.60



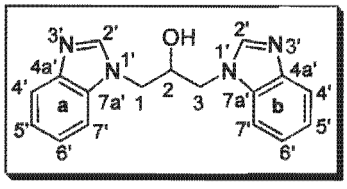
The two methods described in General Procedure G were used for the synthesis of **3.60**, using 12.37 mmol of indole and 37.1 mmol of epichlorohydrin. The product was obtained as a yellow oil (1.5 g, 70%); R_f(EtOAc:Hex 1:9) 0.12; δ_H(400 MHz, $CDCl_3$) 7.66 (d, 1H, *J* 8.0, H-4′), 7.40 (dd, 1H, *J* 1.2, 8.4, H-7′), 7.50 (td, 1H, *J* 1.2, 7.2, H-5′), 7.18 – 7.12 (m, 2H, H-2′, H-6′), 6.55 (d, 1H, *J* 3.2, H-3′), 4.43 (dd, 1H, *J* 3.6, 15.2, H-1α), 4.20 (dd, 1H, *J* 5.2, 15.2, H-1β), 3.24 (m, 1H, H-2), 2.81 (dd, 1H, *J* 4.0, 4.8, H-3α), 2.47 (dd, 1H, *J* 2.8, 4.8, H-3β); δ_C(75 MHz, $CDCl_3$) 136.3, 128.6, 128.1, 121.8, 121.0, 119.6, 109.2, 101.9, 50.8, 47.7, 45.2.

1-(Oxiran-2-ylmethyl)-1*H*-benzo[d]imidazole, 3.63



The two methods described in General Procedure G were used for the synthesis of **3.63**, using 9.67 mmol of benzimidazole and 48.4 mmol of epichlorohydrin. The product was obtained as a yellow oil (0.65 g, 46%); δ_H(400 MHz, $CDCl_3$) 7.90 (s, 1H, H-2′), 7.81 – 7.78 (m, 1H, ArH), 7.46 – 7.43 (m, 1H, ArH), 7.30 – 7.35 (m, 2H, ArH), 4.51 (dd, 1H, *J* 2.8, 15.2, H-1α), 4.13 (dd, 1H, *J* 5.6, 15.2, H-1β), 3.29 (m, 1H, H-2), 2.83 (dd, 1H, *J* 3.6, 4.4, H-3α), 2.49 (dd, 1H, *J* 2.4, 4.4, H-3β); δ_C(75 MHz, $CDCl_3$) 143.6, 143.1, 134.0, 122.9, 122.3, 120.4, 109.6, 50.2, 46.5, 45.1.

1,3-di(1*H*-*Benzo[d]imidazol*-1-*yl*)*propan*-2-*ol*

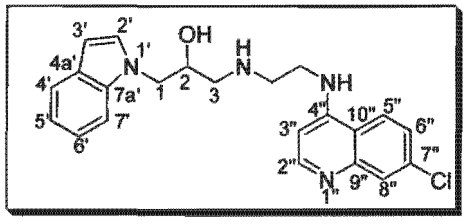


Grey powder, (1.14 g, 46%), m.p. 95 – 97 °C; R_f (EtOAC:Hex 1:4) 0.18; δ_H(400 MHz, $CDCl_3$) 7.56 (s, 2H, H-2′**a**, H-2′**b**), 7.31 (d, 2H, *J* 8.0, H-7′**a**, H-7′**b**), 7.20 (d, 2H, *J* 8.0, H-4′**a**, H-4′**b**), 7.14 (dt, 2H, *J* 1.2, 8.4, H-5′**a**, H-5′**b**), 7.05 (dt, 2H, *J* 1.2, 8.4, H-6′**a**, H-6′**b**), 4.26 (m, 1H, H-2), 4.14 (dd, 2H, *J* 3.6, 14.4, H-1α, H-3α), 4.03 (dd, 2H, *J* 8.4, 14.4, H-1β, H-3β); δ_C(100 MHz, $CDCl_3$) 143.2 (2C), 142.7 (2C), 133.4 (2C), 123.1 (2C), 122.4 (2C), 119.6 (2C), 109.5 (2C), 67.8, 48.8 (2C); HRMS (EI) 292.13208 (M^+ $C_{17}H_{16}N_4O$ requires 292.13241).

All the 1H NMR spectra of the secondary β-amino alcohols revealed a broad NH signal between δ 2.0 and 3 ppm that was obscuring significant signals due to other Hs. Therefore it was necessary to run D_2O experiments in order to rid the spectra of the NH, peak. The following spectroscopic data for all the secondary β-amino alcohols were those obtained following D_2O experiments, thus no couplings to NH were observed.

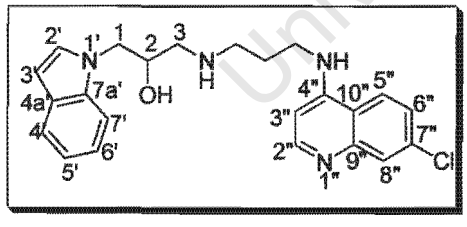
The method used for the synthesis of **3.78** – **3.81** and **3.100** was that described in the General Procedure A, using 0.56 mmol of the epoxide **3.60**.

1-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethylamino*)-3-(1*H*-indol-1-*yl*)*propan*-2-ol, 3.78



Yellow oil (220 mg, 71%); R_f(MeOH:DCM 1:9) 0.13; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3690 (OH), 3409 (NH), 3054 (ArCH), 2986 (CH), 1653 (C=C), 1265 (C-O); δ_H(400 MHz, $CDCl_3$) 8.34 (d, 1H, *J* 5.2, H-2″), 7.87 (d, 1H, *J* 2.0, H-8″), 7.62 (d, 1H, *J* 8.0, H-4′), 7.57 (d, 1H, *J* 9.2, H-5″), 7.35 (d, 1H, *J* 8.4, H-7′), 7.25 (m, 4H, H-2′, H-5′, H-6′, H-6″), 6.50 (d, 1H, *J* 2.8, H-3′), 6.20 (d, 1H, *J* 5.2, H-3″), 4.20 (m, 3H, H-1, H-2), 3.20 (m, 2H, ArNCH_2CH_2N), 2.90 (m, 2H, NCH_2CH_2Ar), 2.73 (d, 1H, *J* 8.8, H-3α), 2.63 (d, 1H, *J* 8.8, H-3β); δ_C(100 MHz, $CDCl_3$) 151.4, 150.3, 149.0, 136.3, 133.6, 129.2, 128.0, 127.1, 124.0 (2C), 120.8, 120.4, 118.6, 117.3, 106.0, 100.0, 98.7, 68.9, 53.0, 50.2, 47.2, 40.6; LRMS (EI) *m/z* 395.2 (86%, M^+ $C_{22}H_{23}ClN_4O$); HPLC Purity: 99%; t_R' = 1.78 min.

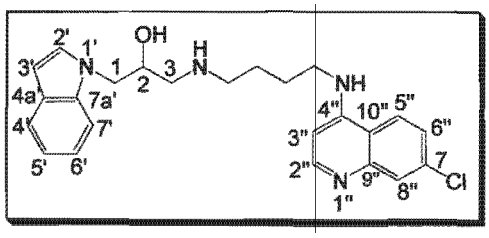
1-(3-(7-*Chloroquinolin*-4-*ylamino*)*propylamino*)-3-(1H-*indol*-1-*yl*)*propan*-2-*ol*. 3.79



Off-white powder (208 mg, 65%), m.p. 139 – 141 °C; R_f(MeOH:DCM 1:9) 0.13; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3690 (OH), 3409 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1649 (C=C), 1265 (C-O); δ_H(300 MHz, DMSO-d_6) 8.35 (d, 1H, *J* 5.7, H-2″), 8.19 (d, 1H, *J* 9.0, H-5″), 7.77 (d, 1H, *J* 2.1, H-8″), 7.50 – 7.24 (m, 3H, H-4′, H-5″, H-6″), 7.30 (d, 1H, *J* 3.3, H-2′), 7.10 (t, 1H, *J* 7.5, H-5′), 7.06 (t, 1H, *J* 7.8, H-6′), 6.46 (d, 1H, *J* 5.7, H-3″), 6.39 (dd, 1H, *J* 0.8, 3.3, H-3′), 4.24 (dd, 1H, *J* 6.4, 14.4, H-1α), 4.07 (dd, 1H, *J* 4.8, 14.4, H-1β), 3.92 (m, 1H, H-2), 3.31 (t,

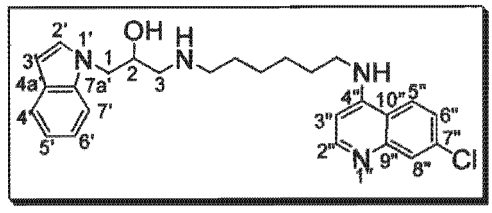
2H, *J* 6.3, ArNCH_2(CH_2)$_2$N), 2.65 (t, 2H, *J* 6.3, NC$H_2$$CH_2$$CH_2$Ar), 2.53 (dd, 2H, *J* 4.8, 7.2, CH_2-3) 2.00 (m, 2H, NCH$_2$C$H_2$$CH_2$Ar); δ_C(75 MHz, DMSO-d_6) 151.9, 150.1, 149.0, 136.1, 133.3, 129.4, 128.0, 127.4, 124.0 (2C), 120.7, 120.2, 118.7, 117.4, 106, 100.0, 98.5, 68.8, 52.9, 50.0, 47.2, 40.8, 27.7; LRMS (EI) *m/z* 409.6 (65%, M^+ $C_{23}H_{25}ClN_4O$); Purity: 94%; t_R' = 1.66 min.

1-(4-(7-*Chloroquinolin*-4-*ylamino*)*butylamino*)-3-(1H-*indol*-1-*yl*)*propan*-2-*ol*, 3.80



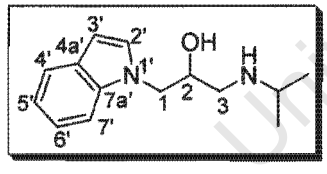
Yellow oil (206 mg, 59%); R_f(MeOH:DCM 1:9) 0.13; IR ν_{max}($CHCl_3$)/cm^{-1} 3690(OH), 3409 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1653 (C=C), 1265 (C-O); δ_H(400 MHz, DMSO-d_6) 8.36 (d, 1H, *J* 5.2, H-2″), 8.25 (d, 1H, *J* 9.2, H-5″), 7.75 (d, 1H, *J* 2.4, H-8″), 7.50 (d, 1H, *J* 7.6, H-4′), 7.45 (dd, 1H, *J* 0.8, 8.4, H-7′), 7.40 (dd, 1H, *J* 2.4, 9.2, H-6″), 7.27 (d, 1H, *J* 3.2, H-2′), 7.07 (t, 1H, *J* 7.2, H-5′), 6.97 (t, 1H, *J* 8.0, H-6′), 6.44 (d, 1H, *J* 5.2, H-3″), 6.38 (dd, 1H, *J* 3.2, CH_2-3′), 4.23 (dd, 1H, *J* 4.8, 14.4, H-1α), 4.05 (dd, 1H, *J* 6.8, 14.4, H-1β), 3.88 (m, 1H, H-2), 3.31 (t, 2H, *J* 7.2, ArNCH_2(CH_2)$_3$N), 2.56 (t, 2H, *J* 7.2, NCH_2[(CH_2)$_3$NAr), 2.50 (dd, 2H, *J* 4.8, 7.2, H-3), 1.68 (m, 2H, ArNCH$_2$C$H_2$$CH_2$N), 1.52 (m, 2H, NCH$_2CH_2$[($CH_2$)$_2$NAr); δ_C(100 MHz, DMSO-d_6) 151.9, 150.1, 149, 136.1, 133.3, 129.3, 128.0, 127.2, 124.0 (2C), 120.8, 120.2, 118.8, 117.4, 106.0, 100.1, 98.5, 68.8, 52.9, 50.0, 47.2, 40.8, 27.8, 24.5; LRMS (EI) *m/z* 451.6 (19%, M^+ $C_{24}H_{27}ClN_4O$); HPLC Purity: 94%; t_R' = 1.51 min.

1-(6-(7-*Chloroquinolin*-4-*ylamino*)*hexylamino*)-3-(1*H*-*indol*-1-*yl*)*propan*-2-*ol*. 3.81



Yellow oil (196 mg, 70%); R_f(MeOH:DCM 1:9) 0.13; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3690(OH), 3409 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1647 (C=C), 1265 (C-O); δ_H(400 MHz, $CDCl_3$) 8.47 (d, 1H, *J* 5.2, H-2″), 7.94 (d, 1H, *J* 2.4, H-8″), 7.63 (m, 2H, H-4′, H-5″), 7.36 (dd, 1H, *J* 1.2, 8.4, H-7′), 7.28 (dd, 1H, *J* 2.4, 8.8, H-6″), 7.19 (t, 1H, *J* 7.2, H-5′), 7.15 (d, 1H, *J* 3.2, H-2′), 7.09 (t, 1H, *J* 8.0, H-6′), 6.49 (dd, 1H, *J* 0.8, 3.2, H-3′), 6.36 (d, 1H, *J* 5.6, H-3″), 4.17 (dd, 2H, *J* 3.2, 5.2, C*H*$_2$-1), 4.17 (m, 1H, H-2), 3.27 (t, 2H, *J* 7.2, ArNC*H*$_2$(CH$_2$)$_5$N), 2.70 (dd, 1H, *J* 3.6, 12.4, H-3α), 2.60 – 2.47 (m, 3H, H-3β, NC*H*$_2$(CH$_2$)$_5$NAr), 1.72 (m, 2H, C*H*$_2$CH$_2$NAr), 1.44 (m, 4H, NCH$_2$C*H*$_2$C*H*$_2$(CH$_2$)$_3$NAr), 1.37 (m, 2H, NCH$_2$(CH$_2$)$_3$C*H*$_2$(CH$_2$)$_2$NAr); δ_C(100 MHz, $CDCl_3$) 152.0, 150.4, 149, 136.3, 133.5, 129.6, 128.0, 127.5, 124.0 (2C), 120.9, 120.5, 118.6, 117.3, 106, 100.0, 98.7, 68.8, 52.9, 50.0, 47.2, 43.1, 29.8, 26.6, 26.7, 27.7; LRMS (EI) *m/z* 451.6 (19%, M^+ $C_{26}H_{31}ClN_4O$); HPLC Purity: 97%; t_R' = 1.75 min.

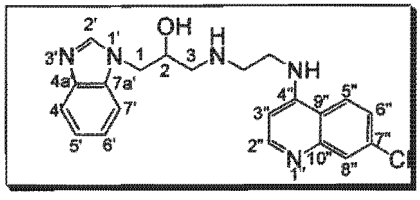
1-(1*H*-*Indol*-1-*yl*)-3-(*isopropylamino*)*propan*-2-*ol*, 3.100



Colourless plates (231 mg, 84%), m.p. 120 – 122 °C; R_f(MeOH:DCM 1:9) 0.36; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3690 (OH), 3409 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1645 (C=C), 1265 (C-O); δ_H(300 MHz, $CDCl_3$) 7.62 (d, 1H, *J* 8.1, H-4′), 7.37 (d, 1H, *J* 8.0, H-7′), 7.20 (t, 1H, *J* 6.9, H-5′), 7.15 (d, 1H, *J* 3.3, H-2′), 7.10 (t, 1H, *J* 8.0, H-6′), 6.50 (d, 1H, *J* 3.3, H-3′), 4.10 (m, 2H, C*H*$_2$-1), 3.99 (m, 1H, H-2), 2.70 (m, 2H, NHC*H*, H-3α), 2.48 (dd, 1H, *J* 5.6, 14.4, H-3β), 1.02 (d, 3H, *J* 6.0, C*H*$_3$), 1.00 (d, 3H, *J* 6.0, C*H*$_3$); δ_C(75 MHz, $CDCl_3$) 136.4, 128.7, 121.6, 120.9, 119.4, 109.4, 101.5, 99.8, 69.2, 50.3, 49.9, 48.9, 22.9, 22.7; LRMS (EI) *m/z* 233 (100%, M^+ $C_{14}H_{20}N_2O$); HPLC Purity: 99%; t_R' = 1.10 min.

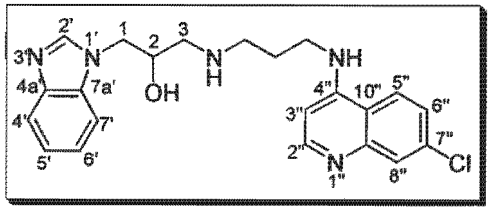
The method employed for synthesis of **3.82 – 3.85** and **3.101** was that described in the General Procedure A, using 0.465 mmol of the epoxide **3.63**.

1-(1*H*-Benzo[d]imidazol-1-yl)-3-(2-(7-chloroquinolin-4-ylamino)ethylamino)propan-2-ol, 3.82



Brown paste (123 mg, 67%); R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}($CHCl_3$)/cm^{-1} 3690(OH), 3409 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1583 (C=N), 1265 (C-O); δ_H(300 MHz, DMSO-d_6) 8.36 (d, 1H, *J* 5.4, H-2″), 8.20 (d, 1H, *J* 9.0, H-5″), 8.15 (s, 1H, H-2′), 7.94 (d, 1H, *J* 2.1, H-8″), 7.58 (m, 2H, H-4′, H-7′), 7.40 (dd, 1H, *J* 9.0, H-6″), 7.16 (m, 2H, H-5′, H-6′), 6.49 (d, 1H, *J* 5.4, H-3″), 4.30 (dd, 1H, *J* 4.8, 14.4, H-1α), 4.12 (dd, 1H, *J* 5.1, 14.4, H-1β), 3.86 (m, 1H, H-2), 3.44 (t, 2H, *J* 6.6, ArNC$H_2$$CH_2$N), 2.86 (dd, 1H, *J* 3.9, 4.8, H-3α), 2.80 (t, 2H, *J* 6.6, NC$H_2$$CH_2$NAr), 2.55 (dd, 1H, *J* 7.2, 14.4, H-3β); δ_C(75 MHz, DMSO-d_6) 151.9, 150.2, 149.0, 144.5, 143.0, 134.4, 133.2, 129.7, 124.0 (2C), 121.5, 120.0, 119.6, 117.9, 115.2, 98.4, 68.2, 52.3, 48.8, 45.7, 43.5; LRMS (EI) *m/z* 396.4 (5%, M^+ + H, $C_{21}H_{22}ClN_5O$ + H); HPLC Purity: 91%; t_R' = 1.23 min.

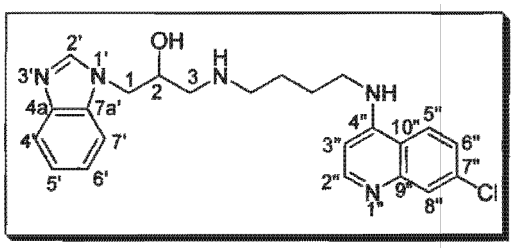
1-(1*H*-Benzo[d]imidazol-1-yl)-3-(3-(7-chloroquinolin-4-ylamino)propylamino)propan-2-ol, 3.83



Pale yellow crystals (122 mg, 64%), m.p. 90 – 93 °C; R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}($CHCl_3$)/cm^{-1} 3690(OH), 3417 (NH), 3050 (ArCH), 2986 (aliphatic CH), 1640 (C=C), 1584 (C=N), 1265 (C-O); δ_H(300 MHz, DMSO-d_6) 8.36 (d, 1H, *J* 5.4, H-2″), 8.20 (d, 1H, *J* 9.0, H-5″), 8.12 (s, 1H, H-2′), 7.92 (d, 1H, *J* 2.1, H-8″), 7.60 (m, 2H, H-4′, H-7′), 7.42 (dd, 1H,

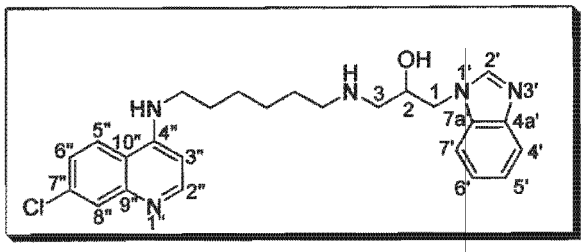
J 2.1, 9.0, H-6″), 7.19 (m, 2H, H-5′, H-6′), 6.47 (d, 1H, *J* 5.4, H-3″), 4.34 (dd, 1H, *J* 3.9, 14.4, H-1α), 4.15 (dd, 1H, *J* 7.2, 14.4, H-1β), 3.93 (m, 1H, H-2), 3.32 (t, 2H, *J* 6.3, ArNCH_2(CH_2)$_2$N), 2.63 (t, 2H, *J* 6.3, NCH_2(CH_2)$_2$NAr), 2.51 (m, 2H, CH_2-3), 1.80 (m, 2H, ArNCH$_2$CH_2CH$_2$N); δ_C(100 MHz, DMSO-d_6) 151.9, 150.1, 149.0, 144.6, 143.2, 134.3, 133.3, 127.2, 123.9 (2C), 121.9, 121.1, 119.2, 117.4, 110.5, 98.5, 68.5, 53.0, 48.6, 47.3, 41.0, 30.4; LRMS (EI) *m/z* 409.9 (45%, M^+ $C_{22}H_{24}ClN_5O$); HPLC Purity: 94%; t_R' = 1.15 min.

1-(1*H*-*Benzo[d]imidazol*-1-*yl*)-3-(4-(7-*chloroquinolin*-4-*ylamino*)*butylamino*)*propan*-2-*ol*, 3.84



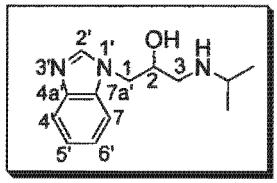
Brown oil (146 mg, 74%); R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}($CHCl_3$)/cm^{-1} 3690 (OH), 3409 (NH), 3052 (ArCH), 2980 (aliphatic CH), 1580 (C=N), 1263 (C-O); δ_H(400 MHz, $CDCl_3$) 8.34 (d, 1H, *J* 5.4, H-2″), 8.21 (d, 1H, *J* 9.0, H-5″), 8.16 (s, 1H, H-2′), 7.90 (d, 1H, *J* 2.0, H-8″), 7.61 (m, 2H, H-4′, H-7′), 7.40 (dd, 1H, *J* 4.0, 8.8, H-6″), 7.17 (m, 2H, H-5′, H-6′), 6.45 (d, 1H, *J* 5.4, H-3″), 4.32 (dd, 1H, *J* 3.9, 14.4, H-1α), 4.12 (dd, 1H, *J* 7.2, 14.4, H-1β), 3.90 (m, 1H, H-2), 3.32 (t, 2H, *J* 6.3, ArNCH_2(CH_2)$_3$N), 2.63 (t, 2H, *J* 6.3, NCH_2(CH_2)$_3$NAr), 2.51 (m, 2H, CH_2-3), 1.80 (m, 2H, N(CH$_2$)$_2$CH_2CH$_2$Ar), 1.60 (m, 2H, NCH$_2$CH_2(CH$_2$)$_2$NAr); δ_C(100 MHz, $CDCl_3$) 151.9, 150.1, 149.0, 144.6, 143.2, 134.3, 133.3, 127.2, 123.9 (2C), 121.9, 121.1, 119.2, 117.4, 110.5, 98.5, 68.5, 53.0, 48.6, 47.3, 41.0, 29.2, 28.6; LRMS (EI) *m/z* 424.4 (2%, M^+ $C_{23}H_{26}ClN_5O$); HPLC Purity: 95%; t_R' = 1.10 min.

1-(1*H*-*Benzo[d]imidazol*-1-*yl*)-3-(6-(7-*chloroquinolin*-4-*ylamino*)*hexylamino*)*propan*-2-*ol*. 3.85



Yellow oil (139 mg, 66%); R_f(MeOH:DCM 1:9) 0.15; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3688(OH), 3411 (NH), 3051 (ArCH), 2983 (aliphatic CH), 1582 (C=N), 1265 (C-O); δ_H(400 MHz, $CDCl_3$) 8.36 (d, 1H, *J* 5.4, H-2″), 8.20 (d, 1H, *J* 9.0, H-5″), 8.12 (s, 1H, H-2′), 7.91 (d, 1H, *J* 2.0, H-8″), 7.60 (m, 2H, H-4′, H-7′), 7.39 (dd, 1H, *J* 2.0, 8.8, H-6″), 7.20 (m, 2H, H-5′, H-6′), 6.47 (d, 1H, *J* 5.4, H-3″), 4.34 (dd, 1H, *J* 3.9, 14.4, H-1α), 4.15 (dd, 1H, *J* 7.2, 14.4, H-1β), 3.93 (m, 1H, H-2), 3.32 (t, 2H, *J* 6.3, ArNCH_2(CH_2)$_5$N), 2.63 (t, 2H, *J* 6.3, NCH_2(CH2)$_5$NAr), 2.51 (m, 2H, CH_2-3), 1.80 (m, 2H, ArNCH$_2$CH_2(CH_2)$_4$N), 1.74 (m, 2H, *J* 6.8, ArNCH$_2$CH_2(CH_2)$_4$)N), 1.62 - 1.49 (m, 4H, ArN(CH_2)CH_2CH_2(CH_2)$_2$N); δ_C(100 MHz, $CDCl_3$) 151.9, 150.1, 149.0, 144.6, 143.2, 134.3, 133.3, 127.2, 123.9 (2C), 121.9, 121.1, 119.2, 117.4, 110.5, 98.5, 68.5, 53.0, 48.6, 47.3, 41.0, 29.5, 28.6, 26.7 (2C); LRMS (EI) *m/z* 452.4 (6%, M^+ $C_{25}H_{30}ClN_5O$); HPLC Purity: 98%; t_R' = 1.02 min.

1-(1*H*-*Benzo[d]imidazol*-1-*yl*)-3-(*isopropylamino*)*propan*-2-*ol*, 3.101

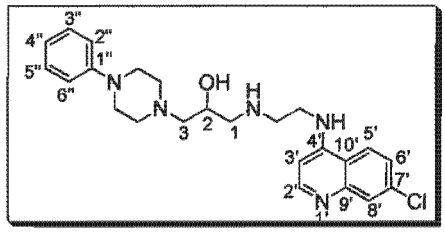


Yellow oil (88 mg, 81%); R_f(MeOH:DCM 1:9) 0.22; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3684(OH), 3429 (NH), 3044 (ArCH), 2985 (aliphatic CH), 1584 (C=N), 1265 (C-O); δ_H(400 MHz, $CDCl_3$) 7.88 (s, 1H, H-2′), 7.66 (d, 1H, *J* 8.0, H-7′), 7.54 (d, 1H, *J* 8.0, H-4′), 7.27 – 7.18 (m, 2H, H-5′, H-6′), 4.22 (dd, 1H, *J* 4.4, 14.4, H-1α), 4.12 (dd, 1H, *J* 7.2, 14.4, H-1β), 3.98 (m, 1H, H-2), 2.75 (m, 2H, H-3α, NHC*H*), 2.48 (dd, 1H, *J* 7.2, 14.4, H-3β), 1.08 (d, 6H, *J* 6.4, C(CH_3)$_2$); δ_C(100 MHz, $CDCl_3$) 144.0, 143.6, 133.4, 122.9, 122.0, 120.0, 109.7, 68.4,

50.0, 49.0, 48.8, 22.5 (2C); LRMS (EI) *m/z* 234.2 (50%, M^+ $C_{13}H_{19}N_3O$); Purity: 94%; t_R' = 0.58 min.

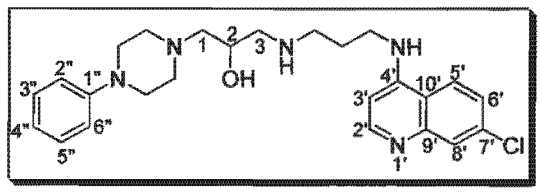
The method employed for the synthesis of **3.86** – **3.89** and **3.102** was that described in the General Procedure A, using 0.69 mmol of epoxide.

1-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethylamino*)-3-(4-*phenylpiperazin*-1-*yl*)*propan*-2-*ol*, 3.86



Yellow oil (200 mg, 66%); R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}($CHCl_3$)/cm^{-1} 3624 (OH), 3419 (NH), 3032 (ArCH), 2987, 2877 (CH), 1647 (C=C), 1567 (C=N); δ_H(300 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.4, H-2′), 7.90 (d, 1H, *J* 2.4, H-8′), 7.77 (d, 1H, *J* 9.0, H-5′), 7.31 – 7.27 (m, 3H, H-3″, H-5″, H-6′), 6.96 – 6.87 (m, 3H, H-2″, H-4″, H-6″), 6.38 (d, 1H, *J* 5.4, H-3′), 3.96 (m, 1H, H-2), 3.42 (m, 2H, PipNCH_2Ar), 3.21 (m, 4H, PipNCH_2, ArNCH_2CH_2N), 3.10 – 2.42 (m, 10H, CH_2-1, CH_2-3, NCH_2CH_2NAr, 2 x PipNCH_2); δ_C(75 MHz, $CDCl_3$) 151.0, 150.7, 147.4, 135.2, 129.1 (2C), 127.3, 125.4, 122.8, 120.0, 117.1, 116.0 (3C), 98.5, 65.6, 62.0, 53.9, 53.5, 49.4, 48.9, 43.4, 30.6, 27.9; LRMS *m/z* 440.5 (18%, M^+ $C_{24}H_{30}ClN_5O$); HPLC Purity: 99%; t_R′ = 1.00 min.

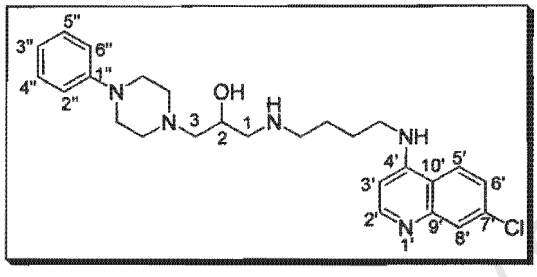
1-(2-(7-*Chloroquinolin*-4-*ylamino*)*propylamino*)-3-(4-*phenylpiperazin*-1-*yl*)*propan*-2-*ol*, 3.87



Orange oil (187 mg, 60%); R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}($CHCl_3$)/cm^{-1} 3620 (OH), 3423 (NH), 3030 (ArCH), 2985, 2879 (CH), 1651 (C=C), 1560 (C=N); δ_H(400MHz, $CDCl_3$) 8.48 (d, 1H, *J* 5.6, H-2′), 7.92 (d, 1H, *J* 2.4, H-8′), 7.78 (d, 1H, *J* 8.8, H-5′), 7.29-

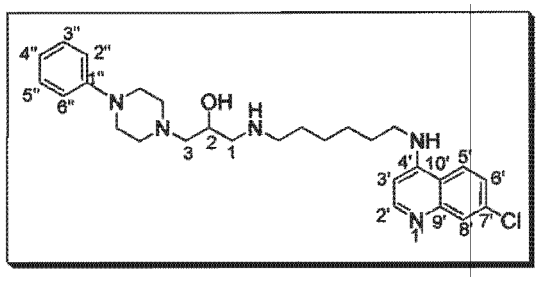
7.25 (m, 3H, H-3″, H-5″, H-6′), 6.94-6.85 (m, 3H, H-2″, H-4″, H-6″), 6.32 (d, 1H, *J* 5.6, H-3′), 3.94 (m, 1H, H-2), 3.40 (m, 2H, PipNCH_2), 3.20 (m, 4H, PipNCH_2Ar, ArNCH_2(CH$_2$)$_2$N), 3.01 – 2.40 (m, 10H, CH_2-1, CH_2-3, NCH_2(CH$_2$)$_2$NAr, 2 x PipNCH_2), 1.94 (quint, 2H, *J* 7.2, NCH$_2$CH_2CH$_2$NAr); δ_C(400MHz, $CDCl_3$) 151.1, 150.5, 147.6, 135.3, 129.1 (2C), 127.1, 125.1, 122.7, 120.0, 117.3, 116.1 (3C), 98.2, 65.7, 62.0, 53.7, 53.3, 49.2, 48.8, 43.2, 30.7, 27.6, 26.4; LRMS (EI) *m/z* 454.4 (27%, M^+ $C_{25}H_{32}ClN_5O$); HPLC Purity: 98%; t_R' = 1.46 min.

1-(2-(7-*Chloroquinolin*-4-*ylamino*)*butylamino*)-3-(4-*phenylpiperazin*-1-yl)*propan*-2-*ol*, 3.88



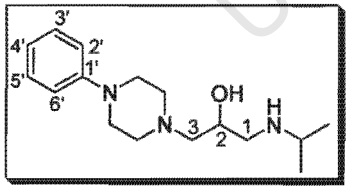
Orange oil (229 mg, 71%); R_f(MeOH:DCM 1:9) 0.15; IR ν_{max} ($CHCl_3$)/cm^{-1} 3618 (OH), 3422 (NH), 3030 (ArCH), 2986, 2877 (CH), 1651 (C=C), 1560 (C=N); δ_H(400 MHz, $CDCl_3$) 8.49 (d, 1H, *J* 5.4, H-2′), 7.92 (d, 1H, *J* 2.1, H-8′), 7.74 (d, 1H, *J* 9.0, H-5′), 7.29 – 7.25 (m, 3H, H-3″, H-5″, H-6′), 6.94 – 6.85 (m, 3H, H-2″, H-4″, H-6″), 6.37 (d, 1H, *J* 5.6, H-3′), 3.88 (m, 1H, H-2), 3.4 (m, 2H, PipNCH_2), 3.20 (m, 4H, PipNCH_2Ar, ArNCH_2(CH$_2$)$_3$N), 3.05 – 2.40 (m, 10H, CH_2-1, CH_2-3, NCH_2(CH$_2$)$_3$NAr, 2 x PipNCH_2), 1.90 (quint, 2H, *J* 7.2, NCH$_2$CH_2(CH$_2$)$_2$NAr), 1.68 (m, 2H, N(CH$_2$)$_2$CH_2CH$_2$Ar); δ_C(100 MHz, $CDCl_3$) 151.1, 150.5, 147.6, 135.3, 129.1 (2C), 127.1, 125.1, 122.7, 120.0, 117.3, 116.3 (3C), 98.2, 65.5, 62.0, 53.5, 53.1, 49.9, 49.0, 43.2, 41.7, 30.7, 27.6, 26.1; LRMS *m/z* 468.6 (6%, M^+ + H $C_{26}H_{34}ClN_5O$); HPLC Purity: 90%; t_R' = 1.32 min.

1-(2-(7-*Chloroquinolin*-4-*ylamino*)*hexylamino*)-3-(4-*phenylpiperazin*-1-*yl*)*propan*-2-*ol*, 3.89



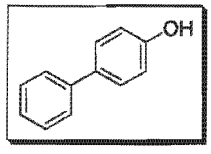
Yellow powder (223 mg, 63%), m.p. 110 – 112 °C; R_f(MeOH:DCM 1:9) 0.15; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3619(OH), 3424 (NH), 3022 (ArCH), 2989, 2887 (CH), 1653 (C=C), 1563 (C=N); δ_H(400 MHz, $CDCl_3$) 8.43 (d, 1H, *J* 5.4, H-2″), 7.94 (d, 1H, *J* 2.1, H-8′), 7.73 (d, 1H, *J* 9.0, H-5′), 7.29-7.23 (m, 3H, H-3″, H-5′, H-6″), 6.93 - 6.82 (m, 3H, H-2″, H-4″, H-6″), 6.35 (d, 1H, *J* 5.6, H-3′), 3.91 (m, 1H, H-2), 3.36 (m, 2H, PipNCH_2Ar), 3.24 (m, 4H, PipNCH_2, ArNC$H_2$$(CH_2)_5$N), 3.06 – 2.41 (m, 10H, CH_2-1, CH_2-3, NC$H_2$$(CH_2)_5$NAr, 2 x PipNC$H_2$), 1.89 (quint, 2H, *J* 7.2, ArNCH$_2$C$H_2$$(CH_2)_4$N), 1.68 (m, 2H, NCH$_2CH_2$$(CH_2)_4$NAr), 1.66 – 1.60 (m, 4H, ArN$(CH_2)_2$CH_2C$H_2$$(CH_2)_2$N); δ_C(100 MHz, $CDCl_3$) 150.9, 150.1, 147.3, 135.1, 129.4 (2C), 127.4, 125.2, 122.8, 120.6, 117.1, 116.4 (3C), 99.2, 65.8, 62.0, 53.7, 53.4, 50.1, 49.4, 48.8, 43.2, 30.8, 30.4, 26.3 (2C), 25.8; LRMS 495.2 (45%, M^+ $C_{28}H_{38}ClN_5O$); HPLC Purity: 97%; t_R' = 1.40 min.

1-(*Isopropylamino*)-3-(4-*phenylpiperazin*-1-*yl*)*propan*-2-*ol*, 3.101



Brown oil (179 mg, 77%); R_f(MeOH:DCM 1:5) 0.25; IR $\nu_{max}(CHCl_3)/cm^{-1}$) 3620 (OH), 3423 (NH), 3030 (ArCH), 2985, 2877 (CH), 1646(C=C); δ_H(400 MHz, $CDCl_3$) 7.25 (m, 2H, ArH), 6.92 (m, 3H, ArH), 3.86 (m, 1H, H-2), 3.20 (m, 4H, 2 x PipNCH_2Ar), 2.84 – 2.38 (m, 9H, 4 x PipNCH_2, NC$H$$(CH_3)_2$), 1.08 (d, 6H, *J* 6.4, CH$(CH_3)_2$); δ_C(100 MHz, $CDCl_3$) 151.1, 129 (2C), 119.0, 116.0 (2C), 65.3, 53.6 (2C), 50.7 (2C), 49.8, 49.1 (2C), 21.3 (2C); LRMS *m/z* 278.7 (100%, M^+ + H $C_{16}H_{27}N_3O$); HPLC Purity: 89%; t_R' = 1.40′.

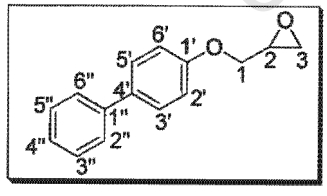
4–Phenylphenol, 3.72

A mixture of 4-bromophenol (0.95 g, 5.5 mmol), phenylboronic acid (0.61g, 5.0 mmol), 

K_2CO_3 (3.45 g, 25 mmol) and tetrakis triphenylphosphine palladium(0) (0.144 g, 0.25 mmol) dissolved in a 1:1 toluene:water mixture (40 ml) was degassed for 10 minutes and refluxed for 14 h under a nitrogen atmosphere. Upon cooling to room temperature the reaction mixture was acidified with 2M HCl to pH ~2, extracted with EtOAc, dried ($MgSO_4$) and evaporated to dryness. The residue was chromatographed on SiO_2 gel (eluent EtOAc:hexane 1:9) to afford the phenol as a cream white gum (0.595 g, 70%), R_f(MeOH:DCM 1:9) 0.10; IR ν_{max}(neat)/cm^{-1} 3400 (OH), 3013 (ArH), 1625 (C=C); δ_H(300 MHz, $CDCl_3$) 7.52 (dd, 2H, *J* 1.2, 8.4, ArH), 7.46 (d, 2H, *J* 8.8, ArH), 7.42 (t, 2H, *J* 7.6, ArH), 7.29 (t, 1H, *J* 8.4, ArH), 6.92 (d, 2H, *J* 8.8, ArH); δ_C(75 MHz, $CDCl_3$) 158.0, 140.0, 134.4 (2C), 132.3 (2C), 128.7, 128.2 (2C), 126.7, 115.0 (2C).

The procedure used for the synthesis of epoxide **3.68** was that desribed in General Procedure G, using 2.15 mmol of the phenol **3.72**.

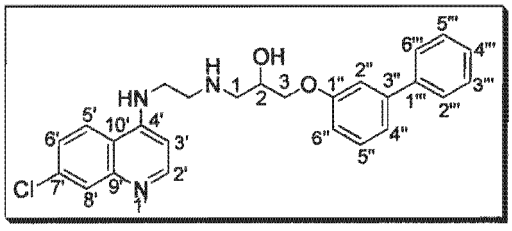
2-(4′-Phenyloxymethyl)oxirane, 3.68b



Colorless oil (485 mg, 73%); R_f(EtOAc:Hex 1:4) 0.20; IR ν_{max}(neat)/cm^{-1} 3006 (ArH), 1634 (C=C), 1239 (C-O); δ_H(400 MHz, $CDCl_3$) 7.55 (dd, 2H, *J* 1.2, 8.4, ArH), 7.46 (d, 2H, *J* 8.8, ArH), 7.42 (t, 2H, *J* 7.6, ArH), 7.29 (t, 1H, *J* 8.4, ArH), 6.92 (d, 2H, *J* 8.8, ArH), 4.07 (dd, 1H, *J* 5.1, 14.4, H-1′α), 3.92 (dd, 1H, *J* 4.8, 14.4, H-1′β), 3.23 (m, 1H, H-2′), 2.82 (dd, 1H, *J* 4.8, 5.1, H-3′α), 2.76 (dd, 1H, *J* 2.7, 5.1, H-3′β); δ_C(75 MHz, $CDCl_3$) 158, 140, 134.4 (2C), 132.3 (2C), 128.7, 128.2 (2C), 126.7, 115 (2C), 69, 50.1, 44.6.

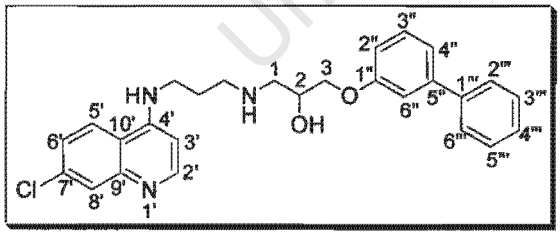
The method used for the synthesis of **3.90 – 3.99**, **3.103** and **3.104** was that described in General Procedure A, using 0.349 mmol of the epoxide.

***1-(2-(7-Chloroquinolin-4-ylamino)ethylamino)-3-(3-phenyloxy)propan-2-ol*, 3.94**



Yellow crystals (89 mg, 57%), m.p. 55 – 58 °C; R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}($CHCl_3$)/cm^{-1} 3604 (OH), 3419 (NH), 3044 (ArCH), 2987 (CH), 1655 (C=C), 1262 (C-O); δ_H(300 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.7, H-2′), 7.88 (d, 1H, *J* 2.1, H-8′), 7.68 – 7.23 (m, 8H, H-5′, H-6′, 6 x ArH), 6.93 (d, 1H, *J* 9.0, ArH), 6.88 (d, 2H, *J* 9.0, ArH), 6.34 (d, 1H, *J* 5.7, H-3′), 4.18 (m, 1H, H-2), 4.05 (dd, 1H, *J* 4.0, 14.4, H-3α), 3.94 (dd, 1H, *J* 7.2, 14.4, H-3β), 3.38 (t, 2H, *J* 6.0, ArNC$H_2$$CH_2$N), 3.18 (t, 2H, *J* 6.0, ArCH_2CH_2N), 2.92 (m, 2H, CH_2-1); δ_C(100 MHz, $CDCl_3$) 158.0, 151.3, 150.2, 148.3, 140.5, 135.1, 134.4, 132.3, 129.9, 128.7, 128.2 (2C), 127.8 (2C), 126.8, 125.3, 121.6, 117.2, 116.3, 113.5, 99.0, 70.6, 68.8, 51.5, 47.5, 42.2; LRMS *m/z* 447 (100%, M^+ $C_{26}H_{26}N_3O_2$); HPLC Purity: 99%, t_R' = 2.64 min.

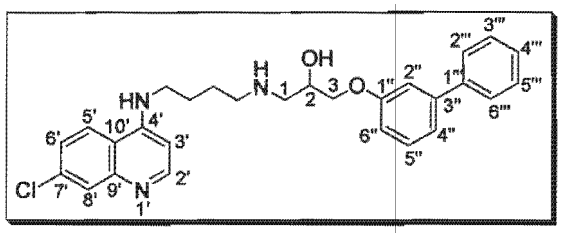
***1-(3-(7-Chloroquinolin-4-ylamino)propylamino)-3-(3-phenyloxy)propan-2-ol*, 3.95**



Yellow oil (122 mg, 76%); R_f(MeOH:DCM 1:9) 0.14; IR ν_{max}(neat)/cm^{-1} 3611 (OH), 3423 (NH), 3034 (ArCH), 2977 (CH), 1653 (C=C), 1260 (C-O); δ_H(300 MHz, $CDCl_3$) 8.42 (d, 1H, *J* 5.7, H-2′), 7.92 (d, 1H, *J* 2.1, H-8′), 7.71 (d, 1H, *J* 9.0, H-5′), 7.57 (d, 2H, *J* 8.4, ArH), 7.42 – 7.06 (m, 7H, H-6′, 6 x ArH), 6.82 (d, 1H, *J* 9.0, ArH), 6.36 (d, 1H, *J* 5.7, H-3′), 4.18 (m, 1H, H-2), 4.06 (d, 2H, *J* 4.4, CH_2-3), 3.23 (q, 2H, *J* 6.0, ArNC$H_2$$(CH_2)_2$N), 2.98 (dd, 1H, *J* 4.0, 12.0, H-1α), 2.81 (dd, 1H, *J* 7.2, 12.0, H-1β), 2.7 (t, 2H, *J* 6.0,

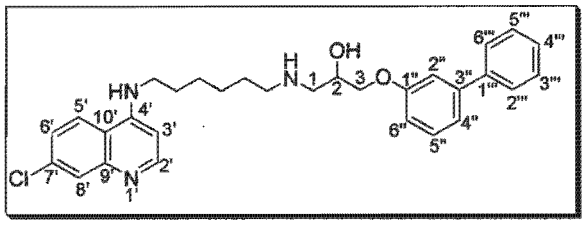
ArN(CH_2)$_2$CH_2N), 1.98 (m, 2H, ArCH$_2$CH_2CH$_2$N); δ_C(75 MHz, $CDCl_3$) 158.8, 151.3, 150.2, 148.5, 140.6, 135.0, 130.3, 129.7, 128.4, 128.2 (2C), 127.4 (2C), 127.0, 125.5, 121.3, 120.2, 117.0, 113.8, 113.2, 98.8, 70.7, 68.8, 52.4, 49.1, 43.3, 30.0; LRMS (EI) *m/z* 463 (79%, M^+ $C_{27}H_{28}ClN_3O_2$); HPLC Purity: 99%, t_R' = 2.59 min.

***1-(4-(7-Chloroquinolin-4-ylamino)butylamino)-3-(3-phenlyloxy)propan-2-ol*, 3.96**



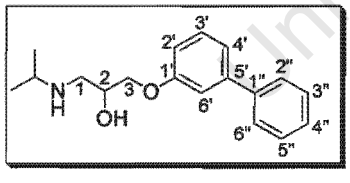
Yellow oil (110 mg, 67%); R_f(MeOH:DCM 1:9) 0.15; IR ν_{max}(neat)/cm^{-1} 3601 (OH), 3412 (NH), 3039 (ArCH), 2982 (CH), 1650 (C=C), 1260 (C-O); δ_H(300 MHz, $CDCl_3$) 8.42 (d, 1H, *J* 5.7, H-2′), 7.95 (d, 1H, *J* 2.1, H-8′), 7.70 (d, 1H, *J* 9.0, H-5′), 7.56 (d, 2H, *J* 8.4, ArH), 7.46 – 7.30 (m, 5H, H-6′, 4 x ArH), 7.20 – 7.06 (m, 2H, ArH), 6.83 (d, 1H, *J* 9.0, ArH), 6.38 (d, 1H, *J* 5.7, H-3′), 4.18 (m, 1H, H-2), 4.06 (d, 2H, *J* 4.4, CH_2-3), 3.23 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_3$N), 2.98 (dd, 1H, *J* 4.0, 12.0, H-1α), 2.83 (dd, 1H, *J* 7.2, 12.0, H-1β), 2.71 (t, 2H, *J* 6.0, NCH_2(CH$_2$)$_3$NAr), 1.82 – 1.80 (m, 2H, ArNCH$_2$CH_2(CH$_2$)$_2$N), 1.61 – 1.59 (m, 2H, NCH$_2$CH_2(CH$_2$)$_2$CH$_2$Ar); δ_C(100 MHz, $CDCl_3$) 159.0, 151.6, 150.0, 148.7, 140.8, 135.0, 130.6, 129.8, 128.7, 128.3 (2C), 127.4 (2C), 127.1, 125.3, 121.2, 120.1, 117.1, 113.6, 113.3, 98.6, 71.0, 68.4, 52.0, 49.1, 43.0, 27.8, 26.0; LRMS (EI) *m/z* 475 (68%, M^+ $C_{28}H_{30}ClN_3O_2$); HPLC Purity: 96%, t_R' = 2.0 min.

***1-(6-(7-Chloroquinolin-4-ylamino)hexylamino)-3-(3-phenyloxy)propan-2-ol*, 3.97**



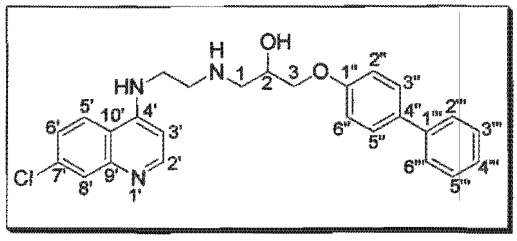
Yellow oil (105 mg, 60%); R_f(MeOH:DCM 1:9) 0.18; IR ν_{max}(neat)/cm^{-1} 3604 (OH), 3418 (NH), 3040 (ArCH), 2980 (CH), 1651 (C=C), 1261 (C-O); δ_H(300 MHz, $CDCl_3$) 8.42 (d, 1H, *J* 5.7, H-2′), 7.95 (d, 1H, *J* 2.1, H-8′), 7.70 (d, 1H, *J* 9.0, H-5′), 7.53 (d, 2H, *J* 8.4, ArH), 7.46 – 7.30 (m, 5H, H-6′, 4 x ArH), 7.18 – 7.05 (m, 2H, ArH), 6.83 (d, 1H, *J* 9.0, ArH), 6.38 (d, 1H, *J* 5.7, H-3′), 4.18 (m, 1H, CH_2-1), 4.06 (d, 2H, *J* 4.4, CH_2-3), 3.23 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_5$N), 2.98 (dd, 1H, *J* 4.0, 12.0, H-1α), 2.82 (dd, 1H, *J* 7.2, 12.0, H-1β), 2.70 (t, 2H, *J* 6.0, NCH_2(CH$_2$)$_5$NAr), 1.80 – 1.78 (m, 2H, ArNCH$_2$NCH_2(CH$_2$)$_4$N), 1.58 (m, 2H, N(CH$_2$)$_2$CH_2(CH$_2$)$_2$NAr), 1.47 – 1.45 (m, 4H, ArN(CH$_2$)$_3$CH_2CH_2CH$_2$N); δ_C(100 MHz, $CDCl_3$) 159.0, 151.6, 150.0, 148.7, 140.8, 135.0, 130.6, 129.8, 128.7, 128.3 (2C), 127.4 (2C), 127.1, 125.3, 121.2, 120.1, 117.1, 113.6, 113.3, 99.2, 70.5, 68.0, 51.8, 49.5, 43.1, 29.4, 28.7, 26.8, 26.5; LRMS (EI) *m/z* 503 (81%, M$^+$ $C_{30}H_{34}ClN_3O_2$); HPLC Purity: 92%, t_R' = 2.32 min.

***1-(Isopropylamino)-3-(3-phenyloxy)propan-2-ol*, 3.104**



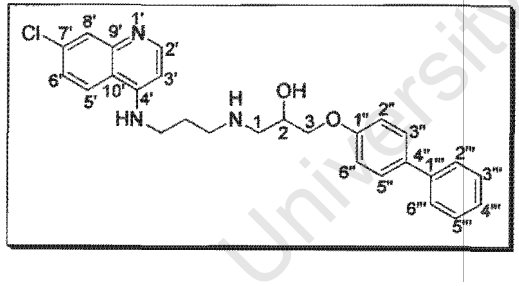
Yellow oil (78.5 mg, 79%); R_f(MeOH:DCM 1:9) 0.21; IR ν_{max}(neat)/cm^{-1} 3604 (OH), 3419 (NH), 3044 (ArCH), 2987 (CH), 1655 (C=C), 1262 (C-O); δ_H(300 MHz, $CDCl_3$) 7.56 (d, 2H, *J* 8.4, ArH), 7.46 – 7.30 (m, 4H, ArH), 7.20 – 7.06 (m, 2H, ArH), 6.83 (d, 1H, *J* 9.0, ArH), 4.28 – 4.0 (m, 3H, H-2, CH_2-3), 2.82 (m, 2H, H-1α, CH(CH$_3$)$_2$), 2.78 (dd, 1H, *J* 7.2, 14.4, H-1β), 1.14 (d, 6H, *J* 6.4, 3 x CH$_3$); δ_C(75 MHz, $CDCl_3$) 159.0, 136.9, 136.1, 129.8, 129.1 (2C), 127.6 (2C), 127.4, 119.1, 113.6, 113.1, 71.0, 67.8, 49.4, 49, 21.0 (2C); LRMS (EI) *m/z* 285 (70%, M$^+$ $C_{18}H_{23}NO_2$); HPLC Purity: 95%, t_R' = 1.34 min.

***1-(2-(7-Chloroquinolin-4-ylamino)ethylamino)-3-(4-phenlyloxy)propan-2-ol*, 3.90**



Yellow oil, (103 mg, 66%); R_f(MeOH:DCM 1:9) 0.10; IR ν_{max}(neat)/cm^{-1} 3598 (OH), 3325 (NH), 3032 (ArH), 2978 (CH), 1263 (C-O); δ_H(300 MHz, $CDCl_3$) 8.44 (d, 1H, *J* 5.4, H-2′), 7.92 (d, 1H, *J* 2.1, H-8′), 7.75 (d, 1H, *J* 9.0, H-5′), 7.54 (dd, 2H, *J* 1.2, 8.4, ArH), 7.44 – 7.23 (m, 6H, H-6′, 5 x ArH), 6.96 (d, 1H, *J* 8.7, ArH), 6.76 (d, 1H, *J* 9.0, ArH), 6.33 (d, 1H, *J* 5.4, H-3′), 4.20 – 3.98 (m, 3H, H-2, C*H*$_2$-3), 3.64 (t, 2H, *J* 6.0, ArNC*H*$_2$CH$_2$N), 3.38 (t, 2H, *J* 6.0, ArNCH$_2$C*H*$_2$N), 2.88 (m, 2H, C*H*$_2$-1); δ_C(75 MHz, $CDCl_3$) 157.8, 151.3, 150.4, 139.9, 134.3, 134.0, 132.2 (2C), 129.2, 128.7, 128.2 (2C), 126.6 (2C), 126.2, 125.1, 122.3, 117.2, 115.0 (2C), 98.6, 70.3, 69.0, 51.5, 47.3, 42.3; LRMS (EI) *m/z* 447 (1005, M^+ $C_{26}H_{26}ClN_3O_2$); HPLC Purity: 95%, t_R' = 2.45 min.

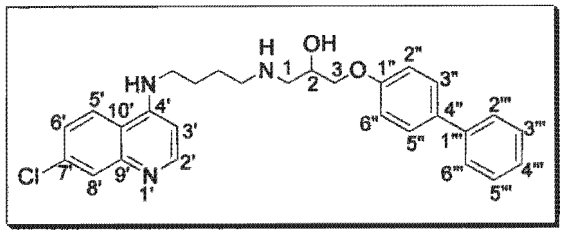
***1-(3-(7-Chloroquinolin-4-ylamino)propylamino)-3-(4-phenlyloxy)propan-2-ol*, 3.91**



Yellow oil, (116 mg, 72%); R_f(MeOH:DCM 1:9) 0.10; IR ν_{max}(neat)/cm^{-1} 3601 (OH), 3315 (NH), 3021 (ArH), 2977 (CH), 1260 (C-O); δ_H(300 MHz, $CDCl_3$) 8.45 (d, 1H, *J* 5.4, H-2′), 7.96 (d, 1H, *J* 2.1, H-8′), 7.72 (d, 1H, *J* 9.0, H-5′), 7.56 (dd, 2H, *J* 1.2, 8.4, ArH), 7.42 – 7.20 (m, 6H, H-6′, 5H, 4 x ArH), 6.98 (d, 1H, *J* 8.7, ArH), 6.79 (d, 1H, *J* 9.0, ArH), 6.30 (d, 1H, *J* 5.4, H-3′), 4.28 – 4.20 (m, 3H, H-2, C*H*$_2$-3), 3.62 (t, 2H, *J* 6.0, ArNC*H*(CH$_2$)$_2$N), 3.40 (t, 2H, *J* 6.0, ArN(CH$_2$)$_2$C*H*$_2$N), 2.82 (m, 2H, C*H*$_2$-1); 1.98 (m, 2H, ArNCH$_2$C*H*$_2$CH$_2$N); δ_C(100 MHz, $CDCl_3$) 158.0, 151.2, 150.3, 140.0, 134.2, 134.2, 132.4 (2C), 129.0, 128.6, 128.4 (2C), 126.5 (2C), 126.3, 125.3,

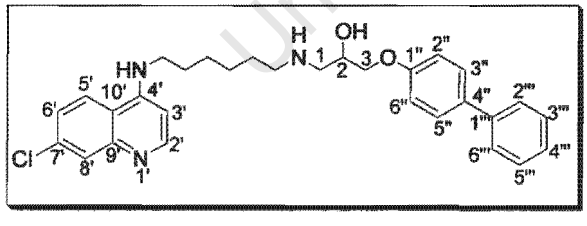
122.2, 117.4, 115.0 (2C), 98.8, 70.4, 68.4, 52.5, 49.3, 43.2, 30.2; LRMS (EI) *m/z* 463 (62%, M^+ $C_{27}H_{28}ClN_3O_2$); HPLC Purity: 90%, t_R' = 2.33 min.

***1-(4-(7-Chloroquinolin-4-ylamino)butylamino)-3-(4-phenyloxy)propan-2-ol*, 3.92**



Yellow oil, (114 mg, 69%); R_f(MeOH:DCM 1:9) 0.11; IR ν_{max}(neat)/cm^{-1} 3609 (OH), 3319 (NH), 3019 (ArH), 2983 (CH), 1265 (C-O); δ_H(300 MHz, $CDCl_3$) 8.48 (d, 1H, *J* 5.4, H-2′), 7.92 (d, 1H, *J* 2.1, H-8′), 7.75 (d, 1H, *J* 9.0, H-5′), 7.54 (dd, 2H, *J* 1.2, 8.4, ArH), 7.47 – 7.18 (m, 6H, H-6′, 5 x ArH), 6.96 (d, 1H, *J* 8.7, ArH), 6.76 (d, 1H, *J* 9.0, ArH), 6.32 (d, 1H, *J* 5.4, H-3′), 4.22 – 4.17 (m, 3H, H-2, CH_2-3), 3.36 (t, 2H, *J* 6.0, $ArNCH_2(CH_2)_3N$), 2.81 (t, 2H, *J* 6.0, $ArN(CH_2)_3CH_2N$), 2.73 (m, 2H, CH_2-1), 1.98 (m, 2H, $ArNCH_2CH_2(CH_2)_3N$), 1.88 (m, 2H, $ArN(CH_2)_2CH_2CH_2N$); δ_C(100 MHz, $CDCl_3$) 158.0, 151.0, 150.3, 139.8, 134.3, 134.1, 132.3 (2C), 129.4, 128.6, 128.4 (2C), 126.7 (2C), 126.3, 125.2, 122.1, 117.0, 114.9 (2C), 99.0, 71.1, 68.6, 52.2, 49.3, 43.0, 27.7, 26.1; LRMS (EI) *m/z* (78%, M^+ $C_{28}H_{30}ClN_3O_2$); HPLC Purity: 92%, t_R' = 2.47 min.

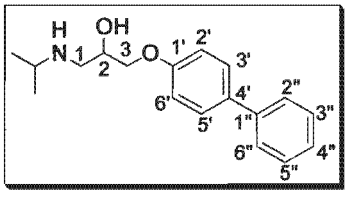
***1-(6-(7-Chloroquinolin-4-ylamino)hexylamino)-3-(4-phenyloxy)propan-2-ol*, 3.93**



Yellow oil, (105 mg, 60%); R_f(MeOH:DCM 1:9) 0.13; IR ν_{max}(neat)/cm^{-1} 3601 (OH), 3323 (NH), 3011 (ArH), 2980 (CH), 1261 (C-O); δ_H(300 MHz, $CDCl_3$) 8.43 (d, 1H, *J* 5.4, H-2′), 7.89 (d, 1H, *J* 2.1, H-8′), 7.78 (d, 1H, *J* 9.0, H-5′), 7.52 (dd, 2H, *J* 1.2, 8.4, ArH), 7.41 – 7.16 (m, 6H, H-6′, 5 x ArH), 6.92 (d, 1H, *J* 8.7, ArH), 6.74 (d, 1H, *J* 9.0, ArH), 6.31 (d, 1H, *J* 5.4, H-3′), 4.23 – 4.15 (m, 3H, H-2, CH_2-3), 3.38 (t, 2H, *J* 6.0, $ArNCH_2(CH_2)_5N$), 2.80 (t, 2H, *J* 6.0, $ArN(CH_2)_5CH_2N$), 2.77 (m,

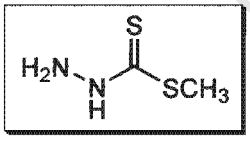
2H, CH_2-1), 1.80 (m, 2H, ArNCH$_2$CH_2(CH$_2$)$_4$N), 1.60 – 1.58 (m, 2H, ArN(CH$_2$)$_4$CH_2CH$_2$N), 1.48 (m, 4H, ArN(CH$_2$)$_2$CH_2CH_2(CH$_2$)$_2$N); δ_C(100 MHz, $CDCl_3$) 157.9, 151.0, 150.3, 140.2, 134.2, 134.0, 132.1 (2C), 129.1, 128.5, 128.1 (2C), 126.5 (2C), 126.1, 125.2, 122.4, 117.1, 115.0 (2C), 98.7, 70.3, 68.0, 51.4, 49.6, 43.3, 29.6, 28.6, 26.5, 26.2; LRMS (EI) *m/z* 503 (87%, M^+ $C_{30}H_{34}ClN_3O_2$); HPLC Purity: 95%, t_R' = 2.56 min.

***1-(Isopropylamino)-3-(4-phenyloxy)propan-2-ol*, 3.104**



White paste, (67 mg, 67%); R_f(MeOH:DCM 1:9) 0.26; IR ν_{max}(neat)/cm^{-1} 3612 (OH), 3317 (NH), 3022 (ArH), 2975 (CH), 1262 (C-O); δ_H(400 MHz, $CDCl_3$) 7.53 (dd, 2H, *J* 1.2, 8.4, ArH), 7.46 (d, 2H, *J* 8.8, ArH), 7.42 (t, 2H, *J* 7.6, ArH), 7.29 (t, 1H, *J* 8.4, ArH), 6.92 (d, 2H, *J* 8.8, ArH), 4.14 – 3.90 (m, 3H, H-2, CH_2-3), 2.98 (dd, 1H, *J* 4.0, 14.4, H-1α), 2.80 (m, 2H, H-1β, CH(CH$_3$)$_2$), 1.14 (d, 6H, *J* 6.4, CH(CH_3)$_2$); δ_C(100 MHz, $CDCl_3$) 157.9, 136.8, 129.4 (2C), 128.6 (2C), 128.0, 127.7 (2C), 127.5, 114.8 (2C), 71.0, 67.8, 49.4, 49.0, 21.0 (2C); LRMS (EI) *m/z* (100%, M^+ $C_{18}H_{23}NO_2$); HPLC Purity: 98%, t_R' = 1.72 min.

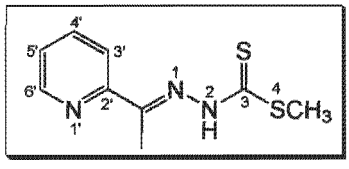
Methyl hydrazinecarbothioate, 4.11



A cooled solution of KOH (19.8 g, 300 mmol) in H_2O (25 ml) and *i*PrOH (20 ml) was treated with $NH_2NH_2.H_2O$ (17 ml). Ice-cold carbon disulfide was added dropwise to the stirred solution while maintaining the temperature below 10 °C. After 2.5 h, ice-cold CH_3I (18.7 ml, 0.3 mol) was added over 2 h. After a further 90 min the white precipitate that formed was filtered under reduced pressure, washed with cold water and recrystallised from DCM to give

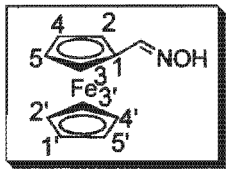
the product as white needles (20.1 g, 55%), m.p. 80 – 82 °C; δ_H(300 MHz, DMSO-d_6) 9.01 (br s, 1H, NH), 4.80 (br s, 1H, NH), 4.20 (br s, 1H, NH), 2.65 (s, 3H, SCH_3).

Methyl-3(-(1-2-pyridyl)ethylidene)carbodithioate, 4.9



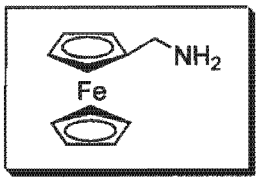
A solution of 2-acetylpyridine (2.10 g, 17.35 mmol) and **4.11** (2.13 g, 17.35 mmol) in MeOH (25 ml) were refluxed for 16 h and the precipitate collected by filtration, affording **4.9** as yellow crystals (3.37 g, 86%), m.p. 127 – 129 °C; R_f(MeOH: DCM 1:99) 0.23; δ_H(300 MHz, $CDCl_3$) 9.99 (br s, 1H, NH), 8.59 (m, 1H, H-6′), 8.18 (m 1H, H-3′), 7.71 (m, 1H, H-4′), 7.29 (m, 1H, H-5′), 2.67 (s, 3H, SCH_3), 2.45 (s, 3H, NCCH_3).

Ferrocenecarboxaldehyde oxime, 4.26



A solution of ferrocene carboxaldehyde (0.64 g, 3.0 mmol) in EtOH (10 ml) was added to a solution of $NH_2OH.HCl$ (0.24g, 3.5 mmol) in H_2O (5 ml) to and the mixture stirred at room temperature. NaOH (2 eq of 10% w/v) was added to the mixture with stirring and the reaction mixture refluxed for 6 h. The cooled reaction mixture was neutralized by adding 1M HCl dropwise and, following cessation of CO_2 evolution extracted with EtOAc (2 x 50 ml). The combined organic fractions were dried (Na_2SO_4) and evaporated *in vacuo*. Concentration under reduced pressure gave the oxime as a red oil, (0.590 mg, 86%); R_f(EtOAc:Hex 2:3) 0.23; δ_H(300 MHz, $CDCl_3$) 8.00 (s, 1H, NC*H*), 4.52 (s, 2H, Cp*H*), 4.34 (s, 2H, Cp*H*), 4.21 (s, 5H, Cp′*H*); δ_C(75 MHz, $CDCl_3$) 149.7, 71.7, 70.2 (2C), 69.2 (5C), 67.8 (2C).

Ferrocenylmethanamine, 4.27

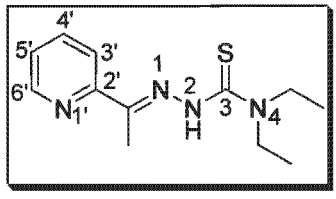


Oxime **4.26** (0.63 g, 2.75 mmol) was dissolved in anhydrous THF then cooled to 0 °C in an ice-water bath and $LiALH_4$ (0.20 g, 5.5 mmol) added portion-wise over 10 min with stirring. The reaction mixture was raised to ambient temperature at which it was left to stir for 30 min before being refluxed for 2 h. After conversion into product (TLC) the reaction mixture was cooled to room temperature, quenched with 10% ice-cold $NaHCO_3$ and extracted with EtOAc (3 x 40 ml). The combined organic fractions were dried ($MgSO_4$), evaporated to dryness and purified by chromatography on SiO_2 (eluent EtOAc:Hex 1:4 then 15% MeOH: DCM) affording the free amine as a brick-red oil (0.42 g, 96%); R_f(MeOH:DCM 1:99) 0.13; δ_H(300 MHz, $CDCl_3$) 4.16-4.10 (m, 9H, 4 x Fc*H*, 5 x Fc'*H*), 3.55 (s, 2H, FcCH_2); δ_C (75 MHz, $CDCl_3$) 91.2, 68.3 (5C), 67.6 (2C), 67.1 (2C), 41.0.

Parallel Solution-Phase Synthesis of thiosemicarbazones and semicarbazones (H)

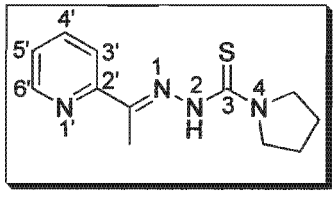
The reactions were performed in parallel on a Carousel Reaction Station®. Scaffold **4.9** (0.20 g, 0.89 mmol), was weighed in each of 12 separate carousel reaction tubes, suspended in MeOH (3 ml) and subsequently heated to achieve complete dissolution prior to addition of the amines. Twelve different amines were then added to the tubes and the resulting reaction mixtures refluxed for 20 h. The reaction mixtures were cooled to room temperature, then filtered and washed with cold EtOH for those that precipitated on cooling or concentrated and recrystallised from Hex/chloroform or EtOH/H_2O).

4,4-Diethyl-1-(1-(pyridin-2-yl)ethylidene)thiosemicarbazone, 4.7a



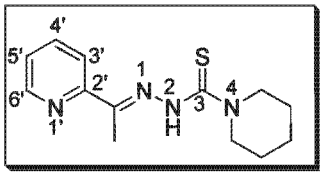
Obtained as a yellow powder (0.213 g, 96%), m.p. (from $CHCl_3$:Hex) 111 – 113 °C, (lit. m.p. 115 – 116 °C);[4] R_f(Et_2O:Hex); 0.39; IR ν_{max}(KBr)/cm^{-1} 3435 (NH), 3035 (Ar CH), 1541 (C=N), 1429 (C=S); δ_H(300 MHz, $CDCl_3$) 8.76 (m, 1H, H-6′), 8.10 (m, 1H, H-3′), 7.78 (m, 1H, H-4′), 7.53 (m, 1H, H-5′), 3.84 (q, 4H, *J* 6.6, $N[CH_2CH_3]_2$), 2.10 (s, 3H, $NCCH_3$), 1.30 (t, 6H, *J* 6.6, $N[CH_2CH_3]_2$); δ_C(75 MHz, $CDCl_3$) 180.1, 155.3, 152.6, 150.0, 135.1, 126.0, 125.1, 45.9 (2C), 19.6 (2C), 12.8; LRMS (EI) *m/z* 250 (76%, M^+); Found: C, 57.27; H 7.47; N 22.18; S 12.41%; Anal. Calc.: C, 57.27; H, 7.47; N, 22.38; S 12.81% for $C_{12}H_{18}N_4S$.

N'-(1-(Pyridin-2-yl)ethylidene)pyrrolidine-1-thiosemicarbazone, 4.7b



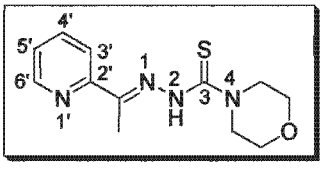
Yellow plates (0.214 g, 97%), m.p. (from $CHCl_3$:Hex) 149 – 151 °C, (lit. m.p. 147 – 148 °C);[4] R_f(Et_2O:Hex 1:1) 0.31; IR ν_{max}(KBr)/cm^{-1} 3433 (NH), 3024 (Ar CH), 1640 (C=C), 1432 (C=S); δ_H(300 MHz, $CDCl_3$) 8.64 (m, 1H, H-6′), 8.10 (m, 1H, H-3′), 7.76 (m, 1H, H-4′), 7.58 (m, 1H, H-5′), 3.96 (m, 4H, $N[CH_2CH_2]_2$), 2.36 (s, 3H, $NCCH_3$), 1.97 (m, 4H, $N[CH_2CH_2]_2$); δ_C(75 MHz, $CDCl_3$) 177.5, 155.2, 153.3, 149.7, 136.1, 126.7, 124.5, 55.4 (2C), 21.8 (2C), 12.8; LRMS (EI) *m/z* 248 (45%, M^+); Found: C, 57.75; H, 6.72; N, 22.23; S 12.54%; Anal. Calc.: C, 58.03; H, 6.49; N, 22.56; S, 12.91% for $C_{12}H_{16}N_4S$.

N'-(1-(Pyridin-2-yl)ethylidene)piperidine-1-thiosemicarbazone, 4.7c



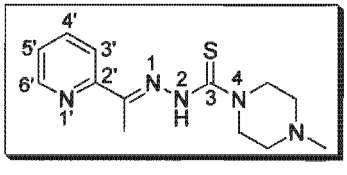
Yellow plates (0.221 g, 95%), m.p. (from $CHCl_3$:Hex) 150 – 152 °C, (lit. m.p. 152 – 153 °C);[4] R_f(Et_2O:Hex 1:1); IR ν_{max}(KBr)/cm^{-1} 3434 (NH), 2980 (aliphatic CH), 1642 (C=N); δ_H(400 MHz, $CDCl_3$) 8.62 (m, 1H, H-6′), 8.00 (m, 1H, H-3′), 7.80 (d, 1H, H-4′), 7.57 (m, 1H, H-5′), 4.00 (m, 4H, N[CH_2CH_2]$_2CH_2$), 2.77 (s, 3H, NCCH_3) 2.60 (m, 6H, N[CH_2CH_2]$_2CH_2$); δ_C(75 MHz, $CDCl_3$) 179.0, 155.8, 153.0, 149.2, 136.6, 126.7, 124.8, 56.5 (2C), 29.0, 23.6 (2C), 13.0; LRMS (EI) *m/z* 262 (68%, M^+); Found: C 59.44, H 5.78, N 20.64, S 11.57%; Anal. Calc.: C, 59.51; H, 6.91; N 21.35; S, 12.22% for $C_{13}H_{18}N_4S$.

***N'*-(1-(*Pyridin-2-yl*)*ethylidene*)*morpholine*-4-*thioemicrabzone*, 4.7d**



Yellow cubes (0.192 g, 93%), m.p. (from $CHCl_3$:Hex) 185 – 187 °C, (lit. m.p. 182 – 185 °C);[4] R_f (Et_2O:Hex 1:1) 0.20; IR ν_{max}(KBr)/cm^{-1} 3436 (br, NH), 3030 (CH), 1642 (C=N), 1430 (C=S); δ_H(300 MHz, $CDCl_3$) 8.60 (m, 1H, H-6′), 7.90 (m, 1H, H-3′), 7.56 (m, 1H, H-4′), 7.28 (m, 1H, H-5′), 4.12 (m, 4H, N[CH_2CH_2]$_2$O), 3.80 (m, 4H, N[CH_2CH_2]$_2$O), 2.60 (s, 3H, NCCH_3); δ_C(75 MHz, $CDCl_3$) 179.5, 156.0, 149.0, 163.2, 126.4, 124.8, 73.2 (2C), 58.8 (2C), 13.1; LRMS (EI) *m/z* 264 (60%, M^+); Found: C, 54.79; H, 6.22; N, 21.19; S 11.79%; Calc.: C, 54.52; H, 6.10; N, 21.19; S 12.13% for $C_{12}H_{16}N_4OS$.

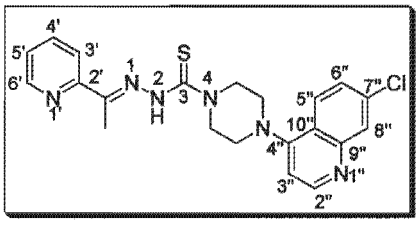
***4-Methyl-N'*-(1-(*pyridin-2-yl*)*ethylidene*)*piperazine*-1-*thiosemicarbazone*, 4.7e**



Yellow crystals (0.21 g, 90%), m.p. (from $CHCl_3$:Hex) 159 – 160 °C, (lit. m.p. 154 – 155 °C)[4]; R_f(MeOH:DCM 9:1) 0.10; IR ν_{max}(KBr)/cm^{-1} 3425 (NH), 3024 (Ar CH), 1640 (C=C), 1435 (C=S); δ_H (300 MHz, $CDCl_3$) 8.78 (m, 1H, H-6′); 8.42 (br s, 1H, NH), 7.8 (m,

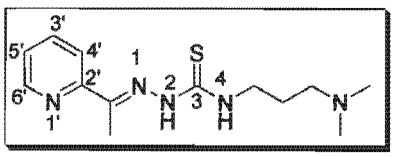
1H, H-3′), 7.50 (m, 1H, H-4′), 7.32 (m, 1H, H-5′), 4.12 (t, 4H, *J* 5.7, 2 x PipNCH_2), 2.45 (m, 7H, 2 x PipNCH_2NCH_3, NCCH_3); δ_C(75 MHz, $CDCl_3$) 178.0, 157.2, 150.2, 137.3, 126.5, 124.5, 120.7, 55.0 (2C), 51.6 (2C), 46.2; LRMS (EI) *m/z* 277 (30%, M^+); Found: C, 56.11; H, 6.77; N, 25.27; S, 10.93%; Anal. Calc.: C, 56.29; H, 6.90; N, 25.25; S, 11.56% for $C_{13}H_{19}N_5S$.

4-(7-*Chloroquinolin*-4-*yl*)-*N*′-(1-(*pyridin*-2-*yl*)*ethylidene*)*piperazine*-1-*thiosemicarbazone*, 4.7f



Off-white powder (0.347 g, 92%), m.p. 213 – 214 °C (from EtOH:H_2O); R_f(EtOAc:Hex 4:1) 0.16; IR ν_{max}(KBr)/cm^{-1} 3433 (NH), 2982 (aliphatic CH), 1640 (C=C), 1432 (C=S); δ_H(400 MHz, $CDCl_3$) 10.42 (br s, 1H, N*H*), 8.72 (m, 1H, H-6′) 8.42 (d, 1H, *J* 5.6, H-2″), 8.32 (d, 1H, *J* 9.0, H-5″), 7.83 (m, 2H, H-3′, H-8″), 7.57 (m, 1H, H-4′), 7.45 (m, 2H, H-5′, H-6″), 6.83 (m, 1H, *J* 5.6, H-3″), 4.42 (m, 4H, 2 x PipNCH_2) 3.28 (m, 4H, 2 x PipNCH_2Ar), 2.65 (s, 3H, NCCH_3); δ_C(75 MHz, $CDCl_3$) 179.2, 155.9, 152.2, 150.7, 149.7, 149.6, 149.2 (2C), 137.2, 128.4, 124.9, 124.6 (2C), 121.6, 118.3, 99.9, 52.6 (2C), 46.9 (2C), 13.0; LRMS (EI) *m/z* 424 (67%, M^+); Found: C, 59.53; H, 4.88; N, 19.24; S 7.10%; Anal. Calc.: C, 59.35; H, 4.98; N, 19.78; S, 7.55% for $C_{21}H_{21}ClN_6S$.

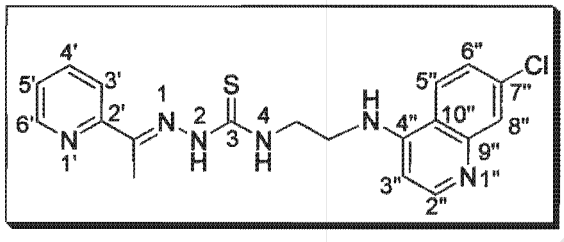
4-(3-(*Dimethylamino*)*propyl*)-1-(1-(*pyridin*-2-yl)*ethylidene*)*thiosemicarbazone*, 4.7g



Orange powder (0.241 g, 97%), m.p. 96 – 98 °C (from $CHCl_3$:Hex); R_f(Et_2O:Hex 1:1) 0.26; IR ν_{max}(CH_2Cl_2)/cm^{-1} 3445 (NH), 3027 (Ar CH), 2805 (aliphatic CH), 1552 (C=N), 1485 (C=S); δ_H(300 MHz, $CDCl_3$) 9.20 (br s, 1H, N*H*-2), 8.64 (t, 1H, *J* 6.3, N*H*-4),

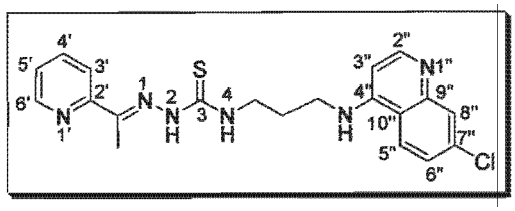
8.59 (m, 1H, H-6′), 8.00 (m, 1H, H-3′), 7.66 (m, 1H, H-4′) 7.28 (m, 1H, H-5′), 3.84 (q, 2H, *J* 6.0, NCH_2(CH_2)$_2$N), 2.48 (q, 2H, *J* 6.0, (CH_3)$_2$NCH_2(CH_2)$_2$N), 2.38 (s, 3H, NCCH_3), 2.26 (s, 6H, N(CH_3)$_2$), 1.81 (m, 2H, NCH$_2$CH_2CH$_2$N); δ_C(75 MHz, $CDCl_3$) 177.6, 154.9, 148.8, 146.9, 135.9, 123.7, 120.1, 59.3, 45.5 (2C), 45.3, 22.5, 11.0; LRMS (EI) *m/z* 279 (84%, M^+); Found: C, 55.81; H, 8.04; N, 24.99; S 10.98%; Anal. Calc.: C, 55.88; H, 7.58; N, 25.07; S, 10.98% for $C_{13}H_{21}N_5S$.

4-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethyl*)-1-(1-(*pyridin*-2-yl)*ethylidene*)*thiosemicarbazone*, 4.7h



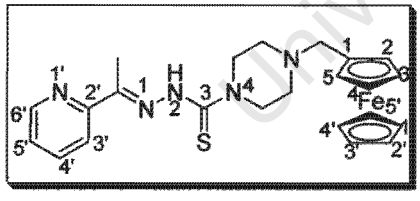
Off-white flakes (0.276 g, 78%), m.p. 205 – 206 °C (from EtOH:H_2O); R_f(MeOH:DCM 1:9) 0.21; IR ν_{max}(KBr)/cm^{-1} 3311 (NH), 3025 (ArCH), 2941 (aliphatic CH), 1588 (C=N), 1435 (C=S); δ_H(400 MHz, DMSO-d_6) 10.45 (br s, 1H, N*H*-2, 8.84 (t, 1H, *J* 6.0, N*H*-4), 8.60 (m, 1H, H-6′), 8.40 (d, 1H, *J* 5.7, H-2″), 8.32 (m, 2H, H-5″, H-8″), 7.80 (m, 2H, H-3′, H-6″), 7.50 – 7.35 (m, 3H, H-4′, H-5′, N*H*Ar), 6.78 (d, 1H, *J* 5.7, H-3″), 3.92 (q, 2H, *J* 6.3, NCH_2CH$_2$NAr), 3.57 (q, 2H, *J* 6.3, NCH$_2$CH_2NAr), 2.30 (s, 3H, NCCH_3); δ_C(100 MHz, DMSO-d_6) 179.2, 155.3, 152.6, 150.9, 149.8, 149.4, 149.2 (2C), 137.0, 128.2, 124.8, 124.7 (2C), 121.4, 118.1, 99.6, 42.6 (2C), 13.0; LRMS (EI) *m/z* 398 (M^+, 72%); Found: C, 57.29; H, 4.77; N, 20.89; S 7.76%; Anal. Calc.: C, 57.21; H, 4.80; N, 21.07; S, 8.04% for $C_{19}H_{19}ClN_6S$.

4-(2-(7-*Chloroquinolin-4-ylamino*)*propyl*)-1-(1-(*pyridin-2-yl*)*ethylidene*)*thiosemicarbazone*, 4.7i



Off-white flakes (0.345 g, 94%), m.p. 161 – 163 °C (from EtOH:H_2O); R_f(Et_2O:Hex) 0.24; IR ν_{max}(KBr)/cm^{-1} 3311 (NH), 3023 (Ar CH), 2943 (aliphatic CH), 1588 (C=N), 1435 (C=S); δ_H(300 MHz, DMSO-d_6) 10.30 (s, 1H, N*H*-2), 8.81 (t, 1H, *J* 6.0, N*H*-4), 8.60 (m, 1H, H-6′), 8.40 (d, 1H, *J* 5.7, H-2″), 8.20 (m, 2H, H-5″, H-8″), 7.81 (m, 2H, H-3′, H-6″), 7.40 (m, 3H, H-4′, H-5′, N*H*Ar), 6.52 (d, 1H, *J* 5.7, H-3″), 3.85 (q, 2H, *J* 6.0, NCH_2(CH_2)$_2$NAr), 3.40 (q, 2H, *J* 6.0, N(CH_2)$_2$CH_2NAr), 2.38 (s, 3H, CH_3), 2.01 (m, 2H, NCH$_2$CH_2CH$_2$NAr); δ_C(75 MHz, DMSO-d_6) 178.1, 154.6, 151.8, 149.9, 149.0, 148.4, 148.2, 136.2, 133.3, 127.4, 124.0, 123.9, 123.8, 120.7, 117.4, 98.6, 41.7 (2C), 27.6, 12.1; LRMS (EI) *m/z* 412 (41%, M^+); Found: C, 58.06; H, 5.18; N, 20.01; S, 7.15%; Anal. Calc.: C, 58.17; H, 5.13; N, 20.35; S, 7.77% for $C_{20}H_{21}ClN_6S$.

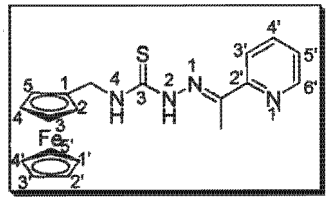
4-*Ferrocenyl*-*N*′-(1-(*pyridin-2-yl*)*ethylidene*)*piperazine*-1-*thiosemicarbazone*, 4.29



Yellow powder (0.344 g, 84%), decomposes at 204 °C; R_f(Et_2O:Hex) 0.19; IR ν_{max}(KBr)/cm^{-1} 3489 (NH), 3039 (Ar CH), 2987 (CH), 1552 (C=N), 1489 (C=S); δ_H(300 MHz, $CDCl_3$) 8.70 (m, 1H, H-6′), 7.86 (m, 1H, H-3′), 7.78 (m, 1H, H-4′), 7.41 (m, 1H, H-5′), 4.28 – 4.20 (m, 13H, 2 x PipNCH_2, 4 x CpH, 5 x Cp′*H*), 3.4 (s, 2H, FcCH_2N), 2.60 – 2.40 (m, 7H, 2 x PipNCH_2, CH_3); δ_C(75 MHz, $CDCl_3$) 185.1, 155.3, 152.6, 150.0, 135.1, 126.0, 121.4, 68.7, 68.3 (2C), 68.0 (5C), 67.4 (2C), 58.0 (2C), 52.3, 51.9 (2C), 12.1; HRMS (EI) *m/z* 461.13366 (M^+ + H, $C_{23}H_{27}FeN_5S$ + H requires 461.13363).

4-*Ferrocenyl*-1-(1-(*pyridin*-2-*yl*)*ethylidene*)*thiosemicarbazone*, 4.30

Green leaves (0.21 g, 60%), decomposes at 139 °C; R_f(Et$_2$O:Hex 1:2) 0.3; IR

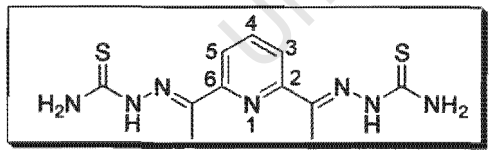


ν_{max}(KBr)/cm^{-1} 3606, 3444 (NH), 3072 (Ar CH), 2954 (aliphatic CH), 1558 (C=N) (Ar C=N), 1460 (C=S); δ_H(300 MHz, $CDCl_3$) 8.78 (br s, 1H, N*H*), 8.78 (m, 1H, H-6′), 7.9 (m, 1H, H-3′), 7.84 (br s, 1H, N*H*), 7.70 (m, 1H, H-4′), 7.26 (m, 1H, H-5′), 4.60 (d, 2H, *J* 5.7, FcCH_2N), 4.27 (d, 2H, *J* 2.1, Cp*H*-2, Cp*H*-5), 4.19 (m, 7H, 2 x Cp*H*, 5 x Cp′*H*), 2.44 (s, 3H, NCCH_3); δ_C(75 MHz, $CDCl_3$) 184.1, 156.0, 150.0, 148.1, 136.2, 124.0, 120.2, 68.5, 68.2 (2C), 68.0 (5C), 67.9 (2C), 44.0, 11.6; HRMS (EI) *m/z* 392.07581 (M^+ + H, $C_{19}H_{20}FeN_4S$ + H requires 392.07580).

Synthesis of Bis-thiosemicarbazones:

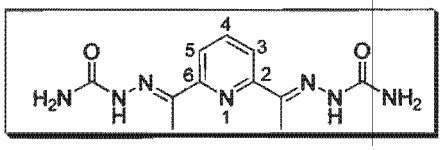
A solution of 2,6-diacetylpyridine (0.886 mmol) in EtOH (15 ml) was condensed with either 1.772 mmol of thiosemicarbazide or semicarbazide hydrochloride and NaOAc (1.772 mmol) and refluxed. The precipitate was filtered and washed with EtOH and H_2O (**4.16b**).

Compound 4.16a



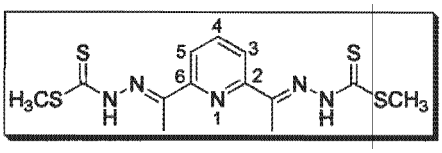
Pale yellow powder (217 mg, 79%) decomposes at 117 °C; R_f(EtOAc:Hex 3:7) 0.35; IR ν_{max}(KBr)/cm^{-1} 3405 (NH), 3012 (Ar CH), 1589 (C=N), 1485 (C=S); δ_H(400 MHz, DMSO-d_6) 10.25 (br s, 2H, N*H*), 8.20 (d, 2H, *J* 6.0, H-3, H-5), 8.10 (br s, 4H, 2 x NH_2), 7.80 (t, 1H, *J* 6.0, H-4), 2.35 (s, 6H, 2 x NCCH_3); δ_C(75 MHz, DMSO-d_6) 179.7 (2C), 154.2 (2C), 148.7 (2C), 137.1 (2C), 121.5, 12.7 (2C); LRMS (EI) *m/z* 310 (67%, M^+ + H). Found: C, 42.70; H, 5.03; N, 30.82; S, 20.73%; Anal. Calc.: C, 42.70; H, 4.89; N, 31.69; S 20.73% for $C_{11}H_{15}N_7S_2$.

Compound 4.16b



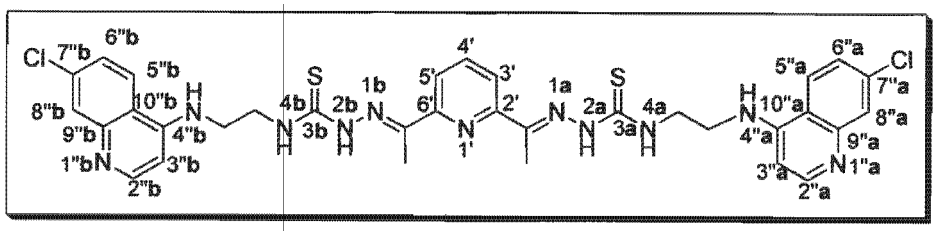
Pale yellow powder (239 mg, 97%), decomposes at 191 °C; R_f(EtOAc:Hex 3:7) 0.35; IR ν_{max}(KBr)/cm^{-1} 3400 (NH), 3032 (Ar CH), 2900 (aliphatic CH), 1677 (C=O), 1587 (C=N); δ_H(400 MHz, DMSO-d_6) 10.25 (br s, 2H, 2 x N*H*) 8.20 (d, 2H, *J* 6.0, H-3, H-5), 8.10 (br s, 4H, 2 x NH_2), 7.8 (t, 1H, *J* 6.0, H-4), 2.35 (s, 6H, 2 x NCCH_3); δ_C(75 MHz, DMSO-d_6) 157.7 (2C), 153.9 (2C), 148.6 (2C), 137 (2C), 121.5, 12.5 (2C); LRMS (EI) *m/z* 278 (68%, M$^+$ + H); Found: C, 47.63; H, 5.42; N, 35.39; Anal. Calc.: C, 47.65; H, 5.45; N, 35.36 for $C_{11}H_{15}N_7O$.

Compound 4.17



Yellow powder (0.263 g, 80%); Decomposes at 139 °C; R_f(Et_2O:Hex 1:19) 0.11; IR ν_{max}(KBr)/cm^{-1} 3400 (NH), 3176 (Ar CH), 2917, 1550 (Ar C=C), 1435 (C=S); 3185 (NH), 3025 (ArCH), 1640 (C=C), 1446 (C=S); δ_H(300 MHz, $CDCl_3$) 12.58 (br s, 2H, 2 x N*H*), 8.10 (d, 2H, *J* 7.8, H-3, H-5), 7.93 (t, H, *J* 7.8, H-4), 3.29 (s, 6H, 2 x SCH_3), 2.52 (s, 6H, 2 x NCCH_3); δ_C(75 MHz, $CDCl_3$) 200 (2C), 153.6 (2C), 151.3 (2C), 137.4 (2C), 120.8, 17.0 (2C), 12.8 (2C); HRMS (EI) 371.03668 (M$^+$ requires *m/z* 371.03659).

Compound 4.18



The method used for the synthesis of **4.18** was that described in General Procedure (H), using (0.16 g, 0.72 mmol) of the 4-aminoquinoline amine **3.52** and **4.17** (0.13 g, 0.36 mmol). The product was obtained as a pale yellow powder (0.22 g, 86%); decomposes at 139 °C; R_f(MeOH:DCM 1:19) 0.24; IR ν_{max}(KBr)/cm^{-1} 3306 (NH), 3022 (ArCH), 2957 (aliphatic CH), 1576 (C=N), 1439 (C=S); δ_H(400 MHz, DMSO-d_6) 10.41 (br s, 2H, N*H*-2**a**, N*H*-2**b**), 8.78 (t, 2H, *J* 6.0, N*H*-4**a**, N*H*-**b**), 8.43 (d, 2H, *J* 5.6, H-2″**a**, H-2″**b**), 8.31 (m, 4H, H-5″**a**, H-5″**b**, H-8″**a**, H-8″**b**), 7.78 (m, 3H, H-3′, H-6″**a**, H-6″**b**), 7.51 – 7.39 (m, 4H, H-4′, H-5′, 2 x NHAr), 6.65 (d, 2H, *J* 5.7, H-3″**a**, H-3″**b**), 3.94 (q, 4H, *J* 6.4, 2 x NCH_2CH$_2$NAr), 3.60 (q, 4H, *J* 6.4, 2 x NCH$_2$CH_2NAr), 2.30 (s, 3H, NCCH_3); δ_C(100 MHz, DMSO-d_6) 179.2 (2C), 155.1 (2C), 153.0 (2C), 1501.1 (2C), 149.8 (2C), 145.2 (2C), 149.2 (2C), 137.0 (2C), 136.8, 128.2 (2C), 125.2 (2C), 124.5 (2C), 121.9 (2C), 117.8 (2C), 99.6 (2C), 42.4 (2C), 13.4 (2C); HRMS (EI) 717.17399 (M^+ $C_{33}H_{33}{}^{35}Cl_2N_{11}S_2$ requires *m/z* 717.17389).

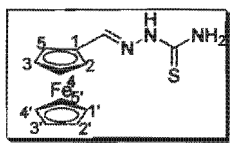
General Methods for the Synthesis of Ferrocenyl Thiosemicarbazones and Semicarbazones (I)

(i) Semicarbazones were prepared as follows. NaOAc (44.4 mg, 0.538 mmol), was added to a solution of semicarbazone hydrochloride (60 mg, 0.538 mmol) in EtOH (5 ml) at room temperature. After stirring for 10 min the ferrocenyl aldehyde/ketone (0.448 mmol) was added and the reaction stirred overnight. Solvent was removed *in vacuo* and the resultant precipitate suspended in water and extracted into DCM. Drying ($MgSO_4$) and concentration under reduced pressure afforded the products in good to excellent yields.

(ii) *Bis*(semicarbazones) were prepared by adding the ferrocenyl aldehyde/ketone to a solution of semicarbazone hydrochloride and NaOAc (88.8 mg, 8.80 mmol) and stirring the reaction mixtures overnight.

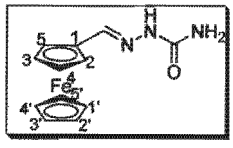
(iii) Thiosemicarbazones and *bis*(thiosemicarbazones) were prepared by refluxing thiosemicarbazide and ferrocenyl ketone/aldehyde in MeOH overnight, followed by removal of solvent under reduced pressure and recrystallisation for appropriate solvent.

1-Ferocenemethylidenethiosemicarbazide, 4.31



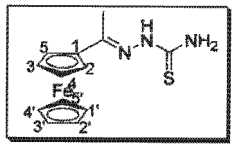
Brick red powder (120 mg, 93%), decomposes at 196 °C; R_f(MeOH:DCM 1:19) 0.21; IR ν_{max}(KBr)/cm^{-1} 3410, 3266 (NH), 3140 (Ar CH), 1595 (C=N), 1480 (C=S); δ_H(300 MHz, $CDCl_3$) 10.2, (br s, 1H, NH), 7.77 (s, 1H, NC*H*), 6.32 (br s, 2H, NH_2), 4.57 (t, 2H, *J* 2.0, Cp*H*-2, Cp*H*-5), 4.42 (t, 2H, *J* 2.0, Cp*H*-3, Cp*H*-4), 4.20 (s, 5H, Cp'*H*); δ_C(75 MHz, $CDCl_3$) 177.6, 145.7, 71.5 (2C), 69.9, 70.0, (2C), 68.1 (5C); HRMS (EI) *m/z* 287.01796 (M^+ + H, $C_{12}H_{13}N_3SFe$ + H requires 287.01795).

1-Ferocenemethylidenesemicarbazide, 4.32



Orange powder (112 mg, 92%), decomposes at 220 °C; R_f(Et_2O:Hex 1:1) 0.18; IR ν_{max}(KBr)/cm^{-1} 3467 (NH), 3143 (Ar CH), 1698 (C=O), 1573 (C=N); δ_H(300 MHz, $CDCl_3$) 9.82 (br s, 1H, NH), 7.67 (s, 1H, NC*H*), 6.20 (br s, 2H, NH_2), 4.6 (t, 2H, *J* 1.8, Cp*H*-2, Cp*H*-5), 4.34 (t, 2H, *J* 1.8, Cp*H*-3, Cp*H*-4), 4.16 (s, 5H, Cp'*H*); δ_C(75 MHz, $CDCl_3$) 157.5, 141.4, 80.1, 70.0 (2C), 69.3 (5C), 67.5 (2C); HRMS (EI) *m/z* 271.04080 (M^+ + H, $C_{12}H_{13}N_3OFe$ + H requires 271.04081).

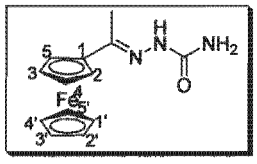
1-(1-Ferocenylethylidene)thiosemicarbazide, 4.33



Brick-red crystals (119 mg, 88%), decomposes at 122 °C; R_f(Et_2O:Hex 1:1) 0.20; IR ν_{max}(KBr)/cm^{-1} 3415 3228 (NH), 2935 (Ar C=C), 1579 (C=N), 1499 (C=S); δ_H(400 MHz, $CDCl_3$) 9.85 (br s, 1H,

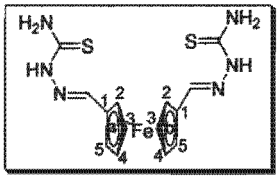
N*H*), 6.21 (br s, 2H, N*H*), 4.60 (t, 2H, *J* 2.0, Cp*H*-2, Cp*H*-5), 4.40 (t, 2H, *J* 2.0, Cp*H*-3, Cp*H*-4) 4.16 (s, 5H, Cp'*H*), 2.10 (s, 3H, CH_3); δ_C(75 MHz, $CDCl_3$) 178.3, 150.3, 140.0, 70.5 (2C), 69.4 (2C), 67.1 (5C), 14.4; HRMS (EI) *m/z* 301.03361 (M^+ $C_{13}H_{15}N_3SFe$ requires 301.03365).

1-(1-Ferocenylethylidene)semicarbazide, 4.34



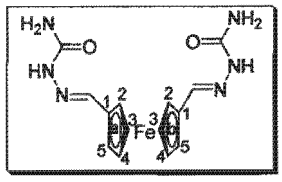
Orange needles (128 mg, 100%), m.p. 191 – 193 °C; R_f(Et_2O:Hex 1:1) 0.18; IR ν_{max}(KBr)/cm^{-1} 3453 (NH), 3206 (Ar CH), 2897, 1685 (C=O), 1564 (C=N); δ_H(300 MHz, $CDCl_3$) 7.98 (s, 1H, N*H*), 6.21 (br s, 2H, NH_2) 4.55 (t, 2H, *J* 1.8, Cp*H*-2, Cp*H*-5), 4.33 (t, 2H, *J* 1.8, Cp*H*-3, Cp*H*-4), 4.16 (s, 5H, Cp'*H*), 2.11 (s, 3H, CH_3); δ_C(75 MHz, $CDCl_3$) 158.0 (C=O), 147.4, 83.3, 69.8 (2C), 69.2 (5C), 66.7 (2C), 14.0; HRMS (EI) *m/z* 285.05645 (M^+ $C_{13}H_{15}N_3OFe$ requires 285.05644).

***Bis*-1,1'-Ferocenemethylidenethiosemicarbazide 4.35**



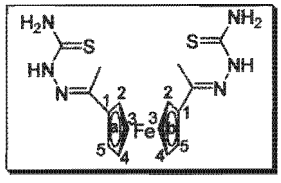
Dark brown powder (165 mg, 95%), decomposes at 187 °C; R_f(Et_2O:Hex 4:5) 0.20; IR ν_{max}(KBr)/cm^{-1} 3396, 3250 (NH), 3150 (Ar CH), 1587 (C=N), 1480 (C=S); δ_H(400 MHz, DMSO-d_6) 11.20 (s, 2H, 2 x N*H*), 8.01 (br s , 2H, 2 x N*H*) 7.80 (s, 2H, 2 x NC*H*), 7.50 (br s, 2H, 2 x N*H*), 6.28 (br s, 4H, 2 x NH_2), 4.70 (t, 4H, *J* 2.0, Cp*H*-2a,b Cp*H*-5a,b), 4.30 (t, 4H, *J* 2.0, Cp*H*-3a,b, Cp*H*-4a,b); δ_C(100 MHz, $CDCl_3$) 177.7 (2C), 143.3 (2C), 80.7 (2C), 71.9 (4C), 69.1 (4C); HRMS (EI) *m/z* 388.02268 (M^+ $C_{14}H_{16}FeN_6S_2$ requires 388.02273).

Bis-1,1'-Ferocenemethylidenesemicarbazide, 4.36



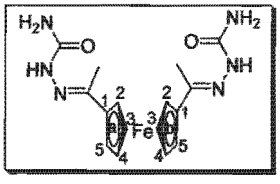
Light brown powder (159 mg, 100%), decomposes at 203 °C; R_f(Et$_2$O:Hex 4:5) 0.19; IR ν_{max}(KBr)/cm^{-1} 3460 (NH), 3154 (Ar CH), 1736, 1679 (C=O), 1589 (C=N); δ_H(400 MHz, DMSO-d_6) 8.80 (br s, 2H, 2 x N*H*), 7.60 (s, 2H, NC*H*), 6.08 (br s, 4H, 2 x NH_2), 4.60 (t, 4H, *J* 2.0, Cp*H*-2a,b Cp*H*-5a,b), 4.31 (t, 4H, *J* 2.0, Cp*H*-3a, b, Cp*H*-4a,b); δ_C(100 MHz, $CDCl_3$) 157.4 (2C), 139.7 (2C), 81.7 (2C), 71.1 (4C), 68.4 (4C); HRMS (EI) *m/z* 356.06819 (M^+ $C_{14}H_{16}FeN_6O_2$ requires 356.06842).

Bis-1,1'-(1,1'-Ferocenylethylidene)thiosemicarbazide, 4.37



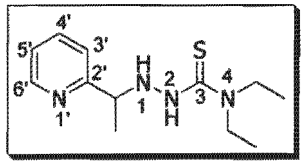
Brick-red powder (186 mg, 100%), m.p. 100 – 102 °C; R_f(Et$_2$O:Hex 4:5) 0.22; IR ν_{max}(KBr)/cm^{-1} 3237 (NH), 3080 (Ar CH), 1538 (C=N), 1495 (C=S); δ_H(400 MHz, $CDCl_3$) 8.80 (s, 2H, 2 x N*H*), 6.21 (br s, 4H, 2 x NH_2), 4.65 (t, 4H, *J* 1.8, Cp*H*-2a,b Cp*H*-5a,b), 4.26 (t, 4H, *J* 1.8, Cp*H*-3a,b Cp*H*-4a,b), 1.98 (s, 6H, 2 x CH_3); δ_C(100 MHz, $CDCl_3$) 178.0 (2C), 149.0 (2C), 84.7 (2C), 71.1 (4C), 68.0 (4C), 14.6 (2C); HRMS (EI) *m/z* 416.05408 (M^+ $C_{16}H_{20}FeN_6S_2$ requires 416.05403).

Bis-1,1'-(1,1'-Ferocenylethylidene)semicarbazide, 4.38



Light brown powder (172 mg, 100%), decomposes at 156 °C; R_f(Et$_2$O:Hex 4:5) 0.21; IR ν_{max}(KBr)/cm^{-1} 3470 (NH), 3163 (Ar CH), 2982, 1718 (C=O) 1579 (C=N); δ_H(400 MHz, $CDCl_3$) 8.82 (s, 2H, 2 x N*H*), 6.20 (s, 4H, 2 x NH_2), 4.65 (d, 4H, *J* 1.8, Cp*H*-2a,b, Cp*H*-5a,b), 4.26 (t, 4H, *J* 1.8, Cp*H*-3a,b Cp*H*-4a,b), 1.98 (s, 6H, 2 x CH_3); δ_C(100 MHz, $CDCl_3$) 157.0 (2C), 146.0 (2C), 86.0 (2C), 70.6 (4C), 68.0 (4C), 14.9 (2C); HRMS (EI) *m/z* 384.09971 (M^+ $C_{16}H_{20}FeN_6O_2$requires 384.09974).

***4,4-Diethyl*-1-(1-(*pyridine*-2-yl)*ethyl*)*thiosemicarbazide*, 4.14**



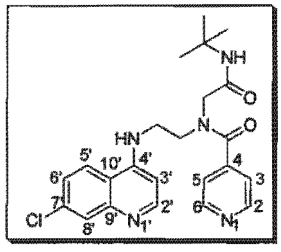
4.7a (130 mg, 0.53 mmol) was dissolved in MeOH (15 ml) and polymer-supported borohydride resin on Amberlite® IRA 400, with a loading capacity of ~2.5 mmol/g added, (0.53 g, 1.33 mmol). The reaction mixture was stirred at room temperature for 48 h on a shaker. After consumption of starting material (TLC) the reaction mixture was filtered and the resins washed with MeOH (3 x 10 ml). Concentration *in vacuo* afforded the product as an orange powder that was recrystallised from $CHCl_3$:Hex to give the *thiosemicarbazide* as orange crystals (104 mg, 78%), m.p. 84 – 87 °C; R_f(DCM:MeOH 23:1) 0.27; IR ν_{max}(KBr)/cm^{-1} 3418 (NH), 3172 (ArCH), 2926 (CH), 1493 (C=S); δ_H(400 MHz, $CDCl_3$) 9.23 (br s, 1H, N*H*-2), 8.80 (m, 1H, H-6′), 7.70 (m, 1H, H-4′), 7.3 (m, 1H, H-3′), 7.17 (m, 1H, H-5′), 5.83 (br s, 1H, N*H*-1) , 4.40 (q, 1H, *J* 6.0, NC*H*CH_3), 3.55 (q, 4H, *J* 6.0, N[C$H_2$$CH_3$]$_2$), 1.45 (d, 3H, *J* 6.0, NCHCH_3, 1.2 (t, 6H, *J* 6.0, N[CH_2CH_3]$_2$); δ_C(75 MHz, $CDCl_3$) 179.0, 162.0, 149.0, 136.5, 122.0, 121.9, 60.3, 45.1, 19.5 (2C), 12.4 (2C), 12.0; *m/z* HRMS (FAB) 252.14076 (M^+ $C_{12}H_{20}N_4S$ requires 252.14087).

Parallel Solution–Phase Synthesis of 4-Aminoquinoline-containing α-Acylamino Amides (J)

A solution of the amine (0.316 mmol) in anhydrous MeOH was condensed with a solution of *para*-formaldehyde (9.5 mg, 0.316 mmol) in MeOH (1 ml) and stirred for 30 minutes; carboxylic acid (0.316 mmol) and isocyanide (0.036 ml, 0.316 mmol) were added in succession to the reaction mixtures with continued stirring at room temperature for 36 – 48 hours. After consumption of starting materials was observed (TLC), the solvent was evaporated to dryness and the residues purified by preparative thin layer chromatography, (eluent MeOH:DCM 3:17).

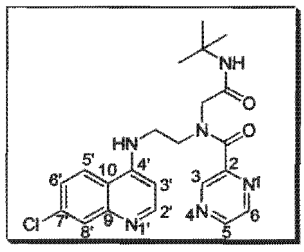
The α-acylamino amides **5.23a** – **5.23j** were revealed as rotational isomers; each chemical shift value in a pair represents the number of Hs stated.

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(2-(7-chloroquinolin-4-ylamino)ethyl)isonicotinamide, 5.23a**



Cream white needles (90.4 mg, 65%), m.p. 106 – 108 °C (from EtOH:H_2O); R_f(CH_2Cl_2:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3393 (NH), 2927 ArCH), 2885 1701 (C=O), (CH), 1686 (conjugated C=O), 1550 (C=N); δ_H(300 MHz, CD_3OD) 8.61/8.28 (d, 2H, *J* 6.0, H-2, H-6), 8.44/8.20 (d, 1H, *J* 5.7, H-2′), 8.25/8.15 (d, 1H, *J* 9.3, H-5′), 7.80/7.76 (d, 1H, *J* 2.4, H-8′), 7.48 (dd, 1H, *J* 2.4, 9.3, H-6′), 7.27/7.10 (d, 2H, *J* 6.0, H-3, H-5), 6.68/6.18 (d, 1H, *J* 5.7, H-3′), 4.22/3.80 (s, 2H, $COCH_2$), 3.78/3.75 (t, 2H, *J* 6.0, $ArNCH_2CH_2N$), 3.65/3.60 (t, 2H, *J* 6.0, $ArNCH_2CH_2N$), 1.30/1.20 (s, 9H, $C(CH_3)_3$); δ_C(75 MHz, CD_3OD) 171.1/170.9, 166.8/166.6, 151.2/151.0, 150.0/149.8 (2C), 149.5/149.4 (2C), 141.8/141.7, 134.2/134.0, 128.1/128.0, 126.0/125.9, 122.6/122.4 (2C), 121.5/121.3, 116.1/116.0, 104.7/104.8, 52.8/52.6, 51.2/51.1, 48.3/48.2, 41.2/41.2, 31.2/31.1 (3C); HRMS (FAB) *m/z* 439.17732 (M^+ + H, $C_{23}H_{26}{}^{35}ClN_5O_2$ + H requires 439.17750).

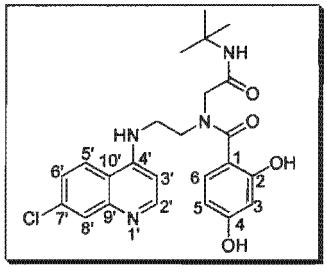
***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(2-(7-chloroquinolin-4-ylamino)ethyl)pyrazine-2-carboxamide, 5.23b**



Cream white paste (96.2 mg, 69%); R_f(DCM:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3288 (NH) 3068 (ArCH), 2926 (CH), 1700 (C=O), 1689 (conjugated C=O), 1590 (C=C); δ_H(300 MHz, CD_3OD) 8.86/8.69(d, 1H, *J* 1.5, H-3), 8.66/8.40 (d, 1H, *J* 2.7,

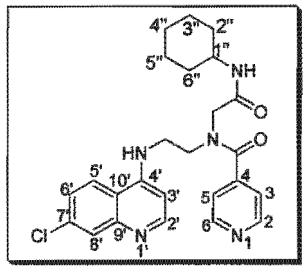
H-6), 8.57/8.38 (dd, 1H, *J* 1.5, 2.7, H-5), 8.39/8.37 (d, 1H, *J* 5.7, H-2′), 8.16/8.13 (d, 1H, *J* 9.0, H-5′), 7.99/7.80 (d, 1H, *J* 2.1, 9.0, H-8′), 7.78/7.42 (dd, 1H, *J* 2.1, H-6′), 6.72/6.61 (d, 1H, *J* 5.7, H-3′), 4.26/4.17 (s, 2H, NCOCH_2), 4.01/3.87 (t, 2H, *J* 6.0, ArNHCH_2CH_2N), 3.80/3.36 (t, 2H, *J* 6.0, ArNCH_2CH_2N), 1.39/1.28 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, CD_3OD) 171.1/170.0, 166.8/166.7, 151.2/151.0, 149.5/149.3 (2C), 147.9/147.8, 144.1/144.0, 143.7/143.5, 141.8/141.7, 134.2/143.1, 128.1/128.0, 126.0/125.9, 121.5/121.3, 116.1/16.0, 104.5/104.4, 52.8/52.6, 51.2/51.1, 48.3/48.1, 41.5/41.4, 30.8/38.6 (3C); HRMS (FAB) *m/z* 441.18072, (M^+ + H, $C_{22}H_{25}{}^{35}ClN_6O_2$ + H requires 441.18056).

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(2-(7-chloroquinolin-4-ylamino)ethyl)-2,4-dihydroxybenzamide, 5.23c**



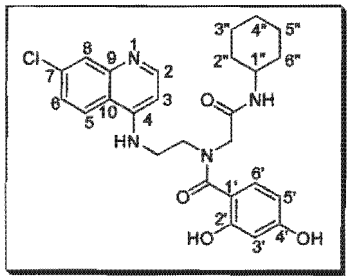
Off-white powder (101 mg, 68%), m.p. (from EtOH:H_2O) 44 – 46 °C; R_f(DCM:MeOH 9:1) 0.12; IR ν_{max}(KBr)/cm^{-1} 3614 (OH), 3290 (NH), 3050 (ArCH), 2927 (CH), 1699 (C=O), 1687 (conjugated C=O), 1587 (C=N); δ_H(300 MHz, CD_3OD) 8.46/8.28 (d, 1H, *J* 5.7, H-2′), 8.18/8.10 (d, 1H, *J* 9.0, H-5′), 7.99/7.80 (d, 1H, *J* 2.4, H-8′), 7.48/7.44 (dd, 1H, *J* 2.4, 9.0, H-6′), 7.33/7.02 (d, 1H, *J* 5.1, H-6), 6.94/6.87 (dd, 1H, *J* 1.2, 5.1, H-5), 6.79/6.65 (d, 1H, *J* 1.2, H-3), 6.60/6.42 (d, 1H, *J* 5.7, H-3′), 4.22/4.12 (s, 2H, NCOCH_2), 3.88/3.85 (t, 2H, *J* 6.0, ArNCH$_2$CH_2N), 3.60/3.51 (t, 2H, *J* 6.0, ArNCH_2CH$_2$N), 1.42/1.31 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, CDO_3D) 170.2/170.0, 167.4/167.3, 159.2/159.0, 157.2/157.1, 152.0/151.9, 150.2/150.0 (2C), 134.6/134.4, 129.5/129.4, 128.4/128.3 (2C), 125.6/125.5, 115.4/115.2, 114.1/114.0, 108.2/108.1, 104.9/105.8, 103.0/102.9, 52.6/52.5, 50.1/50.0, 49.1/49.0, 44.3/44.4, 33.3/33.2 (3C); HRMS (FAB) *m/z* 470.17219 (M^+ $C_{24}H_{27}{}^{35}ClN_4O_4$ requires 470.17208).

***N*-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethyl*)-*N*-(2-(*cyclohexylamino*)-2-*oxoethyl*)-*isonicotinamide*, 5.23d**



White amorphous powder (112 mg, 76%), m.p. 206 – 208 °C (from from EtOH:H_2O); R_f(DCM:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3402 (NH), 3028 (ArCH), 2897 (CH), 1703 (conjugated C=O), 1685 (C=O), 1591 (C=C); δ_H(400 MHz, CD_3OD) 8.60/8.28 (d, 2H, *J* 6.0, H-2, H-6), 8.44/8.39 (d, 1H, *J* 5.6, H-2′), 8.20/8.06 (d, 1H, *J* 9.0, H-5′), 7.83/7.77 (d, 1H, *J* 2.0, H-8′), 7.49/7.47 (dd, 1H, *J* 2.0, 9.0, H-6′), 7.37/7.30 (d, 2H, *J* 6.0, H-3, H-5), 6.70/6.26 (d, 1H, *J* 5.6, H-3′), 4.25/3.93 (s, 2H, NCH_2CO), 3.88/3.85 (t, 2H, *J* 6.0, ArNCH_2CH_2N), 3.59/3.54 (t, 2H, *J* 6.0, ArNCH_2CH_2N), 3.30/3.28 (m, 1H, H-1″), 1.60/1.54 (m, 4H, CH_2-2″, CH_2-6″), 1.20/1.17 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C(75 MHz, CD_3OD) 171.2/171.1, 166.8/166.7, 151.2/151.0, 150.1/150.0 (2C), 149.5/149.3 (2C), 141.8/141.7, 134.2/143.2, 128.1/120, 1276.1/126.0, 122.7/122.5 (2C), 122.1/122.0, 116.2/116.0, 104.6/104.5, 52.8/52.6, 51.2/51.0, 48.3/48.1, 44.4/44.2, 33.1/33.0 (2C), 27.2/27.0, 22.5/22.3 (2C); HRMS (FAB) *m/z* 465.13356 (M^+ + H, $C_{25}H_{28}{}^{35}ClN_5O_2$ requires 465.19315).

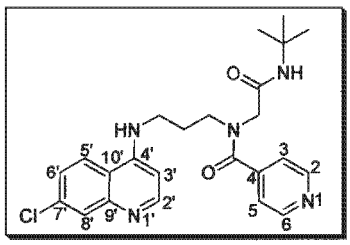
***N*′-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethyl*)-*N*-(2-(*cyclohexylamino*)-2-*oxoethyl*)-2,4-*dihydroxybenzamide*, 5.23e**



White glasses (109 mg, 70%), m.p. 209 – 211 °C (from EtOH:H_2O); R_f(DCM:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3614 (OH), 3393 (NH), 2879 (ArCH), 1699 (C=O), 1689 (conjugated C=O), 1551 (C=N); δ_H(300 MHz, CD_3OD) 8.44/8.27 (d, 1H, *J* 5.7, H-2), 8.20/8.11 (d, 1H, *J* 9.0, H-5), 7.92/7.83 (d, 1H, *J* 2.4, H-8), 7.48/7.42 (dd, 1H, *J* 2.4, 9.0, H-6), 7.33 /7.08(d, 1H, *J* 4.8,

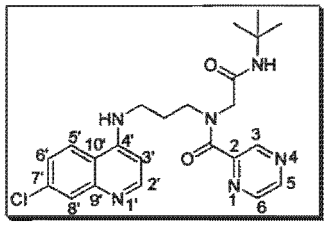
H-6′), 6.94/6.88 (dd, 1H, *J* 1.2, 4.8, H-5′), 6.77/6.71 (d, 1H, *J* 1.2, H-3′), 6.58/6.43 (d, 1H, *J* 5.7, H-3), 4.21/4.12 (s, 2H, NCH_2CO), 3.89/3.84 (t, 2H, *J* 6.0, ArNCH$_2$CH_2N), 3.62/3.53 (t, 2H, *J* 6.0, ArNCH_2CH$_2$N), 3.232/3.30 (m, 1H, H-1″), 1.62/1.60 (m, 4H, CH_2-2″, CH_2-6″), 1.21/1.18 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C(75 MHz, CD_3OD); 170.1/170.0, 167.6/167.4, 159.2/159.0, 157.4/157.3, 152.1/152.0, 150.1/149.8 (2C), 134.6/134.3, 129.5/129.4, 128.4/128.1 (2C), 125.6/125.4, 115.4/115.3, 114.1/114.0, 108.2/108.0, 104.9/104.8, 103.0/102.8, 52.8/52.7, 51.2/51.1, 48.3/48.2, 44.5/44.3, 33.6/33.3 (2C), 27.2/27.0, 23.2/23.0 (2C); HRMS (FAB) *m/z* 496.18770 (M^+ $C_{26}H_{29}{}^{35}ClN_4O_4$ requires 496.18773).

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(3-(7-chloroquinolin-4-ylamino)-propyl)-isonicotinamide, 5.23f**



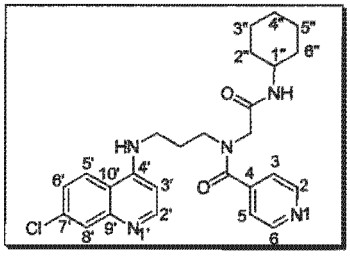
Yellow oil (93.2 mg, 65%), m.p. 90 – 92 °C (from EtOH: H_2O); R_f(DCM:MeOH 9:1) 0.10; IR ν_{max}(KBr)/cm^{-1} 3285 (NH), 3060 (ArCH), 2930 (CH), 1701 (C=O), 1569 (C=N); δ_H(300 MHz, CD_3OD) 8.60/8.29 (d, 2H, *J* 6.0, H-2, H-6), 8.40/8.36 (d, 1H, *J* 5.6, H-2′), 8.24/8.16 (d, 1H, *J* 8.8, H-5′), 7.80/7.76 (d, 1H, *J* 2.0, H-8′), 7.42/7.38 (dd, 1H, *J* 2.0, 8.8, H-6′), 7.28/7.11 (d, 2H, *J* 6.0, H-3, H-5), 6.60/6.23 (d, 1H, *J* 5.6, H-3′), 4.18/4.10 (s, 2H, NCH_2CO), 3.80/3.76 (t, 2H, *J* 6.0, ArN(CH$_2$)$_2$CH_2N), 3.56/3.52 (t, 2H, *J* 6.0, ArNCH_2CH$_2$N), 2.10/2.07 (m, 2H, ArNCH$_2$CH_2CH$_2$N), 1.36/1.30 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, CD_3OD) 171.1/169.9, 166.8/166.7, 151.2/151.0, 150.0/149.8 (2C), 149.5/149.3 (2C), 141.8/141.7, 134.2/134.1, 128.1/128.0, 126.0/125/9, 122.6/122.4 (2C), 121.5/121.4, 116.0/115.9, 104.7/104.6, 52.8/52.7, 51.2/51.0, 48.3/48.1, 41.5/41.4, 31.2/31.0 (3C), 30.1/30.0; HRMS (FAB) *m/z* 454.20019 (M^+ + H, $C_{24}H_{28}{}^{35}ClN_6O_2$ + H requires 454.20097).

***N*-(2-(*tert*-Butylamino)-2-oxoethyl)-*N*-(3-(7-chloroquinolin-4-ylamino)propyl)pyrazine-2-carboxamide, 5.23g**



Pale yellow glasses (90 mg, 63%), m.p. 78 – 80 °C (from EtOH:H_2O); R_f(DCM:MeOH 9:1) 0.10; IR ν_{max}(KBr)/cm^{-1} 3300 (NH), 3070 (ArCH), 2932 (CH), 1709 (C=O), 1687 (conjugated C=O), 1557 (C=N); δ_H(300 MHz, CD_3OD) 8.86/8.67 (d, 1H, *J* 1.5, H-3), 8.64/8.38 (d, 1H, *J* 2.7, H-6), 8.59/8.36 (dd, 1H, *J* 1.5, 2.7, H-5), 8.37/8.24 (d, 1H, *J* 5.7, H-2′), 8.20/8.14 (d, 1H, *J* 9.0, H-5′), 7.96/7.78 (m, 2H, H-6′, H-8′), 6.68/6.32 (d, 1H, *J* 5.7, H-3′), 4.25/4.19 (s, 2H, NCH_2CO), 3.82/3.77 (t, 2H, *J* 6.0, ArN$(CH_2)_2$NCH_2N), 3.58/3.48 (t, 2H, *J* 6.0, ArNC$H_2(CH_2)_2$N), 2.18/2.09 (m, 2H, ArNCH$_2$CH_2CH$_2$N), 1.39/1.27 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, CD_3OD) 171.1/171.1, 166.8/166.7, 151.2/121.0, 149.5/149.3 (2C), 147.8/147.7, 144.1/144.0, 143.7/143.6, 141.8/141.7, 134.2/134.1, 128.1/128.0, 126.0/125.9, 121.5/121.4, 116.0/115.8, 104.6/104.5, 52.8/52.6, 51.2/51.0, 48.3/48.1, 41.5/41.4, 31.2/31.0 (3C), 30.1/30.0; HRMS (FAB) *m/z* 454.18456 (M^+ $C_{23}H_{27}{}^{35}ClN_6O_2$ requires 454.18440).

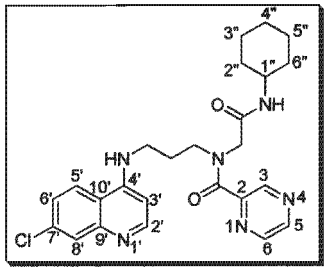
***N*-(3-(7-Chloroquinolin-4-ylamino)propyl)-*N*-(2-(cyclohexylamino)-2-oxoethyl)-isonicotinamide, 5.23h**



Cream white plates (91 mg, 60%), m.p. 101 – 102 °C (from EtOH:H_2O); R_f(DCM:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3298 (NH), 3032 (ArCH), 2926 (CH), 1685 (C=O), 1556 (C=N); δ_H(400 MHz, CD_3OD) 8.60/8.29 (d, 2H, *J* 5.6, H-2, H-6), 8.41/8.37 (d, 1H, *J* 6.0, H-2′), 8.24/8.17 (d, 1H, *J* 9.0, H-5′), 7.80/7.75 (d, 1H, *J* 2.0, H-8′), 7.45/7.41 (dd, 1H, *J* 2.0, 9.0, H-6′), 7.27/7.08 (d, 2H, *J* 6.0, H-3, H-5), 6.64/6.29 (d, 1H, *J* 6.0, H-3′), 4.28/4.19 (s, 2H, NCH_2CO), 3.80/3.76 (t, 2H, *J* 6.0, ArN$(CH_2)_2$NCH_2N),

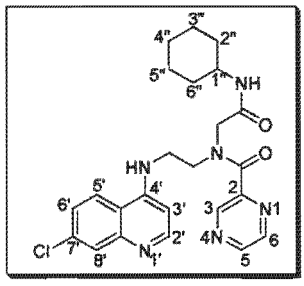
3.54/3.50 (t, 2H, *J* 6.0, ArC*H*$_2$(CH$_2$)$_2$N) 3.30/3.29 (m, 1H, H-1″), 2.10/1.94 (m, 2H, ArNCH$_2$C*H*$_2$CH$_2$N), 1.62/1.63 (m, 4H, C*H*$_2$-2″, C*H*$_2$-6″), 1.20/1.17 (m, 6H, C*H*$_2$-3″, C*H*$_2$-4″, C*H*$_2$-5″); δ_C(75 MHz, CD_3OD) 171.1/170.0, 166.8/166.7, 151.2/151.0, 150.0/149.8 (2C), 149.5/149.3 (2C), 141.8/141.6, 134.2/134.0, 128.1/128.0, 126.0/125.9, 122.5/122.3 (2C), 121.5/121.4, 116.1/116.0, 104.5/104.3, 52.8/52.6, 51.2/51.0, 48.3/48.1, 44.6/44.5, 33.3/33.1 (2C), 29.7/29.5, 27.6/27.5, 22.5/22.3 (2C); HRMS (FAB) *m/z* 479.02869 (M^+ $C_{26}H_{30}{}^{35}ClN_5O_2$ requires 479.02880).

***N*-(3-(7-*Chloroquinolin*-4-*ylamino*)*propyl*)-*N*-(2-(*cyclohexylamino*)-2-*oxoethyl*)*pyrazine*-2-*carboxamide*, 5.23i**



Pale yellow powder (112 mg, 74%), m.p. (from EtOH: H_2O) 100 – 102 °C; R_f(CH_2Cl_2:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3402 (NH), 3023 (ArCH), 2923 (CH), 1699 (C=O), 1682 (conjugated C=O), 1561 (C=N); δ_H(400 MHz, CD_3OD) 8.88/8.58 (d, 1H, *J* 1.6, H-3), 8.64/8.39 (d, 1H, *J* 2.4, H-6), 8.37/8.35 (dd, 1H, *J* 1.6, 2.4, H-5), 8.34/8.20, (d, 1H, *J* 5.6, H-2′), 8.17/8.14 (d, 1H, *J* 9.2, H-5′), 7.98/7.80 (d, 1H, *J* 2.0, H-8′), 7.41/7.39 (dd, 1H, *J* 2.0, 9.2, H-6′), 6.60/6.57 (d, 1H, *J* 5.6, H-3′), 4.28/4.18 (s, 2H, NC*H*$_2$CO), 3.80/3.76 (t, 2H, *J* 6.0, ArN(CH$_2$)$_2$NC*H*$_2$N), 3.51/3.34 (t, 2H, *J* 6.0, ArNC*H*$_2$(CH$_2$)$_2$N), 3.30/3.28 (m, 1H, H-1″), 2.10/1.90 (m, 2H, ArNCH$_2$C*H*$_2$CH$_2$N), 1.66/1.61 (m, 4H, C*H*$_2$-2″, C*H*$_2$-6″), 1.36/1.20 (m, 6H, C*H*$_2$-3″, C*H*$_2$-4″, C*H*$_2$-5″); δ_C(75 MHz, CD_3OD) 171.1/170.0, 166.8/166.7, 151.2/151.1, 149.7/149.5 (2C), 147.9/147.8, 144.2/144.0 (2C), 141.8/141.7, 134.2/134.0, 128.1/128.0, 126.0/125.9, 121.5/121.4, 116.2/116.0, 105.0/104.9, 52.9/52.8, 51.2/51.1, 48.3/48.2, 44.6/44.5, 33.3/33.1 (2C), 28.7/28.6, 27.6/27.5, 22.5/22.3 (2C); HRMS (FAB) *m/z* 481.21124 (M^+ + H, $C_{25}H_{29}{}^{35}ClN_6O_2$ + H requires 481.21186).

***N*-(2-(7-Chloroquinolin-4-ylamino)ethyl)-*N*-(2-(cyclohexylamino)-2-oxoethyl)pyrazine-2-carboxamide, 5.23j**



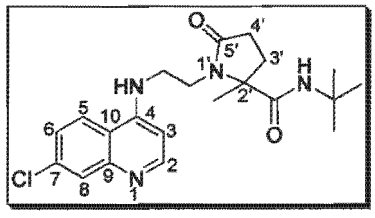
Yellow oil (106 mg, 72%), m.p. (from EtOH:H_2O) 92 – 94 °C; R_f(CH_2Cl_2:MeOH 9:1) 0.11; IR ν_{max}(KBr)/cm^{-1} 3290 (NH), 3071 (ArCH), 2920 (CH), 1700 (C=O), 1559, (C=N); δ_H(400 MHz, CD_3OD), 8.87/8.67 (d, 1H, *J* 1.2, H-3), 8.65/8.40 (d, 1H, *J* 2.8, H-6), 8.54/8.38 (dd, 1H, *J* 1.5, 2.8, H-5), 8.38/8.33 (d, 1H, *J* 5.7, H-2′), 8.11/8.07 (d, 1H, *J* 9.0, H-5′), 7.91/7.78 (d, 1H, *J* 2.0, H-8′), 7.44/7.34 (dd, 1H, *J* 2.0, 9.0, H-6′), 6.72/6.34 (d, 1H, *J* 5.7, H-3′), 4.23/4.16 (s, 2H, NCH_2CO), 4.04/3.95 (t, 2H, *J* 6.0, ArNCH$_2$CH_2N), 3.78/3.66 (t, 2H, *J* 6.0, ArNCH_2CH$_2$N), 3.31/3.29 (m, 1H, NC*H*), 1.70/1.65 (m, 4H, CH_2-2″, CH_2-4″), 1.30/1.27 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C(75 MHz, CD_3OD) 171.3/171.2, 166.8/166.7, 151.1/149.9, 149.6/149.3 (2C), 147.2/147.0, 144.3/144.1, 143.3/143.1, 141.7/141.6, 134.2/134.2, 128.3/128.1, 126.0/125.8, 121.4/121.3, 116.0/115.9, 104.6/104.5, 52.9/52.8, 51.3/51.1, 48.5/48.4, 44.6/44.5, 33.3/33.1 (2C), 27.6/27.6, 22.4/22.2 (2C); HRMS (FAB) *m/z* 467.19237 (M^+ $C_{24}H_{27}{}^{35}ClN_6O_2$ + H requires 467.19263).

General Synthesis of 4-aminoquinoline-containing lactams (K)

Amines **3.52 – 3.55** (0.225 mmol, 1.0 eq) were dispensed in 16 separate vials and dissolved in MeOH (5 ml). A solution of levulinic acid or 4-acteylbutyric acid (0.27 mmol, 1.2 eq) was added *via* micro syringe and the reaction mixtures stirred at room temperature for 30 minutes. At this point isocyanide was added (0.27 mmol, 1.2 eq) and stirring continued for an additional 18 h. After the reactions were deemed complete (TLC) MP-TSOH (0.46 g, 0.675 mmol) was added to each of the vials which were transferred to an orbital shaker. After 1 h, the resins were filtered and washed with

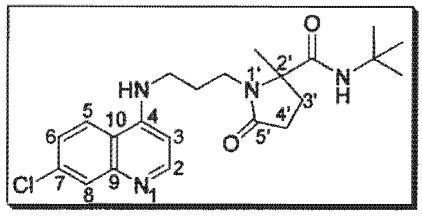
MeOH and suspended in a solution of 3% NH_3 in MeOH and filtered after 30 min of shaking at room temperature. The solvent was removed *in vacuo* and concentrated to afford the lactams.

***N-tert-Butyl*-1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethyl*)-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25a**



White amorphous powder (54 mg, 60%), m.p. 112 – 114 °C; R_f(MeOH:DCM 1:9) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3304 (NH), 3031 (ArCH), 2934 (CH), 1712 (C=O), 1627 (C=C), 1580 (C=N); δ_H(400 MHz, $CDCl_3$) 8.51 (d, 1H, *J* 5.6, H-2), 7.94 (d, 1H, *J* 2.0, H-8), 7.87 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.0, 9.0, H-6), 6.29 (d, 1H, *J* 5.6, H-3), 3.68 (t, 2H, *J* 6.0, ArNCH_2CH_2N), 3.43 (t, 2H, *J* 6.0, ArNCH_2CH_2N), 2.55 – 2.49 (m, 2H, CH_2-4'), 2.32 (m, 1H, H-3α), 1.99 (m, 1H, H-3'β), 1.56 (s, 3H, CCH_3), 1.24 (s, 9H, C(CH_3)$_3$) δ_C(100 MHz, $CDCl_3$) 178.4, 173.0, 151, 150.6, 134.0, 129.0, 126.0, 125.2, 122.0, 117.0, 98.2, 69.3, 52.4, 44.2, 40.3, 33.2, 29.3, 28.5 (3C), 22.6; HRMS (FAB) *m/z* 402.17884, (M^+ $C_{21}H_{27}{}^{35}ClN_4O_2$ requires 402.17874); HPLC Purity: 91%, t_R' = 1.49 min.

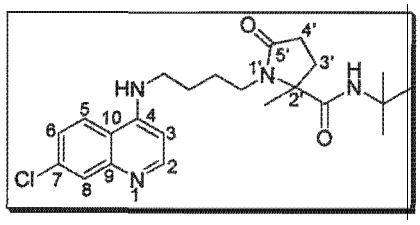
***N-tert-Butyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25b**



Yellow oil (74 mg, 79%); R_f(MeOH:DCM 1:9) 0.21; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 33344 (NH), 3019 (ArCH), 2984 (CH), 1719 (C=O), 1648 (C=C), 1532 (C=N); δ_H(400 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.6, H-2), 7.92 (d, 1H, *J* 2.0, H-8), 7.85 (d, 1H, *J* 9.0, H-5), 7.44 (dd, 1H, *J* 2.0, 9.0, H-6), 6.30 (d, 1H, *J* 5.6, H-3),

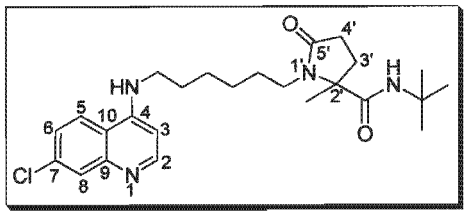
3.66 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_2$N), 3.41 (t, 2H, *J* 6.0, ArN(CH$_2$)$_2$CH_2N), 2.54 – 2.50 (m, 2H, CH_2-4′), 2.33 – 2.21 (m, 1H, H-3′α), 1.97 – 1.88 (m, 1H, H-3′β), 1.84 – 1.80 (m, 2H, ArCH$_2$CH_2CH$_2$N), 1.48 (s, 3H, CCH_3), 1.33 (s, 9H, (CH_3)$_3$); δ_C(100 MHz, $CDCl_3$) 178.6, 173.3, 151.0, 150.7, 134.0, 129.0, 126.2, 125.4, 122.2, 117.3, 98.6, 69.7, 52.3, 44.2, 40.4, 33.2, 30.2, 29.3, 28.4 (3C), 22.3; HRMS (FAB) *m/z* 416.19790, (M^+ $C_{22}H_{29}{}^{35}ClN_4O_2$ requires 416.19837); HPLC Purity: 92%, t_R' = 2.04 min.

N-tert-Butyl-1-(4-(7-chloroquinolin-4-ylamino)butyl)-2-methyl-5-oxopyrrolidine-2-



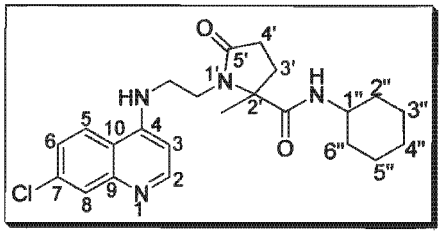
carboxamide, 5.25c White amorphous powder (71 mg, 73%), m.p. 203 – 205 °C; R_f(MeOH:DCM 1:9) 0.21; IR ν_{max}($CHCl_3$)/cm^{-1} 3323 (NH), 3011 (ArCH), 2965 (CH), 1726 (C=O), 1651 (C=C), 1545 (C=N); δ_H(400 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.6, H-2), 7.91 (d, 1H, *J* 2.0, H-8), 7.83 (d, 1H, *J* 9.0, H-5), 7.43 (dd, 1H, *J* 2.0, 9.0, H-6), 6.24 (d, 1H, *J* 5.6, H-3), 3.38 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_2$CH$_2$N), 3.00 (t, 2H, *J* 6.0, ArNCH$_2$(CH$_2$)$_2$CH_2N), 2.51 – 2.38 (m, 2H, CH_2-4′), 2.27 – 2.18 (m, 1H, H-3′α), 1.91 – 1.87 (m, 1H, H-3′β), 1.83 – 1.70 (m, 4H, ArNCH$_2$CH_2CH_2CH$_2$N), 1.48 (s, 3H, CCH_3), 1.39 (s, 9H, C(CH_3)$_3$); δ_C(100 MHz, $CDCl_3$) 178.4, 173.0, 151.0, 150.6, 134.0, 129.0, 126.0, 125.2, 122.0, 117.0, 98.8, 69.3, 52.4, 44.2, 40.3, 33.2, 30.3, 29.3, 29.0, 28.5 (3C), 22.6; HRMS (FAB) *m/z* 430.21355, (M^+ $C_{23}H_{31}{}^{35}ClN_4O_2$ requires 430.21150); HPLC Purity: 90%, t_R' = 2.19 min.

***N-tert-Butyl*-1-(6-(7-*chloroquinolin*-4-*ylamino*)*hexyl*)-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25d**



White amorphous powder (71 mg, 73%), m.p. 203 – 205 °C; R_f(MeOH:DCM 1:9) 0.21; IR ν_{max} ($CHCl_3$)/cm^{-1} 3323 (NH), 3011 (ArCH), 2965 (CH), 1726 (C=O), 1651 (C=C), 1545 (C=N); δ_H(400 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.6, H-2), 7.91 (d, 1H, *J* 2.0, H-8), 7.83 (d, 1H, *J* 9.0, H-5), 7.43 (dd, 1H, *J* 2.0, 9.0, H-6), 6.24 (d, 1H, *J* 5.6, H-3), 3.38 (t, 2H, *J* 6.0, ArNCH_2(CH_2)$_5$N), 3.00 (t, 2H, *J* 6.0, ArN(CH_2)$_5$CH_2N), 2.51 – 2.38 (m, 2H, CH_2-4′), 2.27 – 2.18 (m, 1H, H-3′α), 1.91 – 1.87 (m, 1H, H-3′β), 1.83 – 1.70 (m, 4H, ArNCH$_2$CH_2(CH_2)$_2$CH_2(CH_2)$_2$N), 1.48 (s, 3H, CCH_3), 1.39 (s, 9H, C(CH_3)$_3$), 1.23 – 1.01 (m, 4H, ArN(CH_2)$_2$CH_2CH_2(CH_2)$_2$N; δ_C(100 MHz, $CDCl_3$) 178.5, 172.8, 151.2, 150.7, 134.2, 129.1, 126.2, 125.4, 122.0, 117.0, 99.0, 69.5, 52.5, 44.4, 40.2, 33.4, 30.2, 29.4, 29.0, 28.4 (3C), 22.8; HRMS (FAB) *m/z* 458.24355, (M^+ $C_{25}H_{35}{}^{35}ClN_4O_2$ requires 458.24485); HPLC Purity: 90%, t_R' = 2.19 min.

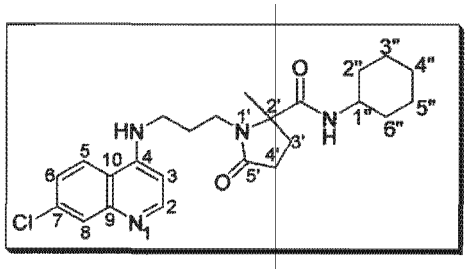
1-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethyl*)-N-*cyclohexyl*-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25e



White amorphous powder (72 mg, 75%), m.p. 210 – 210 °C; R_f(MeOH:DCM 1:9) 0.20; IR ν_{max} ($CHCl_3$)/cm^{-1} 3334 (NH), 3013 (ArCH), 2988 (CH), 1725 (C=O), 1650(C=C), 1555 (C=N); δ_H(300 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.4, H-2), 7.91 (d, 1H, *J* 2.1, H-8), 7.86 (d, 1H, *J* 9.0, H-5), 7.41 (dd, 1H, *J* 2.1, 9.0, H-6), 6.33 (d, 1H, *J* 5.4, H-3), 3.68 (m, 3H, ArNCH_2CH_2N, H-1″), 3.48 (t, 2H, *J* 6.0, ArNCH$_2$CH_2N), 2.52 – 2.50 (m, 2H, CH_2-4′), 2.40 – 2.36 (m, 1H, H-3′α),

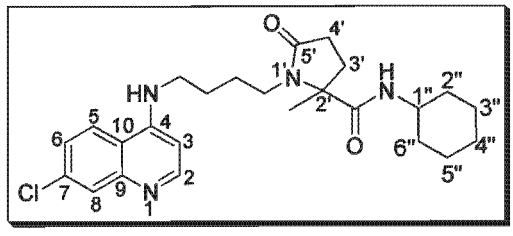
2.10 – 1.99 (m, 1H, H-3′β), 1.78 (br t, 2H, *J* 12.0, C*H*-2″α, C*H*-6″α), 1.59 (m, 5H, C*H*-2″β, C*H*-6″β, CCH_3), 1.26 – 0.99 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C(75 MHz, $CDCl_3$) 178.7, 172.0, 151.1, 150.0, 146.8, 135.0, 126.7, 126.0, 122.5, 117.0, 98.5, 67.8, 48.9, 44.3, 40.2, 33.4, 32.7 (2C), 29.4, 25.2, 24.7 (2C), 22.6; HRMS (FAB) *m/z* 428.19404, (M^+ $C_{23}H_{29}{}^{35}ClN_4O_2$ requires 428.19790); HPLC Purity: 88%, t_R' 2.02 = min.

1-(3-(7-*Chloroquinolin-4-ylamino*)*propyl*)-*N*-*cyclohexyl*-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25f



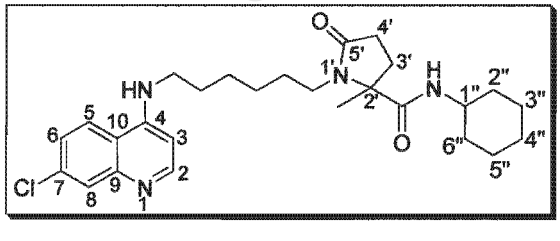
Yellow oil (69 mg, 70%); R_f(MeOH:DCM 1:9) 0.20; IR ν_{max}($CHCl_3$)/cm^{-1} 3309 (NH), 3010 (ArCH), 2973 (CH), 1718 (C=O), 1649 (C=C), 1556 (C=N); δ_H(300 MHz, $CDCl_3$) 8.48 (d, 1H, *J* 5.4, H-2), 7.89 (d, 1H, *J* 2.0, H-8), 7.81 (d, 1H, *J* 9.0, H-5), 7.44 (dd, 1H, *J* 2.1, 9.0, H-6), 6.31 (d, 1H, *J* 5.4, H-3), 3.58 (m, 3H, ArNCH_2(CH_2)$_2$N, H-1″), 3.28 (t, 2H, *J* 6.0, ArN(CH_2)$_2$CH_2N), 2.53 – 2.48 (m, 2H, CH_2-2′), 2.39 – 2.26 (m, 1H, H-3′α), 2.16 – 1.90 (m, 3H, H-3′β, ArNCH_2CH_2CH_2N), 1.78 (br t, 2H, *J* 12.0, C*H*-2″α, C*H*-6″α), 1.60 – 1.40 (m, 5H, C*H*-2″β, C*H*-6″β, CCH_3), 1.38 – 0.98 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C(75 MHz, $CDCl_3$) 178.5, 172.1, 151.3, 150.0, 146.5, 135.2, 126.5, 125.2, 122.7, 117.0, 99.0, 67.6, 49, 44.2, 40.4, 33.5, 32.8 (2C), 30.3, 29.6, 25.1, 24.5 (2C), 22.8, HRMS (FAB) *m/z* 442.21325, (M^+ $C_{24}H_{31}{}^{35}ClN_4O_2$ requires 442.21355); HPLC Purity: 91%, t_R' = 2.35 min.

1-(4-(7-*Chloroquinolin-4-ylamino*)*butyl*)-*N*-*cyclohexyl*-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25g



White amorphous solid (70 mg, 68%), m.p. 182 – 184 °C; R_f(MeOH:DCM 1:9) 0.21; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3331 (NH), 3014 (ArCH), 2984 (CH), 1711 (C=O), 1647 (C=C), 1553 (C=C); δ_H(300 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.4, H-2), 7.91 (d, 1H, *J* 2.0, H-8), 7.86 (d, 1H, *J* 9.0, H-5), 7.41 (dd, 1H, *J* 2.1, 9.0, H-6), 6.33 (d, 1H, *J* 5.4, H-3), 3.68 (m, 3H, ArNC$H_2$$(CH_2)_3$N, H-1″), 3.27 (t, 2H, *J* 6.0, ArN$(CH_2)_3$CH_2N, 2.51 – 2.48 (m, 2H, CH_2-4′), 2.40 – 2.38 (m, 1H, H-3′α), 2.00 – 1.98 (m, 3H, H-3′β, ArCH_2C$H_2$$(CH_2)CH_2$N), 1.78 (m, 4H, C*H*-2″α C*H*-6″α, ArC$H_2$$(CH_2)_2CH_2CH_2$N), 1.61 – 1.48, (m, 5H, C*H*-2″β, C*H*-6″β, CCH_3), 1.38 – 0.98 (m, 6H, CH_2-3″, CH_2-4″, CH_2-5″); δ_C(75 MHz, $CDCl_3$) 178.8, 172.3, 151.3, 150.2, 146.5, 135.2, 126.8, 125.7, 122.4, 117.2, 98.7, 67.7, 48.6, 44.4, 40.6, 33.5, 32.4 (2C), 30.1, 29.3, 29, 25.3, 24.5 (2C), 22.3; HRMS (FAB) *m/z* 456.23033, (M^+ $C_{25}H_{33}{}^{35}ClN_4O_2$ requires 456.22920); HPLC Purity: 95%, t_R' = 2.90 min.

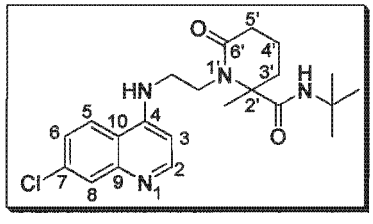
1-(6-(7-*Chloroquinolin-4-ylamino*)*hexyl*)-*N*-*cyclohexyl*-2-*methyl*-5-*oxopyrrolidine*-2-*carboxamide*, 5.25h



Yellow foam (84mg, 77%); R_f(MeOH:DCM 1:9) 0.23; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3323 (NH), 3026 (ArCH), 2954 (CH), 1716 (C=O), 1652 (C=C), 1583 (C=N); δ_H(300 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.4, H-2), 7.92 (d, 1H, *J* 2.0, H-8), 7.81 (d, 1H, *J* 9.0, H-5), 7.41 (dd, 1H, *J* 2.1, 9.0, H-6), 6.33 (d, 1H, *J* 5.4, H-3), 3.43 (m, 3H, ArNC$H_2$$(CH_2)_5$N, NC*H*), 3.22 (t, 2H, *J* 6.0, ArN$(CH_2)_5$CH_2N), 2.50 – 2.46 (m, 2H, CH_2-4′), 2.41 – 2.39 (m, 1H, H-

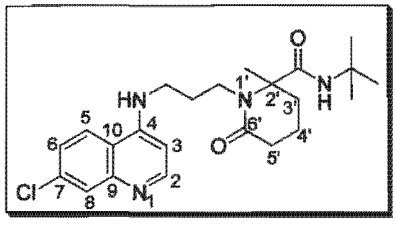
3′α), 2.00 – 1.98 (m, 3H, H-3′β, $ArCH_2CH_2(CH_2)_4N$), 1.70 – 1.62 (m, 4H, C*H*-2″α, C*H*-6″α, $ArCH_2(CH_2)_4CH_2CH_2N$), 1.59 (m, 5H, C*H*-2″β, C*H*-6″β, CCH_3), 1.25 – 0.98 (m, 10H, CH_2-3″, CH_2-4″, CH_2-5″, $ArN(CH_2)_2CH_2CH_2(CH_2)_2N$); δ_C(75 MHz, $CDCl_3$) 178.6, 172.0, 151.4, 150.0, 146.7, 135.0, 126.3, 125.4, 122.5, 117.0, 98.9, 67.8, 48.7, 44.3, 40.2, 33.4, 32.3 (2C), 31.5, 29.5, 28.2, 26.7, 26.5, 25.2, 24.7 (2C), 22.8; HRMS (FAB) *m/z* 484.26050, (M^+ $C_{27}H_{37}{}^{35}ClN_4O_2$ requires 484.26024); HPLC Purity: 94%, t_R' = 2.46 min.

***N*-*tert*-Butyl-1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethyl*)-2-*methyl*-6-*oxopiperidine*-2-*carboxamide*, 5.25i**



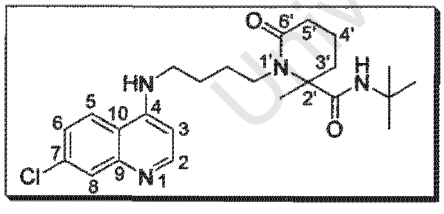
White gum (70 mg, 75%); R_f(MeOH:DCM 1:9) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3317 (NH), 3030 (ArCH), 2974 (CH), 1715 (C=O), 1646 (C=C), 1578 (C=N); δ_H(300 MHz, $CDCl_3$) 8.44 (d, 1H, *J* 5.7, H-2), 8.08 (d, 1H, *J* 9.0, H-5), 7.9 (d, 1H, *J* 2.1, H-8), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 6.26 (d, 1H, *J* 5.4, H-3), 3.25 (t, 2H, *J* 6.0, $ArNCH_2CH_2N$), 3.0 (t, 2H, *J* 6.0, $ArNCH_2CH_2N$), 2.56 – 2.40 (m, 2H, CH_2-5′), 2.20 – 2.14 (m, 2H, CH_2-3′), 1.80 – 1.74 (m, 2H, CH_2-4′), 1.55 (s, 3H, CCH_3), 1.32 (s, 9H, $C(CH_3)_3$); δ_C(75 MHz, $CDCl_3$) 178.7, 172.4, 151.6, 150.4, 147.9, 134.8, 126.3, 125.7, 122.3, 117.4, 98.2, 66.5, 51.4, 42.7, 41.0, 36.1, 34.0, 29.0, 28.6 (3C), 17.4; HRMS (EI) 416.19785 (M^+ $C_{22}H_{29}{}^{35}ClN_4O_2$ requires 416.19790); HPLC Purity: 91%, t_R' = 2.12 min.

N-tert-Butyl-1-(3-(7-chloroquinolin-4-ylamino)propyl)-2-methyl-6-oxopiperidine-2-carboxamide, 5.25j



Cream white paste (69 mg, 71%); R_f(MeOH:DCM 1:9) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3322 (NH), 3035 (ArCH), 2956 (CH), 1721 (C=O), 1647 (C=C), 1583 (C=N); δ_H(300 MHz, $CDCl_3$) 8.38 (d, 1H, *J* 5.7, H-2), 8.10 (d, 1H, *J* 9.0, H-5), 7.88 (d, 1H, *J* 2.1, H-8), 7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 6.34 (d, 1H, *J* 5.7, H-3), 3.26 (t, 2H, *J* 6.0, ArNCH_2(CH_2)$_2$N), 3.10 (t, 2H, *J* 6.0, ArN(CH_2)$_2$CH_2N), 2.57 – 2.40 (m, 2H, CH_2-5′), 2.20 – 2.12 (m, 2H, CH_2-3′), 1.94 – 1.89 (m, 2H, CH_2-4′), 1.83 – 1.72 (m, 2H, ArNCH$_2$C*H*CH$_2$N), 1.49 (s, 3H, CH_3), 1.38 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, $CDCl_3$) 178.8, 172.2, 151.5, 150.2, 147.6, 134.7, 126.4, 125.8, 122.6, 117.2, 98.6, 66.5, 51.7, 42.5, 42.1, 36.3, 34.2, 30.4, 29.2, 28.8 (3C), 17.7; HRMS (EI) 430 (M^+ $C_{23}H_{31}{}^{35}ClN_4O_2$ requires 430.21355); HPLC Purity: 96% , t_R' = 1.81 min.

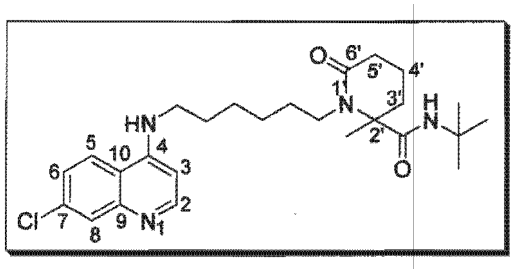
***N-tert-Butyl*-1-*(4-(7-chloroquinolin-4-ylamino)butyl)-2-methyl-6-oxopiperidine*-2-*carboxamide*, 5.25k**



Cream white paste (69 mg, 69%); R_f(MeOH:DCM 1:9) 0.21 IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3332 (NH), 3027 (ArCH), 2966 (CH), 1715 (C=O), 1636 (C=C), 1582 (C=C); δ_H(300 MHz, $CDCl_3$) 8.37 (d, 1H, *J* 5.7, H-2), 8.06 (d, 1H, *J* 9.0, H-5), 7.81 (d, 1H, *J* 2.1, H-8), 7.38 (dd, 1H, *J* 2.1, 9.0, H-6), 6.24 (d, 1H, *J* 5.7, H-3), 3.22 (t, 2H, *J* 6.0, ArNCH_2(CH_2)$_3$N), 3.11 (t, 2H, *J* 6.0, ArN(CH_2)$_3$CH_2N), 2.53 – 2.41 (m, 2H, CH_2-5′), 2.22 – 2.13 (m, 2H, CH_2-3′), 1.97 – 1.95 (m, 2H, CH_2-4′), 1.85 – 1.74 (m, 4H, ArNCH$_2$CH_2CH_2N), 1.52 (s, 3H, CH_3), 1.34 (s, 9H, C(CH_3)$_3$); δ_C(75 MHz, $CDCl_3$) 178.5, 172.6, 151.3, 150.5, 147.7, 134.2, 126.4, 125.5, 122.4, 117.2, 98.3,

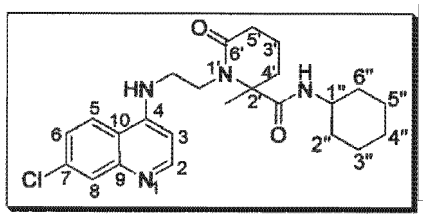
66.3, 51.5, 42.8, 41.2, 36.3, 34.2, 30.5, 29.1, 28.7, 28.8 (3C), 17.9; HRMS (EI) 444.23007 (M^+ $C_{24}H_{33}{}^{35}ClN_4O_2$ requires 444.22920); HPLC Purity: 87%, t_R' = 1.95 min.

***N-tert-Butyl*-1-(6-(7-*chloroquinolin*-4-*ylamino*)*hexyl*)-2-*methyl*-6-*oxopiperidine*-2-*carboxamide*, 5.25l**



White paste (79 mg, 74%); R_f(MeOH:DCM 1:9) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3319 (NH), 3031 (ArCH), 2987 (CH), 1707 (C=O), 1653 (C=C), 1567 (C=N); δ_H(300 MHz, $CDCl_3$) 8.35 (d, 1H, *J* 5.7, H-2), 8.0 (d, 1H, *J* 9.0, H-5), 7.88 (d, 1H, *J* 2.1, H-8), 7.41 (dd, 1H, *J* 2.1, 9.0, H-6), 6.28 (d, 1H, *J* 5.7, H-3), 3.30 (t, 2H, *J* 6.0, ArNC$H_2$$(CH_2)_5$N), 3.00 (t, 2H, *J* 6.0, ArN$(CH_2)_5$CH_2N), 2.56 – 2.40 (m, 2H, CH_2-5′), 2.20 – 2.14 (m, 2H, CH_2-3′), 2.05 – 2.01 (m, 2H, CH_2-4′), 1.90 – 1.80 (m, 2H, ArNCH$_2$C$H_2$$(CH_2)_4$N), 1.82 – 1.70 (m, 4H, ArN$(CH_2)_2$CH_2C$H_2$$(CH_2)_2$N), 1.62 – 1.53 (m, 5H, ArN$(CH_2)_2$C$H_2$$(CH_2)_2N, CCH_3$), 1.30 (s, 9H, C(C$H_3$)$_3$); δ_C(75 MHz, $CDCl_3$) 178.8, 172.6, 151.7, 150.5, 147.8, 134.7, 126.5, 125.8, 122.4, 117.6, 99.0, 66.6, 51.3, 42.8, 41.3, 36.5, 34.3, 31.8, 29.3, 28.8 (3C), 28.1, 26.5, 26.6, 17.7; HRMS (EI) 472.26001 (M^+ $C_{26}H_{37}{}^{35}ClN_4O_2$ requires 472.26050); HPLC Purity: 92%, t_R' = 1.93 min.

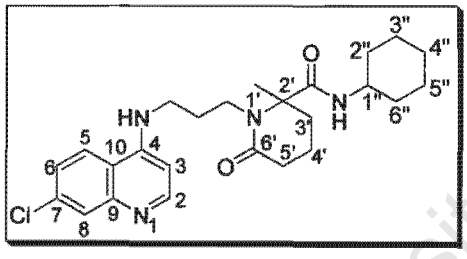
1-(2-(7-*Chloroquinolin*-4-*ylamino*)*ethyl*)-*N*-*cyclohexyl*-2-*methyl*-6-*oxopiperidine*-2-*carboxamide*, 5.25m



Off-white crystals (65 mg, 66%); R_f(MeOH:DCM 1:9) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3331 (NH), 3015 (ArCH), 2976 (CH), 1722 (C=O), 1617 (C=C), 1559 (C=N); δ_H(300 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.4, H-2), 7.91 (d,

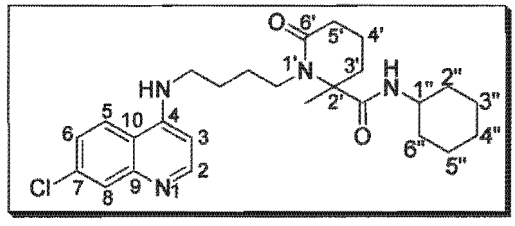
1H, *J* 2.0, H-8), 7.81 (d, 1H, *J* 9.0, H-5), 7.41 (dd, 1H, *J* 2.1, 9.0, H-6), 6.33 (d, 1H, *J* 5.4, H-3), 3.68 (m, 1H, H-1″), 3.32 (t, 2H, *J* 6.0, ArNCH_2CH_2N), 3.10 (t, 2H, *J* 6.0, ArNCH$_2$CH_2N), 2.57 – 2.42 (m, 2H, CH_2-5′), 2.20 – 2.12 (m, 2H, CH_2-3′), 1.81 – 1.70 (m, 4H, C*H*-2″α, C*H*-6″α, CH_2-4′), 1.64 – 1.60 (m, 5H, C*H*-2″β C*H*-6″β, CCH_3), 1.25 – 1.22 (m, 3H, C*H*-3″α, C*H*-4″α, C*H*-5″α), 1.22 – 1.01 (m, 3H, C*H*-3″β, C*H*-4″β, C*H*-5″β); δ_C(75 MHz, $CDCl_3$) 178.4, 172.0, 151.3, 150.2, 146.9, 135.2, 126.6, 125.5, 122.7, 117.3, 98.8, 66.4, 49.8, 44.6, 43.9, 36.0, 33 (2C), 32.6, 30.0, 25, 24.8 (2C), 17.8; HRMS (EI) 442.21343 (M^+ $C_{24}H_{31}{}^{35}ClN_4O_2$ requires 442.21355); HPLC Purity: 95%, t_R' = 2.14 min.

***1-(3-(7-Chloroquinolin-4-ylamino)propyl)-N-cyclohexyl-2-methyl-6-oxopiperidine-2-carboxamide*, 5.25n**



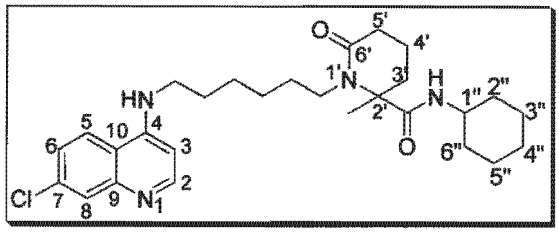
Cream white paste (74 mg, 72%); R_f(MeOH:DCM 1:9) 0.20; IR ν_{max}($CHCl_3$)/cm^{-1} 3327 (NH), 3027 (ArCH), 2988 (CH), 1718 (C=O), 1630 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) 8.43 (d, 1H, *J* 5.4, H-2), 7.85 (d, 1H, *J* 2.0, H-8), 7.78 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 6.30 (d, 1H, *J* 5.4, H-3), 3.66 (m, 1H, H-1″), 3.30 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_2$N), 3.11 (t, 2H, *J* 6.0, ArN(CH$_2$)$_2$CH_2N), 2.56 – 2.43 (m, 2H, CH_2-5′), 2.20 – 2.12 (m, 2H, CH_2-3′), 1.99 – 1.97 (m, 2H, CH_2-4′), 1.84 – 1.71 (m, 4H, C*H*-2″α, C*H*-6″α, CH_2-4′), 1.67 – 1.65 (m, 5H, C*H*-2″β, C*H*-6″β, CCH_3), 1.26 – 1.24 (m, 3H, C*H*-3″α, C*H*-4″α, C*H*-5″α), 1.10 – 1.00 (m, 3H, C*H*-3″β, C*H*-4″β, C*H*-5″β); δ_C(75 MHz, $CDCl_3$) 178.3, 172.2, 151.4, 150.3, 146.8, 135.1, 126.5, 125.6, 122.8, 117.5, 98.4, 66.7, 49.4, 44.8, 43.3, 36.2, 33.0 (2C), 32.4, 30.3, 26.5, 25.2, 24.7 (2C), 18.0; HRMS (EI) 456.22954 (M^+ $C_{25}H_{33}{}^{35}ClN_4O_2$ requires 456.22920); HPLC Purity: 94%, t_R' = 2.51 min.

1-(4-(7-*Chloroquinolin-4-ylamino*)*butyl*)-*N*-*cyclohexyl*-2-*methyl*-6-*oxopiperidine*-2-*carboxamide*, 5.25o



Off-white gum (78 mg, 74%); R_f(MeOH:DCM 1:9) 0.21; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3322 (NH), 3302 (ArCH), 2974 (CH), 1721 (C=O), 1627 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) 8.44 (d, 1H, *J* 5.4, H-2), 7.90 (d, 1H, *J* 2.0, H-8), 7.86 (d, 1H, *J* 9.0, H-5), 7.39 (dd, 1H, *J* 2.1, 9.0, H-6), 6.28 (d, 1H, *J* 5.4, H-3), 3.68 (m, 1H, H-1″), 3.33 (t, 2H, *J* 6.0, ArNCH_2(CH$_2$)$_3$N), 3.11 (t, 2H, *J* 6.0, ArN(CH$_2$)$_3$CH_2N), 2.53 – 2.44 (m, 2H, CH_2-5′), 2.3 – 2.13 (m, 2H, CH_2-3′), 1.80 – 1.72 (m, 6H, C*H*-2″α, C*H*-6″α, CH_2-4′, ArNCH$_2$CH_2(CH$_2$)$_2$N), 1.66 – 1.63 (m, 7H, C*H*-2″β, C*H*-6″β, ArN(CH$_2$)$_2$CH_2CH$_2$N, CCH_3), 1.24 – 1.22 (m, 3H, C*H*-3″α, C*H*-4″α, C*H*-5″α), 1.0 – 0.98 (m, 3H, C*H*-3″β, C*H*-4″β, C*H*-5″β); δ_C(75 MHz, $CDCl_3$) 178.2, 172.1, 151.2, 150.3, 146.7, 135.3, 126.4, 125.5, 122.9, 117.5, 99.0, 66.3, 49.5, 44.4, 41.3, 36.3, 33.4 (2C), 32.2, 30.3, 27.3, 25.6, 25.2, 24.7 (2C), 18.3; HRMS (EI) 470.24466 (M^+ $C_{26}H_{35}{}^{35}ClN_4O_2$ requires 470.24485); HPLC Purity: 91%, t_R' = 2.08 min.

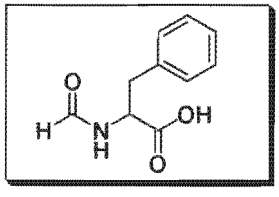
1-(6-(7-*Chloroquinolin-4-ylamino*)*hexyl*)-*N*-*cyclohexyl*-2-*methyl*-6-*oxopiperidine*-2-*carboxamide*, 5.25p



Off-white hygroscopic solid (81 mg, 72%); R_f(MeOH:DCM 1:9) 0.22; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3319 (NH), 3011 (ArCH), 2984 (CH), 1734 (C=O), 1622 (C=C), 1558 (C=N); δ_H(300 MHz, $CDCl_3$) 8.44 (d, 1H, *J* 5.4, H-2), 7.93 (d, 1H, *J* 2.0, H-8), 7.80 (d, 1H, *J* 9.0, H-5), 7.38 (dd, 1H, *J* 2.1, 9.0, H-6), 6.30 (d, 1H, *J* 5.4, H-3), 3.60 (m, 1H,

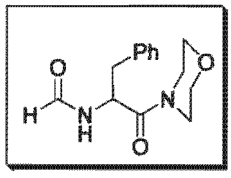
H-1″), 3.34 (t, 2H, *J* 6.0, ArNC*H*$_2$(CH$_2$)$_5$N), 3.12 (t, 2H, *J* 6.0, ArN(CH$_2$)$_5$C*H*$_2$N), 2.57 – 2.42 (m, 2H, C*H*$_2$-5′), 2.20 – 2.12 (m, 2H, C*H*$_2$-3′), 1.80 – 1.70 (m, 6H, C*H*-2″α, C*H*-6″α, C*H*$_2$-4′, ArNCH$_2$C*H*$_2$(CH$_2$)$_4$N), 1.65 – 1.63 (m, 5H, C*H*-2″β, C*H*-6″β, CC*H*$_3$), 1.40 – 1.36 (m, 6H, ArNCH$_2$CH$_2$(C*H*$_2$)$_3$CH$_2$N), 1.23 – 1.18 (m, 3H, C*H*-3″α, C*H*-4″α, C*H*-5″α), 1.10 – 0.99 (m, 3H, C*H*-3″β, C*H*-4″β, C*H*-5″β); δ_C(75 MHz, $CDCl_3$) 178.1, 172.2, 151.1, 150.4, 146.7, 135.3, 126.5, 125.4, 122.6, 117.1, 98.6, 66.5, 49.6, 44.5, 43.7, 36.2, 33.1 (2C), 32.3, 30.6, 30.3, 27.7, 27.4, 26.4, 25.0, 24.5 (2C), 18.1; HRMS (EI) 498.27656 (M^+ $C_{28}H_{39}{}^{35}ClN_4O_2$ requires 498.27612); HPLC Purity: 93%, t_R' = 2.36 min.

2-Formylamino-3-phenylpropionic acid, 5.98



Ac_2O was added dropwise to a solution of phenylalanine (2.00 g, 12.11 mmol) dissolved in 85% formic acid at 0 °C. After 15 minutes of stirring, the temperature of the reaction mixture was elevated to room temperature with continued stirring for an additional 18 hours. Following consumption of amino acid (TLC) the reaction mixture was diluted with H_2O and the solvent removed on a rotary evaporator to yield a residue that was recrystallised from water affording the formamide **5.98** as white needles (1.64 g, 74%), m.p. 188 – 189 °C (lit. m.p. 187 – 188 °C);[5] R_f(EtOAc:Hex 2:3) 0.15; δ_H(300 MHz, $CDCl_3$) 8.15 (s, 1H, NC*H*O), 7.3 (m, 5H, ArH), 4.9 (t, 1H, *J* 7.2, NC*H*CH$_2$Ar), 3.8 (d, 2H, *J* 7.2, NCHC*H*$_2$Ar); δ_C(75 MHz, $CDCl_3$) 171.1, 169.0, 143.0, 130.1 (2C), 129.4, 128.0 (2C), 56.4, 53.2.

_N_-(Benzyl-2-morpholin-4-yl-2-oxo-ethyl) formamide, 5.97



Method (a)

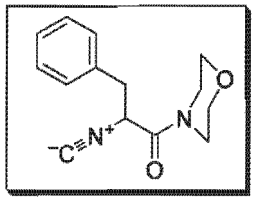
In a 250ml round bottomed flask containing a solution of 2-formylamino-3-phenyl-propionic acid **5.98** (1.8 g, 10 mmol) in methylene chloride (50 ml) was added in succession Et_3N (1.67ml, 12 mmol), 1-hydroxybenzotriazole, HOBt, (1.62 g, 12 mmol), 1-(3-dimethylaminopropyl)-3-ethyl carbodiimide hydrochloride, EDC, (2.30 g, 12 mmol) and morpholine. The reaction mixture was stirred at room temperature overnight; the solvent was removed under reduced pressure to yield a residue that was purified by flash chromatography (eluent Hex:EtOAc 1:1, then MeOH:DCM 1:49). The product was obtained as a yellow oil (2.48 g, 94%).

Method (b)

A suspension of **5.98** (0.90 g, 5.0 mmol) in anhydrous DCM (50 ml) was stirred a room temperature and *N,N*-carbonyldiimidazole (0.81 g, 5 mmol) added at once. Anhydrous morpholine (0.44 ml) was added after 10 min *via* syringe and the clear solution was stirred for 30 min, evaporated to dryness and purified by flash chromatography eluting with 4% MeOH in DCM.

The amide was obtained as a yellow oil (1.31 g, 100%); R_f(EtOAc:Hex 2:3) 0.22; δ_H(300 MHz, $CDCl_3$) 8.15 (s, 1H, NC*H*O), 7.27 – 7.19 (m, 5H, ArH), 5.20 (m, 1H, NC*H*CH_2Ar), 3.86 (m, 4H, N[CH_2CH_2]$_2$O), 3.20 (m, 6H, N[CH_2CH_2]$_2$O, ArCH_2); δ_C(75 MHz, $CDCl_3$) 166.0, 163.8, 134.8, 129.0 (2C), 128.8 (2C), 127.3, 66.1 (2C), 65.9, 55.3 (2C), 46.3.

1-Morpholin-4-yl-3-phenylpropan-1-one-2-isocyanide, 5.94

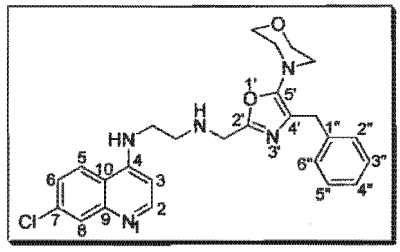


A stirred solution of *N*-(Benzyl-2-morpholin-4-yl-2-oxo-ethyl) formamide (1.17 g, 4.46 mmol) and Et_3N (3.1 ml, 22.32 mmol) in DCM (60 ml) was cooled to –25 °C and $POCl_3$ (1.25 ml, 13.4 mmol) added dropwise. The reaction mixture was stirred at –25 °C for an additional 2.5 hours, then a solution of $NaHCO_3$ (20% w/v) was added dropwise so as to maintain the reaction temperature at –25 °C. After 3 hours of stirring at low temperature, the reaction mixture was raised to room temperature and stirring continued overnight. The aqueous layer was separated and extracted with DCM (2 x 30 ml); the combined organic phases were washed with brine, dried ($MgSO_4$) and evaporated. Column chromatography on SiO_2 gel (eluent EtOAc:Hex 1:2) afforded the isocyanide upon evaporation of the solvent as a brick-red oil (1.05 g, 98%); R_f(Hex:EtOAc) 0.24; IR ν_{max}(neat)/cm^{-1} 3012 (Ar CH), 2145 (NC), 1712 (C=O), 1661 (Ar C=C); δ_H(300 MHz, $CDCl_3$) 7.30 (m, 5H, ArH), 5.5 (m, 1H, NC*H*), 3.84 (m, 4H, N[CH_2CH_2]$_2$O), 3.60 – 3.20 (m, 6H, N[CH_2CH_2]$_2$O, ArCH_2); δ_C(75 MHz, $CDCl_3$) 163.4, 158.0, 135.0, 129.4 (2C), 129.0 (2C), 127.7, 66.4, 65.9, 55.1, 46.2, 42.9, 39.0.

General Procedure for the Parallel Solution-Phase synthesis of Aminooxazoles (L)

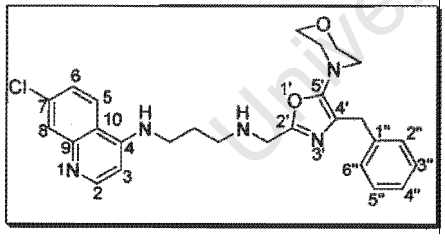
The reactions were performed in parallel on a Carousel Reaction Station®. A mixture of aldehyde (0.90 mmol) and amine (0.45 mmol) were heated at 45 °C for 30 min before addition of isocyanoacetamide **5.94** (0.10g, 0.41 mmol). After stirring for 12 h at this temperature, the reaction mixtures were cooled to room temperature, evaporated *in vacuo* and purified by column chromatography on SiO_2 gel eluting with 2 – 10% MeOH:DCM.

***N*-(2-((4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*methylamino*)*ethyl*)-7-*chloroquinolin*-4-*amine*, 5.115a**



Orange amorphous solid (150 mg, 70%), m.p. 80 – 82 °C; R_f(DCM:MeOH 19:1) 0.21; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3363 (NH), 2985, 2860, (CH), 1611 (C=C), 1582 (C=N), 1219 (C-O); δ_H(300 MHz, $CDCl_3$) 8.48 (d, 1H, *J* 5.4, H-2), 7.93 (d, 1H, *J* 2.1, H-8), 7.80 (d, 1H, *J* 8.7, H-5), 7.38 – 7.15 (m, 6H, H-6, 5 x ArH), 6.33 (d, 1H *J* 5.4, H-3), 3.88 (s, 2H, NCH_2CO), 3.80 (s, 2H, $ArCH_2$), 3.70 (t, 4H, *J* 4.8, $N[CH_2CH_2]_2O$), 3.42 (t, 2H, *J* 6.0, $ArNHCH_2CH_2N$, 3.08 (t, 2H, *J* 6.0, NCH_2CH_2Ar), 2.95 (t, 4H, *J* 4.8, $N[CH_2CH_2]_2O$); δ_C(75 MHz, $CDCl_3$) 161.0, 152.0, 151.6, 150.1, 148.8, 139.1, 134.4, 128.0 (5C), 126.2, 125.1, 124.3, 121.8, 117.3, 98.8, 66.7 (2C), 51.7, 51.0 (2C), 45.1, 42.3, 31.6; HRMS (FAB) *m/z* 477.19267, (M^+ $C_{26}H_{28}{}^{35}ClN_5O_2$ requires 477.19282); HPLC Purity: 93%, t_R' = 1.84 min.

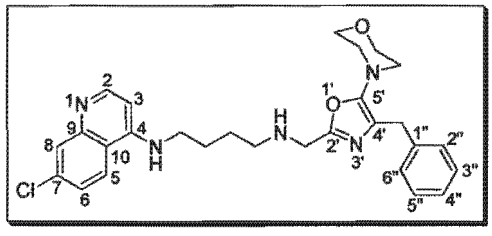
***N*-(2-((4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*methylamino*)*propyl*)-7-*chloroquinolin*-4-*amine*, 5.115b**



Orange oil (150 mg, 68%); R_f(DCM:MeOH 19:1) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3279 (NH), 3031 (Ar CH), 2921, 2858 (CH), 1611 (C=C), 1534 (C=N), 1220 (C-O); δ_H(400 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.6, H-2), 7.91 (d, 1H, *J* 2.0, H-8), 7.64 (d, 1H, *J* 8.7, H-5), 7.38 – 7.16 (m, 6H, H-6, 5 x ArH), 6.30 (d, 1H *J* 5.6, H-3), 3.84 (s, 2H, $NHCH_2CO$), 3.80 (s, 2H, $ArCH_2$), 3.68 (t, 4H, *J* 4.8, $N[CH_2CH_2]_2O$), 3.38 (t, 2H, *J* 6.0, $ArNHCH_2(CH_2)_2N$), 2.94 (t, 2H, *J* 6.0, $NHCH_2(CH_2)_2Ar$), 2.89 (t, 4H, *J* 4.8, $N[CH_2CH_2]_2O$), 1.90 (quint, 2H, *J* 6.8, $ArNCH_2CH_2CH_2N$); δ_C(100 MHz, $CDCl_3$) 161.0, 152.0, 151.3, 150.4, 148.78, 139.0, 134.2, 128.0 (5C), 126.1, 125.3,

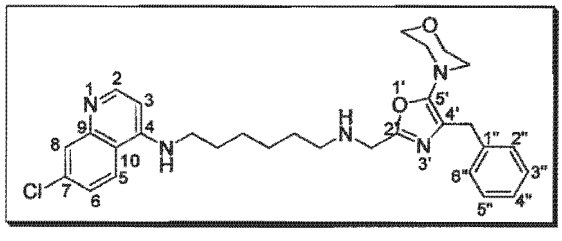
124.1, 121.6, 117.0, 98.5, 66.5 (2C), 51.7, 51.2 (2C), 45.2, 42.2, 31.8, 26.9; HRMS (FAB) *m/z* 491.21015, (M^+ $C_{27}H_{30}{}^{35}ClN_5O_2$ requires 491.20999); HPLC Purity: 95%, t_R' = 1.80 min.

***N*-(2-((4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*methylamino*)*butyl*)-7-*chloroquinolin*-4-*amine*, 5.115c**



Orange oil (141 mg, 62%); R_f(DCM:MeOH 19:1) 0.23; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3327 (NH), 3031 (ArCH) 2922, 2860 (CH), 1611 (C=C), 1582 (C=N), 1218 (C-O); δ_H(300 MHz, $CDCl_3$) 8.48 (d, 1H, *J* 5.4, H-2), 7.93 (d, 1H, *J* 2.1, H-8), 7.70 (d, 1H, *J* 8.7, H-5), 7.37 – 7.19 (m, 6H, H-6, 5 x ArH), 6.34 (d, 1H *J* 5.4, H-3), 3.79 (s, 2H, NCH_2CO), 3.78 (s, 2H, $ArCH_2$), 3.71 (t, 4H, *J* 4.8, $N[CH_2CH_2]_2O$), 3.29 (t, 2H, *J* 6.0, $ArNHCH_2(CH_2)_3N$), 2.95 (t, 2H, *J* 6.0, $NHCH_2(CH_2)_3Ar$), 2.73 (t, 4H, *J* 4.8, $N[CH_2CH_2]_2O$), 1.85 (quint, 2H, *J* 6.6, $ArNCH_2CH_2(CH_2)_2N$), 1.69 (quint, 2H, *J* 6.6, $ArN(CH_2)_2CH_2CH_2N$); δ_C(75 MHz, $CDCl_3$) 160.6, 151.7, 151.2, 150.8, 148.1, 139.6, 135.2, 128.2 (5C), 126.2, 125.1, 124.3, 121.8, 117.4, 98.8, 66.5 (2C), 52.7, 51.0 (2C), 47.1, 43.3, 31.6, 27.8, 26.6; HRMS (FAB) *m/z* 505.22673, (M^+ $C_{28}H_{32}{}^{35}ClN_5O_2$ requires 505.22580); HPLC Purity: 94%, t_R' = 1.93 min.

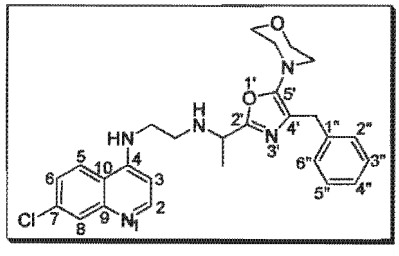
***N*-(2-((4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*methylamino*)*hexyl*)-7-*chloroquinolin*-4-*amine*, 5.115d**



Yellow oil (93 mg, 39%); R_f(DCM:MeOH 19:1) 0.22; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3448 (NH), 2933, 2860 (CH), 1611 (C=C), 1582 (C=N), 1223 (C-O); δ_H(400 MHz, $CDCl_3$) 8.46 (d, 1H, *J* 5.6, H-2), 7.97 (d, 1H, *J* 2.0, H-8), 7.77 (d, 1H, *J* 8.8, H-5), 7.39 – 7.19 (m,

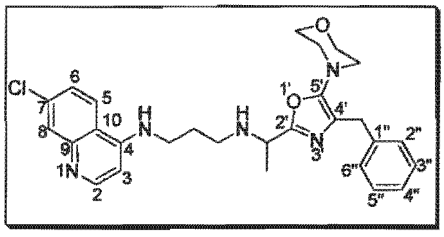
6H, H-6, 5 x ArH), 6.34 (d, 1H *J* 5.6, H-3), 3.89 (s, 2H, NHCH_2CO), 3.81 (s, 2H, ArCH_2), 3.72 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 3.45 (t, 2H, *J* 6.0, ArNCH_2CH_2(CH_2)$_5$)N), 3.09 (t, 2H, *J* 6.0, NHCH_2(CH_2)$_5$Ar), 2.91 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 1.88 (quint, 2H, *J* 6.8, ArNCH$_2$CH_2(CH_2)$_4$)N), 1.46 (quint, 2H, *J* 6.8, ArN(CH_2)$_2$CH_2(CH_2)$_2$N), 1.38 – 1.36 (m, 4H, ArN(CH_2)$_3$CH_2CH_2CH$_2$N); δ_C(100 MHz, $CDCl_3$) 161.0, 152.0, 151.6, 150.1, 148.4, 139.5, 135.1, 128.0 (5C), 126.2, 125.1, 124.1, 121.5, 117.3, 98.1, 66.7 (2C), 52.4, 51.2 (2C), 45.1, 42.3, 31.0, 30.6, 26.7 (2C), 26.0; HRMS (FAB) *m/z*, 533.25675 (M^+ $C_{30}H_{36}{}^{35}ClN_5O_2$ requires 533.25536); HPLC Purity: 91%, t_R' = 2.01 min.

***N*-(2-(1-(4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*ethylamino*)*ethyl*)-7-*chloroquinolin*-4-*amine*, 5.115e**



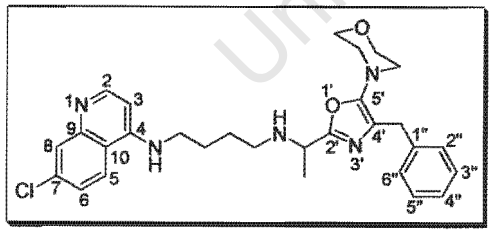
Yellow oil (150 mg, 68%); R_f(DCM:MeOH 19 23:1) 0.24; IR ν_{max}($CHCl_3$)/cm^{-1} 3400 (NH), 3053 (ArCH), 2974, 2861 (CH), 1638 (C=C), 1529 (C=C), 1211 (C-O); δ_H(300 MHz, $CDCl_3$) 8.44 (d, 1H, *J* 5.4, H-2), 7.94 (d, 1H, *J* 2.1, H-8), 7.74 (d, 1H, *J* 9.0, H-5), 7.25 – 7.15 (m, 6H, H-6, 5 x ArH), 6.28 (d, 1H *J* 5.4, H-3), 3.87 (q, 1H, *J* 7.2, NHC*H*CH_3), 3.77 (s, 2H, ArCH_2), 3.64 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 3.45 (t, 2H, *J* 6.0, ArNHCH_2CH$_2$N), 3.03 (t, 2H, *J* 6.0, NHCH_2CH$_2$NAr), 2.85 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 1.50 (d, 3H, NCHCH_3); δ_C(75 MHz, $CDCl_3$) 160.0, 152.0, 151.6, 150.0, 148.7, 139.2, 134.8, 128.5 (5C), 126.2, 125.0, 124.3, 121.8, 117.3, 98.8, 66.7 (2C), 51.7, 50.9 (2C), 45.1, 42.5, 31.6, 20.3; HRMS (FAB) *m/z* 491.20648, (M^+ $C_{27}H_{30}{}^{35}ClN_5O_2$ requires 491.20832); HPLC Purity: 96%, t_R' = 1.93 min.

***N*-(2-(1-(4-Benzyl-5-morpholinooxazol-2-yl)ethylamino)propyl)-7-chloroquinolin-4-amine, 5.115f**



Cream white crystals (116 mg, 51%), m.p 72 – 74 °C; R_f(DCM:MeOH 19:1) 0.24; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3287 (NH), 2983, 2859 (CH), 1612 (C=C), 1536 (C=N), 1223 (C-O); δ_H(300 MHz, $CDCl_3$) 8.44 (d, 1H, *J* 5.6, H-2), 7.94 (d, 1H, *J* 2.0, H-8), 7.74 (d, 1H, *J* 9.0, H-5), 7.26 – 7.15 (m, 6H, H-6, 5 x ArH), 6.28 (d, 1H, *J* 5.6, H-3), 3.87 (q, 1H, *J* 7.2, NHC*H*CH_3), 3.77 (s, 2H, ArCH_2), 3.64 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 3.45 (t, 2H, *J* 6.0, ArNHCH_2(CH_2)$_2$N), 2.89 (t, 2H, *J* 6.0, NHCH_2(CH_2)$_2$NAr), 2.85 (t, 4H, *J* 4.8, N[C$H_2$$CH_2$]$_2$O), 1.87 (quint, 2H, *J* 6.6, ArNCH_2C$H_2$$CH_2$N), 1.45 (d, 3H, NCHC$H_3$); δ_C(75 MHz, $CDCl_3$) 160.4, 151.9, 150.9, 150.0, 147.9, 139.3, 135.2, 128.4 (5C), 126.2, 125.0, 124.5, 122.3, 117.3, 98.1, 66.7 (2C), 52.4, 50.9 (2C), 47.2, 43.9, 31.8, 27.5, 20.1; HRMS (FAB) *m/z* 505.2259, (M^+ $C_{28}H_{32}{}^{35}ClN_5O_2$ requires 505.22580); HPLC Purity: 97%, t_R' = 1.94 min.

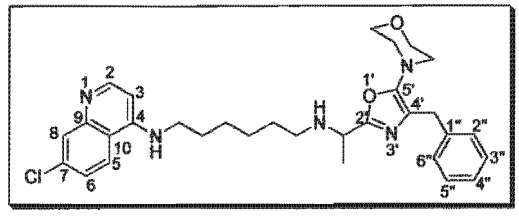
***N*-(2-(1-(4-Benzyl-5-morpholinooxazol-2-yl)ethylamino)butyl)-7-chloroquinolin-4-amine, 5.115g**



Yellow oil (156 mg, 67%); R_f(DCM:MeOH 19:1) 0.26; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3409 (NH), 3029 (ArCH), 2988, 2843 (CH), 1644 (C=C), 1583 (C=N), 1217 (C-O); δ_H(400 MHz, $CDCl_3$) 8.45 (d, 1H, *J* 5.6, H-2), 7.91 (d, 1H, *J* 2.0, H-8), 7.72 (d, 1H, *J* 8.8, H-5), 7.29 – 7.15 (m, 6H, H-6, 5 x ArH), 6.33 (d, 1H, *J* 5.2, H-3), 3.84 (q, 1H, *J* 7.2, NHC*H*CH_3), 3.79 (s, 2H, ArCH_2), 3.69 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 3.25 (t, 2H, *J* 6.0, ArNHCH_2(CH_2)$_3$N), 2.94 (t, 2H, *J* 6.0, NHCH_2(CH_2)$_3$NAr), 2.73 (t, 4H, *J* 4.8, N[C$H_2$$CH_2$]$_2$O), 1.77 (quint, 2H, *J* 6.60,

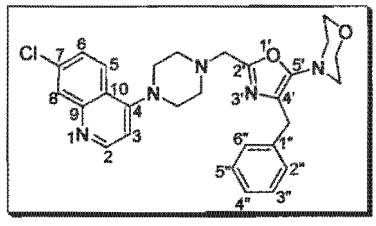
ArNCH$_2$C*H*$_2$(CH$_2$)$_2$N), 1.64 (quint, 2H, *J* 6.6, ArN(CH$_2$)$_2$C*H*$_2$CH$_2$N), 1.44 (d, 3H, *J* 7.2, NCHC*H*$_3$); δ_C(100 MHz, $CDCl_3$) 160.4, 151.9, 150.9, 149.9, 147.9, 139.3, 135.2, 128.4 (5C), 126.2, 125.0, 124.5, 122.3, 117.3, 98.1, 66.7 (2C), 52.4, 50.9 (2C), 47.2, 43.9, 31.8, 27.5, 26.4, 20.1; HRMS (FAB) *m/z* 519.23781, (M^+ $C_{29}H_{34}{}^{35}ClN_5O_2$ requires 519.24010); HPLC Purity: 96%, t_R' = 2.10 min.

***N*-(2-(1-(4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*ethylamino*)*hexyl*)-7-*chloroquinolin*-4-*amine*, 5.115h**



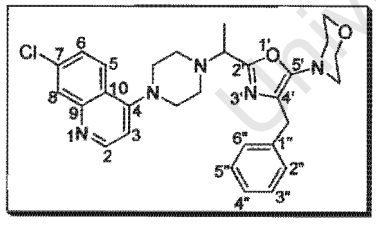
Yellow oil (106 mg, 43%); R_f(DCM:MeOH 19:1) 0.26; IR ν_{max}($CHCl_3$)/cm^{-1} 3364 (NH), 3049 (ArCH), 2985, 2864 (CH), 1612 (C=C), 1583 (C=N), 1220 (C-O); δ_H(400 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.6, H-2), 7.95 (d, 1H, *J* 2.0, H-8), 7.65 (d, 1H, *J* 8.8, H-5), 7.34 (dd, 1H, *J* 2.0, 8.8, H-6), 7.25 – 7.15 (m, 5H, ArH), 6.37 (d, 1H *J* 5.6, H-3), 3.82 (q, 1H, *J* 7.2, NHC*H*CH$_3$), 3.80 (s, 2H, ArC*H*$_2$), 3.70 (t, 4H, *J* 4.8, N[CH$_2$C*H*$_2$]$_2$O), 3.28 (t, 2H, *J* 6.0, ArNC*H*$_2$(CH$_2$)$_5$)N), 2.96 (t, 4H, *J* 4.8, N[C*H*$_2$CH$_2$]$_2$O), 2.54 (t, 2H, *J* 6.0, NHC*H*$_2$(CH$_2$)$_5$Ar), 1.74 (quint, 2H, *J* 6.8, ArNCH$_2$C*H*$_2$(CH$_2$)$_4$)N), 1.49 (quint, 2H, *J* 6.6, ArN(CH$_2$)$_2$C*H*$_2$(CH$_2$)$_2$N), 1.40 (d, 3H, *J* 7.2, NCHC*H*$_3$), 1.33 (m, 4H, ArN(CH$_2$)$_3$C*H*$_2$C*H*$_2$CH$_2$N); δ_C(100MHz, $CDCl_3$) 160.4, 151.9, 150.9, 150, 147.9, 139.3, 135.2, 128.4 (5C), 126.2, 125, 124.5, 122.3, 117.3, 98.1, 66.7 (2C), 52.4, 50.9 (2C), 47.2, 43.9, 31.8, 26.5 (2C), 25.9, 20.1; HRMS (FAB) *m/z* 547.25277 (M^+ $C_{30}H_{38}{}^{35}ClN_5O_2$ requires 547.25883); HPLC Purity: 94%, t_R' = 2.10 min.

4-(4-(4-*Benzyl*-5-*morpholinooxazol*-2-*yl*)*methyl*)*piperazin*-1-*yl*)-7-*chloroquinoline*, 5.116a



Brown oil (210 mg, 93%); R_f(DCM:MeOH 49:1) 0.18; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3054 (ArCH), 2986, 2839 (CH), 1640 (C=C), 1577 (C=N), 1221 (C-O); δ_H(400 MHz, $CDCl_3$) 8.68 (d, 1H, *J* 5.2, H-2), 8.05 (d, 1H, *J* 2.0, H-8), 7.90 (d, 1H, *J* 8.7, H-5), 7.41 (dd, 1H, *J* 2.0, 8.8, H-6), 7.30 – 7.15 (m, 5H, 5 x ArH), 6.81 (d, 1H *J* 5.2, H-3), 3.84 (s, 2H, NCH_2CO), 3.72 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 3.70 (s, 2H, ArCH_2), 3.28 (t, 4H, *J* 5.2, 2 x PipNHCH_2), 3.00 (t, 2H, *J* 4.8, N[CH_2CH_2]$_2$O), 2.84 (t, 4H, *J* 5.2, 2 x PipNCH_2); δ_C(100 MHz, $CDCl_3$) 161.0, 152.0, 151.6, 150.1, 148.8, 139.3, 134.6, 128.1 (5C), 126.2, 125.1, 124.3, 121.8, 117.3, 98.8, 66.7 (2C), 52.3, 51.7, 51.0, 50.8, 45.1 (2C), 31.6; HRMS (FAB) *m/z* 503.20832, (M^+ $C_{28}H_{30}{}^{35}ClN_5O_2$ requires 503.20880); HPLC Purity: 99%, t_R' = 2.03 min.

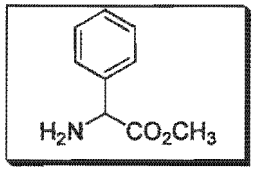
4-(4-(1(4(-*Benzyl*-5-*morpholinooxazole*-2*yl*)*ethyl*)*piperazine*-1-*yl*)-7-*chloroquinoline*, 5.116b



Brick-red oil (202 mg, 87%); R_f(DCM:MeOH 49:1) 0.20; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3031 (ArCH), 2979, 2841 (CH), 1642 (C=C), 1584 (C=N)n 1224 (C-O); δ_H(300 MHz, $CDCl_3$) 8.66 (d, 1H, *J* 5.2, H-2), 8.05 (d, 1H, *J* 2.0, H-8), 7.92 (d, 1H, *J* 8.7, H-5), 7.41 (dd, 1H, *J* 2.0, 8.8, H-6), 7.29 – 7.16 (m, 5H, ArH), 6.74 (d, 1H *J* 5.2, H-3), 3.84 (q, 1H, *J* 7.2, NHC*H*CH_3), 3.72 (t, 4H, *J* 4.8, N[CH_2CH_2]$_2$O), 3.71 (s, 2H, ArCH_2), 3.28 (t, 4H, *J* 5.2, 2 x PipNCH_2), 3.00 (t, 2H, *J* 4.8, N[CH_2CH_2]$_2$O), 2.84 (t, 4H, *J* 5.2, N[CH_2CH_2]$_2$O), 1.49 (d, 3H, *J* 7.2, NCHCH_3); δ_C(75 MHz, $CDCl_3$) 160.0, 152.0, 151.6, 150.0, 148.7, 139.2, 134.8, 128.5 (5C), 126.2, 125.0, 124.3, 121.8, 117.3, 98.8,

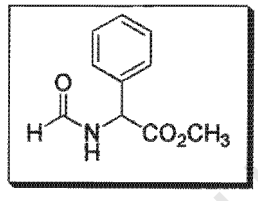
66.7 (2C), 52.8, 51.4, 50.9, 50.6, 45.4 (2C), 31.5, 20.3; HRMS (FAB) *m/z* 517.22378, (M^+ $C_{29}H_{32}{}^{35}ClN_5O_2$ requires 517.22445); HPLC Purity: 98%, t_R' = 2.18 min.

Methyl 2-amino-2-phenylacetate, 5.47



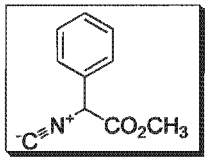
A suspension of phenylglycine (1.8 g, 11.9 mmol) in MeOH (50 ml) was cooled to 0 °C in an ice-water bath and $SOCl_2$ added dropwise over 10 min. The clear solution was elevated to ambient temperature and stirred for 2 h, continuously evaporated with DCM and redissolved in MeOH. K_2CO_3 (3.29 g, 23.8 mmol) was added in one portion and the suspension stirred for 20 min, filtered, dried ($MgSO_4$) and concentrated *in vacuo* to afford the aminoester as a white amorphous powder (1.96 g, 100%); R_f(DCM: MeOH 1:9) 0.23; δ_H(300 MHz, $CDCl_3$) 7.49 (m, 5H, 5 x ArH), 5.20 (s, 1H, ArC*H*), 3.81 (s, 3H, OCH_3); δ_C(75 MHz, $CDCl_3$) 161.0, 135.7, 129.0 (2C), 128.4, 127.0 (2C), 54.9, 52.8.

Methyl 2-formamido-2-phenylacetate, 5.48



A cooled solution of Ac_2O:HOOCH (1:1.1) was added to a suspension of the amino acetate **5.47** (11.76 g, 66 mmol) in anhydrous DCM (100 ml), and the mixture refluxed for 2 h. Upon cooling, the solvent was continuously removed until a pale yellow oil formed. The oil was concentrated under reduced pressure and used deemed pure; it was used in the next step without further purification. Yellow oil, (12.75 g, 100%); R_f(EtOAc:Hex 1:2) 0.22; δ_H(300 MHz, $CDCl_3$) 8.20 (s, 1H, NC*H*O), 7.45 (m, 5H, 5 x ArH), 5.24 (s, 1H, ArC*H*), 3.80 (s, 3H, OCH_3); δ_C(75 MHz, $CDCl_3$) 171.0, 160.1, 136.0, 129.2 (2C), 128.6, 127.1 (2C), 55.0, 53.0.

Methyl 2-isocyano-2-phenylacetate, 5.44

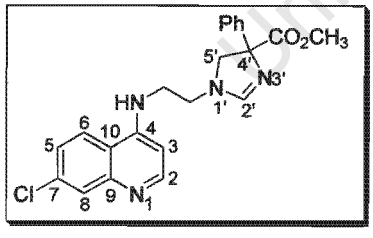


The method used for the synthesis of **5.94** was adapted for the synthesis of the isocyanoacetate **5.44**, using 65.64 mmol of the formamide. The isocyanoactetate was obtained as a yellow oil (2.3 g, 20%); R_f(EtOAc:Hex 1:2) 0.22; IR ν_{max}(Film)/ cm^{-1} 3024 (Ar CH), 2145 (NC), 1712 (C=O), 1661 (Ar C=C); δ_H(300 MHz, $CDCl_3$) 7.43 (m, 5H, 5 x ArH), 5.4 (s, 1H, ArC*H*), 3.79 (s, 3H, OCH_3).

General Procedure for the parallel synthesis of Imidazolines 5.60 – 5.74 (M)

A solution of the amine (0.848 mmol) was condensed with aldehyde (0.929 mmol, 1.1 eq) in MeOH (8 ml) at 45 °C for 30 min, then phenylisocyanoacetate **5.44** (0.93 mmol) was added to each of the reaction mixtures. The reactions were stirred for 2 h, after which TLC indicated complete conversion into products. The solvent was removed *in vacuo* and the products purifieed by column chromatography on SiO_2 gel eluting with 0 – 10% MeOH/DCM.

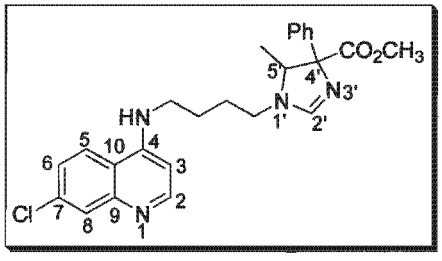
***Methyl*-1,1-(2-(7-*chloroquinolin*-4-*ylamino*)*ethyl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 5.60**



Pale yellow foam (290 mg, 84%); R_f(MeOH:DCM 1:9) 0.25; IR ν_{max}($CHCl_3$)/cm^{-1} 3418 (NH), 3042 (ArCH), 2954 (aliphatic CH), 1728 (C=O), 1659 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.7, H-2), 7.98 (d, 1H, *J* 2.1, H-8), 7.70 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.20 (m, 5H, 5 x PhH), 6.98 (s, 1H, H-2′), 6.30 (d, 1H, *J* 5.7, H-3), 4.40 (d, 1H, *J* 9.6, H-5′α), 3.69 (s, 3H, OCH_3), 3.30 (d, 1H, *J* 9.6, H-5′β), 3.29 – 3.15 (m, 4H, $ArNCH_2CH_2N$); δ_C(75 MHz, $CDCl_3$) 173.3, 156.7, 154.0, 151.0, 149.0, 148.0, 142.0, 134.0, 128.6 (2C), 127.6, 125.5, 125.3

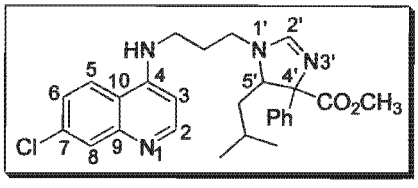
(2C), 121.0, 117.0, 99.0, 80.1, 57.6, 53.0, 46.6, 42.8; HRMS (EI) 408.13406, (M^+ $C_{22}H_{21}{}^{35}ClN_4O_2$ requires 408.13530); HPLC Purity: 92%; t_R' = 2.43 min.

***Methyl*-1-(4-(7-*chloroquinolin*-4-*ylamino*)*butyl*)-5-*methyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 5.61**



Pale yellow foam (165 mg, 43%); R_f(MeOH:DCM 1:9) 0.27; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3428 (NH), 3030 (ArCH, 2951 (aliphatic CH), 1733 (C=O), 1649 (C=C), 1580 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.54/8.41 (d, 1H, *J* 5.7, H-2), 7.93/7.91 (d, 1H, *J* 2.1, H-8), 7.76/7.66 (d, 1H, *J* 9.0, H-5), 7.42/7.29 (dd, 1H, *J* 2.1, 9.0, H-6), 7.36 – 7.27 (m, 5H/5H, 5 x PhH), 7.24/7.1 (s, 1H, H-2'), 6.40/6.16 (d, 1H, *J* 5.7, H-3'), 4.56/3.97 (q, 1H, *J* 6.6, H-5'), 3.69/3.67 (s, 3H, OCH_3), 3.4/3.25 (m, 4H, $ArNCH_2NCH_2N$), 2.0/1.92 (quint, 2H, *J* 7.2, $ArNCH_2CH_2(CH_2)_2N$), 1.71/1.63 (m, 2H, $ArN(CH_2)_2CH_2CH_2N$), 1.36/0.64 (d, 3H, *J* 6.6, $CHCH_3$); δ_C(75 MHz, $CDCl_3$) 174.0, 156.0, 151.0, 149.0, 148.8, 142.7, 137.2, 135.1, 128.3 (2C), 126.0 (2C), 126.1, 125.4, 121.0, 117.1, 98.9, 82.7, 59.8, 52.9, 41.8, 40.3, 30.0, 26.8, 14.5; HRMS (EI) 450.18224, (M^+ $C_{25}H_{27}{}^{35}ClN_4O_2$ requires 450.18225); HPLC Purity: 95%; t_R' = 2.42 min.

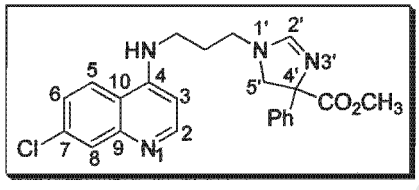
***Methyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-5-*isobutyl*-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 5.62**



Cream white foam (238 mg, 56%); R_f(MeOH:DCM 1:9) 0.29; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3427 (NH), 2963 (aliphatic CH), 1713 (C=O), 1642 (C=C), 1583 (C=N); δ_H(300 MHz, $CDCl_3$) (major/minor) 8.55/8.53 (d, 1H, *J* 5.7, H-2), 7.96/7.93 (d, 1H, *J* 2.1,

H-8), 7.74/7.63 (d, 1H, *J* 9.0, H-5), 7.44/7.42 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.26 (m, 5H/5H, 5 x PhH), 7.06/7.04 (s, 1H, H-2′), 6.40/6.21 (d, 1H, *J* 5.7, H-3), 3.58/3.42 (m, 1H, H-5′), 3.68/3.37 (s, 3H, OCH_3), 3.38/3.29 (m, 2H, $ArNCH_2(CH_2)_3N$), 3.27/3.25 (m, 2H, $ArN(CH_2)_3CH_2N$), 3.22/3.18 (m, 1H, $CH(CH_3)_2$), 2.5/2.20 (m, 2H, CH_2CH), 2.05/1.90 (m, 2H, $ArNCH_2CH_2CH_2N$), 1.09/0.97 (d, 3H, *J* 6.6, CH_3), 0.96/0.42 (d, 3H, *J* 6.6, CH_3); δ_C(75 MHz, $CDCl_3$) 174.2, 156.0, 154.1, 149.2, 148.6, 142.6, 137.4, 135.0, 128.3 (2C), 126.4 (2C), 126.1, 125.5, 121.0, 117.2, 98.9, 84.4, 74.0, 66.1, 51.6, 43.0, 40.0, 28.0, 26.8, 21.3, 17.2; HRMS (EI) (M^+ $C_{27}H_{31}{}^{35}ClN_4O_2$ requires 478.21355); HPLC Purity: 99%; t_R' = 2.42 min.

***Methyl*-1-(3-(7-*chloroquinolin*-4-*ylamino*)*propyl*)-4-*phenyl*-4,5-*dihydro*-1*H*-*imidazole*-4-*carboxylate*, 5.63**



Yellow oil (278 mg, 78%); R_f(MeOH:DCM 1:9) 0.26; IR $\nu_{max}(CHCl_3)/cm^{-1}$ 3229 (NH), 3054 (ArCH), 2986 (aliphatic CH), 1728 (C=O), 1658 (C=C), 1582 (C=N); δ_H(300 MHz, $CDCl_3$) 8.50 (d, 1H, *J* 5.7, H-2), 7.98 (d, 1H, *J* 2.1, H-8), 7.70 (d, 1H, *J* 9.0, H-5), 7.40 (dd, 1H, *J* 2.1, 9.0, H-6), 7.38 – 7.20 (m, 5H, 5 x PhH), 7.00/6.98 (s, 1H, H-2′), 4.4 (d, 1H, *J* 9.6, H-5′α, 3.69 (s, 3H, OCH_3), 3.30 (d, 1H, *J* 9.6, H-5′-β), 3.28 – 3.13 (m, 4H, $ArNCH_2CH_2N$), 1.70 (m, 2H, $ArNCH_2CH_2CH_2N$); δ_C(75 MHz, $CDCl_3$) 173.3, 156.7, 154.2, 151.0, 149.0, 148.0, 142.0, 134.0, 128.6 (2C), 127.6, 125.5, 125.3 (2C), 121.0, 117.1, 99.2, 80.1, 57.6, 53.2, 46.6, 42.8, 26.0; HRMS (EI) 422.15023, (M^+ $C_{23}H_{22}{}^{35}ClN_4O_2$ requires 422.15095); HPLC Purity: 92%; t_R' = 2.44 min.