Design and Synthesis of Quinoline, Cinchona Alkaloids and Other Potential Inhibitors of Leishmaniasis

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Statement of Originality

The content of this thesis has not previously been submitted for a degree or diploma in any university. To the best of my knowledge and belief, the dissertation contains no material previously published or written by another person except where due reference is made in the dissertation itself.

Kristie Anne Reynolds BSc (Hons)

Preface

Unless otherwise stated, the results in this thesis are those of the author. Parts of this work have appeared elsewhere.

Refereed Journal Publications

Reynolds, Kristie A., Young, David J., Loughlin, Wendy A. *Synthesis*, **2010**, 21, 3645-3648.

Conference Presentations

Posters

3D at the Cove, Drug Discovery and Development Conference, Corun Cove, 13-17 July 2008 – 'A Limitation of the Skraup-Doebner-Miller Reaction for the Synthesis of Bioactive Quinolines.' Kristie A. Reynolds, Wendy A. Loughlin and David J. Young.

Seminars

Eskitis Student Symposium, March 2010 – 'Developing New Chemotherapies for Leishmaniasis'

RACI - BBOCS (Brisbane Biological and Organic Chemistry Symposium), 27 November 2009, Institute for Glycomic, Gold Coast – 'Aspects of the Doebner-Miller Reaction for the Synthesis of Bioactive Quinolines'

Southern Highlands Conference on Heterocyclic Chemistry, 27-29 August 2010, Moss Vale, NSW – 'Developing New Chemotherapies for Leishmaniasis' Kristie A. Reynolds, Wendy A. Loughlin and David J. Young.

Graduate School of the Chinese Academy of Sciences, 2010 International Student Forum, 25-28 September 2010, Beijing, China – 'Developing New Chemotherapies for Leishmaniasis' Kristie A. Reynolds, Wendy A. Loughlin and David J. Young.

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Abbreviations

+ve	positive
^{13}C	carbon
AIDS	acquired immune deficiency syndrome
AmB	amphotericin B
ATP	adenosine-triphosphate
BM	bone marrow
BnBr	benzyl bromide
Boc	*tert*-butyl carbamate
Boc_2O	Boc anhydride
BOP	benzotriazole-1-yl-oxy-tris-(dimethylamino)-phosphonium
BuLi	butyllithium
CAM	cerium ammonium molybdate
$CDCl_3$	deuterated chloroform
CH_2Cl_2	Dichloromethane
$CHCl_3$	Chloroform
CL	cutaneous leishmaniasis
cm^{-1}	wave numbers
CRC	concentration response curve
Cys	cysteine
D_2O	deuterated water
DCM	dichloromethane
DIC	*N*,*N'*-diisopropylcarbodiimide
DIPEA	diisopropylethylamide
DMF	dimethylformamide
DMSO	dimethyl sulfoxide
DMSO-d_6	deuterated dmso
DNA	deoxyribonucleic acid
DTT	dithiothreitol
EDG	electron donating group

equiv.	equivalent
ESMS	electrospray mass spectroscopy
Et_3N	triethylamine
EtO_3	diethyl ether
EtOAc	ethyl acetate
EWG	electron withdrawing group
FAD	flavin adenine dinucleotide
FTIR	fourier transform infrared
G	Grams
g-COSY	gradient correlation spectroscopy
g-HMBC	gradient heteronuclear multiple bond correlation spectroscopy
g-HSQC	gradient heteronuclear single quantum correlation spectroscopy
Glu	glutamic acid
Gly	glycine
GR	glutathione reductase
GSH	glutathione
Gsp	glutathiospermidine
GSSG	glutathione disulphide
H	hour(s)
H_2O	Water
H_2O_2	hydrogen peroxide
H_2SO_4	sulphuric acid
HAART	highly active antiretroviral therapy
HCl	hydrochloric acid
HEPES	4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid
HEPPS	3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid
His	histidine
HIV	human immunodeficiency virus
HMQC	heteronuclear Multiple Quantum Coherence
HOBt	hydroxybenzotriazole
HPLC	high performance liquid chromatography
HPMA	*N*-(2-hydroxypropyl)methacrylamide

HRMS	high resolution mass spectroscopy
Hz	Hertz
IC_{50}	half maximal inhibitory concentration
Ile	isoleucine
INF-γ	interferon gamma
IPA	isopropyl alcohol
IR	infrared spectroscopy
K_2CO_3	potassium carbonate
KBr	potassium bromide
Kg	kilogram
K_i	enzyme binding constant
K_m	dissociation constant
KOH	potassium hydroxide
L- AmB	liposomal-amphotericin B
LipDH	lipoamine dehydrogenase
LogP	logarithm of the octanol / water partition coefficient
LogS	compound solubility
LPS	lipopolysaccharide
Lys	Lysine
M	Molar
m-	*Meta*
m.p.	melting point
MCL	mucocutaneous leishmaniasis
m-CPBA	*meta*-chloroperoxybenzoic acid
M-CSF	macrophage colony stimulating factor
MeI	methyl iodide
MeOH	methanol
MeOH-d_4	deuterated methanol
Mg	milligrams
$MgSO_4$	magnesium sulphate
MHz	megahertz
Min	minutes

mL	millilitres
mM	millimolar
Mmol	millimoles
MOA	mode of action
MOI	multiplicity of infection
Mol	moles
MS	mass spectroscopy
MW	molecular weight
Na_2CO_3	sodium carbonate
NADH	nicotinamide adenine dinucleotide
NADPH	nicotinamide adenine dinucleotide phosphate
NaH	sodium hydride
$NaHCO_3$	sodium hydrogen carbonate
$NaNO_2$	sodium nitrite
NaOH	sodium hydroxide
NAPDH	nicotinamide adenine dinucleotide phosphate
nM	nanomolar
NMR	nuclear magnetic resonance
NO	nitric oxide
o-	*ortho*
O.N.	over night
^oC	degrees celcius
OHNH	hydrogen bond donor
ON	hydrogen bond acceptor
p-	*para*
PBS	phosphate buffer solution
Pd/C	palladium on carbon
Phe	phenylalanine
PhLi	phenyllithium
PhMe	toluene
Ppm	parts per million
Pro	proline

PVA	pentavalent antimonies
PyBOP	benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate
QSAR	quantitative structure activity relationship
r.t.	room temperature
RNA	ribonucleic acid
ROTB	rotational bonds
RPMI	Roswell Park Memorial Institute
SAR	structure activity relationship
Sb	antimony
t- or *tert*-	*tertiary*
$T(SH)_2$	trypanothione (reduced)
TBME	*tert*-butylmethoxy ether
TFA	trifluoroacetic acid
THAB	tetrahexyl ammonium bromide
THF	tetrahydrofuran
Thr	threonine
TIPS	triisopropysilane
TLC	thin layer chromatography
TPSA	total polar surface area
Trx	thioredoxin
TrxR	thioredoxin reductase
Try	tryptophan
TryR	trypanothione reductase
TryS	trypanothione synthase
TS_2	trypanothione (oxidised)
US	United States
UV	ultraviolet
Val	valine
-ve	negative
VL	visceral leishmaniasis
V_{max}	maximum reaction rate

^{1}H	proton
μg	microgram
μL	microliters
μM	micromolar
μmol	micromoles

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Finally, to Matt, thank you for your unwavering support, love, care and friendship throughout this ordeal. I will cherish you forever. Thank you.

Abstract

The synthesis and biological activity of Trypanothione mimics and a series of quinoline derivatives designed as potential antileishmanial chemotherapies is reported.

The biosynthesis of trypanothione is a unique pathway for parasites such as *Leishmania* and trypanosomes. A structure-guided design of trypanothione mimics led to the identification of 4 target compounds. Compounds **93** and **94** were designed to mimic the left-hand side chain, while compounds **105** and **106** mimicked side chains of molecule **59**. The selected side chain mimics have not been previously assessed for their potential as TryR inhibitors. The left-hand side chain mimics **93**/**94** were synthesised from thioamine **85** and bromopropylamine **89** in six steps and in an overall yield of 2.3%. Side chain mimics **105**/**106** were synthesised from cysteine methyl ester **103** in five steps and in an overall yield of 3.5%. Five mimics, **93**, **94**, **98**, **105** and **106**, were tested *in vitro* against *Leishmania major*, however none exhibited activity. Preliminary enzymatic assays of mimics **94** and **106** against Trypanothione synthase indicated a degree of activity for **94** (inhibition >45 μM).

The Doebner-Miller synthesis of quinolines under modified biphasic conditions was investigated. Crotonaldehyde, reacted readily with aniline to produce 2-methyl quinoline. However, cinnamaldehyde and other γ-substituted α,β-unsaturated aldehydes yielded complex mixtures with substituted anilines to provide only trace quantities of quinolines. The Doebner-Miller reaction under these conditions is only suitable for sterically accessible α,β-unsaturated aldehydes.

A generalised SAR for quinolines active against leishmaniasis was developed from relevant literature. A series of 23 quinoline derivatives were generated using the Doebner-Miller reaction, nucleophilic addition of organometallic reagents to quinoline or palladium-catalyzed direct arylation of quinoline n-oxides. A total of 32 quinoline derivatives were designed varying from mono- to di-substituted quinolines, quinoline-*N*-oxides, and cinchona alkaloids. The derivatives **116**-**119**, **125**-**127**, **135**-**136**, **147**-**149**, **151**-**152**, **160**, **162**, **165**-**166**, **168**-**170** and **172**-**182** were screened against *L. major* infected macrophages and some SARs determined. Addition of a methoxy group at the 6-position of the quinoline promoted activity (*c.f.* **117** IC_{50} = 71.4 μM, **116** >200 μM).

Benzyl protection of the alcohol in the side chain of the cinchona alkaloid derivatives also led to improved activity (*c.f.* **170** IC_{50} = 1.83 μM, **172** 10% at 24 μM). Compounds **169, 170 177, 117** gave IC_{50} values ranging from 0.49 μM to 71.4 μM. Maximal inhibitions were also found for compounds **165**, **172**, **176**, **174** and **175** in the mid-low micromolar ranges. Cinchona alkaloid **177** (IC_{50} = 0.49 μM) was identified as the most potent compound from this series of compounds, and suggests cinchona derivatives have potential as chemotherapeutic agents for leishmaniasis.

Chapter One

Introduction

Leishmaniasis is a category of clinical diseases produced by at least 17 species of the protozoan *Leishmania*.[1] The spectrum of clinical manifestations caused by *Leishmania* species can be clinically divided into cutaneous and visceral diseases:

- *Leishmania major*, *L. tropica* and *L. mexicana* are etiologically responsible for cutaneous leishmaniasis (CL) local infections of skin subcutaneous tissue, and regional lymph nodes. This form of leishmaniasis is generally self healing.
- Mucocutaneous leishmaniasis (MCL) is caused by *L. braziliensis* and leads to metastatic infections of the oronasal mucosa.
- Visceral leishmaniasis (VL), or kala azar, caused by *L. donovani* and *L. infantum*, results in disseminated infection of visceral organs (e.g. liver, spleen and bone marrow) of the mononuclear phagocyte system.[2]

Typically leishmaniasis causes lesions at the site of infection, which can lead to erosion and disfigurement. For example, destruction of the palette of the mouth can occur with MCL. VL is the most serious form of the disease and can be fatal in two to three years. Fatality can be quicker if the subject suffers from any infections of the immune system, such as human immunodeficiency virus (HIV).[3]

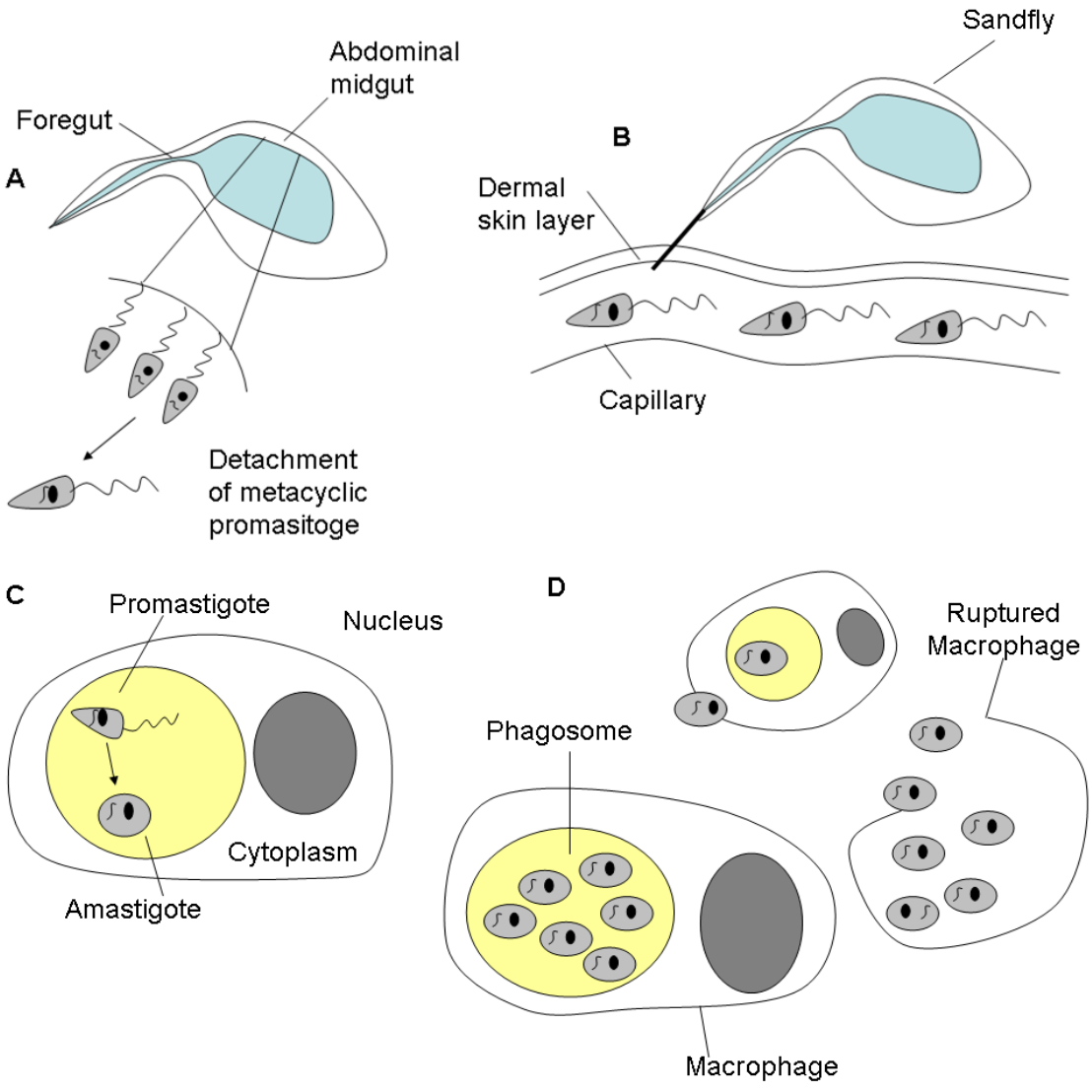
Leishmaniasis is a world-wide vector borne disease. It affects 88 countries; 72 developing countries and 13 countries which are among the least developed countries. VL occurs in 65 countries with the majority (90%) of cases occurring in poor rural and suburban areas of Bangladesh, India, Nepal, Sudan and Brazil. By contrast 90% of CL cases occur in 7 countries: Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria.[4] The World Health Organisation diagnoses over 40,000 new cases of human leishmaniasis every year.[2] Most of these occur in Asia, Africa, the Mediterranean basin, the Middle East and the Americas.[2] Leishmaniasis, as a collective disease, currently affects 12 million people, with 350 million at risk of contracting the disease. There is an annual estimated incidence of 1.5-2 million cases (1-1.5 million for CL and 500 000 for VL).[4,5]

1.1 Stages of leishmaniasis

Leishmania parasites are typically transmitted via the female sandfly of the genus *Phlebotomus* during a blood meal.[6] The female sandfly becomes infected after feeding on an animal or human affected by the disease. The parasites are passed on when the sandfly bites its next victim.[4] The epidemiology is extremely diverse: 20 *Leishmania*

species are pathogenic for humans, combined with 30 sandfly species as proven vectors. The two main epidemiological entities include zoonotic, which includes animal reservoir hosts in the transmission cycle and anthroponotic, in which man is the sole source of infection for the vector.[4] Several studies indicate that there are specific relationships between *Leishmania* parasite and their *Phlebotomine* vectors which varies depending on the parasite-vector pairs involved.[7]

Leishmania parasites have a digenetic life cycle (Figure 1) that alternates between an extracellular promastigote (flagellate stage) in its sandfly vector and an intracellular amastigote (nonflagellate stage) within mammalian macrophages.[8] The parasite inhabits its vector and effectively exists as a dividing non-infectious procyclic promastigote, which is attached to the epithelial cells of the midgut.[9] The *Leishmania* parasite multiplies in the gut of the sand fly and becomes infective 8–20 days later.[4]



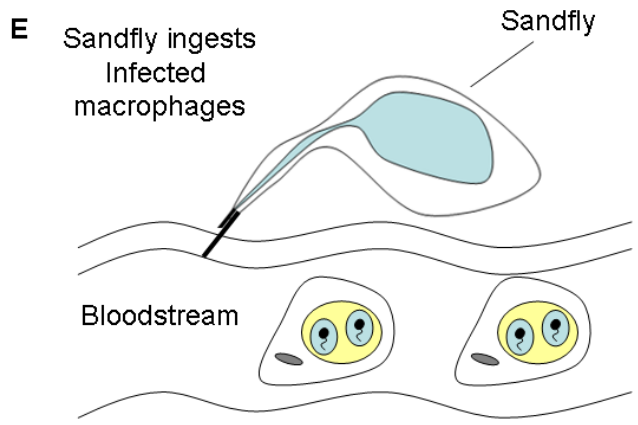


Figure 1 Development stages of Leishmania. (A) Abdominal midgut of a *Leishmania*-infected sandfly. Metacyclic promastigotes detach from the gut wall and migrate to the foregut. (B) Parasite injection into mammalian bloodstream. (C) Promastigotes bind to macrophages and are taken up into a phagosome where promastigotes transform to amastigotes. (D) Amastigotes multiply within the macrophage and then are released from ruptured host cells thus spreading infection. (E) The cycle then repeats when a sandfly takes a bloodmeal from an infected host.

During the process of metacyclogenesis (the conversion of procyclic promastigotes to metacyclic promastigotes) the promastigotes cease to divide, detach themselves from the epithelial cells of the midgut and migrate to the mouth of the sandfly (Figure 1 (A)).[10] The process by which transmission of the parasite into the mammalian host occurs remains poorly understood. The accumulation of metacyclic promastigotes in the anterior regions of the sandfly gut may not be sufficient to allow transmission into the vertebrate host.[7] It has been proposed that, in addition to the presence of virulent promastigotes, there is the formation of a biological plug (within the stomodeal valve) which impairs blood uptake.[7,11] As the fly attempts to dislodge this plug it promotes the regurgitation of the metacyclic promastigotes from the foregut and behind the stomodeal valve initiating the bloodmeal.[7] The highly motile and virulent metacyclic promastigotes are then inoculated into the microscopic wound produced when sandflies feed (Figure 1 (B)).[12] As the macrophages are attracted to the wound, the parasites attach themselves and enter the macrophages via a receptor mediated process (Figure 1 (C)).[12,13] Promastigotes are subsequently taken up by phagocytosis and within 12-14 h of residing within the macrophages the promastigote differentiates into a non-flagellate amastigote.[13] As the phagosome matures the amastigotes multiply and eventually the host cell lyses releasing the amastigotes which spread and colonise other macrophages (Figure 1 (D)).[13] The process is then restarted when a sandfly takes a bloodmeal from an

infected host ingesting macrophages containing amastigotes which further differentiate into promastigotes and attach themselves to the wall of the midgut (Figure 1 (E)).

Cases of leishmaniasis have increased rapidly over the last 10 years. Most developing countries are also struggling with endemic HIV/AIDS which has led to a sharp increase in co-infection rates of leishmaniasis and HIV/AIDS.[14] The HIV/AIDS pandemic over the past 20 years has modified the spectrum of leishmaniasis both clinically and epidemiologically.[15] To date there are cases reported in 35 countries and the increase in co-infection has made it more difficult to manage VL infection.[15] Both diseases usually depress the immune response and increase the infection of the other, causing higher probability of relapse and clinical progression in the diseases. A sizable proportion of VL patients infected with HIV fail to demonstrate anti-leishmanial antibodies which implies that VL in HIV positive patients may be the consequence of either a new infection or a reactivation of a latent infection.[15,16] The availability of highly active anti-retroviral therapy (HAART) in Europe has led to far fewer cases of new leishmanial infections in HIV-positive patients, but this treatment is only sparsely available in the majority of developing countries.

1.2 Current chemotherapies

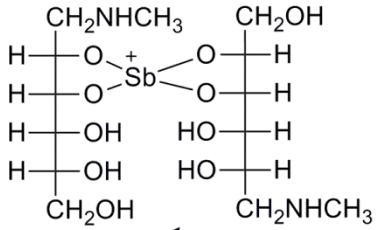
Despite the progress made on the biochemical and biophysical properties of *Leishmania* parasites, the absence of effective vaccines and vector control programmes makes chemotherapy the most widely used treatment for this disease.[17] Chemotherapies include Amphotericin B (AmB),[18] pentavalent antimonies (PVAs),[19] Pentamidine,[20] Paromomycin,[21] Imiquimod,[21] Sitamaquine[22] and orally active drugs such as Miltefosine.[17,20] Most of the treatments are toxic, expensive and require long-term (~28 days) daily injections generally associated with long hospital visitations.[20]

Most *Leishmania* cases reported are zoonotic, where acquired drug resistance is not generally considered. However, in areas with anthroponotic VL, such as India, acquired resistance to pentavalent antimonials (PVAs) has occurred and efficient monitoring of drug resistance is required.[19] Individual species of *Leishmania* parasites (*L. major* or *L. donovani*) tend to have a higher efficacy for certain drugs e.g. AmB is used to treat VL over the cutaneous disease types. This identifies that although *Leishmania* parasites are ultrastructurally the same, their biochemistry differs between species.[23] Therefore, in

treating patients and in the design of new chemotherapeutics these, differences need to be considered as well as the immune status of the patient, and the pharmacokinetic properties of the drugs themselves.[19] Therefore there is a high necessity for drug candidates that are less toxic, orally available, affordable and which avoid the known resistance mechanism.

1.2.1 Pentavalent Antimony

The primary drugs currently in use are PVAs which include meglumine antimoniate (Glucantime; **1**)[24] and sodium stibogluconate (Pentostam, 2,4:2',4'-*O*-(oxydistibylidyne)bis[D-gluconic acid] Sb,Sb'-dioxide trisodium salt nonahydrate; **2**).[17] These drugs are used in long-term treatment regimes and possess the disadvantage of drug resistance.[1] PVAs exhibit variable efficacy with a cure rate above 90% for some forms of leishmaniasis. Resistance towards PVA drugs is growing, with 60% of VL cases in North Bihar, India not responding to PVAs.[17,19] Despite this, PVAs remain the therapeutic mainstay in all regions of the developing world. Treatment with antimonials, such as **2**, is not very practical.[22,25] Both compounds **1** and **2** require either intravenous or intramuscular injections with doses of 20 mg/kg once daily for 28 days in a hospital environment. Drug toxicities are common producing many side effects including nausea, pancreatitis, abdominal pain and cardiotoxicity resulting in arrhythmia.[20,26-30] The purchase of PVAs can also be expensive. This is illustrated by the cost of one course of Pentosam, which was approximately US$200 per patient in 2006. Recently, generic formulations have proven effective and are much more affordable (sodium antimony gluconate US$13 per person in 2006).[20,23]



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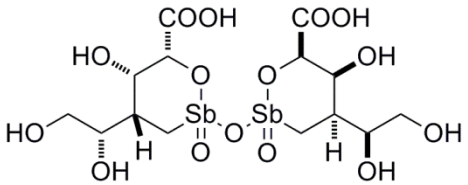


2

The mode of action of PVA based drugs is poorly understood and the identity of the biological species that PVAs act on is uncertain. The original consensus was that antimony acts upon the bioenergetic targets of the *Leishmania* parasite by inhibiting glycolysis, fatty acid β-oxidation and inhibiting ADP phosphorylation.[22] Sb(III) based drugs may also cause non specific blocking of thiol groups in amastigotes and cause the

inhibition of DNA topioisomerse I.[29] Results also suggested that Sb(III) compromises the thiol redox potential of the cell by inducing the efflux of intracellular thiols (trypanothione disulfide ($T(S)_2$) and glutathione disulfide) and by inhibiting trypanothione reductase (TryR), thus further disturbing the thiol redox potential of the cell and removing trypanothione from several, essential metabolic processes.[1,22,31]

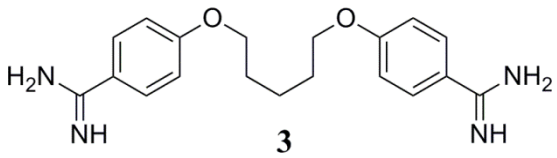
In order to be active, it is likely that Sb(V) has to be converted to Sb(III), however the site of this conversion is unknown.[1] Recently it has been reported that a parasite-specific enzyme (TDR1) catalyses the conversion of Sb(V) to Sb(III) using glutathione as a reducing agent.[32] In addition, a new enzyme (ACR2), which reduces Sb(III) was characterised in *Leishmania*. ARC2 increases the sensitivity of *Leishmania* cells to Sb(V).[1] Evidence has been found to suggest that Sb(III) enters *Leishmania* cells principally though an aquaglyceroporin named AQP1.[33] The level of expression of the AQP1 transporter may be linked to resistance regulation of the parasite to Sb(III).[33] It was also found that DNA fragmentation was induced when *L. infantum* amastigotes were treated with low concentrations of Sb(III), which suggests the appearance of late events of apoptosis.[33]

The first *Leishmania* TryR structure which demonstrates the mechanism of TryR inhibition by Sb(III) was described recently.[34] Upon NADPH reduction of TryR the trivalent antimony binds to the protein, thus affecting inhibition of protein activity.[34] Sb(III) binds directly to Cys52, Cys57, Thr335 and His461' of TryR preventing hydride transfer from the protein to trypanothione and therefore the reduction of trypanothione. Inhibition of TryR prevents the regulation of the cellular redox equilibrium in *Leishmania* parasites thus impairing the defence of the parasite against oxidative stress. This occurs by a shift in the equilibrium towards the disulphide form of trypanothione, thus disturbing the thiol redox potential of the cell.[34]

1.2.2 Pentamidine

A second line drug, Pentamidine (4-[5-(4-carbamimidoylphenoxy) pentoxyl benzenecarboximidamide, **3**) is an aromatic diamidine that is toxic for a number of protozoa and fungi including *Leishmania*. Pentamidine **3** was originally used in antimonial-resistant cases of leishmaniasis,[17] but its use has now been reduced due to high toxicity, long-term parenteral administration and a reduced cure rate, (now 70% down from more than 95% in the early 1980's).[19,35] The mechanism of action for **3** has

not yet been fully established. Research so far has demonstrated that **3** has an ability to bind to kinetoplast DNA and affect the membrane potential of the inner mitochondrial membrane.[19,28,36] Parasite resistance has also been associated with intracellular changes in arginine or polyamine transporter concentrations.[1] Recent studies have indicated that pentamidine gathers in the mitochondria enhancing the efficacy of mitochondrial respiratory chain complex II inhibitors.[1] The use of **3** has always been restricted. This has led to the gradual abandonment of **3** as a second-line treatment due to side effects such as considerable toxicity, hypotension, hypoglycaemia, diabetes and nephrotoxicity.[37]



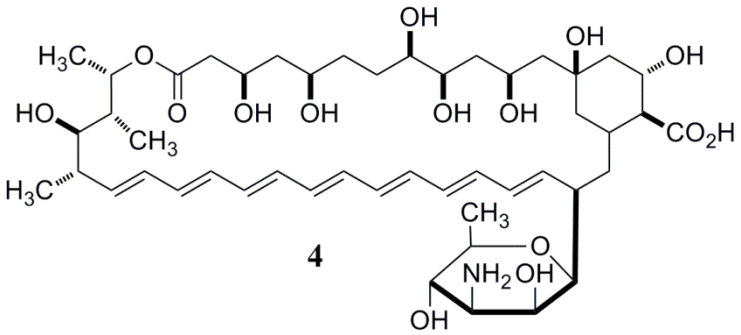
3

Between 1991 and 1997 a randomised clinical trial was carried out on patients unresponsive to Sb(V) in India, where 2 or 4 mg/kg of pentamidine was given intramuscularly on alternate days for 30 days.[38] Patients receiving 2 mg/kg were also given alloputinol 15 mg/kg/day during the treatment period. Combinational therapy cured 91% compared to 74% of patients with pentamidine alone.[38] Pentamidine has shown to be effective in the treatment of CL and MCL, requiring fewer injections over a shorter time period with minimal toxicity.[38]

1.2.3 Amphotericin B

Over the past decade Amphotericin B deoxycholate (AmB; **4**) has been used as an alternative anti-leishmanial therapeutic to pentavalent antimony, especially in areas where there are high levels of Sb(V) resistance.[39] AmB (**4**) has a treatment regime comparable to that of antimonials, where 15-20 infusions of the drug at a dose of 0.75-1.0 mg/kg are administered either once daily or on alternate days.[20,22] A cure rate of up to 98% in untreated patients and >90% in patients unresponsive to previous antimonial treatment was observed.[26,27,40] Resistance to **4** does not appear to have emerged rapidly.[39] However, **4** has a high toxicity and its use can result in severe side effects; high fever, thrombophlebitis (inflammation of a vein with blood clot formation inside the vein at the site of inflammation), myocarditis, renal dysfunction, hypokalemia

(condition of below normal levels of potassium in the blood serum) and death. Thus **4** can only be administered in a hospital setting.[22,26,41]



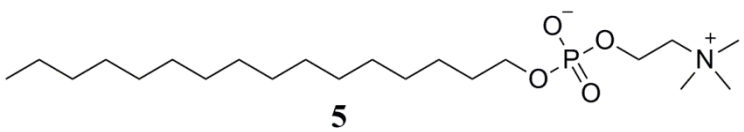
4

AmB (**4**) interacts with fungal membrane sterols and preferentially with ergosterol. Like fungi, *Leishmania* parasites also have ergostane based sterols as their major membrane sterol and this likely explains the efficacy of **4** against leishmaniasis.[17,42] The mechanism of action for **4** involves binding to both ergosterol (fungal cells) and cholesterol (mammalian cells) producing pores out of which cell contents leak, leading to cell death.[43,44] Liposome-encapsulated **4** (L-AmB) recognises the ergosterol precursors, to which they are more specific, causing disruption of the membranes.[45,46] L-AmB also recognises receptors on specific cells leading to a reduction in the rate transfer of **4** to the toxic site while not decreasing its effectiveness at the active site. This leads to an increase in the therapeutic index of the drug and a reduction in the toxic effects.[47] The lipid molecules of L-AmB are also phagocytosed by macrophages and so the drug is delivered to exactly where the parasites reside.[48,49] Newer lipid formulations of **4** retain their antifungal activity while drastically decreasing toxicity and making it safer to administer **4**,[26,28,50,51] and possible to administer high doses of **4** lipid formulations over short durations (1–5 days).[52] Nevertheless lipid formulations of this drug come at an unaffordable price for developing and emerging countries. This limits the use of this type of drug in countries where need for treatment is greatest.[26] In India during 2006, the cost of a single course of treatment for **4**, AmBisone and Abelcet was US$417, US$872 and US$974 respectively.[22] L-AmB is now available at a 90% reduced preferential cost of US $20.00 per 50 mg vial. This is still both unaffordable and beyond the means of patients in many regions of India. Attempts have been made to provide patients with inexpensive lipid formulations by combining commercially available lipid emulsions with the drug. These preparations generally lack uniformity of components as well as quality control.[52] Currently, **4** as a lipid emulsion has been

licensed for use within India for VL treatment. Preclinical studies showed 80 times more tolerance of **4** with lipid emulsion in mice then conventional **4**.[52]

1.2.4 Miltefosine

Miltefosine (**5**), a former antitumor agent, is the first orally-active drug that has been found to possess antileishmanial efficacy.[21,26] Given orally for 28 days, it cures approximately 94% of patients suffering from VL, as shown in a study based in India.[19] The orally active drug has proven safe and effective in children suffering from VL. The only side effects observed are mild to moderate gastrointestinal toxicities which induce vomiting and diarrhoea and renal toxicity. These symptoms are treatable and reversible.[22,25] This novel oral drug is commonly used for treatment of VL, and has been successful in immunocompetent and immunocompromised patients (e.g. HIV patients). However, studies have shown the unresponsiveness of New World *Leishmania* species, such as, *L. braziliensis*, *L. guyanensis* and *L. Mexicana.*[35]



5

There are two major drawbacks to compound **5**. It is a teratogen, which cannot be given to any woman of child bearing age, and compound **5** has a long half life (150-200 h), which means that resistance could develop quickly if compound **5** is not used in conjunction with other drugs.[21,22,41] The current suggested mode of action is associated with changes in alkyl-lipid metabolism, and phospholipid biosynthesis. Miltefosine (**5**) causes modulation of cell surface receptors, inositol metabolism, phospholipase activation, protein kinase C and other mitogenic pathways in the promastigote stage, eventually causing apoptosis.[35] Miltefosine (**5**) seems to block sterol biosynthesis of *Trypanosoma cruzi*, either of the epimastigote or the amastigote forms of the parasite.[1] *Trypanosoma* and *Leishmania* parasites are classified under the common family level of *Trypanosomatidae*, and thus share similar biological behaviour (Figure 2).

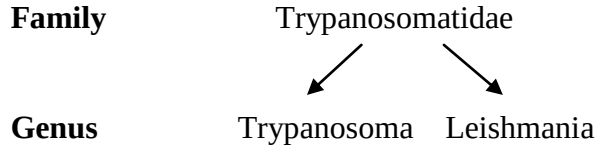
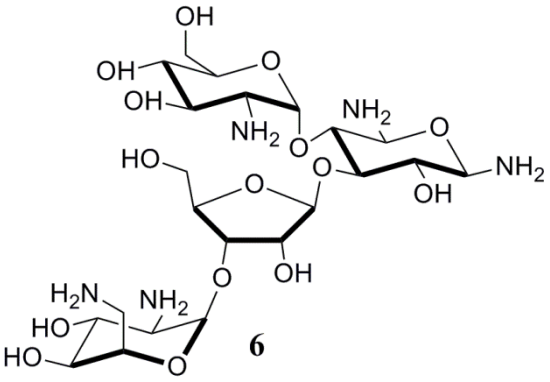


Figure 2 The relationship between *Trypanosoma* and *Leishmania*

1.2.5 Paromomycin

Paromomycin (**6**) is an aminoglycosidic antibiotic produced by *Streptomyces riomosus var. Paromomycinus*.[35] Compound **6** has been used to treat both VL, in parenteral formulation, and CL in topical and parenteral formulations.[53] There are three paromomycin ointments currently in use for CL; paromomycin 15% with methylbenzethonium chloride 12%, paromomycin 15% with urea 10% and paromomycin with gentamicin 0.5%.[35] These preparations have shown variable results according to the epidemiological situation and the *Leishmania* species involved.[35] Paromomycin (**6**) is poorly absorbed after oral administration, however it is rapidly absorbed from intramuscular injections with peak plasma concentration occurring within 30-90 minutes (min) and a volume of distribution of 25% of body weight.[35] The half-life of **6** varies between 2-3 h (h) if patients have normal renal function. Side effects associated with **6** include ototoxicity (damage to the cochlea or auditory nerve and sometimes the vestibular system), and liver problems. Problems associated with ointment formulations include skin rashes and local pruritus (localised itching).[35]



6

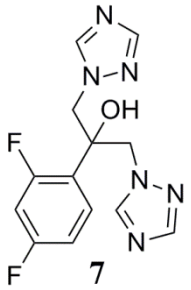
The mode of action of **6** is well known. Paromomycin (**6**) acts as an inhibitor in protein synthesis through its interaction with ribosomal RNA subunits.[53] Three mechanisms of aminoglycoside resistance in prokaryotes have been recognised and include reduced uptake or decreased cell permeability, alterations at the ribosomal binding sites, or production of aminoglycoside modifying enzymes.[53] However, the mechanism associated with *Leishmania* species requires additional understanding. Studies have suggested the involvement of mitochondrial membrane potential, ribosomes and respiratory dysfunction.[53] Paromomycin (**6**) is also known to inhibit protozoan protein synthesis by binding to the 30S ribosomal subunit. Interfering with protein synthesis initiation by fixing the 30S-50S ribosomal complex at the start codon of mRNA, ultimately causing accumulation of abnormal initiation complexes.[35]

1.2.6 Future treatments

Advances in the pharmacology of leishmaniasis are under constant change due to the need for improved drugs. Current research is based around a number of factors, which may provide more efficient and available leishmanial treatment; (a) knowledge of the *Leishmania* parasite genome, (b) drug information used for other infection or pathologies, (c) new chemotherapies based on rational drug design and (d) natural product isolation.[35]

1.2.6.1 *Azoles*

Imidazoles and triazoles are well recognised antifungal agents which are well tolerated by patients. As anti-leishmanial drugs, these heterocycles have shown inhibition against certain *Leishmania* species by inhibiting 14α-demethylase, and thus interfering with cell membrane biosynthesis. Flucanazole (**7**) has been used to treat Old World *L. Major* and ketoconazole (**8**) in New World *L. panamensis* and *L. mexicana*.[54,55] Itrakonazole has been used against Old and New World *Leishmania* with low efficacy and posaconazole, which has not yet passed clinical trials, has shown activity against *L. amazonensis*.[35]



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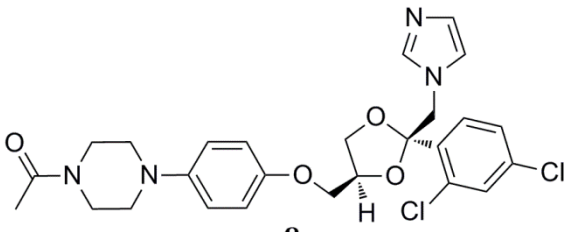

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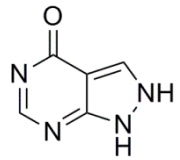
1.2.6.2 *Allopurinol*

Allopurinol (**9**), a xanthine oxidase inhibitor, was identified over 30 years ago for the treatment of CL due to its oral bioavailability and use in other clinical indications.[35] It has been investigated in clinical trials for CL and VL with unsuccessful results.[56] Allopurinol (**9**) and its major metabolic product oxipurinol inhibit adenylosuccinate synthetase, a mediator in the conversion of inosonic acid to adenosine monophosphate.[57] Allopurinol (**9**) is selectively incorporated into the nucleic acid of trypanosomatid parasites through hydrolysis to allopurinol ribosides. Through this process an analogue of inosine is incorporated instead of ATP into leishmanial RNA, interfering with normal protein synthesis. Therefore, adenylosuccinate synthetase or the adenine phosphoribosyltransferase are a probable target.[35,57]



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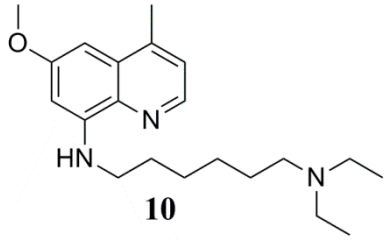
1.2.6.3 *Sitamaquine*

Sitamaquine (**10**) is an orally active 8-aminoquinoline analogue (8-aminoquinoline (8-[6-(diethylamino)hexyl]amino)-6-methoxy-4-methylquinoline) known as WR 6026.[35]



10

The molecular target of this cationic molecule is not known. Sitamaquine (**10**) is thought to act as a prodrug with the resultant metabolic product responsible for antiparasitic activity.[19] In addition **10** induces a rapid collapse of the mitochondrial inner membrane potential of *L. donovani* promastigotes, promoting fast and extensive alkalinisation of *L. donovani* acidocalcisomes.[58] Sitamaquine (**10**) has also been shown to interact with mitochondrial and other lipid membranes and result in alteration of parasite morphology.[59,60] Animal studies have shown positive results in the treatment and prophylaxis of VL, however in clinical trials after 28 days of treatment low efficacy was observed.[35] While **10** was generally well tolerated by patients, side effects regarding adverse renal events have been observed.[61] Studies have also shown that significant amounts of **10** and/or its metabolites remain in the liver following a single oral administration.[62] Sitamaquine (**10**) has also been used in a cross disease study where use of **10** in an HIV-positive patient with VL indicated that the patient responded to the use of sitamaquine.[63]

1.2.6.4 *Antimonial-encapsulated liposomes*

Liposomes have been used in helping decrease the toxicity of **4** as well as for improving the effectiveness of antimonials against VL. There are four reasons that liposomes are appropriate carrier systems for antimonials; (a) they effectively encapsulate and retain large amounts of water-soluble compounds, (b) they have a tendency to be captured by

the macrophages of the reticuloendothelial system, which are the same cells that harbor *Leishmania* parasites, (c) they result in fewer side effects and (d) they have high versatility with respect to lipid composition, volume and composition of internal compartment, vesicle size and lamellarity.[33] In terms of parasite suppression in the liver and/or spleen, liposome-encapsulated meglumine antimoniate and sodium stibogluconate administered intravenously were more than 700 times more potent compared to the free drugs.[33] These recent achievements expose new directions for the improvement of anitmonial therapies.

At present it is difficult to identify and distinguish any specific starting point in the development of novel treatments for leishmaniasis. The existing focus is based around new chemotherapeutics or the development of chemotherapies taken from other sources such as imidazoles (antifungal agent) and paromomycin (antibacterial agent) rather then elucidating the current mechanism of actions for known chemotherapies. Current research has focused on determining the mechanism of the older more toxic drugs such as the PVAs and Pentamidine. Little and speculative information is available for the mode of action of newer chemotherapeutics, such as Miltefosine (**5**) and Sitamaquine (**10**). This provides opportunities for using a rational based design for new therapeutics against leishmaniasis.

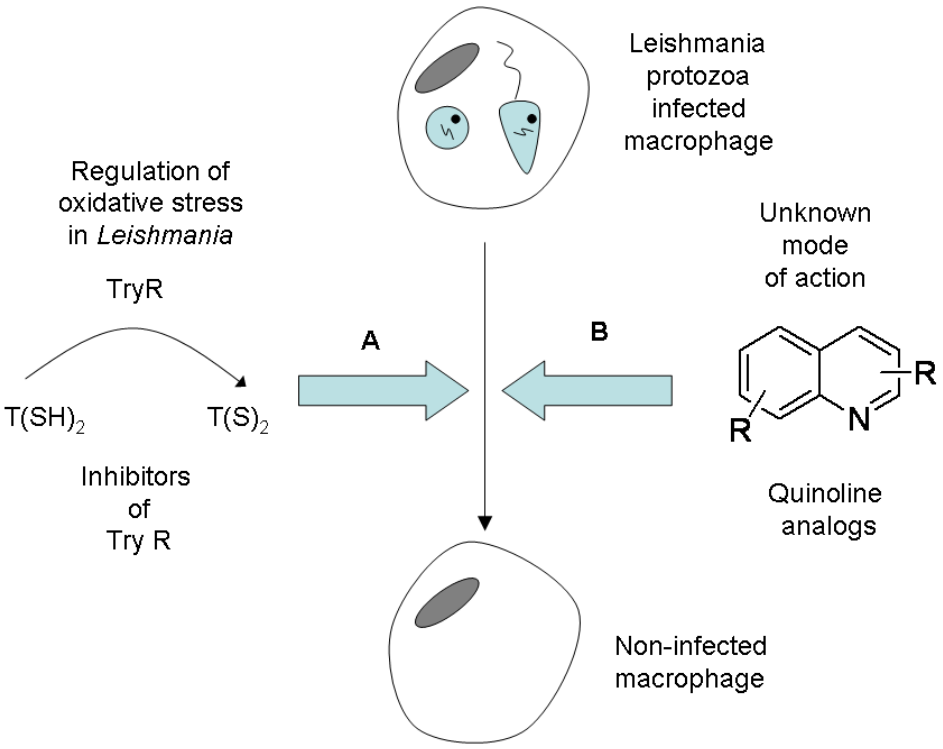


Figure 3 Schematic representation of thesis project.

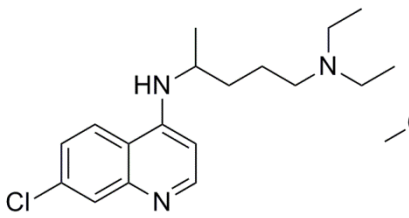
The current project seeks to progress the identification of new anti-leishmanial compounds. Two major approaches were considered in the context of the above discussion. One approach was to develop the structural diversity through a modified structure activity relationship (SAR) study of quinoline compounds, which are known anti-leishmanial compounds with an unknown mode of action (Figure 3B). The other approach was to use a structure-guided design of TryR, a key enzyme involved in regulation of oxidative stress in the *Leishmania* parasite (Figure 3A). Without regulation the parasite will die.

1.3 Quinoline

The heterocycle quinoline is a well established feature in a variety of naturally occurring and medicinally active compounds.[64,65] The isolation of quinoline/quinoline-containing compounds, such as cinchona alkaloids and those of the camptotheca family, have been successfully used to treat parasitic infections such as malaria[66] and leishmaniasis.[67] Quinoline derivatives are actively used in cancer[68,69] and heart disease treatment,[70] and exhibit a range of antitubercular,[71] antimalarial,[72] antiallergic and antiasthmatic activites.[73] Quinoline derivatives remain an area of active research for antiparasitic treatments.[74]

1.3.1 Antimalarials

Derivatives of the quinoline family of drugs have been in use for antimalarial treatment for over 300 years.[75] Cross application of antimalarial quinoline compounds for treatment of *Leishmania* has resulted from understanding that the common target is a parasite and that approval for therapeutic use was already obtained.



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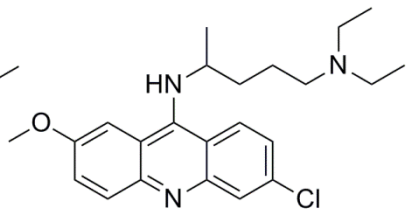

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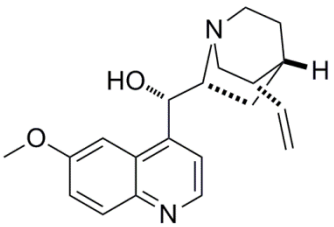
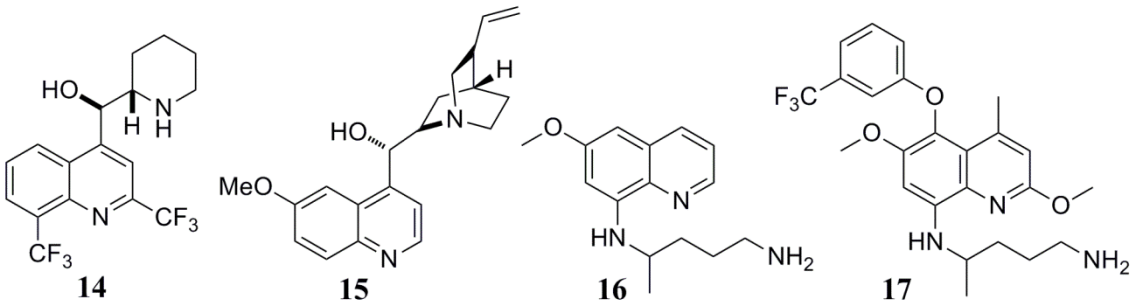


13

Many of these quinoline-related compounds were synthesised in Germany during the 1930's which lead to the introduction of chloroquine (**11**) as the primary treatment for malaria.[2] Subsequently, Quinacrine (**12**) replaced **11** as the drug of choice for malarial

treatment. In the 1960's quinine (**13**), an effective anti-malarial originally isolated from the bark of the American cinchona tree, was reintroduced due to the widespread resistance of chloroquine.[2] The most widely used anti-malarial quinolines include chloroquine (**11**), mefloquine (**14**), quinine (**13)**, quinidine (**15**), and primaquine (**16**), which include effective anti-malarials for both treatment and prophylaxis.[76] The mechanism of action of the 4-aminoquinolines is unclear despite their high efficacy against malaria.[75]



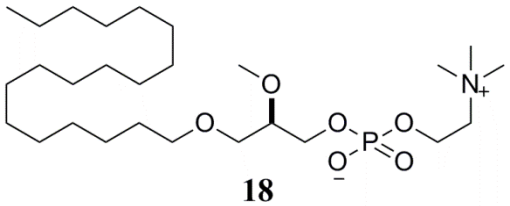
Chloroquine (**11**) increases the pH of lysosomes of mammalian cells as well as the phagocytic vesicles of *Plasmodium* species. The mode of action of **11** is thought to involve in part alkalinisation of these vesicles. Chloroquine (**11**) may also intercalate into DNA as well as bind to ferriprotoporphyrin IX which is produced by haemoglobin degradation in the phagocytic vesicles of malaria parasites.[77-79] Mefloquine (**14**) is effective against malaria yet developed resistance and undesirable side effects quickly leading to its declined use.[76] The 8-aminoquinoline **16** is the only 8-substituted derivative in clinical use and is the optimum choice for the fundamental cure of relapsing malaria. Due to this factor other 8-aminoquinolines have been synthesised and evaluated for their activity against several *Plasmodium* species *in vivo*. Based on these results tafenoquine (**17**) was chosen for clinical evaluation against relapsing malaria cases.[80]

Due to resistance many of these drugs are conversely used in conjunction with antibiotics and artemisinin derivatives (anti-malarials) due to both single and multi-drug resistance worldwide.[64,81] As previously mentioned the mode of action by which quinoline derivatives affect malarial parasites is yet to be identified.[78]

1.3.2 Cinchona Alkaloids

The cinchona alkaloids are commercially the most important alkaloid family and continue to attract attention among organic chemists. Cinchona alkaloids are isolated from the bark of several species of Cinchona and Remeyia trees.[65] During the 17th century, Europeans became aware of the action of the powdered bark against fever caused by malaria.[65,82]The major component, quinine, was isolated and has since been the most widely used drug, particularly as an anti-malarial. The role of the alkaloids widened throughout chemistry when their potential in various organocatalytic transformations was noted, acting as nucleophilic, phase transfer and chiral-base catalysts.[83]

The specific mechanism of the alkaloids as anti-malarial agents is largely unknown.[82] A number of postulates have been suggested including DNA intercalation inhibiting both DNA and RNA synthesis.[2] However, this theory has since been abandoned. Other theories include alkalization of parasite acid vesicles causing parasite death.[76] Quinine (**13**) is a blood schizontocide (kills erythrocytic stage of the parasite), which potentially inhibits haem polymerase contributing to parasite death and is curative to *Falciparum* malaria but only provides prophylaxis against *Vivax* malaria.[76,82] Quinidine (**15**) and its stereoisomer **13** act as ion channel blockers, with the most notable mechanism of **15** being the possibility to alter diastolic depolarisation due to its interaction with the M-gate in the fast Na^+ channel.[84] Both **13** and **15** as well as other anti-malarials, **12**, **14** and edelfosine (**18**) inhibit choline transport in *Leishmania* parasites.[85] In doing this, they kill the promastigote form of *Leishmania in vitro* in low μM ranges, and more importantly lead to a possible mechanism of action.[85] These alkaloids also act on oxidative metabolism producing significant effects in lipid peroxidation.[84]



18

1.3.3 Anti-leishmanials

Representative compounds with *in vitro* or *in vivo* activity against *Leishmania* include phenolic compounds, terpenoids, quinones and alkaloids. Most of these major classes of

natural products have demonstrated various activities against *Leishmania* parasites. The most promising candidates belong to the alkaloid family, and as previously mentioned, alkaloids such as the anti-malarial quinine have inhibited choline transport in *L. major* promastigotes.[85]

Several studies have focused on the activity of variously substituted quinoline compounds, including individual and multiple substitutions. The large number of individually substituted quinolines focus on 2- and 8-substitution. The 2-substituted quinoline alkaloid natural products were originally isolated from the Bolivian medicinal plant, *Galipea longiflora* (Rutaceae).[86]

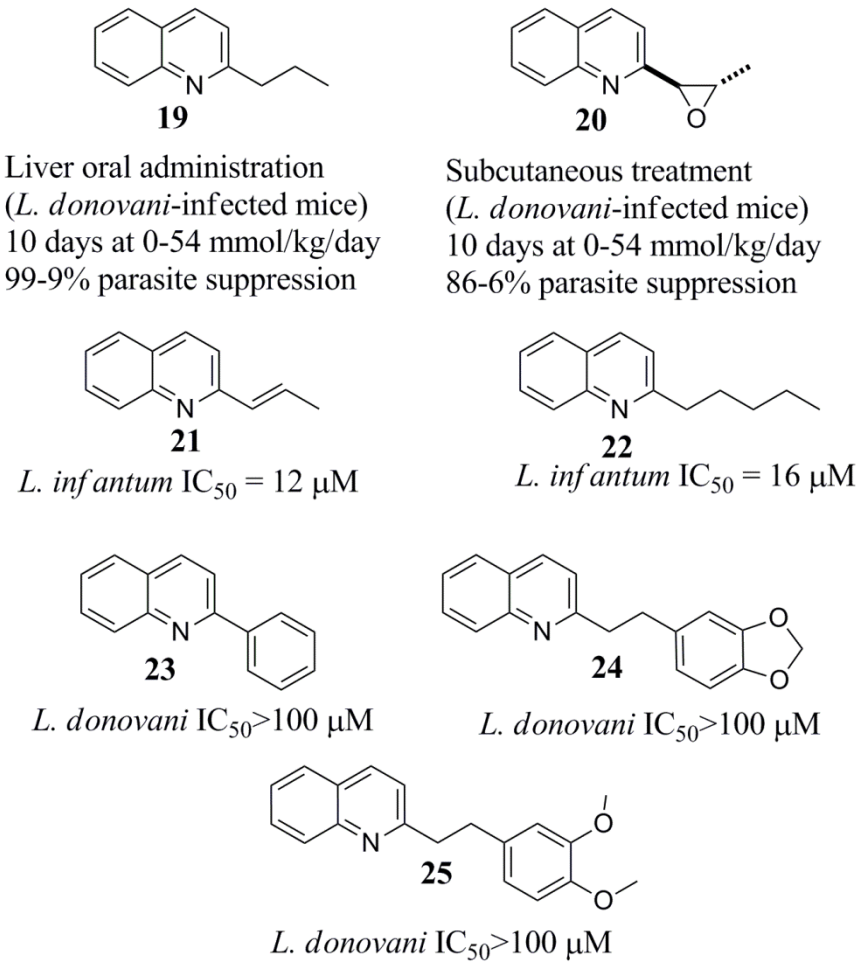
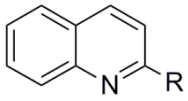


Figure 4 Various quinolines with activity against *Leishmania* parasites

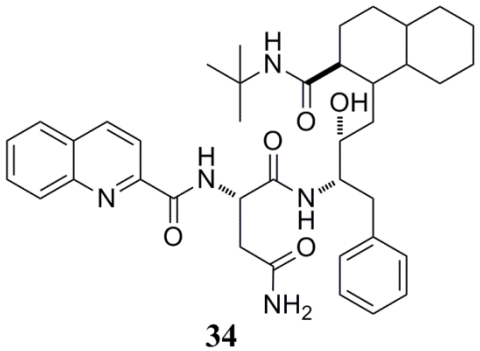
These structurally simple quinolines show potent oral activity against both CL[67,87] and VL.[58] A variety of substitutions at the 2-position of the quinoline ring has been extensively studied due to the potent activity seen with isolated natural products 2-*n*-propylquinoline (**19**), 2-(l',2'-trans-epoxypropyl)quinoline (chimanine D) (**20**), 2-(E)-

prop-1'-enylquinoline (chimanine B) (**21**), 2-*n*-pentylquinoline (**22**), 2-phenylquinoline (**23**), and 2-(3,4-methylenedioxyphenyl ethyl) quinoline (**24**) (Figure 4).[88,89]

The anti-leishmanial efficacies were evaluated in BALB/c mice infected with *L. amazonensis*, *L. venezuelensis*[87,90] or *L. donovani*.[89] Three-carbon chain quinolines, **19** and **21**, were more potent then *N*-methylglucamine antimonite while other quinoline alkaloids such as **20**, **24** and 2-(3,4-dimethoxyphenylethyl)quinoline (**25**, were as effective as the reference drug in *L. amazonensis* or *L. venezuelensis* infected BALB/c mice.[67,87] The potent activity of **19** was further increased in analogs where the 2-substituent was -C≡CH, -CH=CHC(O)H, -CH=CHCH$_2$OH, or -CH=CHCH$_2$OCH$_3$.



26 R = CH=CHCH$_2$OH; IC$_{50}$ = 2 μM (*L. infantum*)
12.5mg/kg, ~60-70% parasite burden (Liver, in vivo)
27 R = CH=CHCH$_3$; IC$_{50}$ = 12 μM (*L. infantum*)
28 R = CH=CHPh; IC$_{50}$ = 11 μM (*L. infantum*)
29 R = CH=CHCOH; IC$_{50}$ = 2 μM (*L. infantum*)
30 R = CH=CHBr; IC$_{50}$ = 2 μM (*L. infantum*)
31 R = CH=CBr$_2$; IC$_{50}$ = 2 μM (*L. infantum*)
32 R = CH=CHQuinoline; IC$_{50}$ = 8 μM (*L. infantum*)
33 R = C≡CH; IC$_{50}$ = <5 μM (*L. infantum*)

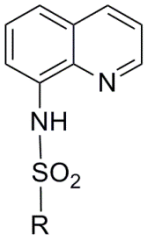


34
L. major at 50 μM 48% of control grown

Compounds **26**-**33** are significantly more effective than meglumine antimoniate in reducing the parasite burden in both the liver and spleen.[86,88] Quinoline **26** was the most promising compound against CL and VL and **26** along with compounds **27**-**33** and

displayed double the antileishmanial and antiviral activities compared to **4**, suggesting their potential use as a treatment for co-infection of *Leishmania*-human immunodeficiency virus.[86] The 2-aminoquinoline, saquinavir (**34**), also demonstrated inhibitory effects against *Leishmania* parasites with increased activity against *L. major* parasite growth (17% to 65% over 2 to 3 days) yet little effectiveness against *L. infantum* (5% to 34%).[58]

While a number of studies have focused on 2-substituted quinolines, there have been few studies on the 3-, 5- or 6-substituted quinolines and limited *in vitro* activity is seen against a variety of *Leishmania* species (*L. amazonensis*, *L. infantum*, *L. major*, *L. mexicana* and *L. donovani*).[88,91] Conversely, the 8-substituted quinolines, like the 2-substituted, have a more diversified outlook. Individually substituted 8-sulfonylamide or substituted 8-boronic acid quinolines show predominantly high *in vitro* activity against *Leishmania* parasites.[92,93] The variety of substituted sulfonylamides **35**-**37** studied show activities twice that of **4** (0.1-10 μM) in *L. chagasi* parasites. Compounds **38**-**40** similarly substituted with a sulfonylamide exhibit activities ranging from IC_{50} values of 6.27 to 14.47 μM and 2.18 to 13.96 μM against *L. amazonensis* and *L. chagasi* respectively.[74]



35 R = 3-bromo-2-chloropyridine; IC_{50} = 0.56 μM (*L. chagasi*)

36 R = 3,5-difluorobenzene; IC_{50} = 0.45 μM (*L. chagasi*)

37 R = 2,3-dibromothiophene; IC_{50} = 0.53 μM (*L. chagasi*)

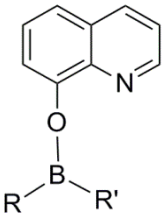
38 R = C_6H_4-C_6H_5; IC_{50} = 2.18 μM (*L. chagasi*)

39 R = 4-dimethylnaphthalen-1-amine; IC_{50} = 3.53 μM (*L. chagasi*)

40 R = *p*-propylbenzene; IC_{50} = 13.96 μM (*L. chagasi*)

The 8-boronic acid quinoline analogs **41**-**46** show similar activities to the sulfonylamides. These compounds contain varying side chains attached to the quinoline moiety via a boronic acid linker. The side chains linked from the boronic acid consists of vinyl or aryl (phenyl, furanyl, pyridinyl or dihydrooxazolyl) substitution at the R or

R’ position. The aryl components are further substituted with either amino chains, halogens or cyano groups.[94] Over 70 compounds similar to boronic acid compounds **41**-**46** were tested and IC_{50} values were observed ranging from 0.20-0.80 μg/ml against *L. donovani* amastigotes cultured from golden hamsters.[94]



41 R = 2-F-4-Cl-C_6H_3, R' = 3-F-C_6H_4; IC_{50} = 0.02 μg/ml (*L. donovani*)

42 R = 3-Me-4-Cl-C_6H_3, R' = 4-CN-C_6H_4; IC_{50} = 0.07 μg/ml (*L. donovani*)

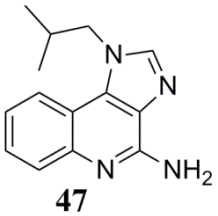
43 R = 3-CN-4-F-C_6H_3, R' = vinyl; IC_{50} = 0.19 μg/ml (*L. donovani*)

44 R = 3,5-di-F-C_6H_3, R' = vinyl; IC_{50} = 0.21 μg/ml (*L. donovani*)

45 R = 3-CN-C_6H_4, R' = vinyl; IC_{50} = 0.25 μg/ml (*L. donovani*)

46 R = 4-Cl-C_6H_4, R' = 4-OMe-3-F-C_6H_3; IC_{50} = 0.29 μg/ml (*L. donovani*)

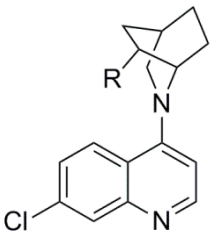
Both *in vitro* and *in vivo* studies have included a range of di-, tri- and tetra-substituted quinoline derivatives that could be considered derivatives of the potential frontline anti-leishmanial lead compounds primaquine (**16**) and sitamaquine (**10**). However, there are a few exceptions, such as chloroquine (**11**),[95] indolylquinoline,[96] imiquimod (**47**)[97] and mefloquine (**14**).[98]



47

Chloroquine (**11**), a 4,7-disubstituted quinoline, is an anti-malarial agent that has a growing widespread resistance worldwide.[76] Chloroquine (**11**) has shown partial *in vitro* efficacy against promastigotes and amastigotes of *L. mexicana mexicana* and *L. donovani*. In *L. donovani* amastigotes compound **11** inhibited survival by 55% at 3 μM.[99] However, **11** did not inhibit *in vivo L. donovani* parasite multiplication in hamster livers when administered at 100 mg/kg/day for 12 days[95] or *L. major* in TFW mice when administered at 90 mg/kg/day for 5 days.[98] Due to slight efficacy *in vitro* further

4,7-substituted isoquinuclidine quinoline analogues, derived from **11**, were synthesised and displayed potent *in vitro* antileishmanial activity.[100] The isoquinuclidine 7-chloro quinolines analogs, **48**, **49**, and **50**, inhibited *L. donovani* promastigote growth comparable to that of pentamidine (2.8 μg/mL) but all are less potent than amB (IC_{50} 0.18 μg/mL).[100]



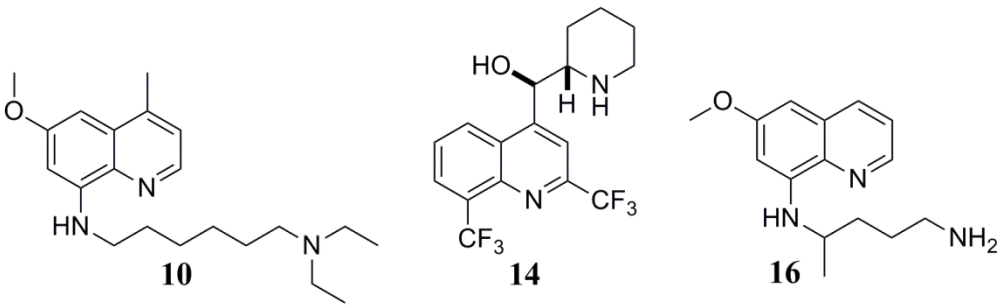
48 R = $CH(NH_2)Et$; IC_{50} = 1.9 μM (*L. donovani*)

49 R = $CH(NH_2)Pr$; IC_{50} = 3.8 μM (*L. donovani*)

50 R = CH_3; IC_{50} = 3.0 μM (*L. donovani*)

Previously mentioned **16** is a 6,8-substituted aminoquinoline commonly used in the treatment of *P. vivax* malaria and has shown high potency against *L. donovani* amastigotes.[101] The high toxicity of **16** has also been evaluated against three species of axenically cultured *Leishmania* amastigotes. Amastigotes and promastigotes of *L. amazonensis* and *L. infantum* showed little divergence in chemosensitivity to **16**, while *L. mexicana* amastigotes demonstrate approximately a 2-fold greater susceptibility than promastigotes.[102] Studies into primaquine-loaded polyisohexylcyanoacrylate nano particles have identified improved *in vitro* anti-leishmanial activity with *L. donovani* infected macrophages by 21-fold compared to **16** alone.[103]

Sitamaquine (**10**), a previously discussed 4,6,8-aminoquinoline, was originally synthesised by the U.S. Army and evaluated for its antiparasitic activity. Of the library of compounds synthesised by the US Army, **10** was found to have a higher activity compared to the analog primaquine against *Leishmania*. Sitamaquine (**10**) was further used in clinical evaluation and was found to exhibit variable cure rates and mixed results in clinical trials.[80,103] Sitamaquine (**10**) is the most studied example of a tri-substituted leishmanicidal quinoline analog and has an EC_{50} of 5.09 μM and an EC_{90} of 20.33 μM *in vitro* against *L. donovani*.[104]



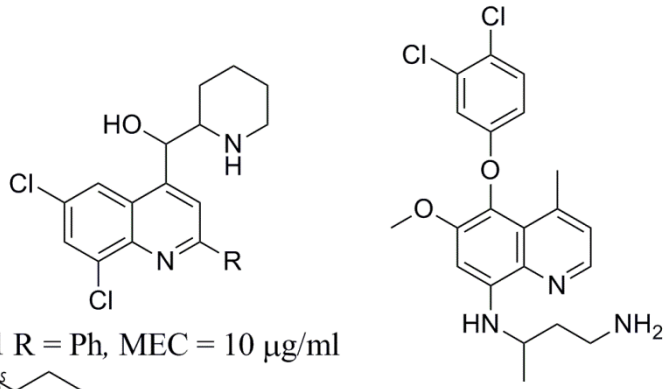
10 **14** **16**

Analogs of **10** bearing structural variations in the 8-[6-(diethylamino)hexyl]amine side chain, such as a di-*n*-propylamino, substituted-1-piperazine or related heterocycle unit, are active against *L. donovani* in hamsters, although generally of a lower the potency compared to the parent drug.[105,106] However, a number of analogs in this category including 5-methoxy sitamaquine were significantly more potent than the parent drug against *L. major* and *L. m. amazonensis* in random-bred albino mice.[107]

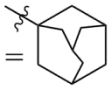
Another anti-malarial quinoline, mefloquine (**14**), has been tested against various types of *Leishmania* species *in vivo*. Mefloquine (**14**) displayed moderate activity against *L. major* in TFW mice when administered at 70 mg/kg/day over 5 days.[98] However, **14** was not efficient in the treatment of New World CL in a non-blinded, therapeutic trial conducted in Columbia where 1.25-1.5 grams of **14** was given as a single oral dose or as 250 mg a day for 5-6 days,[108] whereas a trial in Ecuador gave a cure rate of 68% in patients (11 patients) over four weeks.[109,110] Of the two trials, including **14**, a direct comparison might have been complicated by issues such as patient compliance, early vomiting, superinfection of lesions, a potential difference in drug bioavailability due to methods of drug manufacture, and classification of lesions at a point in time (lesion might initially enlarge before healing). Tetra-substituted quinoline analogs have been tested with varying results dependent on the substitution pattern.

The 2,4,6,8-substituted quinoline compounds **51** and **52** are substituted analogs of **10**, which differ at the 2-position containing a phenyl or tricyclic ring. Both compounds **51** and **52** are more toxic then primaquine.[111] 4,5,6,8-Substituted aminoquinoline, NPC1161 (**53**) is another anti-malarial drug which has a favourable toxicity relative to that of primaquine, as well as a known anti-leishmanial activity against *L. Donovani*.[112,113] Attachment of the water soluble copolymer *N*-(2-hydroxypropyl)methacrylamide (HPMA) to **53** containing variations in the spacer of HPMA showed effective reductions in parasite numbers *in vitro*.[112] NPC1161 (**53**) was

effective in killing all parasites *in vitro* at 30 mg/ml although it was toxic to macrophages at the same dose.[112]



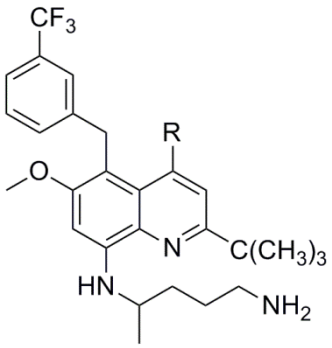
51 R = Ph, MEC = 10 μg/ml

52 R =  , MEC = 10-100 μg/ml;

53

L. donovani

Penta-substituted compounds **54**, **55** and **56** showed variable activities with an IC_{50} comparable to pentamidine (IC_{50} = 3.4 μg/mL) and IC_{90} values ranging from 6.5-6.7 μg/mL compared to that of pentamidine 8.0 μg/mL. However, all compounds were less potent than AmB (IC_{50} = 0.17 and IC_{90} = 1.7 μg/mL).[114]



54 R = H; IC_{50} = 3.0 μM
55 R = CH_3; IC_{50} = 3.4 μM
56 R = C_2H_5; IC_{50} = 2.9 μM
L. donovani

The mechanism of how quinoline drugs work to kill *Leishmania* parasites remains unknown. Nevertheless, there is some data concerning the mode of action (MOA) of **10** (previously described) and a few quinolines, such as imiquimod (**49**), which verifies such compounds as potent inducers of alpha interpheron and cytokinesin in both *in vitro* and *in vivo* experiments.[58] The research into the anti-leishmanial activities of quinolines has identified numerous substituted quinoline compounds that have shown activity both

in vitro and *in vivo*.[105,115-118] Many of these compounds have been substituted at the 2-, 3- or 8-position of the quinoline ring. Nevertheless, many of these active *in vitro* compounds are not progressed through to clinical trials. This may be due to issues such as absorption, distribution, metabolism, elimination or toxicology. This concludes the review of quinoline containing compounds with degrees of parasitic inhibition. The second focus of this project is the trypanothione system in parasites.

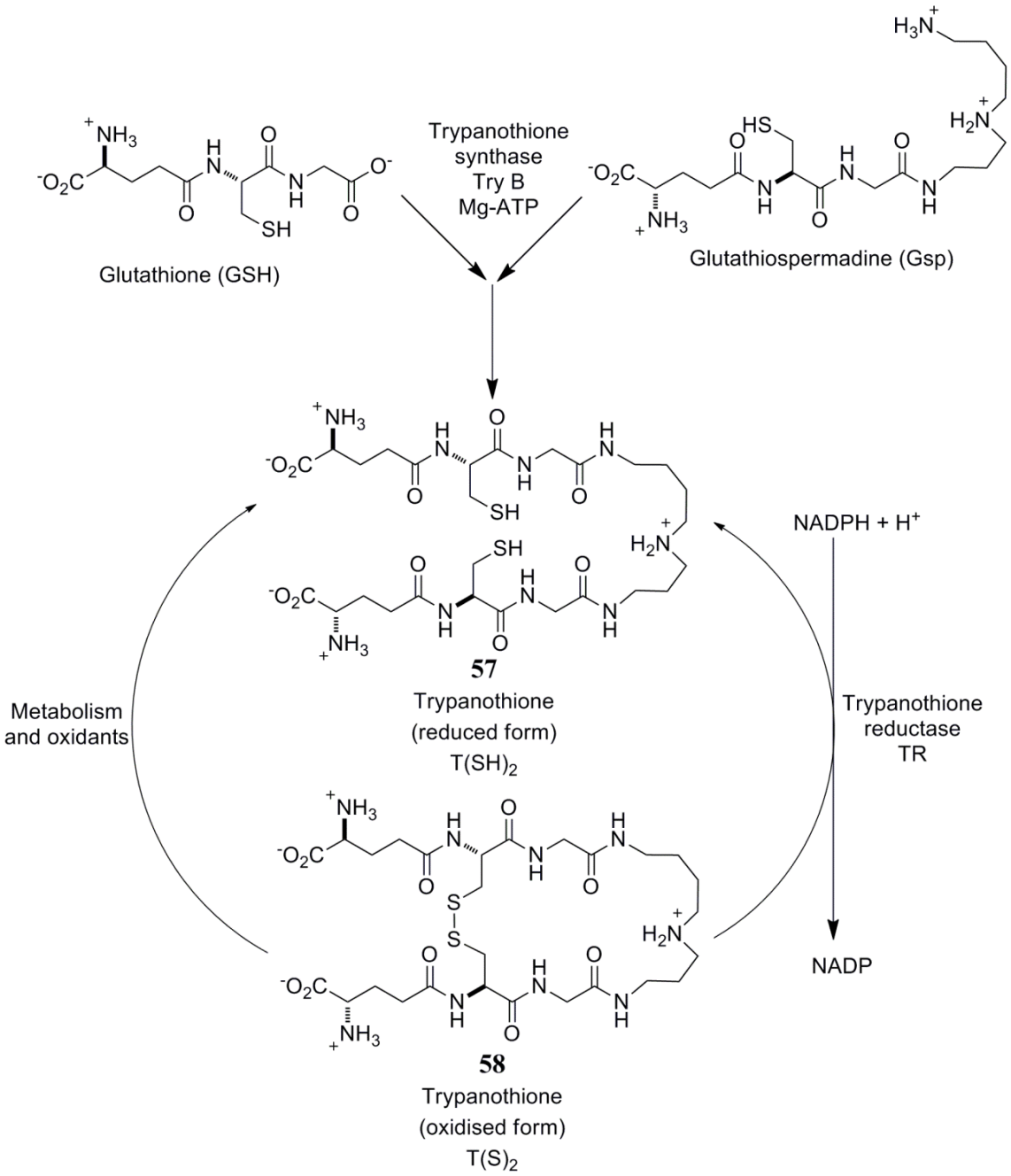
1.4 Trypanothione System in Parasites

Glutathione (GSH)/glutathione reductase (GR) and thioredoxin (Trx)/thioredoxin reductase (TrxR) systems maintain the intracellular thiol redox homeostasis of most eukaryotic organisms.[119] The trypanosomatids, which include *Leishmania,* show a number of biochemical, morphological and genetic differences to eukaryotes.[119] One noticeable difference is the thiol redox metabolism pathway. The trypanosomatids do not rely on the GSH/GR system to keep a redox balance, but rather on the dithiol, trypanothione ($T(SH)_2$) and TryR system.[120] The trypanothione system is absent in eukaryotes. TryR is the only enzyme which provides reducing equivalents within the parasite and it is extremely sensitive to oxidative stress. Therefore the components of this metabolism are an attractive drug target molecule.[120,121] The current thinking is that a thorough analysis of the trypanothione metabolism and its control mechanisms will further reveal additional unique features and assume new targets for selective antiparasitic drug development.[121]

Leishmania promastigotes avoid the lethal effects of lysosomal enzymes and free radicals produced within macrophages by halting phagosomal maturation.[119] During normal phagocytosis (fusion of vesicles, such as lysosomes) the phagosome is a prerequisite for successful eradication of invading microorganisms. Trypanothione performs a variety of functions and is essential for parasite survival for two reasons: (a) trypanothione plays a crucial role in regulating cellular redox equilibrium and (b) pathogenic parasites have no alternate mechanism to protect against the oxidative stress.[122] These functions are fulfilled in other ways by different organisms. Parasites are exposed to various reactive oxygen species, which are generated by the host defence system.[123] Trypanothione helps 'protect' the parasite from excessive oxidative stress by maintaining intracellular redox balance.[124] It does this via reduction to $T(SH)_2$ (**57**) and reoxidation to the disulphide TS_2 (**58**) via enzymatic transformation (Scheme 1)**.** This

parasite system is less efficient than the mammalian glutathione peroxidase in detoxifying hydroperoxide, but has a much broader substrate specificity by also reducing lipid hydroperoxide.[123]

Both nitric oxide (NO), a highly reactive short-lived molecule, and its metabolites (peroxynitrite) are toxic to several microorganisms. The production of NO, for instance by macrophages, is an important part of the innate immune response.[119] During phagocytosis macrophages produce peroxynitrate (superoxide reacted with nitrous oxide) a strong oxidizing and nitrating agent, and hydroxyl radicals as a first line defence.

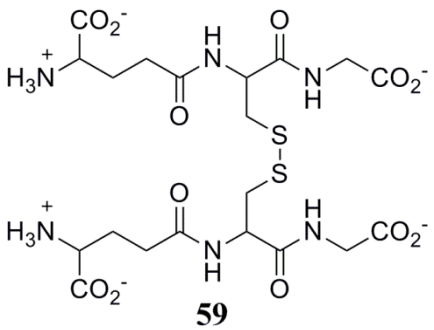


Scheme 1 Trypanothione cycle present in the parasite *Leishmania*

Under normal conditions many oxidant species are maintained at intracellular steady-state concentration by various enzymes and low molecular antioxidants. An imbalance in this steady-state leads to oxidative stress which may be deleterious or even lethal.[57] Trypanothione is fuelled by the cascade of two distinct oxidoreductases which when working in concert, reduce hydroperoxides at the expense of trypanothione. This system in parasites is quite distinct from the analogous mammalian system.[125] Several studies have shown that killing of intracellular *L. major* by macrophages requires NO production.[119]

Both, GR and TryR are members of the well-characterised family of FAD-dependent NADPH oxidoreductases. Mechanistically and structurally TryR and human GR are closely related. For example, there is a 41% sequence identity between *Trypanosoma congolense* TryR and human GR.[120,126] The most important difference between parasite and host enzymes is their mutually exclusive substrate specificity which is based on the respective charge distribution of their active sites.[131] It has been shown in studies that human GR has a 9000-fold preference for $G(SH)_2$ over $T(SH)_2$ based on V_{max}/K_m values.[120,127] GR and TryR are diametric molecules whose subunits fold into four domains: (a) the FAD-binding, (b) the NADPH-binding, (c) the central, and (d) the interface domains (Figure 5).

The nucleotides are kept separate from the disulphide substrates by the isoalloxazine ring of FAD. This geometry is characteristic of all FAD-dependent NADPH oxidoreductases.[120] The active site of human GR differs to that of TryR in that the hydrophobic pocket of the latter complements the spermidine portion of the substrate. In GR this region is comprised of polar and positively charged side chains to neutralise the carboxylate ion sites of glutathione disulphide (GSSG) (**59**).[121]



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However, for trypanothione disulphide (TS_2) (**58**) in TryR it is hydrophobic and negatively charged. This is a large discriminating factor for specific inhibitors of TryR versus GR.[126] There is also a second hydrophobic pocket, known as the Z site tentatively located from graphic analysis of the region Phe396. This site was discovered for TryR when the alternate substrate *N*,*N*-bis(benzyloxycarbonyl)-L-cysteinylglycyl-3-(dimethylamino) propyl amide disulfide was found.[121]

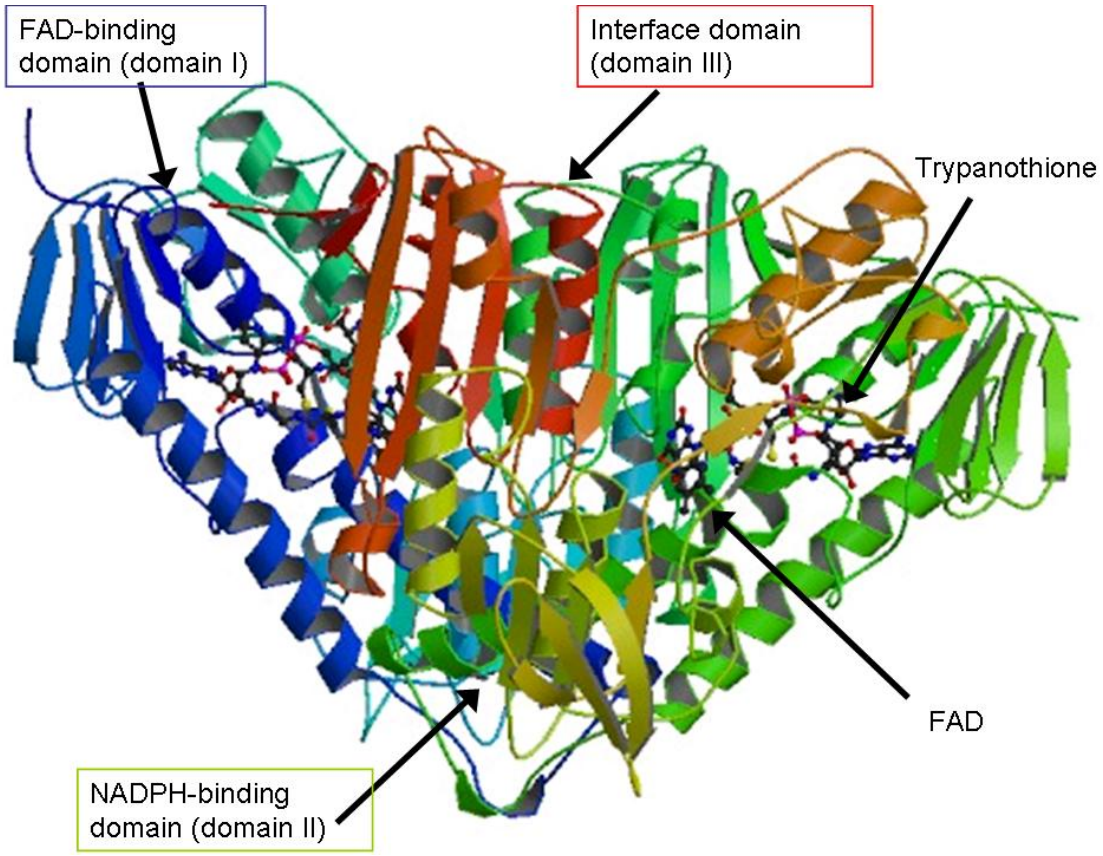


Figure 5 Organisation of the TryR dimer of *T. cruzi* crystal structure (PDB code: 1bzl): FAD and $T(S)_2$ molecules shown as ball and stick. The three distinct domains from each subunit are coloured as: the FAD-binding domain (domain I) is in blue (left-hand side of dimer); the NADPH-binding domain (domain (II) is in yellow (bottom of dimer) and the interface domain is in red/green (top middle of dimer).

The enzyme TryR and $T(SH)_2$ (**57**) play an important role in maintaining an intracellular reduced environment and in protecting these parasites against oxidative damage arising both internally, as a result of their aerobic metabolism and externally, by the immune response of the mammalian host.[128] The last two steps of trypanothione biosynthesis are the most relevant ones in terms of molecular targets, because they involve enzymes that have no mammalian counterparts.

1.4.1 The Active Site of TryR

Recent availability of the crystal structure of TryR with TS_2 has allowed for structure based identification and development of several small molecule inhibitors.[129] The crystal structure of TryR and $T(SH)_2$ (PDB code: 1bzl; Figure 6) and molecular detail provides information regarding the shape and electrostatic nature of the active site, the substrate conformation and the enzyme-ligand interactions.[130]

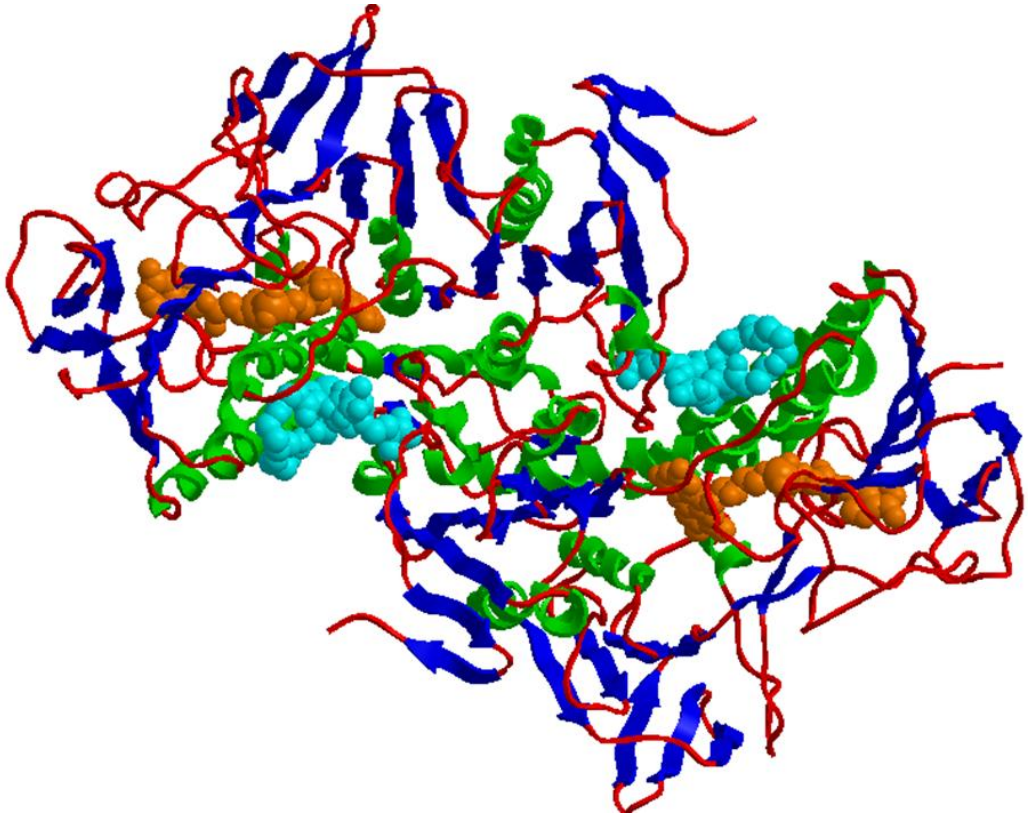
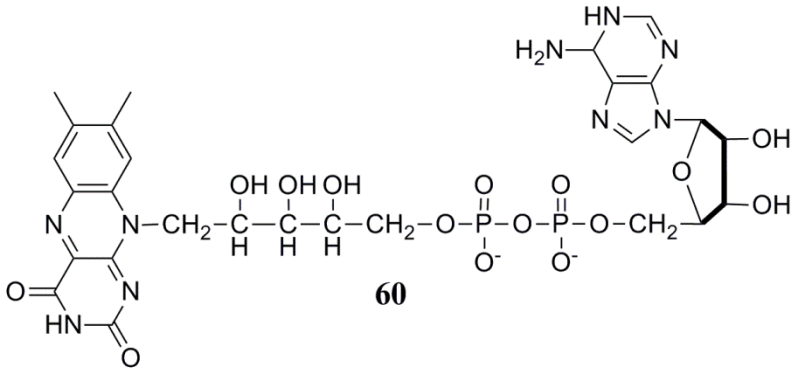


Figure 6 Crystal structure (PDB code: 1bzl) of FAD (**60**) and $T(SH)_2$ in the enzyme TryR (TryR (green, blue and red ribbon), orange structure FAD, light-blue structure $T(SH)_2$).

The disulfide-substrate-binding site is ~15 Å wide and deep, 20 Å in length and is formed by residues from the FAD-binding domain and the interface domain of the partner subunit.[130] The redox-active disulfides (Cys53 and Cys58) and active-site base (His461) residues are involved in catalysis and are located at the bottom of the cleft near the isoalloxazine ring of FAD (**60**).[130]



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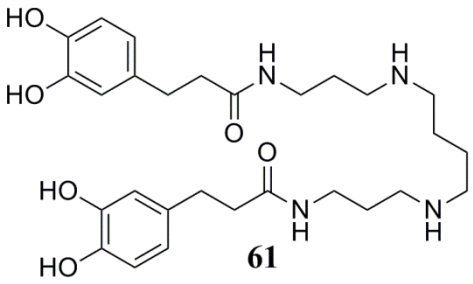
TS_2 binds asymmetrically in the rigid TryR active site adopting a U-shape whereas the tripeptide moiety adopts an extended conformation.[130] There are 25 amino acid residues implicated in the binding of TS_2 to TryR by either hydrogen bonding and/or van der Waals interactions (Table 1).

Table 1 The Van der Waals interaction residues between TS_2 and TryR.

TS_2 residue	TryR residue
GluI	Pro336, Ile339, Gly459', His461', Glu466
CysI	Val54, Tyr111, Thr335, Ile339, His461'
GlyI	Ser15, Leu18, Tyr111, Ile339
Spm	Leu18, Glu19, Trp22, Ser110, Tyr111
GluII	Val54, Val59, Lys62, Phe396, Lys399, His461', Pro462, Thr463'
CysII	Val59, Ile107, His461'
GlyII	Ile107

1.4.2 Inhibitors of TryR

A variety of molecules have been identified as inhibitors of TryR, most notably hydrophobic linear polyamine derivatives, such as bis(tetrahydrocinnamoyl) spermine, Kukoamine A (**61**) and the recently identified spermidine-based macrocyclic alkaloid, Lunarine (**62**).[129,131] Numerous studies into TryR inhibitors have been completed with the main focus being reversible and competitive inhibitors.



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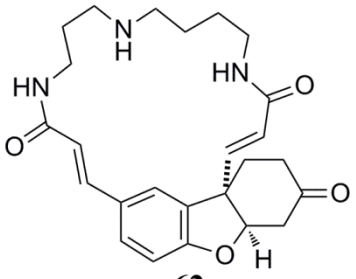


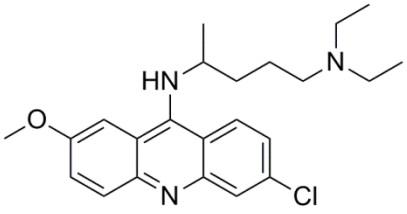
62

1.4.2.1 *Irreversible inhibitors*

Lunarine (**62**) is a competitive, irreversible inhibitor of TryR in its reduced state.[129,131] The suggested mechanism of action for Lunarine (**62**) involves conjugate addition to one of the unsaturated amide moieties within the macrocycle by a redox-active cysteine residue within the active site of TryR (Cys53).[131] This mechanism is supported by the requirement for the enzyme to be in the reduced form and the presence of a potential Michael acceptor unit within the inhibitor.[131] Recent preliminary studies have identified that the presence of basic and phenyl groups in an inhibitor molecule are crucial for TryR inhibition. For example, some ferroquine analogues, aromatic quinolines, ferrocenyl and urea substituted groups which also inhibit TryR, possess a basic amine centre but lack the tricyclic acridine moiety.[132]

1.4.2.2 *Reversible Inhibitors*

Reversible inhibitors that have received popular focus are the tricyclic compounds, quinacrine (**12**), phenothiazine (**63**) and imipramine (**64**).[133,134] The first competitive inhibitor of this type, **12**, showed a K_i value of 25 μM against *T. cruzi*.[133] These tricyclics moieties interact with the polyamine hydrophobic binding site within the TryR active site making them specific inhibitors of TryR over human GR.[133,134] This interaction has been shown in the crystal structure complex of quinacrine with TryR.[133,135]



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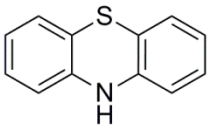

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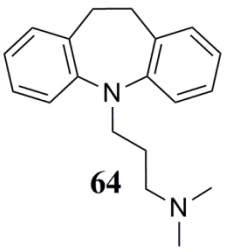

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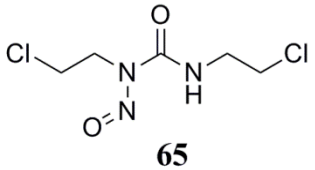
The chloro, methoxy and alkylamino functional groups of **12** are all involved in the binding of **12** to the active site of TryR. The acridine ring of **12** is fixed within the active site and is located close to the hydrophobic wall formed by Trp21 and Met113, while the alkylamino side chain points towards the side chain of Glu18 and is thought to have some effect on the orientation of binding.[133,134] The methoxy group of **12** has a kinetic effect on binding and is found within hydrogen bonding distance of the side chain oxygen Ser109. The chloro group of **12** is within the area of the ring nitrogen of Trp21, which also has a positive effect on the binding kinetics.[135] Some hydrophobically functionalised phenothiazines may also bind within an additional hydrophobic cavity (not used in $T(S)_2$ binding) known as the Z site.[134] Recent studies have synthesised quinacrine derivatives as potential TryR inhibitors. The IC_{50} values for these compounds were an order of magnitude lower than quinacrine itself and had significant activity against *L. donovani* and *T. brucei* in culture.[133]

Other known reversible inhibitors include polyamine based inhibitors. Compound **61** is an example which inhibits *Crithidia fasciculate* TryR with K_i and $K_{i'}$ values of 1.8 and 1.3 μM.[133] Spermine derivatives however have been shown to be more effective, with K_i values in the sub-micromolar range being obtained. In obtaining these values the spermine backbone was substituted with two 2-aminophenylsulphide moieties resulting in an inhibitor with a K_i of 400 nM.[133] By comparison, using 1,4-bis(3-aminopropyl)piperazine to attach two diphenylsulphide moieties gave derivatives that retained their potency of TryR inhibition.[133]

1.4.2.3 *Competitive inhibitors*

In terms of strictly competitive inhibitors, low nanomolar potencies may be required to maintain intracellular inhibition in high concentrations. This is due to the millimolar levels of $T(S)_2$ accumulating because of TryR inhibition. Compounds which inactivate TryR in irreversible or a tight-binding manner are attractive alternatives for inhibition of TryR.[132] To date there are only a few irreversible inhibitors of TryR such as Carmustine (**65**). Carmustine (**65**) is a nitrosourea drug that is not selective for TryR and also inactivates human GR.



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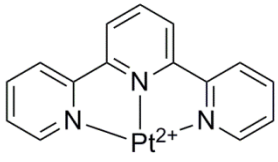

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Carmustine (**65**) acts syncatalytically by carbamoylating an active site cysteine residue which is accessible when NADPH reduces the enzyme.[133] A newer class of irreversible inhibitors include the (2,2':6',2"-terpyradine) platinum (II) (**66**) complexes. Inactivation of *T. cruzi* TryR by **66** is achieved by the complex being fixed to TryR due to coordination of the platinum ion to Cys52. This is also accompanied by increase oxidase activity.[133] Studies have also shown **66** does not affect human GR.

1.4.2.4 *Subversive substrates*

Subversive substrates (turncoat inhibitors) of TryR which have been identified include nitrofuranes and naphthoquinones. The subversive substrates are reduced by flavoenzymes (TryR, lipoamine dehydrogenase (LipDH) and GR) when the ligand inhibits the physiological reaction of the enzyme promoting an unwanted side reaction.[136] Therefore in a single-electron step the respective radical further reacts with molecular oxygen yielding superoxide anion radicals.[133] Simultaneously, the physiological reduction of $T(S)_2$ is inhibited, NADPH and O_2 are exhausted and the thiol/disulphide ratio is lowered (Figure 6). The subversive substrate is then regenerated by the reaction of the radical intermediate with molecular oxygen. Overall, the compounds act as a catalyst generating oxidative stress having a strong impact on the redox metabolism within the parasites.[133,136] The turncoat inhibitor menadione (**67**) acts as an inhibitor of TryR while furthermore catalysing the reduction of O_2 to give a superoxide radical. Binding of **67** to TryR changes the function of the enzyme from antioxidative to prooxidative (Figure 7).

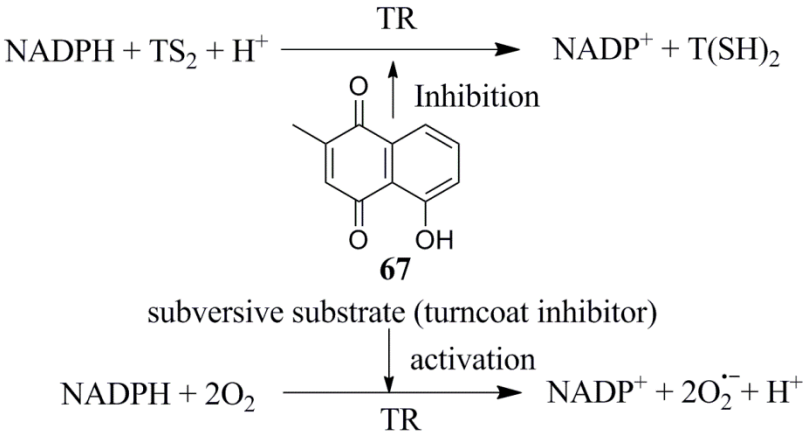
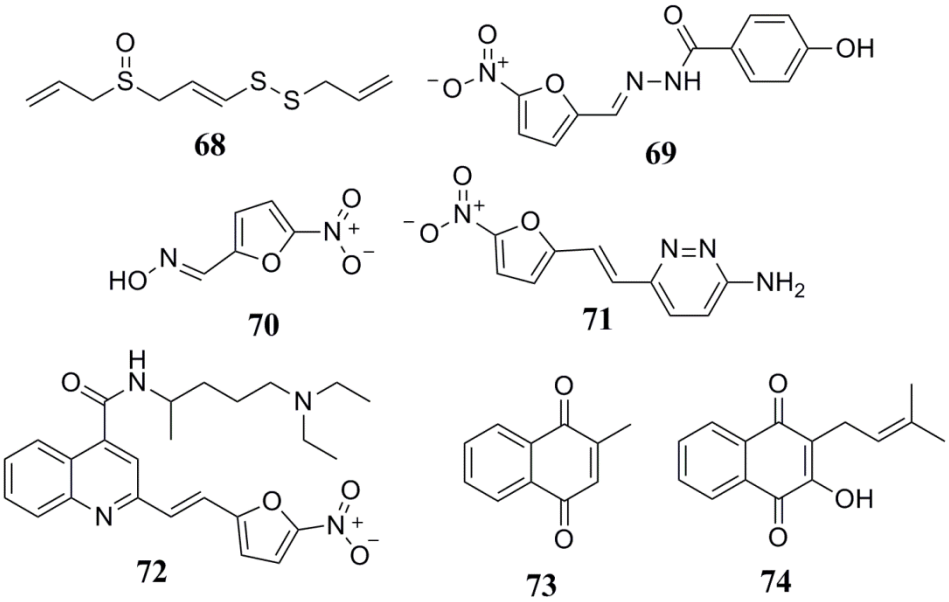


Figure 7 Inhibition of TryR by **67**

Ajoene (**68**), the major sulphur component in garlic, has shown to be a subversive substrate and covalent inhibitor of both TryR and GR with modified enzymes showing an increase in oxidase activity.[133,137] Nitrofuran derivatives Nifuroxazide (**69**), Nifuroxime (**70**) and Nifurprazine (**71**) are efficient subversive substrates of *T. cruzi* LipDH, although weaker for TryR and ineffective for GR. While Chinifur (**72**), another nitrofuran derivative, is both an inhibitor and a more efficient subversive substrate of TryR over LipDH or GR.[138] The naphthoquinone drugs, such as, **67**, Plumbagin (**73**), Lapachol (**74**) and other 1,4-naphthoquinones display notable trypanocidal activities. However, **67**, **73** and **74** act as inhibitors and subversive substrates for both TryR and GR but not LipDH, whereas 1,4-naphthoquinones are largely reversible inhibitors and subversive substrates of GR.[133] Currently there is no evidence as to the binding mode of turncoat inhibitors.



Human GR crystal structures of numerous un-/non-competitive or mixed type inhibitors have been repeated, with the compounds seen to bind within a large cavity at the interface of the homodimeric protein.[139] When GR is bound with **67**, low occupancies at the NADPH and glutathione binding sites were seen. It is possible to speculate that a possible binding site for naphthoquinones is the respective large cavity at the two-fold axis of TryR. Molecular modelling has also suggested that this site of the enzyme also exerts preference for positively charged ligands.[133]

1.5 Project aims

Trypanothione mimics and quinoline analogues were identified as potential target compounds that may provide potent inhibitors of leishmaniasis. The two-fold approach in this project thus includes a structure-guided design of TryR inhibitors, as well as a fragment-guided design of substituted quinolines based on existing bioactive compounds. Therefore, the primary aims of this project are:

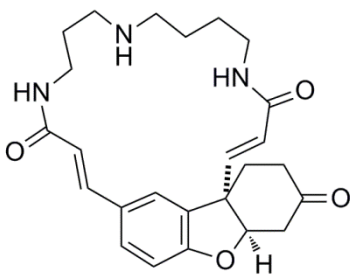
- To explore new chemical structures as potential agents against *Leishmania*
- To design and synthesise novel TryR inhibitors, specifically structural mimics of the left-hand side and bottom-side of $T(S)_2$
- To examine whether a structure activity relationship (SAR) can be established for reported and synthesised novel quinoline compounds.
- To develop and synthesise a variety of novel substituted quinolines and cinchona alkaloids.
- To biologically validate by enzymatic and cellular methods the synthetic TryR inhibitors and substituted quinolines obtained as potential inhibitors of *Leishmania*.

Chapter Two

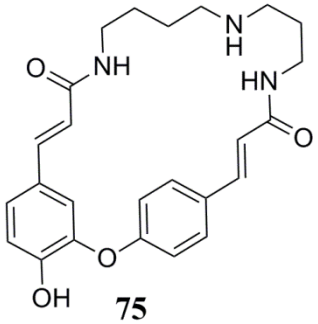
Synthesis and Design of Trypanothione Mimics

Survival of *Leishmania* parasites within host macrophages requires parasites to withstand the natural defence system associated with host mammalian cells. Trypanothione is the key molecule associated with this oxidative stress defence mechanism. Trypanothione metabolism is an attractive target for drug design as the trypanothione cycle is only present within the parasite and not the mammalian host. *Leishmania* parasites have no alternative protective measure against oxidative stress. An intracellular redox balance is maintained by reducing ($T(SH)_2$) or oxidising (TS_2) trypanothione via enzymatic transformation with TryR (TryR) (Scheme 1, Section 1.4).

The X-ray crystal structure of TyrR with TS_2 has been determined (PDB code: 1bzl) and provides information regarding the shape and electrostatic nature of the enzyme active site, as well as the conformation of the substrate and enzyme interactions (refer to figure 6, pg 29).[130] The size of the active site makes it challenging to develop small molecular mimics of TS_2. Two natural products, lunarine (**62**) and cadabacine (**75**), have been identified as potential inhibitors of TryR based on the active site information.[130] Simple inhibitors, such as the tricyclics are reviewed in Section 1.4.2; however, none have proved suitable as anti-leishmanial drugs.



62



75

A preliminary structural analysis of TS_2-TyrR binding was based on the reported X-ray data of the TS_2-TryR complex (PDB code: 1bzl). From this data, key interactions were identified leading to the design of target compounds that should maintain interactions. The key features incorporated included hydrogen binding interactions of Gly_{459} and Ser_{470} of TryR with the top-side chain of TS_2 as well as the interactions between Ser_{464} and Leu_{399} of TryR with the bottom-side chain of TS_2. In addition, deactivating the disulphide cleavage site of TS_2 and replacing it with a -S-CH_2- group should allow the mimic to interact with the active site His_{461} residue yet prevent cleavage of the molecule.

Previous work has focused on glutathiospermidine (Gsp) mimics (**76**-**84**) and their potency against glutathiospermidine synthase[122] as well as right hand-side chain mimics (**76**-**80**) of the trypanothione molecule.[124,140] All of the mimics **76**-**84** in Figure 8 are the most active to date against TryR (TS_2 mimics) and TryS (Gsp mimics), however, none have moved forward as potential treatments for parasitic diseases, *Leishmania* or *Trypanosoma*. Compounds **79**-**80** were modest competitive inhibitors of *T. cruzi* TryR, as were the initial leads **81**-**84** against *T. cruzi* TryS.

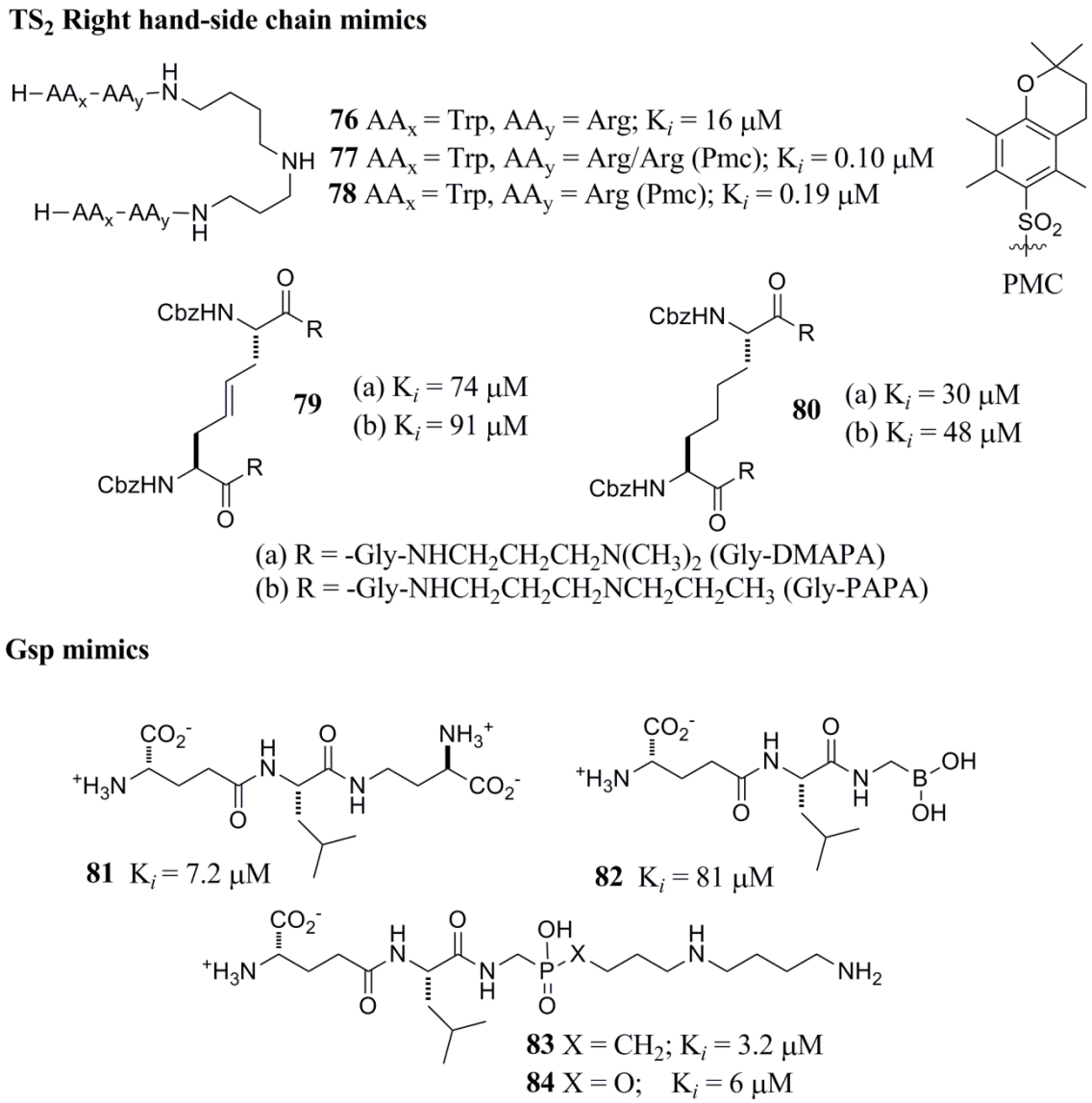


Figure 8 Potency of mimics against TryR for TS_2 mimics and against TryS for Gsp mimics[122,124,140]

In this chapter, a structure guided design and synthesis of potential TryR inhibitors is investigated. Three compounds, a TS_2 left-hand side mimic and two TS_2 side chain mimics were the targets of this investigation (Figure 9).

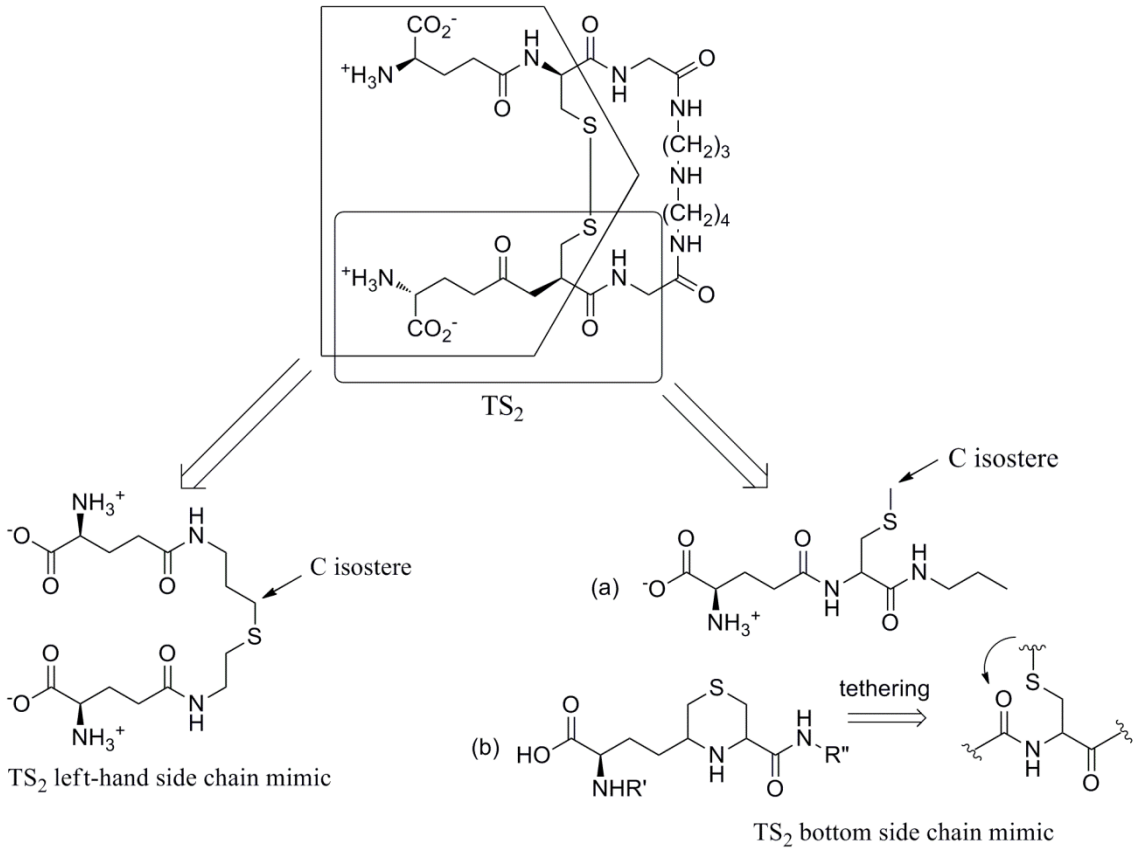


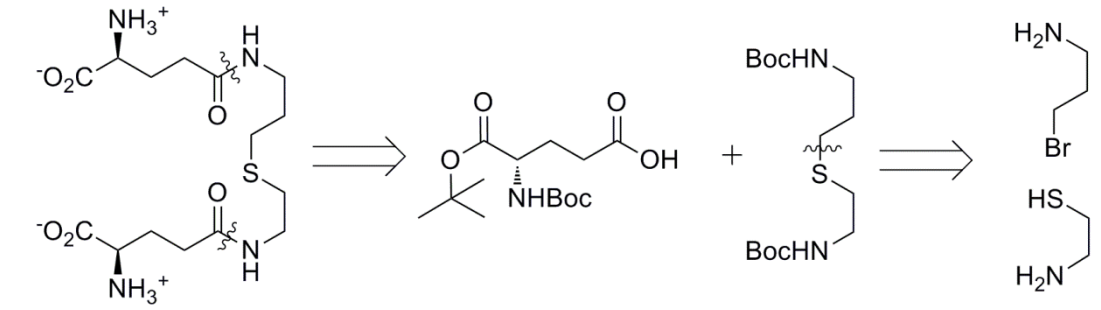
Figure 9 Proposed mimics of TS_2

2.1 Trypanothione mimics

The synthesis of trypanothione mimics was approached using a solution phase peptide techniques involving the selective protection of all functional groups not being used in amide bond generation. Protecting groups for solution phase peptide synthesis have been reviewed elsewhere.[141] The protecting group chosen for this project was *tert*-butyl carbamate (Boc) group as it is stable towards most nucleophiles and bases. *tert*-Butyl carbamate is easily cleaved under anhydrous acidic conditions producing *tert*-butyl cations which lose a proton to yield methylpropene.

2.1.1 Synthesis of TS_2 left-hand side chain mimic

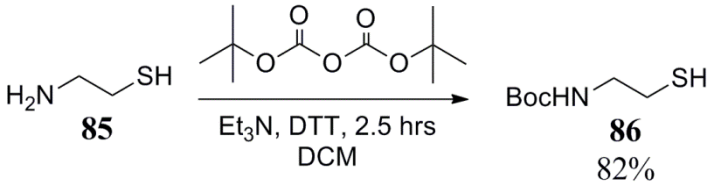
The macrocyclic ring was removed in the TS_2 left-hand side chain mimic. Maintenance of the groups with sites for hydrogen bonding should constrain the mimic in a fold-back conformation when complexed within the enzyme. A retrosynthesis is shown in Scheme 2. The synthesis would incorporate coupling of Boc-protected cysteamine hydrochloride and Boc-protected 3-bromopropylamine hydrobromide. Deprotection, coupling, using Boc- and *tert*-butyl-protected L-glutamic acid, followed by deprotection would then be to give the target TS_2 left-hand side mimic.



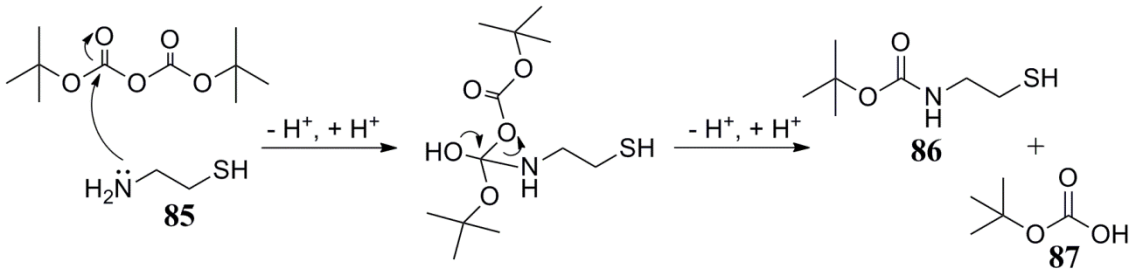
Scheme 2 Retrosynthetic analysis of TS_2 left-hand side mimic

2.1.1.1 *N-Boc Protection*

The first reaction towards the TS_2 left-hand side chain mimic was the Boc-protection of cysteamine hydrochloride **85**. Commercially available cysteamine hydrochloride **85** (1 equiv.) was treated with triethyl amine (Et_3N) (2.1 equiv.) in dichloromethane (DCM), to generate the free base. *tert*-Butyl dicarbonate (Boc_2O) (1 equiv.) and dithiotheitol (DTT) (10%) were added to the mixture and reacted for 2.5 h. Purification of the crude residue resulted in the isolation of Boc-protected cysteamine **86** (82%) (Scheme 3).



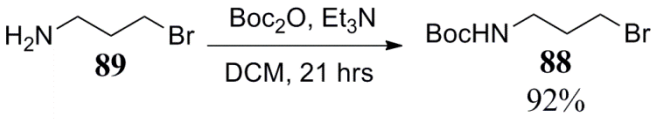
Scheme 3



Scheme 4

A mechanism for the formation of compound **86** is suggested in Scheme 4. Nucleophilic attack of the amine **85** on *tert*-butyl dicarbonate would be followed by elimination of carbonic acid (**87**) which would be expected to collapse to *t*-BuOH and CO_2. Dithiotheitol was used to prevent oxidation of the thiol to the disulphide (Scheme 4).

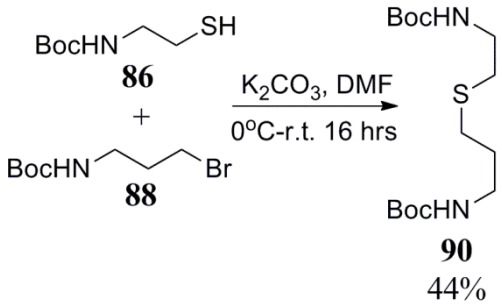
The next target compound was 3-*tert*-butyl-3-bromopropylcarbamate **88**. 3-Bromopropylamine hydrobromide **89** (1 equiv.), was treated with Et_3N (2.4 equiv.), followed by the addition of Boc_2O (1.2 equiv.) in DCM for 21 h. Purification by column chromatography gave Boc-protected bromopropylamine **88** (92%) (Scheme 5). The structure of **88** was confirmed by ^1H NMR spectroscopy. A strong CH_3 signal was observed due to *t*-butyl group.



Scheme 5

2.1.1.2 *Thio ether formation*

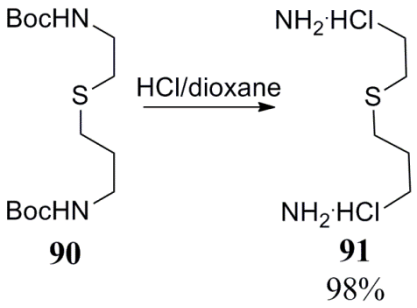
With compounds **86** and **88** in hand, the coupling of Boc-protected cysteamine **86** and Boc-protected bromopropylamine **88** to form thio ether **90** was examined (Scheme 6). Boc-protected cysteamine **86** (1 equiv.) and amine **88** (1 equiv.) were mixed with potassium carbonate (K_2CO_3) in DMF at 0 ºC and then left for 16 h at room temperature. Purification of the crude mixture by column chromatography and recrystallisation (ethyl acetate:hexane) provided compound **90** in 44% yield. Formation of di-Boc-thioether **90** proceeds via an S_N2 route.



Scheme 6

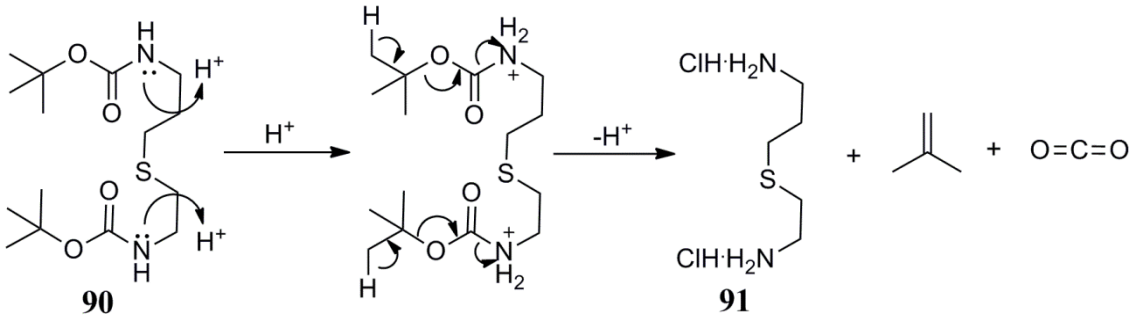
2.1.1.3 *Deprotection of amine groups*

Global Boc removal from of compound **90** was required before amide coupling could take place. Removal of the Boc-protecting groups was undertaken by adding excess HCl/dioxane (4 M, 6 equiv.) to di-Boc-thioether **90** (1 equiv.) under an inert atmosphere for 1 h at room temperature (Scheme 7). Dihydrochloride salt **91** was obtained pure (98%) as a white salt.



Scheme 7

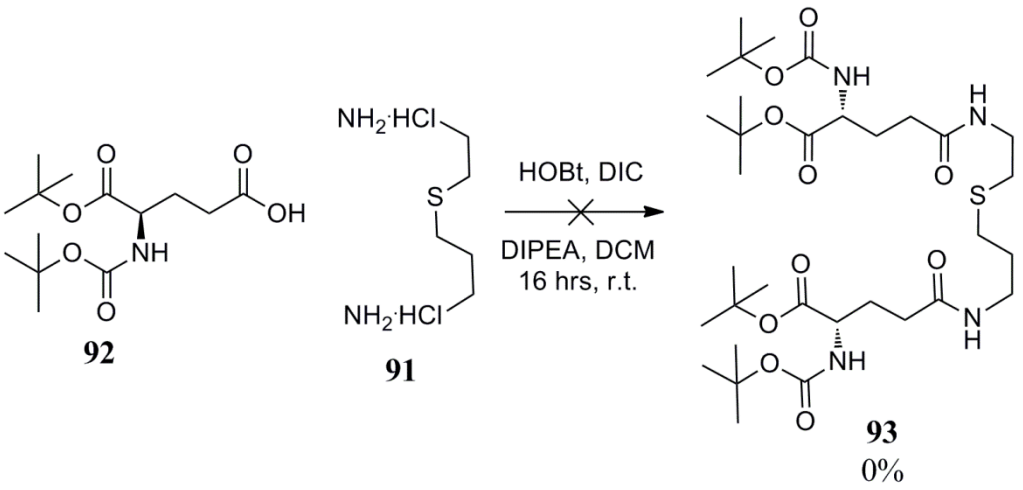
Protonation of the amine facilitates the elimination of methylpropylene and carbon dioxide (Scheme 8).



Scheme 8

2.1.1.4 *Coupling to form the amide bond*

In the next step, coupling of **91** with Boc-L-glutamic acid 1-*tert*-butyl ester (**92**) was required to form the key amide bonds. Initially, dihydrochloride salt **91** (1 equiv.) was reacted with glutamic acid **92** (2 equiv.) in DCM and cooled to 0 °C. Hydroxybenzotriazole (HOBt) (2.4 equiv.), carbodiimide (DIC) (2.2 equiv.) and *N*,*N*-diisopropylethylamine (DIPEA) (14 equiv.) were added to the mixture and reacted for 16 h at room temperature (Scheme 9). Boc-protected glutamic acid should undergo nucleophilic attack at the centre carbon of DIC. HOBt then couples to stabilise the amino acid and prevent racemisation. The amine nucleophile should then couple via the activated ester to displace the HOBt group. Chromatography of the crude material yielded a complex mixture. To identify whether compound **93** was present in this mixture 2D NMR spectroscopy (Heteronuclear Multiple Bond Coherence (gHMBC) and Heteronuclear Single Quantum Coherence (gHSQC)) was carried out.



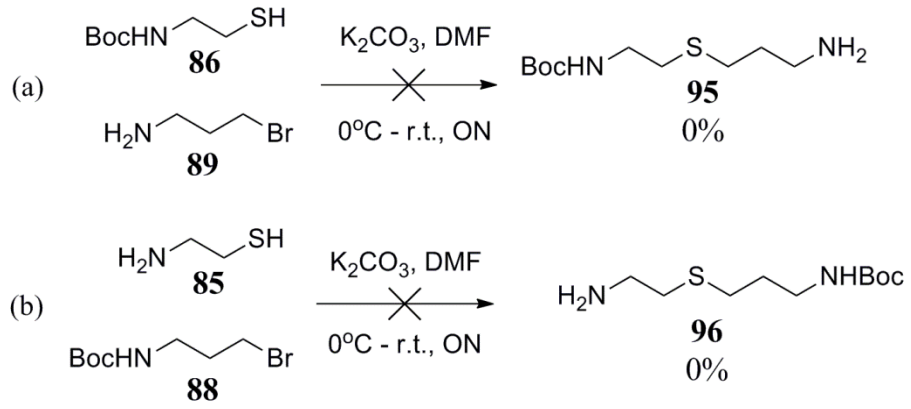
Scheme 9

The gHSQC and gHMBC spectroscopic experiments displayed a number of correlations that could be identified with sections of compound **93**. However, the absence of the Boc-carbonyl group, as well as lack of proton exchange of amide protons indicated that the Boc-protecting group had been removed during synthesis. To ensure complete deprotection and establish whether the target compound was present the above product mixture (Scheme 9) was added to a mixture of cooled HCl/dioxane (4 M, 6 equiv.) and triisopropylsilane (TIPS) (0.5%), and reacted under an inert atmosphere at room temperature for 1 h. Analysis of the crude residue by ^{1}H NMR spectroscopy and mass spectrometry indicated no formation of the expected deprotected product, glutamic acid hydrochloride salt **94** (Scheme 13). Likewise treatment of **91** with with sodium hydroxide to generate the free amine and isolation of the amine prior to reaction with glutamic acid **92** (2 equiv.), HOBt (2.4 equiv.), DIC (2.2 equiv.) and DIPEA (14 equiv.) also gave a complex mixture. Due to the large amounts of byproducts, particularly dicyclohexyl urea, isolated during purification and the difficulty of removing these byproducts an improved synthesis of **93** was required.

2.1.1.5 *Mono-Boc protection*

The direct approach to the target mimic using the diamino dihydrochloride salt, and *in situ* generation of the corresponding diamine was not successful. Attention was then turned to the synthesis of the mono amines **95** and **96** for use in subsequent trial couplings with Boc-glutamic acid. Either Boc-protected thiol **86** or Boc-protected bromine **88** was reacted with bromopropyl amine **89** or thioamine **85** respectively (Scheme 10 (a), (b)). Each reaction used conditions outlined for thio ether formation

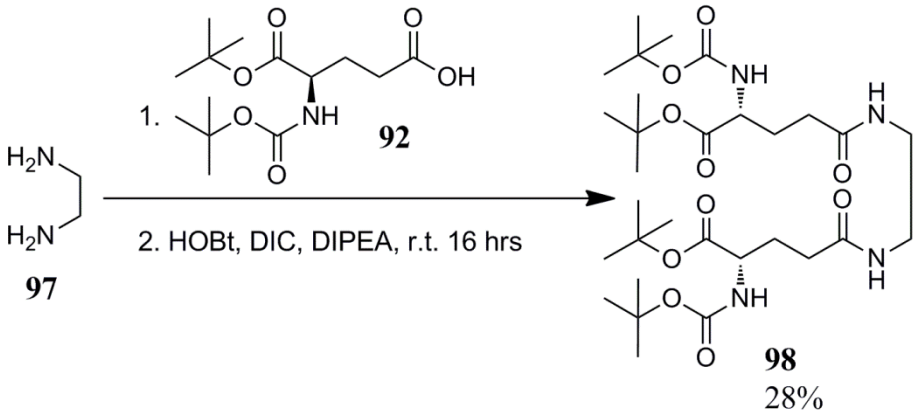
identified in Section 2.1.1.3. No identifiable products were isolated in either reaction (a) or reaction (b) (Scheme 10).



Scheme 10

2.1.1.6 *Model study*

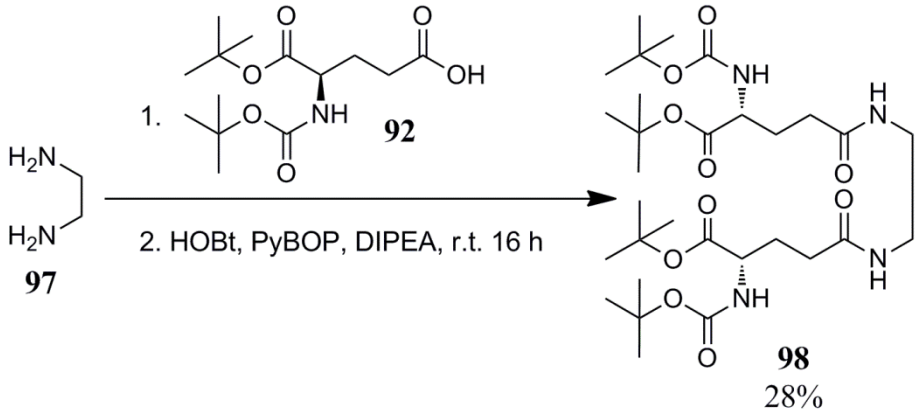
Next, a model study was undertaken using the commercially available ethane-1,2-diamine (**97**). The benefit of using the simple diamine **97** was the availability of the free amine. It was thought that the free amine form of **91** was not generated under the reaction conditions. Diamine **97** (1 equiv.) and glutamic acid (**92**) were reacted under the same conditions detailed previously. Analysis by mass spectrometry and ^{1}H NMR spectroscopy of the crude residue identified formation of dimer **98** but with byproducts that hampered purification (Scheme 11).



Scheme 11

The formation of unwanted urea byproducts from the DIC coupling reagent prompted a reconsideration of the coupling reagents used. Benzotriazolyloxy-tris[pyrrolidino]-phosphonium hexafluorophosphate (PyBOP) was chosen.

With a new coupling reagent in hand, glutamic acid **92** was reacted with DIPEA in cooled DCM under an inert atmosphere. HOBt (2.4 equiv.) and PyBOP (1 equiv.) were added and the reaction mixture stirred for 30 min at room temperature. Diamine **97** (1 equiv.) was added to the reaction mix under an inert atmosphere and reacted at room temperature for 16 h (Scheme 12). Purification of the crude mixture by column chromatography isolated diamide **98** with a slight impurity observed in the ^{1}H NMR spectrum. Semi-preparative HPLC was used to isolate pure diamide **98** in an isolated yield of 28%. This reaction established successful reaction conditions for use in the synthesis of the target compound **93**.



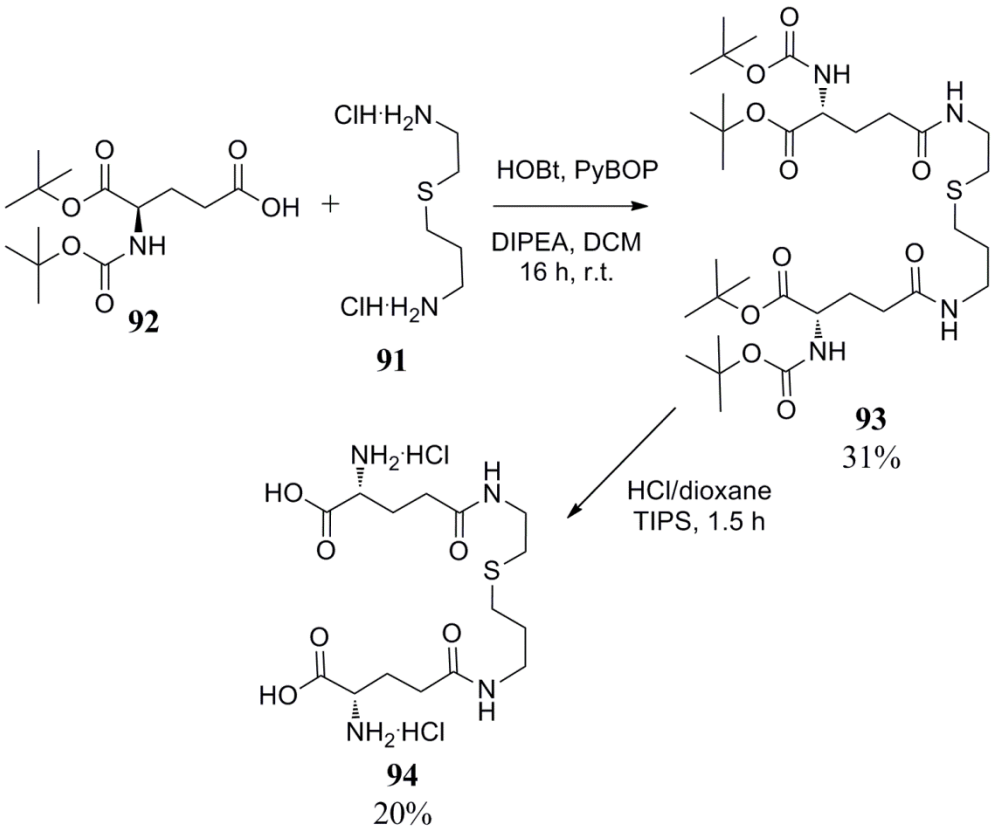
Scheme 12

2.1.1.7 *Diamide coupling of diamine hydrochloride salt **91***

The success of the above reaction indicated that it was necessary to generate the free amines of hydrochloride salt **91** and use PyBOP as the new coupling reagent. To generate the free amine, amine hydrochloride salt **91** (1 equiv.) and DIPEA (7 equiv.) were cooled in DMF under an inert atmosphere and reacted for 1 h at room temperature. In a separate reaction, glutamic acid **92** (2 equiv.) and DIPEA (7 equiv.) were reacted in DMF. HOBt (2.4 equiv.) and PyBOP (2 equiv.) were then added to the glutamic acid reaction mixture. Next, the two reaction mixtures were combined and reacted for 16 h under an inert atmosphere (Scheme 13). ^{1}H NMR spectroscopy identified diamide **93** contaminated with about 10% impurities. Purification of the **93** mixture by silica gel column chromatography and attempts at recrystallization were unable to remove the impurities. Instead, semi-preparative HPLC gave pure diamine **93** in an isolated yield of 31%.

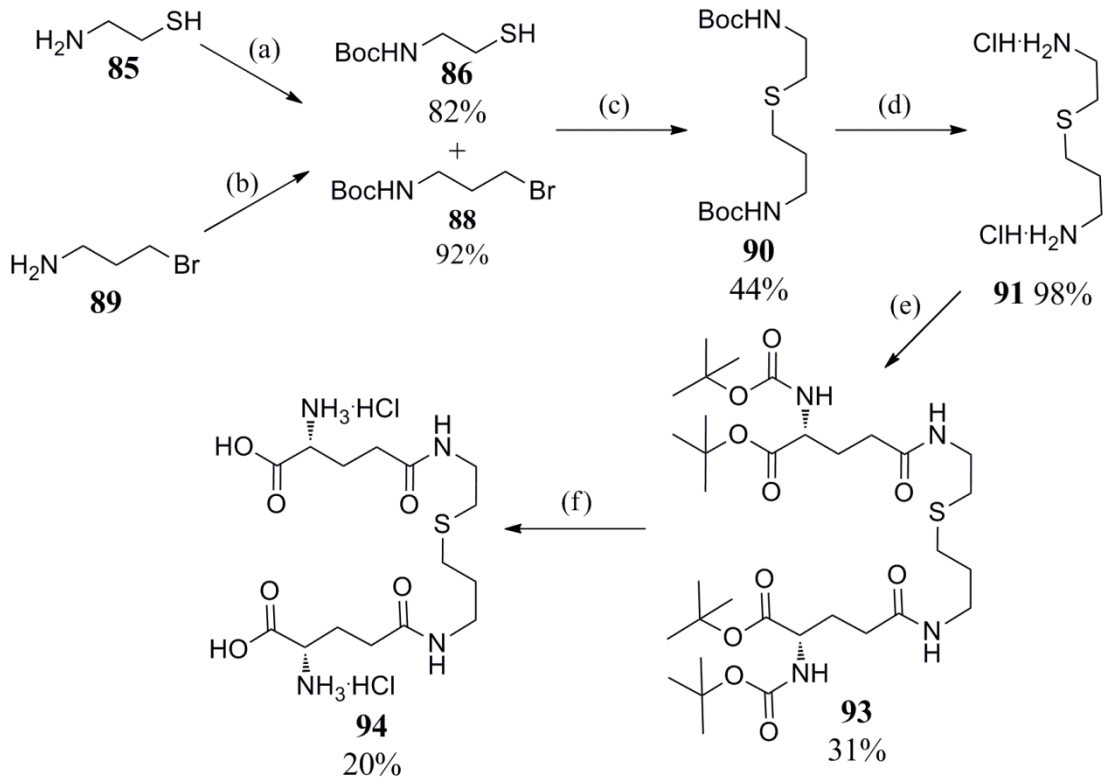
2.1.1.8 *Deprotection of diamide **93***

Diamide **93** (containing minor impurities) was deprotected in an attempt to remove the impurities. Deprotection of the Boc- and *tert*-butyl carbamate followed procedures previously outlined. Compound **93** (1 equiv.) was reacted with cooled HCl/dioxane (4 M, 6 equiv.) and TIPS (0.5%) under an inert atmosphere (Scheme 13). Analysis of the crude residue by ^{1}H NMR spectroscopy and mass spectrometry indicated formation of glutamic acid hydrochloride salt **94**. Purification of hydrochloride salt **94** by semi-preparative HPLC gave pure hydrochloride salt **94** in an isolated yield of 20%.



Scheme 13

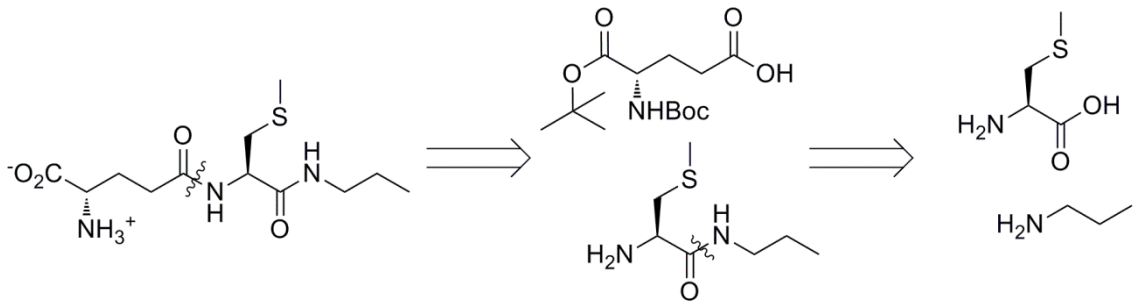
In summary, the synthesis of compound **94** was achieved in 6 steps from amines **85** and **89** in an overall yield of 2.3% (Scheme 14).



Scheme 14 Synthetic route for the preparation of TS_2 left-hand side chain mimic **94**. Reagents and conditions: (a) Et_3N, DTT, Boc_2O, DCM, r.t., 2.5 h. (b) Et_3N, Boc_2O, DCM, r.t., 21 h (c) K_2CO_3, DMF, 0 ºC-r.t., 16 h (d) HCl/dioxane (4 M), r.t., 1 h (e) *i.* Boc-L-glutamic acid 1-tert-butyl ester, DIPEA, DCM; *ii.* HOBt, PyBOP, DIPEA, r.t. 16 h (f) HCl/Dioxane (4 M), TIPS, r.t. 1 h.

2.2 Synthesis of TS_2 side chain mimic

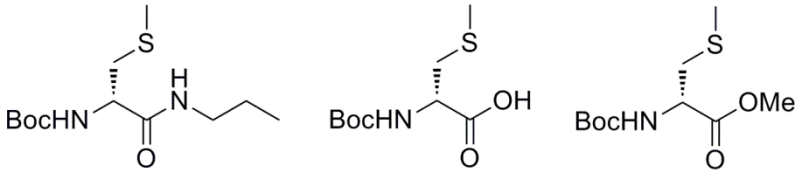
The next mimic considered was the TS_2 side chain mimic. Key features are functional groups that maintain hydrogen bonding interactions and a sulphur group for active site interactions. These potential inhibitor mimics require hydrogen bonding interactions across the side-chain of TS_2 as well as interaction with Lys_{402} and a side chain carbonyl of TS_2. A retrosynthetic analysis of target TS_2 side chain mimic (Target 1) is provided in Scheme 15.



Scheme 15 Retrosynthetic analysis of TS_2 side chain mimic (Target 1)

2.2.1 Proposed synthesis of a TS_2 side chain mimic (a)

Amide **99** could be obtained either by standard peptide coupling of acid **100** or aminolysis of methyl ester **101**. Thus the S-methyl ester **101** and Boc-S-methyl cysteine **100** were the initial synthetic targets.

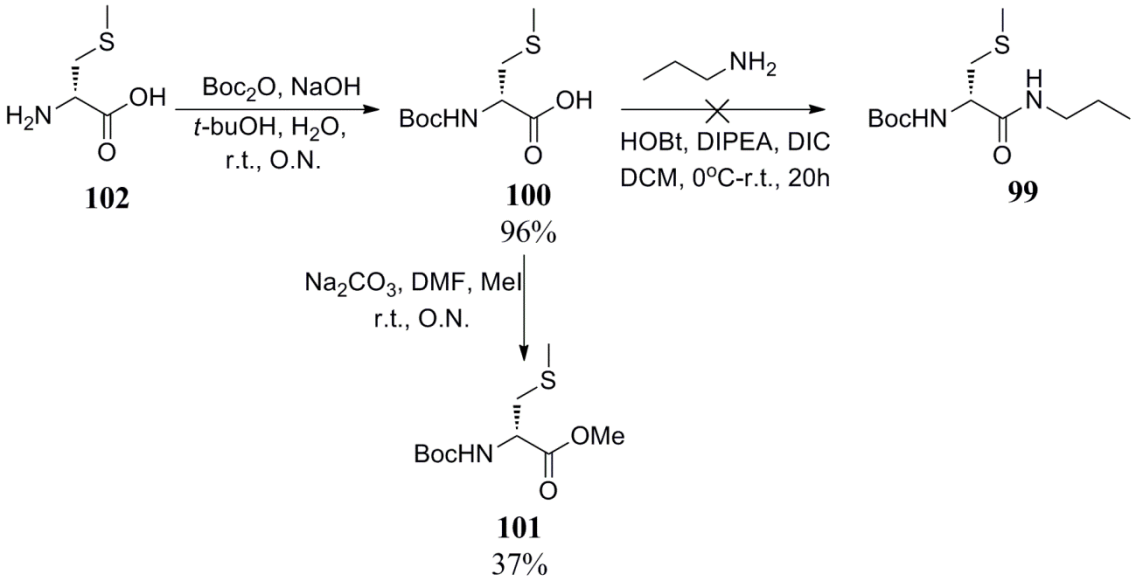


99 **100** **101**

2.2.2 Formation of compound 99

2.2.2.1 *N-Boc protection of S-methyl-L-cysteine **102***

Protection of S-methyl-L-cysteine (**102**) was required to prevent further side-reactions later in the synthetic sequence. The amine group of **102** was Boc-protected by reaction of cysteine **102** (1 equiv.), Boc_2O (1 equiv.), with sodium hydroxide (1 equiv.), in water (5 mL) and *tert*-butanol (2 mL) (Scheme 16). Equal equivalents of base were used to avoid racemisation of cysteine **102**. Boc-protected cysteine **100** was obtained in an excellent yield of 96%.



102 **100** 96% **99**

101 37%

Scheme 16

2.2.2.2 *Amide coupling*

Attempts to generate amide **99** from cysteine **100** proved difficult. Initially, cysteine **100** (1 equiv.) was reacted with propylamine (1 equiv.), DIC (1.1 equiv.), DIPEA (7 equiv.) and HOBt (1.2 equiv.) in DCM under an inert atmosphere for 20 h (Scheme 16; Table 3, Entry 1). Analysis of the crude mixture by ^{1}H NMR spectroscopy indicated that starting materials as well as a large amount of urea were present. The byproducts were unable to be removed from the mixture using column chromatography and the desired amide **99** was not isolated.

Due to the lack of formation of amide **99**, aminolysis of ester **101** was considered. Methyl iodide (MeI) (5 equiv.) was added to a solution of cysteine **100** (1 equiv.) and sodium bicarbonate ($NaHCO_3$) (2 equiv.) in DMF and reacted for 16 h. Purification of the crude mixture by column chromatography gave ester **101** in moderate yield (30%) (Scheme 16; Table 2, Entry 1). Reaction optimisation of **101**, using a stronger base, sodium carbonate (Na_2CO_3), and reduced equivalents of methyl iodide (2.5 equiv.) gave ester **101** in increased yield (37%) (Table 2, Entry 2). Increasing the scale (0.24 g to 5 g) of the reaction, using the above conditions, decreased the formation of ester **101** (20%) (Table 2, Entry 3). In a further reaction, a temperature of 65 ºC (Table 2, Entry 4) was used in order to completely dissolve the base. However, ester **101** (20%) was still isolated in only low yield. The formation of S-methyl ester **101** could not be achieved on a larger scale by methylation of acid **100** using methyl iodide under these conditions.

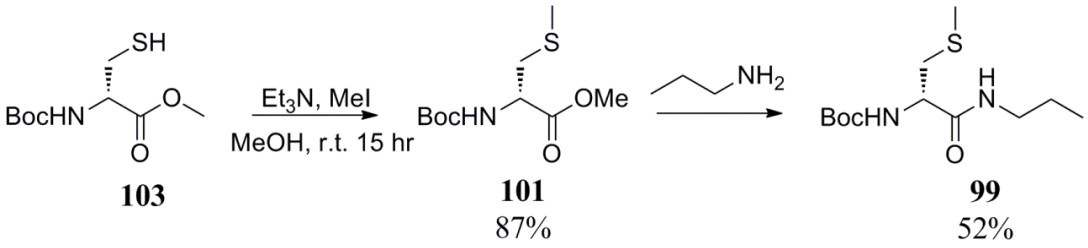
Table 2 Different reaction conditions for the esterification of **100**

Entry	Conditions	Yield (%)
1	$NaHCO_3$, MeI (5eq), DMF, r.t., O.N.	30
2	Na_2CO_3, MeI (2.5eq), DMF, r.t, O.N.*	37
3	Na_2CO_3, MeI, DMF, r.t, O.N.*†	20
4	Na_2CO_3, MeI, DMF, 65^{o}C, O.N.*†	20

*Base and acid **100** left to stir for 30 min before addition of the halide. †Large scale.

Since the esterification procedure was low yielding and the use of diazomethane is hazardous, an alternate route was considered. The commercial availability of thiol **103** allowed for a methyl group to be added via the thiol group rather than the carboxylic acid. Thiol **103** (1 equiv.) was reacted with triethylamine (2.7 equiv.) and methyl iodide

(1.3 equiv.) in methanol for 16 h at room temperature (Scheme 17). Analysis of purified S-methyl ester **101** by ^{1}H NMR spectroscopy indicated ester **101** in high yield (87%).



103 **101** 87% **99** 52%

Scheme 17

2.2.2.3 *Amide bond formation*

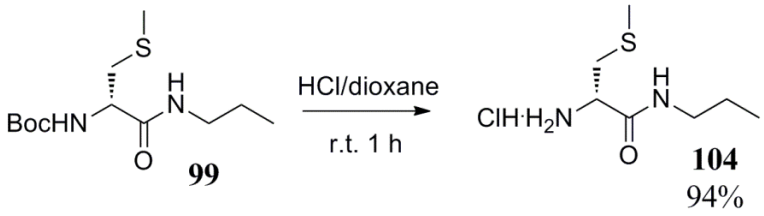
With the ester **101** in hand, formation of amide **99** was re-examined. Ester **101** was treated with equal equivalents of propylamine (1 equiv.) and methanol (MeOH) (1 equiv.) (Table 3, Entry 2). Purification of the residue obtained by column chromatography gave amide **99** in low yield (18%) along with unreacted starting material ester **101** (72%). Further attempts to generate amide **99** via the above method proved ineffective. Instead, ester **101** (1 equiv.) was reacted neat with propylamine (1 equiv.) in a direct aminolysis reaction. Amide **99** was obtained in moderate yield (52%) (Table 3, Entry 3) (Scheme 17).

Table 3 Different reaction conditions for propylamine addition

Entry	Reactant	Reagents	Yield (%)
1	**100**	Propylamine, DCC, DIPEA, HOBt,	0
2	**101**	Propylamine, MeOH	18
3	**101**	Propylamine	52

2.2.2.4 *N-Boc deprotection of amide **99***

The Boc-protecting group of amide **99** was then deprotected by treatment with HCl/dioxane (4 M). ^{1}H NMR spectroscopy identified formation of propylamide hydrochloride salt **104** (94%) in high yield (Scheme 18).

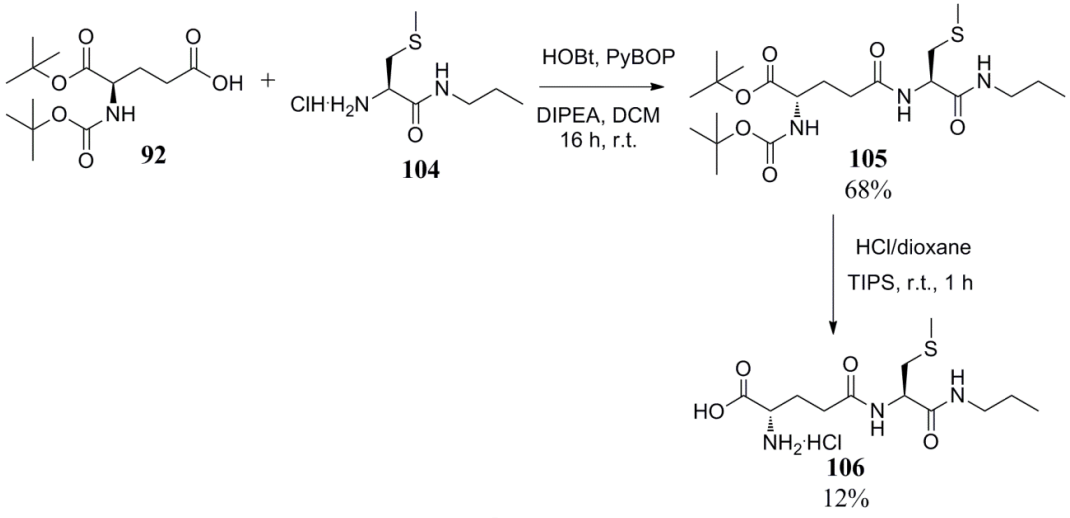


Scheme 18

2.2.2.5 *Formation of amides **105** and **106***

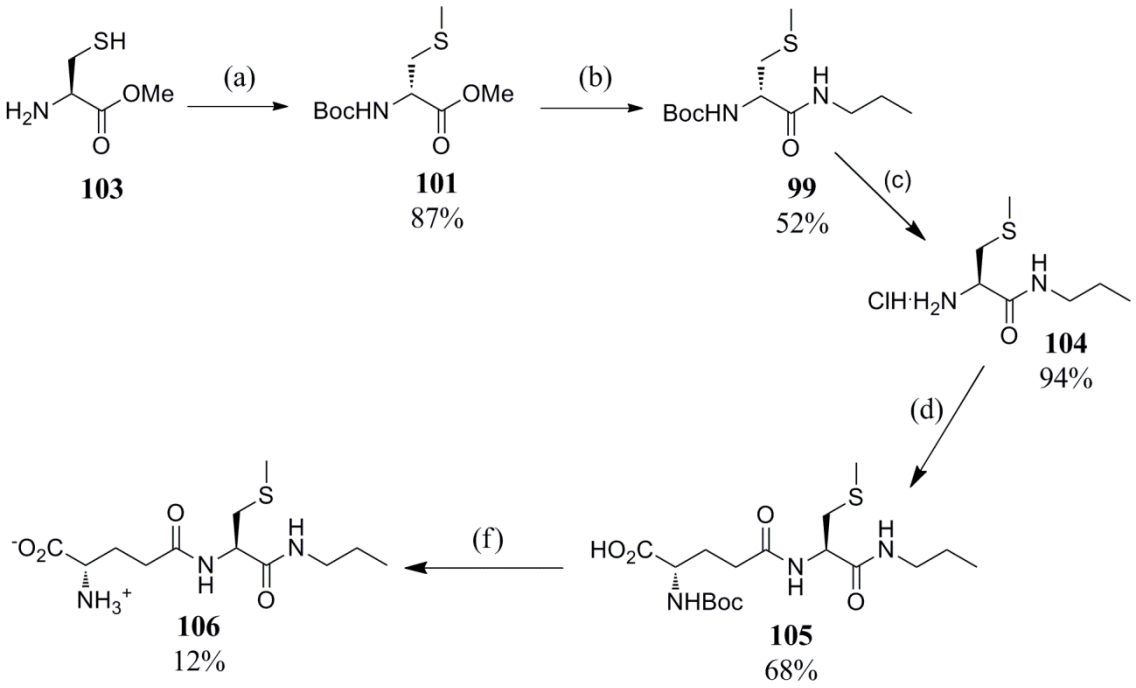
Application of the chemistry used for the synthesis of amide **93** and amide hydrochloride salt **94** was now considered for the synthesis of amide **105** and amide hydrochloride salt **106** (Scheme 19). Initially the free amine of amide hydrochloride salt **104** (1 equiv.) was generated by reacting **104** with DIPEA (3.5 equiv.) in DMF. In a separate reaction, glutamic acid **92** (1 equiv.), DIPEA (3.5 equiv.), HOBt (1.2 equiv.) and PyBOP (1 equiv.) were reacted. The two mixtures were combined and reacted for 16 h. ^{1}H NMR spectroscopy of the crude residue identified the presence of amide **105** contaminated with 12% impurities. Purification of **105** by silica gel column chromatography and attempts at recrystallisation were unsuccessful in removing the impurities. Instead, semi-preparative HPLC gave pure amide **105** in an isolated yield of 68%.

Amide **105** was deprotected with HCl/dioxane (6 equiv.) and TIPS (0.5%) at room temperature (Scheme 19). Analysis of the crude residue by ^{1}H NMR spectroscopy and mass spectrometry indicated formation of glutamic acid hydrochloride salt **106**. Purification of hydrochloride salt **106** by semi-preparative HPLC gave the target hydrochloride salt **94** in an isolated yield of 12%.



Scheme 19

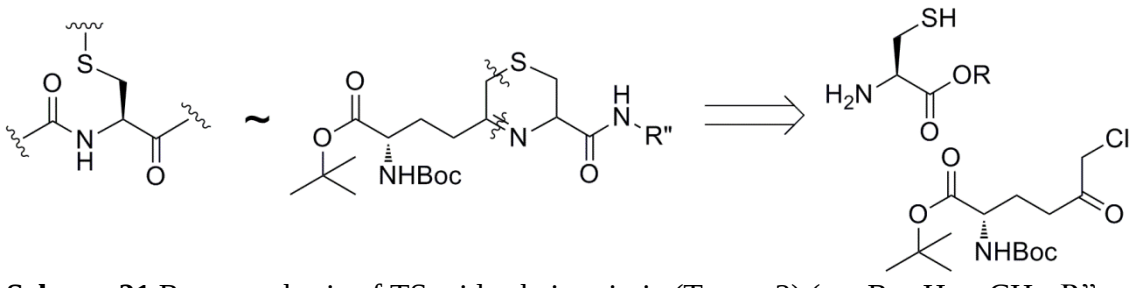
In summary, the synthesis of compound **106** was achieved in 5 steps from cysteamine **103** in an overall yield of 3.5% (Scheme 20).



Scheme 20 Synthetic route for the preparation of TS_2 side chain mimic **106**. Reagents and conditions: (a) MeOH, Et_3N, MeI, r.t., 16 h (b) propylamine, r.t., 16 h (c) HCl/Dioxane (4M), r.t., 1 h, (d) *i*. Boc-L-glutamic acid 1-*tert*-butyl ester, DIPEA, DCM, *ii*. HOBt, PyBOP, DIPEA, DCM, r.t. 16 h (e) HCl/Dioxane (4M), TIPS, r.t. 1 h.

2.3 Trial study for synthesis of a TS_2 side chain mimic (Target 2)

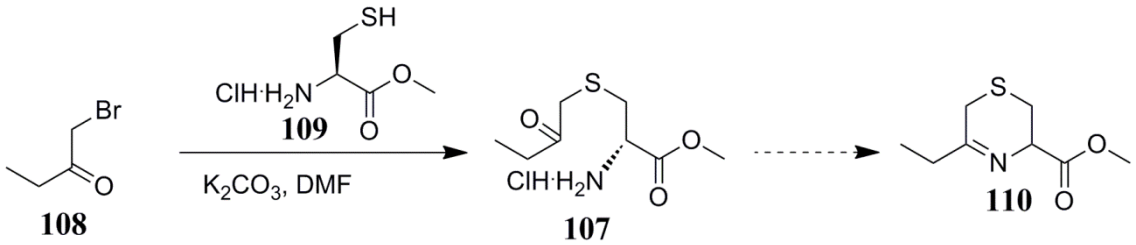
The second approach to the TS2 side chain mimic formation involves the use of thiazines. Thiazines provide a nonpeptide heterocyclic analog in which the peptide backbone is tethered. The inclusion of the thiazine moiety restricts the conformation of the peptide backbone, while allowing for the peptide to still retain its functionality. Thiazines can be conveniently accessed from the cyclocondensation of a chloromethyl ketone with a cysteine methylester (Scheme 21).



Scheme 21 Retrosynthesis of TS_2 side chain mimic (Target 2) (eg: R = H or CH_3, R" = CH_3).

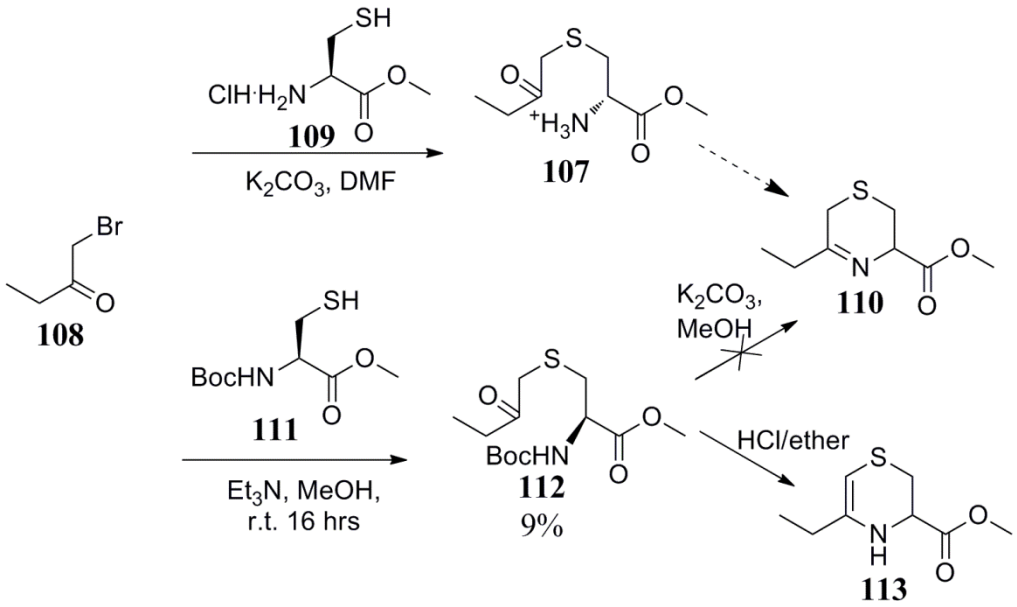
2.3.1 Methyl-2-amino-3-((2-oxobutyl)thio)propanoate hydrochloride (107)

A trial study (Scheme 22) for the synthesis of the second TS_2 side chain mimic (Figure 9b) was attempted first. Reaction of commercially available 1-bromo-2-butanone (**108**) (1 equiv.) with potassium carbonate (1.1 equiv.) and cysteine hydrochloride salt **109** in DMF for 16 h (Scheme 22) gave a complex mixture.



Scheme 22

Whereas when boc-protected cysteine **111**, bromoketone **108** and Et_3N were reacted for 16 h, protected amine **112** (9%) was obtained. Treatment of **112** with potassium carbonate (4 equiv.) in methanol at 50 ºC gave recovered starting material **112** (Scheme 23).



Scheme 23

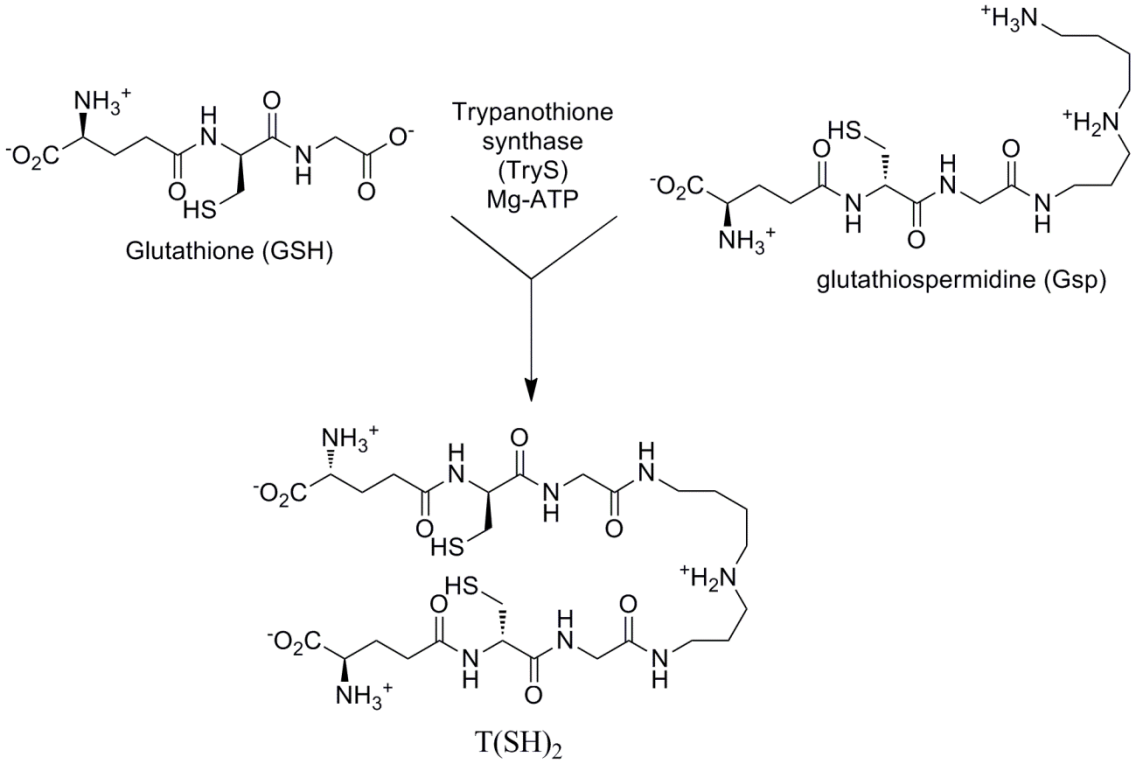
The lack of imine formation **110** was likely due to the protecting group on the amine of **112** remaining intact. Deprotection of amine **112** with HCl/ether (1M, 10 equiv.) for 2.5 h gave an inseperable mixture of hydrochloride salt **107** (37%) and alkene **113** (62%). Amine **112** (1 equiv.), potassium carbonate (4 equiv.) and methanol were irradiated in a

microwave at 100 ºC for 10 min. Analysis of the crude mixture by ^{1}H NMR spectroscopy showed no identifiable products.

In situ generation (with K_2CO_3) of the free amine of deprotected **112** was unsuccessful, and attempts to deprotonate amine hydrochloride salt **107** with other bases, DIPEA and potassium hydroxide, and isolate the free amine resulted in complex mixtures. Other targets were now considered.

2.4 Antiparasitic Studies

Trypanothione inhibitors **93**, **94**, **105**, **106** and **98** were assessed for their ability to inhibit the growth of *L. major* MHOM/IL/81/FE/BNI amastigotes in mouse macrophage cells by the candidate (K. Reynolds) under the supervision of Associate Professor Heinrick Korner at James Cook University, Townsville. Inhibitiors **94** and **98** were also assessed for their inhibition of *T. brucei* trypanothione synthase (TryS). TryS is the adjacent key enzyme in the metabolic pathway and is responsible for the formation of $T(SH)_2$ from the precursors glutathione and glutathiospermidine (Scheme 24). TryS provides an alternate control point for the production of trypanothione. TryS assays were undertaken by Associate Professor David J. Young at the Southwestern Medical Centre, Dallas under the guidance of Professor. Margaret Phillips.



Scheme 24

2.4.1 Nitrite and parasite inhibition assays

Trypanothione mimics (**93**, **94**, **98**, **105** and **106**) were screened against *L. major* promastigotes to determine inhibition concentrations. This was followed by nitrite assays to determine the production of NO by macrophage cells. The activities of the mimics **93**, **94**, **98**, **105** and **106** trypanothione inhibitors are outlined in Table 4. Compounds **93**, **94** and **98** were found to be cytotoxic against the parasites at 100 μM. However, inhibitors **105** and **106** showed a maximal inhibition at 60% at 100 μM and 50% at 100 μM respectively.

The low activities identified for compounds **93**, **94**, **98**, **105** and **106** against *L. major* could be due to the inability of the compounds to reach the active site in the cellular assay. The nitrite assays for compounds **93**, **94**, **98**, **105** and **106** also showed no nitrite production indicating they are not activating TryR or any other biological process at a cellular level.

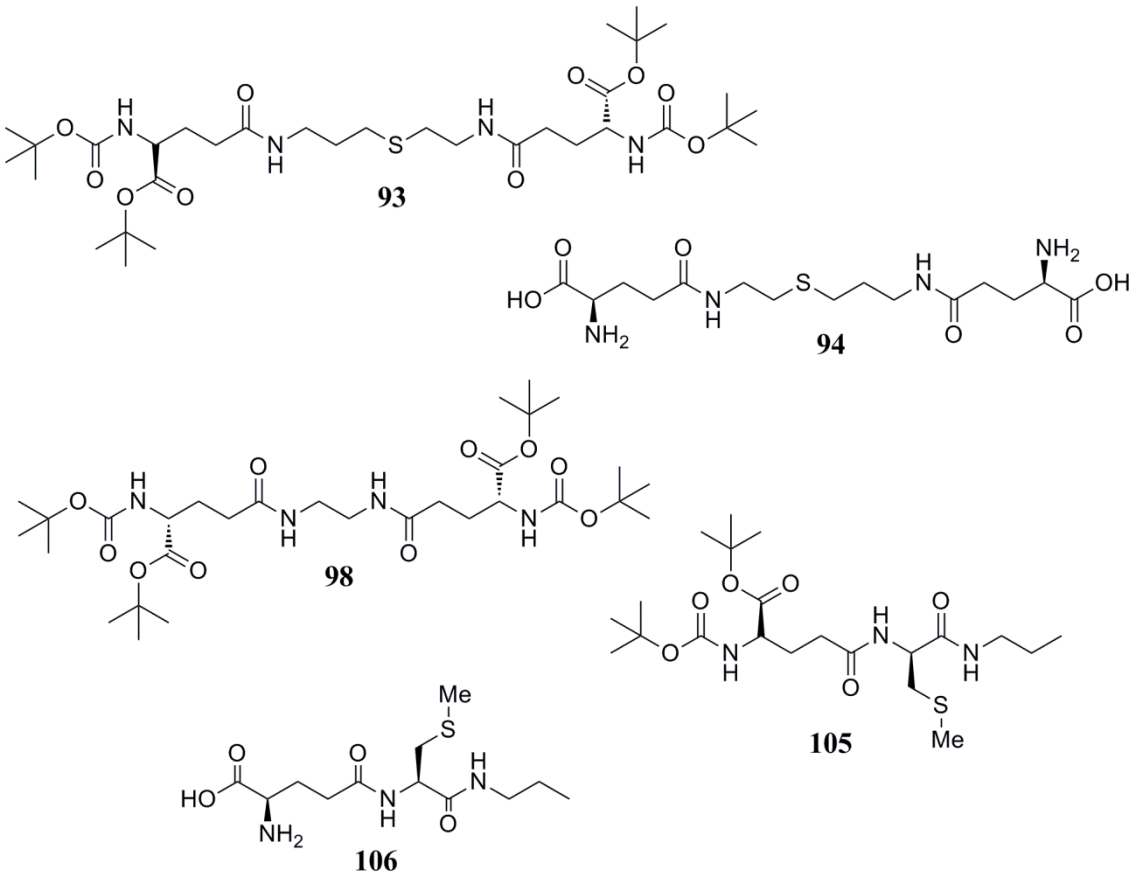


Table 4 Inhibition values and Lipinski calculations of compounds **93**, **94**, **98**, **105** and **106** tested against *L. major*

Compound	*Inhibition value (μM)*	*MW*	*LogP*	*LogS*	*# ROTB*‡	*# ON*‡	*# OHNH*‡	*# Lipinski Violations*
93	100†	704.41	3.41	-5.84	25	14	4	3
94	100†	394.47	2.73	-4.97	21	14	4	3
98	100†	630.77	2.73	-4.97	21	14	4	3
105	60% at 100	461.25	2.25	-4.56	15	9	3	0
106	50% at 100	306.39	-4.16	-4.34	10	7	6	1

†Cytotoxic, ‡ROTB (rotational bonds), ON (hydrogen bond acceptor), OHNH (hydrogen bond donor)[142]

2.4.2 Trypanothione Synthase Assay

TryS enzyme assays were used to screen trypanothione mimics **94** and **106**. Preliminary inhibition studies of compounds **94** and **106** indicated that compound **106** was inactive and that compound **94** partially inhibited *T. Bruncei* TryS at >45 μM. A K_i was not obtained for compound **94**.

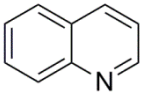
2.5 Conclusion

Two novel tryanothione mimics **94** and **106** were designed *de novo*. Mimic **94** was successfully synthesised in 6 steps with an overall yield of 2.3%. Mimic **106** was synthesised in 5 steps with an overall yield of 3.5%. Unfortunately, thiazine mimic **110** (Target 2) was unable to be synthesised due to its instability. Both the protected and deprotected mimics **93**, **94**, **105** and **106** along with compound **98**, were tested for intracellular activity against *L. major*. However, all five compounds proved inactive, likely due to the inability on the compound to reach the TryR target site within the parasite. Two mimics, **94** and **106**, were assessed for inhibition of *T. brucei* TryS. Compound **94** showed partial inhibition at >45 μM. In future work, all five compounds should be tested against *Leishmania* and / or *Trypanosoma* TryR.

Chapter Three

Quinoline Synthesis Using the Doebner-Miller Reaction

Quinoline compounds have been used in a variety of chemical processes for over 100 years and have attracted wide interest from synthetic and medicinal chemists. The quinoline (**114**) ring occurs in a range of biologically active natural products and drug-like compounds as discussed in Chapter One.[64,65,143] Quinoline is used as a catalyst, a corrosion inhibitor (in dye manufacturing) as part of agricultural chemicals and in many synthetic processes. As well as being actively used in pharmaceutical treatments, quinolines exhibit a range of antiparasitic activities in malaria,[144] tuberculosis[71,145] and leishmaniasis[74,146] (Chapter One). Our interest in the quinoline ring systems lies in the wide ranging bioactivity of such heterocycles against the parasitic disease leishmaniasis as discussed in Section 1.3.

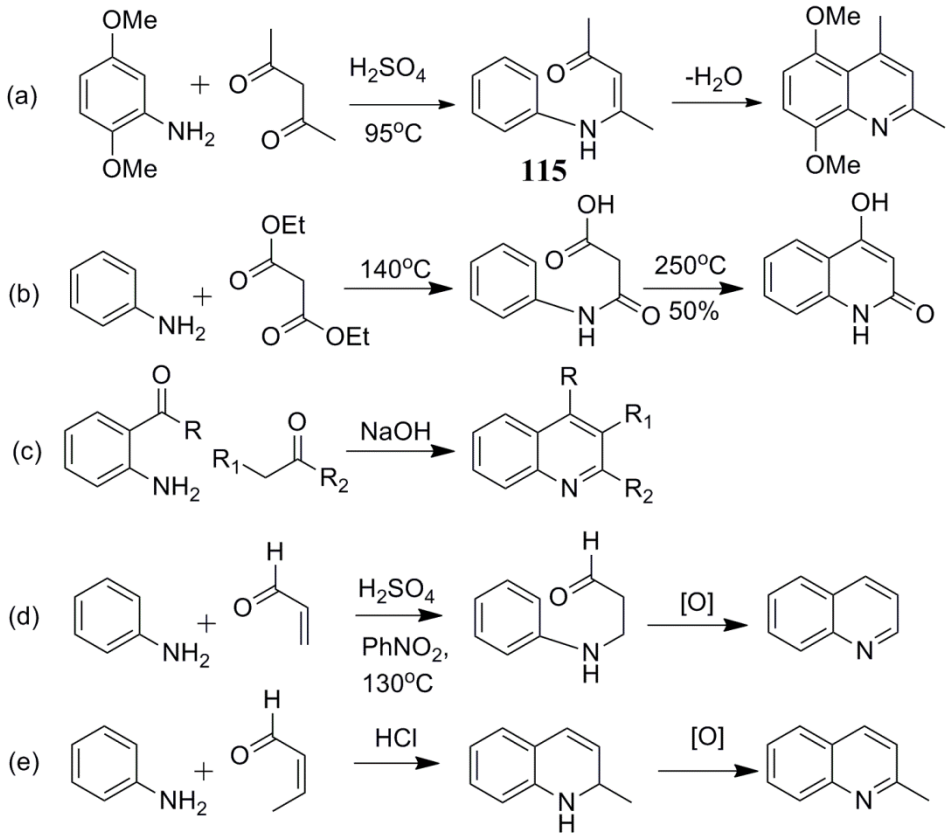


114

Syntheses have been developed for quinoline and its analogues. Some common approaches to access a quinoline ring system are based on the cyclisation of non-heterocyclic precursors (Scheme 25). Condensation of aniline with a 1,3-dicarbonyl compound gave a phenylamino intermediate (**115**) which was cyclised with acid using the Combes synthesis[147-149] (Scheme 25 (a)). If the 1,3-dicarbonyl component is at the 1,3-keto acid oxidation level, the product formed is a quinolone, as in the Conrad-Limpach-Knorr Reaction[147,148,150] (Scheme 25 (b)). The Friedlander synthesis involves condensation of *o*-aminobenzaldehyde with an aldehyde or ketone in the presence of base (Scheme 25 (c)).[151] This synthesis is valuable for the preparation of quinolines substituted at the 3-position, which are difficult to access via other methods.[152] In both the Skraup reaction[147,153,154] and Doebner-Miller synthesis[155] condensation of aniline with α,β-unsaturated carbonyl compound in the presence of an oxidizing agent gives quinoline derivatives (Scheme 25 (d), (e)). The functionalization of quinolones is also achieved by the conversion of quinoline-N-oxides to the 2-aminoquinoline,[156] further allowing for Suzuki-type couplings to take place,[157] or by means of ring functionalization using Grignard reactions from the heteroaromatic tosylate.[158]

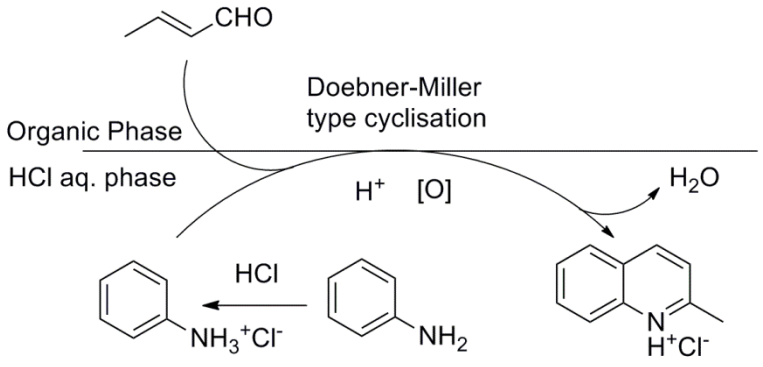
All of the synthetic protocols mentioned in Scheme 25 use harsh reaction conditions and are generally low yielding. In choosing appropriate reaction conditions for this project, an advanced review of the literature was undertaken and a convenient synthesis of quinoline compounds was identified. A modified Doebner-Miller reaction was selected

first, which uses a 2-phase system (aniline and hydrochloric acid (HCl, 10 M)), rather than a single phase acid solution, to help reduce polymerization (Scheme 26). The reaction also incorporated a phase transfer catalyst to promote a reaction between the aniline and unsaturated aldehyde situated in the other solvent layer.[159]



Scheme 25

In this chapter, the use of the modified Doebner-Miller synthesis for the formation a range of substituted quinoline derivatives is decribed. The synthesis of a library of quinoline derivatives that had not previously been tested for their bioactivity against *Leishmania* parasites is also described.

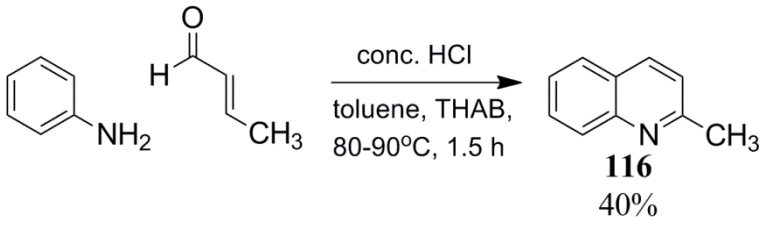


Scheme 26

3.1 Quinoline synthesis

3.1.1 Synthesis of 2-methyl quinoline (116)

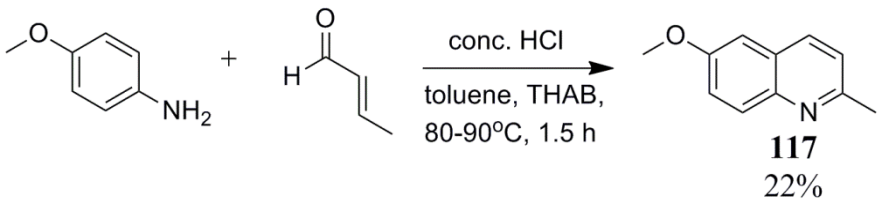
We initially focused on the direct formation of quinoline **116** from aniline and crotonaldehyde in a biphasic mixture of toluene and HCl (10 M) with a phase transfer catalyst, tetrahexylamonium bromide (THAB) (Scheme 27). Toluene was added to a mixture of aniline (1 equiv.) and THAB (5 mol%) in concentrated hydrochloric acid (10 M). The mixture was heated to reflux at 80-90 ºC. Crotonaldehyde (2 equiv.) was added to the reaction mixture at reflux and the mixture reacted for 1.5 h (Scheme 27). The boiling point of crotonaldehyde is 101-102 ºC and would azeotrope with the refluxing toluene and water. Purification of the crude mixture by column chromatography provided methylquinoline **116** (40%) in moderate yield (Scheme 27).



Scheme 27

3.1.2 Synthesis of 6-methoxy-2-methyl quinoline (117)

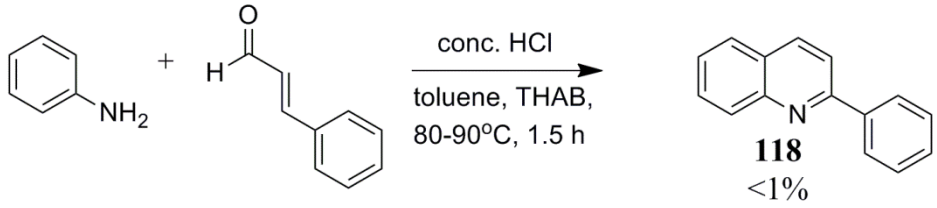
p-Anisidine (1 equiv.) was reacted with crotonaldehyde (2 equiv.) under the conditions in Scheme 28. Partial purification of the crude material by column chromatography gave a mixture. Analysis of the mixture by ^{1}H NMR spectroscopy identified the presence of methoxyquinoline **117** and what appeared to be polymerised byproduct in the δ 1-4 ppm region of the ^{1}H NMR spectrum. Further purification by column chromatography gave methoxyquinoline **117** (22%). The reduced yields observed for quinolines **116** (40%) and **117** (22%) was interesting as literature reports the reaction to give reduced amounts of polymerisation as well as simple purification of desired quinoline derivatives, this however was not observed here.[72]



Scheme 28

3.1.3 Synthesis of 2-phenyl quinoline (118)

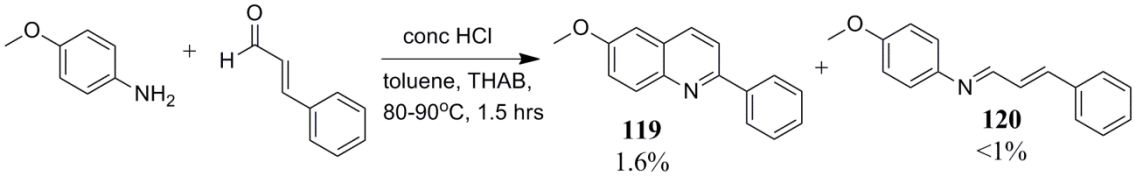
Generation of 2-aryl substituted quinolines for the quinoline library was also desired. Aniline (1 equiv.) and cinnamaldehyde (2 equiv.) were reacted under the conditions in Scheme 29. Analysis of the partially purified mixture by ^{1}H NMR spectroscopy identified very little formation of phenylquinoline **118** and a large amount of polymerised material in the ^{1}H NMR spectrum. Further partial purification by silica gel column chromatography as well as recrystallisation isolated an impure sample of phenylquinoline **118** (<1%) (Scheme 29).



Scheme 29

3.1.4 Synthesis of 2-phenyl-6-methoxy quinoline (119)

Again, the decrease in quinoline product formation proved disappointing and led to a questioning of the literature reaction conditions. As part of this investigation, generation of methoxy phenylquinoline **119** was considered. The methoxy group may promote formation of reaction intermediates through inductive stabilisation. *p*-Anisidine (1 equiv.) was reacted with cinnamaldehyde (2 equiv.) under the conditions in Scheme 30.



Scheme 30

^{1}H NMR spectroscopic analysis of the crude residue identified polymerised byproduct and what appeared to be compounds **119**. Purification of the crude mixture by column chromatography established two products by ^{1}H NMR spectroscopic analysis. The formation of conjugated imine **120** indicated that the reaction to form the quinoline **119** had not gone to completion, conjugated imine **120** (<1%) and methoxy phenylquinoline **119** (1.6%).

Figure 10 shows the distinctive difference between the conjugate imine **120** and quinoline **119**. The peaks identified at δ 7.08, 7.35-7.44 and 8.45 ppm are indicative of the conjugated imine functionality of **120**. The identification of the formation of **120** helps to discern a mechanim for the Doebner-Miller reaction.

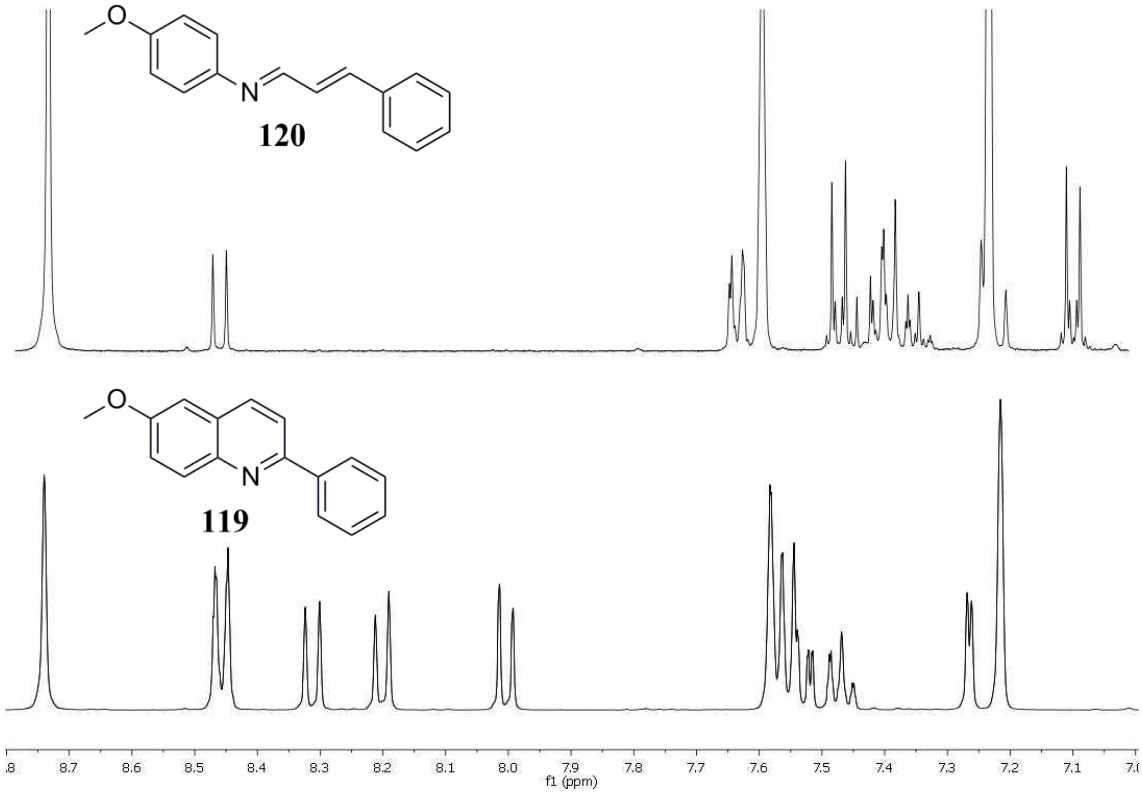
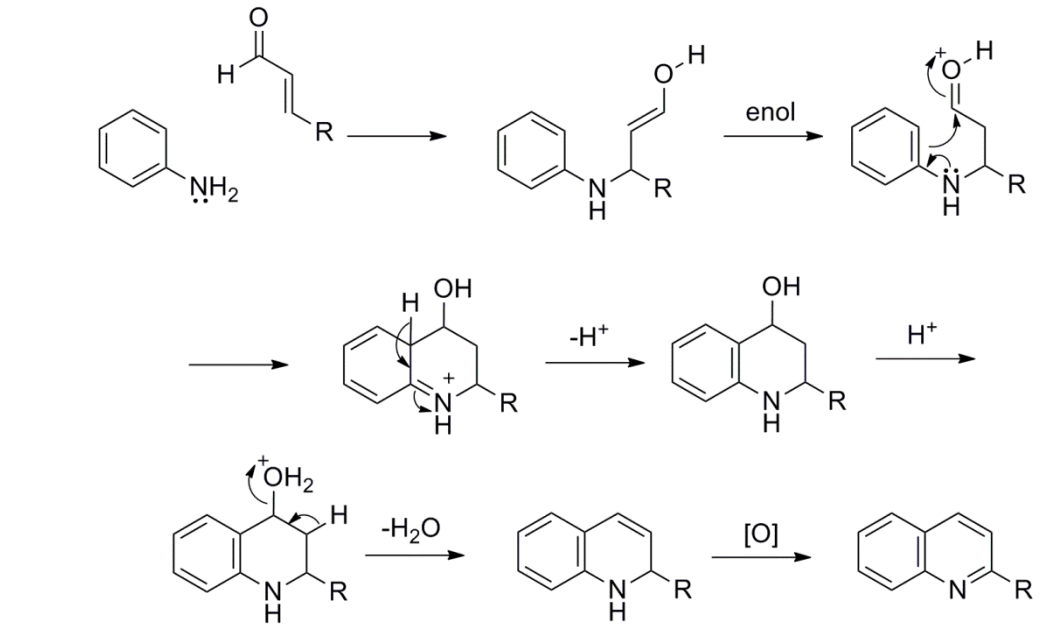


Figure 10 Expansion of (a) ^{1}H NMR spectrum (400 MHz, 25 ºC, pyridine-d_5) of imine **120** and (b) ^{1}H NMR spectrum (400 MHz, 25 ºC, pyridine-d_5) of quinoline **119**.

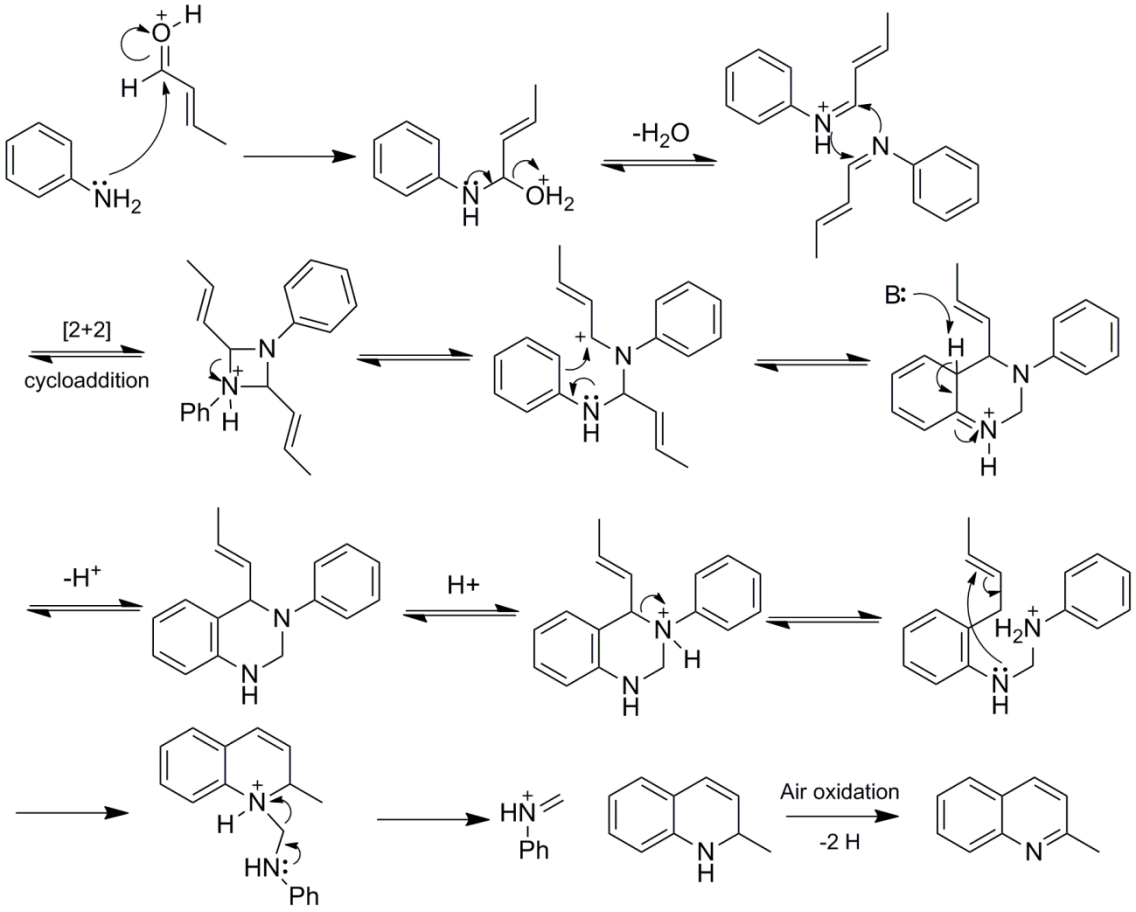
Currently, there are two suggested mechanisms reported for the synthesis of quinolines. The first is known as the Skraup synthesis,[160] which undergoes a 1,4-condensation of aniline to the α,β-unsaturated aldehyde to generate the enol. Intermolecular cyclisation generates the cyclised imine. Loss of hydrogen produces the quinolinol intermediate. Protonation followed by loss of a water molecule generates the dihydroquinine which is finally oxidised to the quinoline product (Scheme 31).

Another suggested Doebner-Miller reaction mechanism[161] however, involves a Michael addition between aniline and the α,β-unsaturated aldehyde, to form a conjugated imine. The conjugated imine reacts with a second imine molecule in a [2+2] cycloaddition which ring opens due to ring strain, allowing for further intermolecular cyclisation. Ring opening of the cyclised intermediate allows for re-aromatisation and therefore

elimination of the second equivalent of aniline to give the dihydroquinoline. This is then oxidised to give the final quinoline product (Scheme 32).



Scheme 31



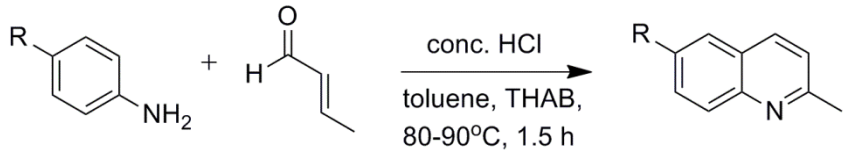
Scheme 32

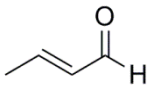
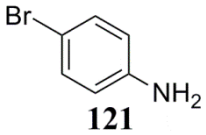
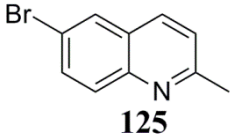
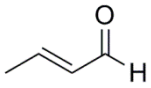
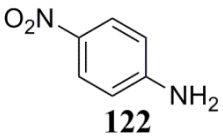
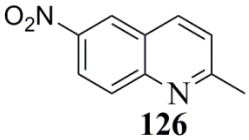
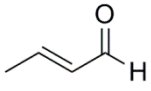
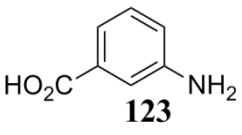
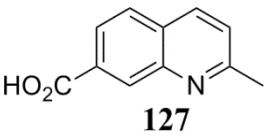
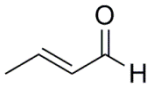
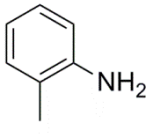
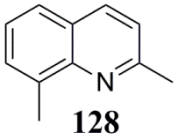
The formation of the conjugated imine identified in the Doebner-Miller mechanism (Scheme 32) seems to be associated with the formation of quinoline **119** along with the conjugated imine **120**. However, the formation of other quinolines may proceed by either mechanism. Steric hindrance at the γ-position of the α,β-unsaturated aldehyde, (eg with cinamaldehyde) may force the reaction to go via the Doebner-Miller mechanism rather than the Skraup mechanism. However, crotonaldehyde is not sterically hindered at the γ-position.

3.1.5 Synthesis of quinolines: electronic effects associated with aniline

Difficulties with the formation of quinolines **118** and **119** compared to quinolines **116** and **117** led us to look at the effects of different electron donating (EDG) and electron withdrawing groups (EWG) on aniline. It was important to continue to consider target quinolines that had not been previously tested against *Leishmania*. Thus aniline derivatives **121-124** were reacted with crotonaldehyde. Table 5 shows the quinoline derivatives synthesised from aniline derivatives **121-124** and their corresponding yields.

Table 5 Reaction of crotonaldehyde with anilines **121-124**.



α,β-unsaturated aldehyde	Aniline	Electronic properties	Quinoline	Yield
	 **121**	Halogen	 **125**	17
	 **122**	EWG	 **126**	23
	 **123**	EWG	 **127**	Complex mixture
	 **124**	EDG	 **128**	7

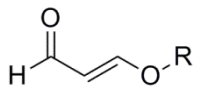
Quinolines **125**, **126** and **128** were synthesised in low to moderate yields, while quinoline **127** was not isolated (Table 5). The variation in yield is probably not due to the electronic effects associated with the aniline starting materials. If electron donating and electron withdrawing effects correlated with quinoline formation, then quinolines **126** and **127** would likely have comparable yields. However, this was not the case. Instead, yield variation may be linked back to purification methods. Purification of dimethyl quinoline **128** by column chromatography was repeated three or four times before ^{1}H NMR spectroscopy confirmed isolation of dimethyl quinoline **128**. On the other hand, bromoquinoline **125** required purification by column chromatography to remove the majority of polymerised material followed by recrystallisation to remove any extra impurities. Similarly nitroquinoline **126** was purified by recrystallisation.

3.1.6 Effect of steric/electronic substitution at the γ-position of α,β-unsaturated aldehyde

Quinolines **116**-**117** and **125**, **126** and **128** were synthesised in low to moderate yields yet quinolines **119**, **118** and **127** were not able to be synthesised. An assessment of both the electronic substitution and steric substitution at the γ-position of α,β-unsaturated aldehyde was then undertaken.

3.1.6.1 *Electronic substitution effects*

To adequately test the effects of electronic properties at the γ-position of α,β-unsaturated aldehyde, a suitable α,β-unsaturated aldehyde similar to **129** was considered. A commercially available α,β-unsaturated aldehyde with these properties was desirable, however no such compound could be sourced.

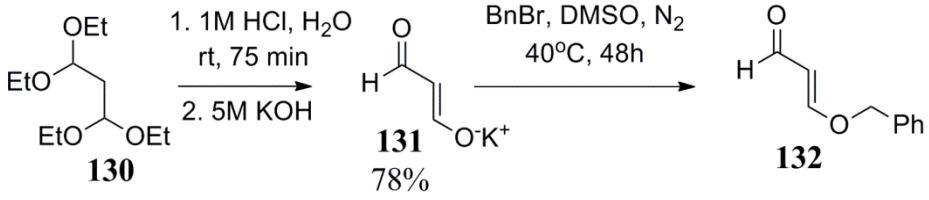


129

R = alkyl or aryl chain

Ideally, a benzyloxy group at the γ-position of **129** would provide an example with modified steric and electronic substitution at the γ-position and there was literature precedence for its formation. Hydrolysis of tetraethoxypropane **130** (1 equiv.) with hydrochloric acid, followed by treatment with potassium hydroxide gave potassium salt **131** (78%) (Scheme 33). Sodium salt **131** (1 equiv.) and benzyl bromide (1 equiv.) were

reacted in DMF at room temperature and for 2 h (Table 6, Entry 1). After workup, examination of the crude material by ^{1}H NMR spectroscopy identified starting materials. The reaction was repeated with equal equivalents in THF and bought to reflux at 60 ºC (Table 6, Entry 2). However, ^{1}H NMR spectroscopy identified the recovery of benzyl bromide (BnBr).



Scheme 33

The lack of formation aldehyde **132** led to an NMR study of the reaction. Equal equivalents of potassium salt **131** and benzyl bromide were added to either MeOH-d_4 (1 mL) or DMSO-d_6 (1 mL) in a 5 mm NMR tube and the mixture vortexed. Spectra were aquired at 0, 24 and 48 h (Table 6, Entry 3 and 4). ^{1}H NMR spectroscopy identified partial formation (75%) of aldehyde **132** at 24 and 48 h in MeOH-d_4. However, at 24 h in DMSO-d_6 partial formation (90%) of aldehyde **132** was observed in the ^{1}H NMR spectra.

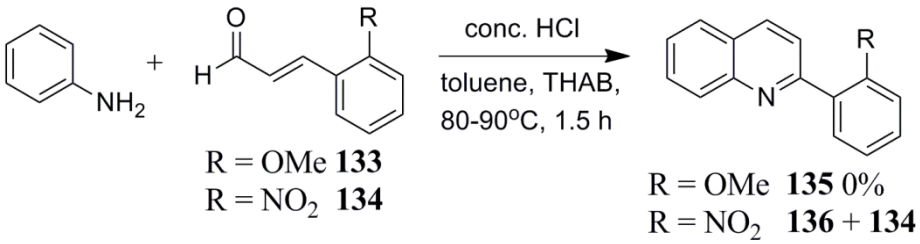
Table 6 Reaction conditions for generating aldehyde **132**

Attempt	Time (hr)	Temperature (ºC)	Reaction Concentration (M)	Solvent	Inert atmosphere	Yield (%)
1	2	0-25	2.73	DMF‡	x	0
2	2	0-60	1.4	THF‡	x	0
3 (NMR)	0, 24, 48	25	0.7	MeOH-d_4	x	75*
4 (NMR)	0, 24, 48	25	0.7	DMSO-d_6	x	90*
5	16	25	6.9	DMSO‡	x	0
6	16	25	3.5	DMSO‡	x	0
7	16	40	3.5	DMSO‡	x	0
8	48	40	0.7	DMSO‡	x	0
9	48	40	0.7	DMSO‡	y	0

‡Anhydrous solvent. *estimated yield obtained from the NMR sample.

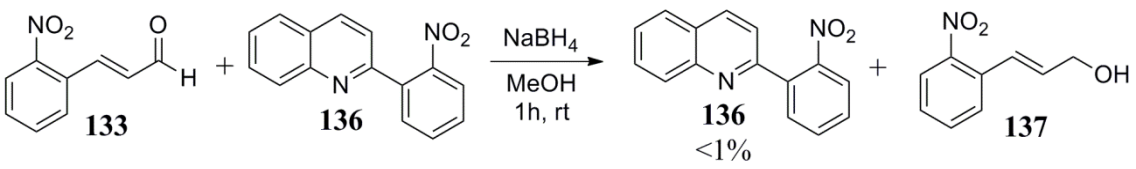
In an attempt to convert the NMR results to a preparative scale, sodium salt **131** (1 equiv.) and benzyl bromide (1 equiv.) were reacted in anhydrous dimethyl sulfoxide (DMSO) for 16 h at room temperature (Table 6, Entry 5). However, ^{1}H NMR spectroscopy only identified recovered starting materials upon workup. The reaction was repeated decreasing the concentration of the reaction again (Table 6, Entry 6). Further attempts to generate aldehyde **132** included heating the reaction mixture to 40 ^{o}C for 48 h (Table 6, Entry 7), completing the reaction under an inert atmosphere (Table 6, Entry 8), as well as changing the concentration to that of the ^{1}H NMR experiment under an inert atmosphere for 24 h (Table 6, Entry 9).

Failure to generate any of the α,β-unsaturated aldehyde **132** and the unavailability of commercial products of this nature lead to the inclusion of electron donating aldehyde **133** and electron withdrawing aldehyde **134** in the study. Each of the commercially available α,β-unsaturated aldehydes **133** and **134** were reacted with aniline under the general conditions (Scheme 34).



Scheme 34

Purification of the crude mixture from the reaction between aldehyde **134** and aniline indicated no formation of methoxyphenyl quinoline **135** by ^{1}H NMR spectroscopy (Scheme 34). However, purification and analysis of the crude mixture from the reaction of aldehyde **133** and aniline by ^{1}H NMR spectroscopy identified a discrete 2:3 mixture consisting of aldehyde **133** and nitrophenyl quinoline **136** respectively. The mixture of **133** and **136** were reduced with sodium borohydride in methanol (Scheme 35).

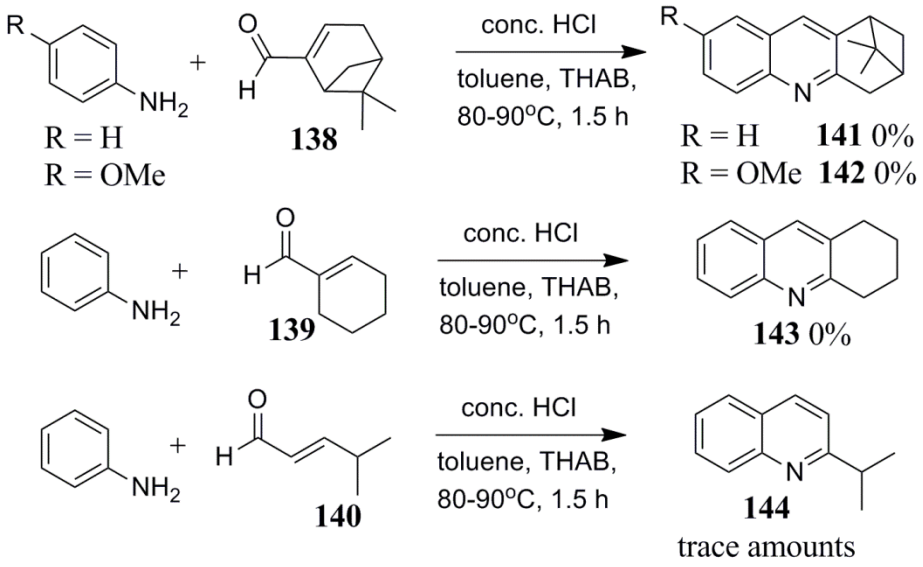


Scheme 35

Reduction of **133** would give **137** and this was discarded. Nitrophenyl quinoline **136** was isolated by column chromatography in very poor yield (<1%).

3.1.6.2 *Steric substitution effects*

In order to fully compare steric effects at the γ-position of α,β-unsaturated aldehyde, a range of commercially available substituted or β-unsaturated aldehydes, **138**-**140**, were considered. Aldehyde **138** is the most sterically hindered followed by **139** and then aldehyde **140**.

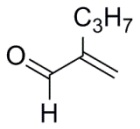


Scheme 36

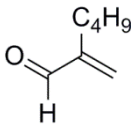
Each of the aldehydes **138**-**140** (2 equiv.) were reacted with aniline (1 equiv.) under the general reaction conditions (Scheme 36). Aldehyde **138** was also reacted with *p*-anisidine under conditions given in Scheme 36. Crude residues were all analysed by ^{1}H NMR spectroscopy and mass spectrometry. The reaction between aldehyde **138** and aniline identified only recovery of starting material. Similarly the reaction of *p*-anisidine and aldehyde **138**, as well as the reaction of aniline and aldehyde **139** gave complex mixtures. ^{1}H NMR spectroscopy suggested that quinoline **144** had formed in the reaction. However, **144** could not be separated from polymerised material using column chromatography.

The above results identified that the bulky substituents at the γ-position in the α,β-unsaturated aldehyde prevented formation of the desired quinoline. Investigation of the literature identified that these results are consistent with other examples previously

reported, in which only unsubstituted α,β-unsaturated aldehydes, such as **145** and **146**, gave quinolines with aniline or substituted anilines under similar conditions.[162,163]



145



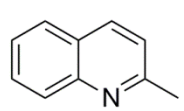
146

3.2 Conclusion

The generation of quinolines using reactions such as the Doebner-Miller reaction needs to be carefully considered. Although the reactions are simple and work well for α,β-unsaturated aldehydes that are not sterically hindered at the γ-position, such reactions use harsh conditions and regardless of the use of a 2-phase system, and generate large amounts of polymerised byproduct. The polymerised products are often difficult to remove from the quinolines. In the case of nitroquinoline **136**, the quinoline was only successfully isolated if a separate reaction was used to reduce the aldehyde.

The formation of the conjugated imine **120** and the outcome observed with bulky aldehydes lends support to the Dobner-Miller reaction mechanism. The formation of products from unhindered crotonaldehyde suggests a mechanism invoking conjugate addition of the aniline to the α,β-unsaturated aldehyde Michael acceptor. Further mechanistic studies are required to identify if using non-steric aldehydes like crotonaldehyde cause the reaction to proceed via the simpler Skraup mechanism or if they too follow the Doebner-Miller mechanism.

The following quinoline derivatives were successfully synthesised; **116**-**119**, **125**, **126, 128** and **136**. None of these quinolines have hitherto been screened for biological activity against *Leishmania*. The results of this screening are described in Chapter Five.



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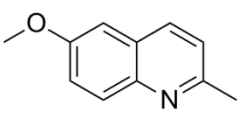
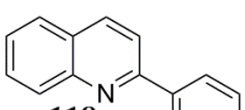
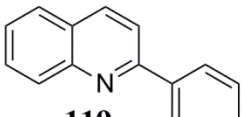


117



118



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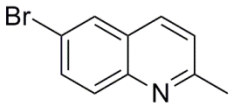

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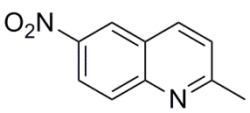
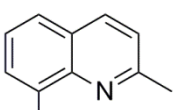
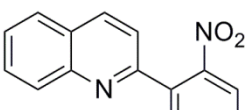


126



128



136

Chapter Four

Synthesis of Quinoline and Cinchona Alkaloid Analogs

As previously discussed (Section 1.3) both quinoline analogues and cinchona alkaloids have a broad range of bioactivity against leishmaniasis and other parasitic deseases including malaria (Figure 11).[65,76,85,88] In this chapter, syntheses were devised to expand the current quinoline library, including cinchona alkaloid derivatives.

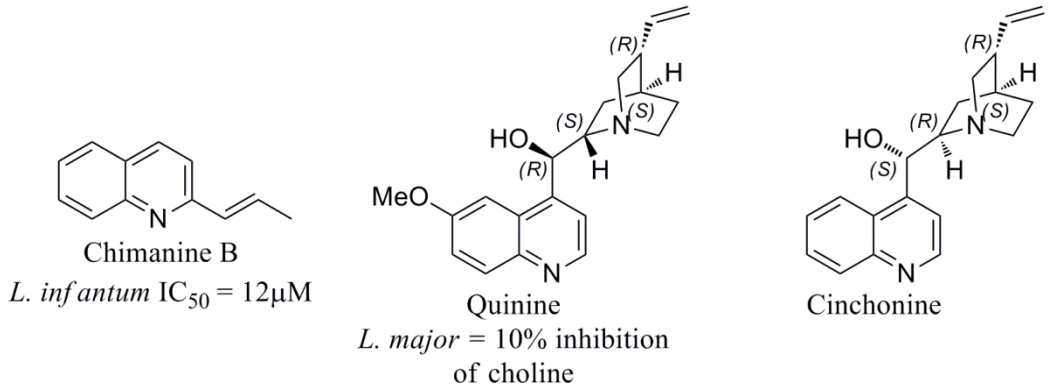


Figure 11 Representative quinoline or cinchona alkaloids

Quinolines substituted at positions 2- or 2,6-substituted together with analogs of cinchonine and quinine (Figure 12) were chosen for study based on inhibition data and basic leishmanicidal SARs obtained from the literature (Chapter One).

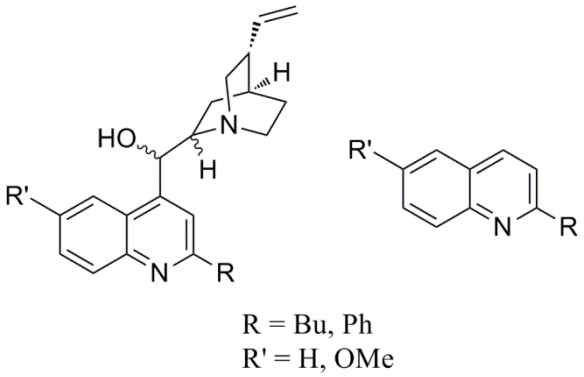
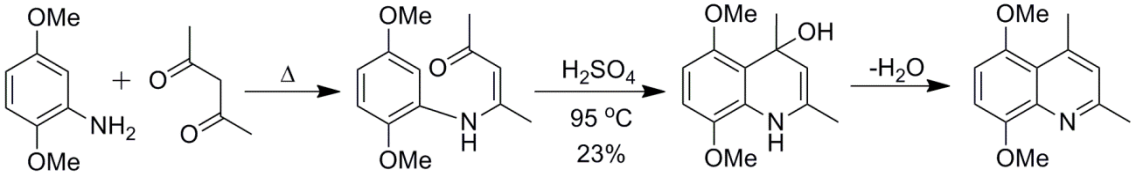


Figure 12 Exemplars of target substituted analogs

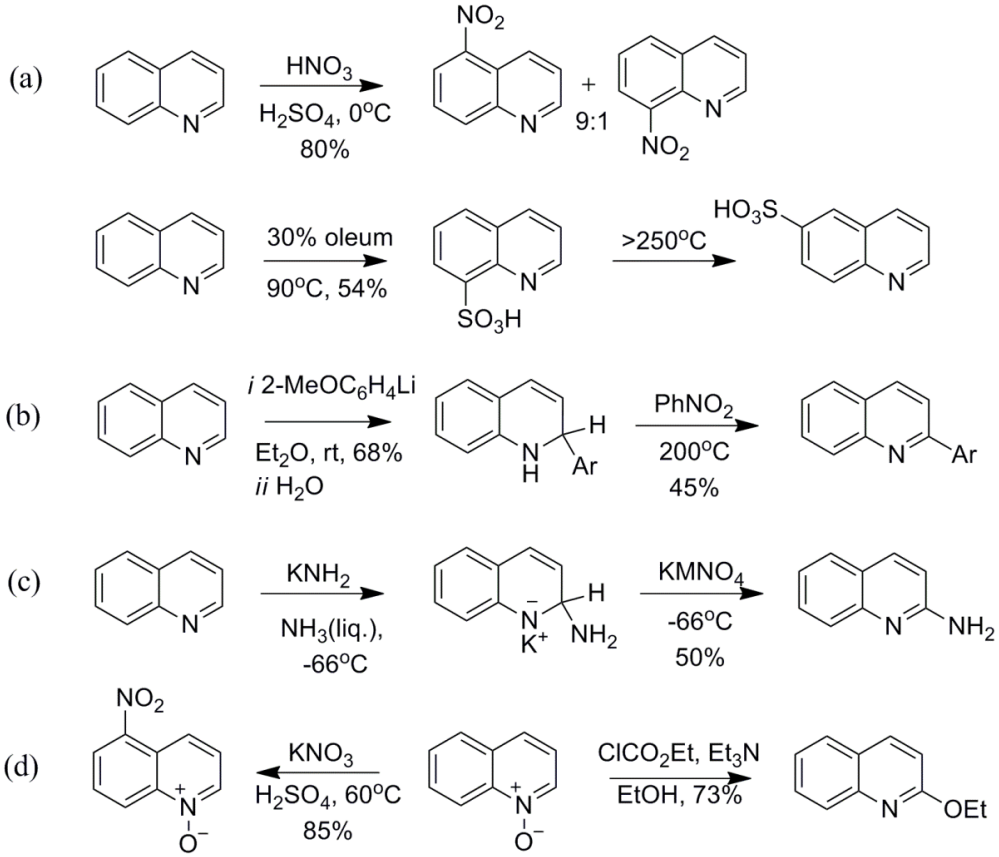
Generalised approaches to quinoline analogues are based on the use of non-heterocyclic precursors such as the Combes synthesis[147-149] (Scheme 37), Conrad-Limpach-Knorr reaction.[147,148,150] the Skraup[147,153,154] or Doebner-Miller synthesis[155] (Chapter Three). In addition to these methods, quinolines can be substituted by electrophilic reactions[147] such as nitration[163,164] or sulphonation;[165,166] by nucleophilic reactions[147] such as alkylation[167] or arylation[168] with organometallic reagents; amination,[169] and nitration,[170]

or by formation of *N*-oxides[147,171] which are subsequently reduced to give a substituted quinoline (Scheme 38).



Scheme 37

Many of these synthetic protocols suffer from harsh reaction conditions, low yields and / or the use of hazardous reagents. This is particularly notable with reactions using non-heterocyclic precursors (as previously discussed in Chapter Three).



Scheme 38

In this chapter, some synthetic methods were explored to form a variety of substituted quinoline and cinchona alkaloids. These methods include the use of simple quinoline starting reagents including the parent quinoline, 6-methoxy quinoline and quinoline-1-oxide, and their reaction with organometallic reagents. The reaction of quinoline with various organometallic reagents is relatively limited but can be successful for particular

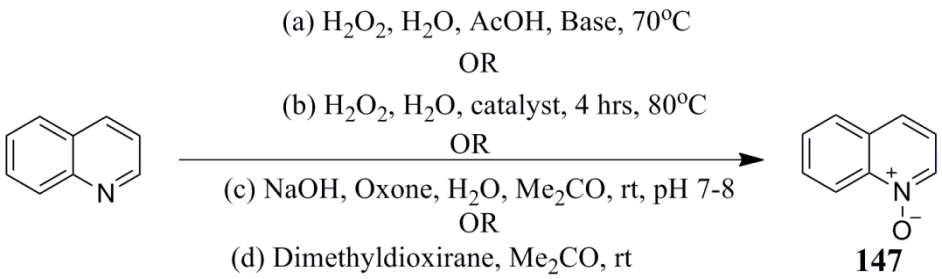
substrates.[172] The use of the corresponding *N*-oxide as starting material is a common feature of these protocols.[173]

4.1 Direct synthesis of substituted quinolines

The direct formation of 2-substituted quinoline and cinchona alkaloid derivatives from quinoline-1-oxide (**147**) and (2*S*,4*R*,8*S*)-2-((*R*)-hydroxy(1”-oxidoquinolin-4”-yl)methyl) -8-vinylquinuclindine 1-oxide (**148**) was examined. The starting point was synthesis of the required *N*-oxides. It was proposed that the *N*-oxides would be used for direct 2-substitution with Grignard or lithium reagents.

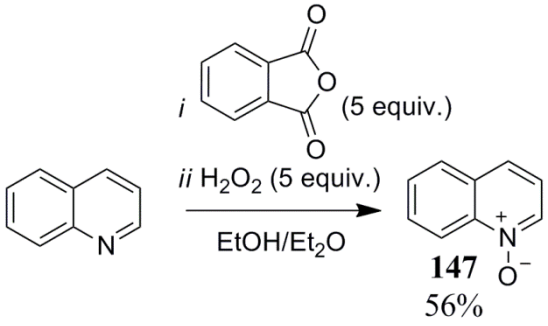
4.1.1 Synthesis of quinoline-1-oxide

Oxidation of quinolines can be achieved in various ways (Scheme 39).[156,174-178] Oxidation with perphthalic acid has the advantage of *in situ* generation.



Scheme 39

The perphthalic acid solution was generated *in situ* from hydrogen peroxide (H_2O_2) and phthalic anhydride in diethyl ether at 0 ºC (Scheme 40). Treatment of quinoline with perphthalic acid (2 equiv.) solution in methanol under an inert atmosphere for 16 h gave quinoline-1-oxide in an isolated yield of 32%.



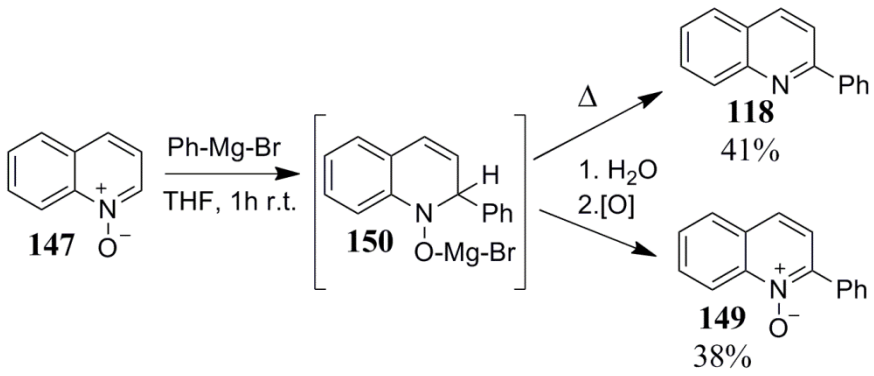
Scheme 40

In an attempt to increase the yield of quinoline-*N*-oxide **147** the equivalents of perphthalic acid were increased. Using 5 equivalents of the peracid gave an improved yield of 56%.

4.1.2 Synthesis of 2-substituted quinolines using quinoline-1-oxide

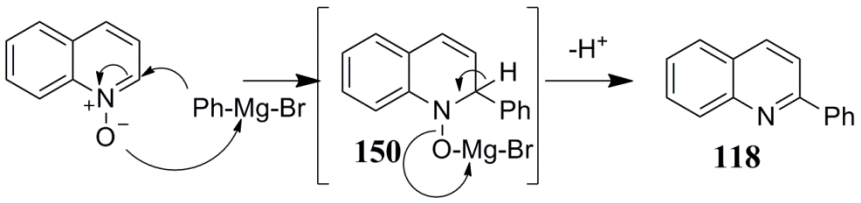
4.1.2.1 *Synthesis of 2-phenylquinoline (**118**)*

The initial approach to 2-substituted quinolines was via reaction of quinoline-1-oxide with an appropriate Grignard reagent. 2-Phenylquinoline was previously generated via the Doebner-Miller reaction in low yields (Section 3.13). The Grignard approach offers a more general procedure.[179] A phenylmagnesium bromide solution was generated *in situ* by reacting magnesium turnings with phenyl bromide in dry THF over 30 minutes. Quinoline-*N*-oxide **147** (1 equiv., added slowly over 15 minutes) was treated with the solution of phenylmagnesium bromide (3 equiv.) in dry THF for 1 hour under an inert atmosphere. Upon workup quinoline **118** was isolated in a moderate yield (41%) (Scheme 41). Analysis of the crude product mixture by ^{1}H NMR spectroscopy identified that phenyl quinoline-*N*-oxide **149** (38%) was also generated within the reaction. It is assumed this occurs when competing hydrolysis of the intermediate **150** is favoured by an oxidation reaction during workup (Scheme 41).



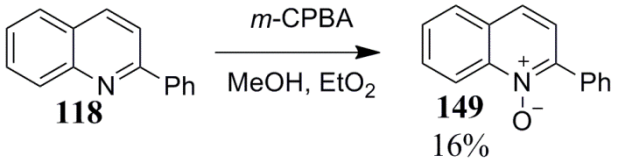
Scheme 41

A mechanism for the formation of quinoline **118** is suggested in Scheme 42. Coordination of the oxygen atom to the Grignard reagent positions the phenyl group for attack at the electrophilic 2-position of the quinoline ring. Elimination allows for the recovery of aromaticity and removal of the oxygen-magnesium bromide species affords the final quinoline product.



Scheme 42

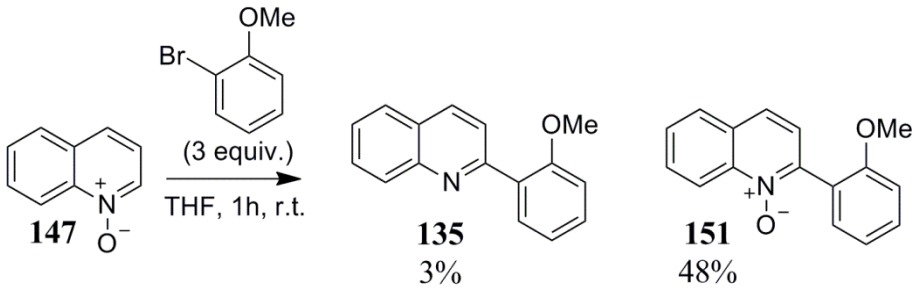
Next, phenyl quinoline-*N*-oxide **149** was synthesised via a separate route from quinoline **118** (Scheme 43). A solution of quinoline **118** (1 equiv.) in methanol was added dropwise to a cooled solution of *m*-chloroperoxybenzoic acid (*m*-CPBA) (5 equiv.) in diethyl ether and left to react at room temperature under an inert atmosphere for 16 h. Purification by silica gel column chromatography gave phenyl quinoline-*N*-oxide **149** (16%).



Scheme 43

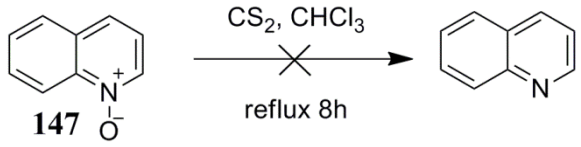
4.1.2.2 *Synthesis of 2-(2-methoxyphenyl) quinoline (**135**)*

The success of the Grignard reaction for the preparation of 2-phenyl quinoline encouraged the use of this approach for the preparation of other substituted quinolines. Quinoline-*N*-oxide **147** (1 equiv.) was reacted with a solution of 2-methoxybenzenemagnesium bromide in THF (3 equiv., generated *in situ*) under similar conditions to those previously described (Scheme 44).



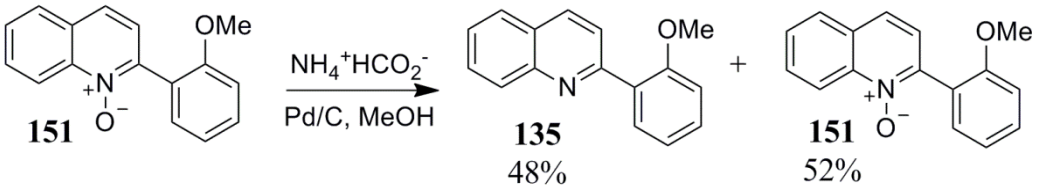
Scheme 44

^{1}H NMR spectroscopic analysis of the crude mixture indicated the presence of both quinoline **135** and *N*-oxide **151**. Purification of the crude mixture by column chromatography gave quinoline **135** (3%) and *N*-oxide **151** (48%) (Scheme 45). The low yield of quinoline **135** prompted an effort to reduce *N*-oxide **151**. This was initially attempted using carbon disulphide which has been reported to readily reduce *N*-oxides (Scheme 45).[180] Carbon disulfide (1 equiv.) was added to a cooled solution of *N*-oxide **151** (1.4 equiv.) in chloroform ($CHCl_3$) and the mixture reacted for 1 hour at 0 ºC, then heated at reflux for 8 h. Analysis of the crude mixture by ^{1}H NMR spectroscopy indicated the presence of only *N*-oxide **147**.



Scheme 45

Due to the toxicity of carbon disulphide other possible reactants were sought. Review of the literature identified a rather simple reduction utilising ammonium formate and palladium-on-carbon.[177] This procedure was applied to the reduction of *N*-oxide **151**. Ammonium formate (10 equiv.) was added to a stirred solution of *N*-oxide **151** (1. equiv) and palladium-on-carbon (10%) in methanol under an inert atmosphere for 2 h. Analysis of the crude product indicated a 48% conversion of *N*-oxide **151** to quinoline **135**. Purification by silica gel column chromatography (DCM:acetone; 9:1) gave quinoline **135** (48%) and recovered *N*-oxide **151** (52%) (Scheme 46).



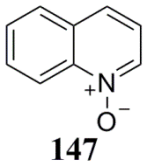
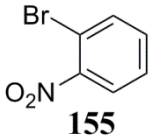
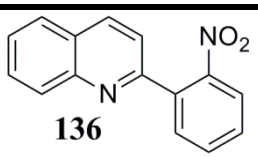
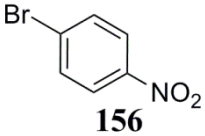
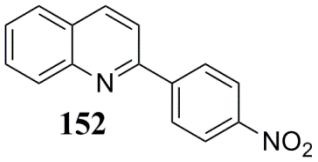
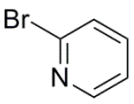
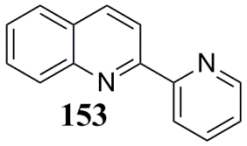
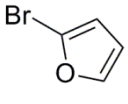
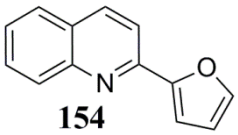
Scheme 46

4.1.2.3 *Attempted synthesis of quinolines **136**, **152**, **153** and **154***

Next, other new 2-substituted quinolines targets were selected based on a SAR analysis of the literature. The previous conditions for the Grignard reaction were repeated substituting the bromide compound with 1-bromo-2-nitrobenzene (**155**), 1-bromo-4-

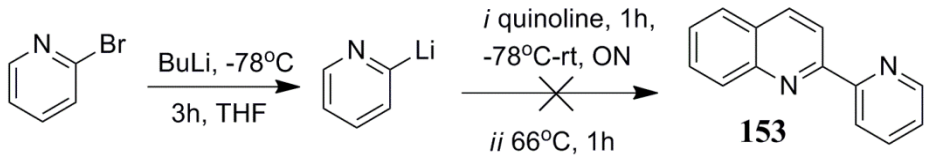
nitrobenzene (**156**), 2-bromopyridine (**157**) and 2-bromofuran (**158**). Unfortunately, none of the target quinolines (**136** and **152-154**) or their corresponding *N*-oxides were obtained, recovering only starting materials in each case (Table 7). Investigation into the literature discovered various reasons for the inability to generate Grignard reagents of **155-158**. The highly basic nature and reactivity of Grignard reagents toward many functional groups does not allow for Grignard formation with reactive functional groups such as a nitro group.[181] Paul Knochel *et al*[182] have developed an alternate strategy by preparing relatively unreactive organometallic reagents incorporating the desired functional groups, then to effect a metal-metal exchange (using zinc and tin or copper) to form a reactive analogue in the presence of an electrophilic co-reactant. The formation of furan and pyridine Grignard reactions is more complex. The generation of 2-pyridylmagnesium bromide is difficult to form due to 2-pyridylmagnesium bromide breaking down and coating the magnesium and quenching the reaction. Continual addition of ethyl bromide and the formation of ethylmagnesium bromide maintains a clean magnesium surface allowing for formation of both ethylmagnesium bromide and 2-pyridylmagnesium bromide.[183] While furan compounds, like 2-bromofuran are relatively inert which decreases the likelyhood of Grignard formation.[184]

Table 7 Attempted reaction of aryl Grignard generated from aryl bromide with quinoline-*N*-oxide **147**

N-Oxide	Aryl Bromide	Target Product	Yield (%)
 **147**	 **155**	 **136**	0
	 **156**	 **152**	0
	 **157**	 **153**	0
	 **158**	 **154**	0

Next, the synthesis of the organolithium reagents of the bromide compounds for subsequent organometallic reaction was attempted. The literature reports the formation

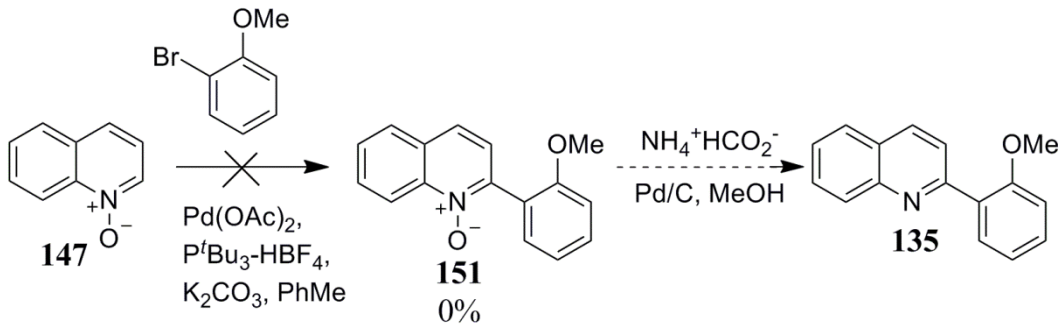
of organolithium reagents by reacting organobromides in THF with *n*-butyllithium at -80 ºC under an inert atmosphere.[185]



Scheme 47

Aryl bromide **157** was treated with butyllithium under the reported conditions. Quinoline was further reacted under gentle reflux under an inert atmosphere (Scheme 47). However, stabilisation of the reaction temperature at -80 ºC was unable to be achieved. Both mass spectrometry and ^{1}H NMR spectroscopy identified only starting materials were isolated.

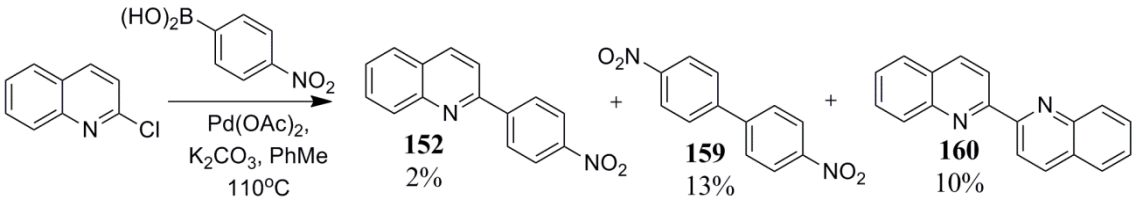
Regioselective palladium catalysed arylation of pyridine-*N*-oxides has been reported.[177,186] The similarity of pyridine-*N*-oxides and quinoline-*N*-oxides suggested that the reaction could be applied to synthesise the target compounds. Thus 2-bromoanisole (0.3 M) in dry toluene was reacted with palladium (II) acetate (5 mol%), tri-*tert*-butylphosphine tetrafluoroborate (5 mol%), potassium carbonate (2 equiv.) and quinoline-*N*-oxide **147** (3 equiv.) under an inert atmosphere at 110 ºC for 16 h (Scheme 48). Analysis of the crude product by ^{1}H NMR spectroscopy revealed a complex mixture. No identifiable product could be isolated by chromatography.



Scheme 48

Next, the use of a Suzuki coupling reaction to generate the desired quinoline products was investigated (Scheme 49).[187] The Suzuki coupling reaction is the reaction of an aryl- or vinyl-boronic acid with an aryl- or vinyl-halide catalysed by a palladium

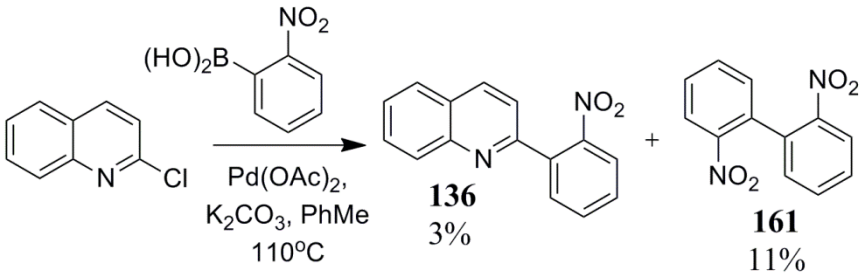
complex. It is widely used to synthesise poly-olefins, styrenes, and substituted biphenyls, and has been extended to incorporate alkyl bromides.[188,189] Palladium (II) acetate (5 mol%), potassium carbonate (2 equiv.) and 4-nitrophenylboronic acid (2 equiv.) were combined. A solution of 2-chloroquinoline (1 equiv., 0.3 M) and dry toluene was then added and reacted under an inert atmosphere at 110 ºC for 16 h.



Scheme 49

Analysis of the crude material by ^{1}H NMR spectroscopy identified a number of byproducts and potentially the product quinoline **152**. Purification by silica gel column chromatography gave 3 products; quinoline **152** (combined with **159**), biphenyl **159** (8%), and biquinoline **160** (10%). Another purification step was required to separate quinoline **152** from biphenyl **159**. Alumina gel column chromatography using hexane:DCM (4:1) with 2% ethyl acetate, gave **152** (2%) and **159** (5%).

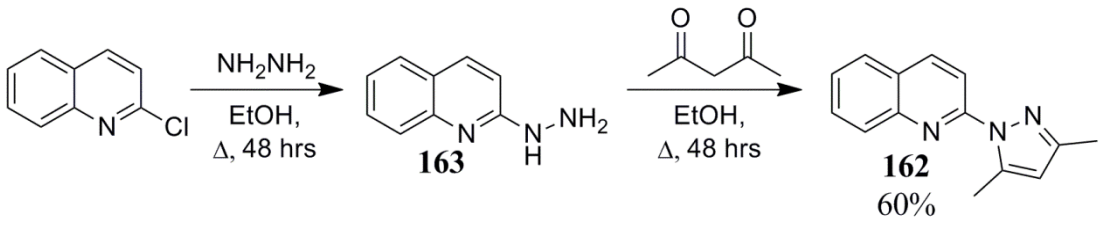
Repeating the above synthetic procedure with 2-nitrophenyl boronic acid instead of 4-nitrophenyl boronic acid gave quinoline **136** also in a very poor yield of 3% along with biphenyl **161** (11%). The decreased yield was caused by difficulties in separating quinoline **136** from biphenyl **161** (Scheme 50).



Scheme 50

4.1.2.4 *Synthesis of 2-(3,5-dimethyl-1H-pyrazol-1-yl)quinoline (**162**)*

The novel quinoline **162** was synthesised via formation of the hydrazine **163**. Reaction of **163** with acetylacetone via condensation, and imine bond, formation gave the pyrazole ring (Scheme 51).



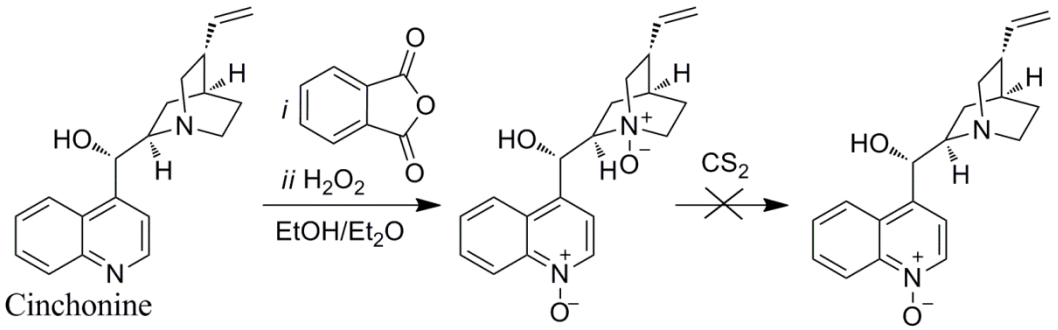
Scheme 51

Thus 2-chloroquinoline (1 equiv.) was reacted with hydrazine (40 equiv.) under an inert atmosphere for 48 h. Subsequent reaction of the crude quinoline **163** with acetylacetone gave quinoline **162** in an isolated yield of 60%. Formation of the quinoline pyrazole ring was confirmed by the presence of a singlet at ^{1}H NMR δ 6.030 ppm due to H4' and peaks at δ 109.5, 142.3 and 150.1 ppm due to C4', C3' and C5' respectively in the ^{13}C NMR spectrum.

4.2 Direct synthesis of cinchona alkaloid derivatives

4.2.1 Synthesis of cinchonine-1-oxide (148)

Attention was now turned towards the direct synthesis of cinchona alkaloid derivatives. With an oxidation procedure of quinolines in hand, the cinchona alkaloid, cinchonine, was used to synthesise a variety of 2-substituted alkaloids. Cinchonine was oxidised using perphthalic acid as previously described (Section 4.1.1, page 76). The quinuclidine nitrogen is more reactive than the quinoline nitrogen and so both must be oxidised and then the quinuclidine-*N*-oxide selectively reduced. Cinchonine-*N*,*N*'-dioxide (**148**) was isolated and purified by column chromatography as a mixture of the dioxide (85%) and cinchonine (15%) A trial reaction using the mixture of **148** and cinchonine was carried out to establish whether the dioxide **148** could be selectively reduced to the monoxide **164** using carbon disulfide (Scheme 52).[180] Purification of the **148** mixture by silica gel column chromatography and attempts at recrystalisation were unable to remove the 15% of starting cinchonine. Instead semi-preparative HPLC gave pure dioxide **148** in an isolated yield of 15%.

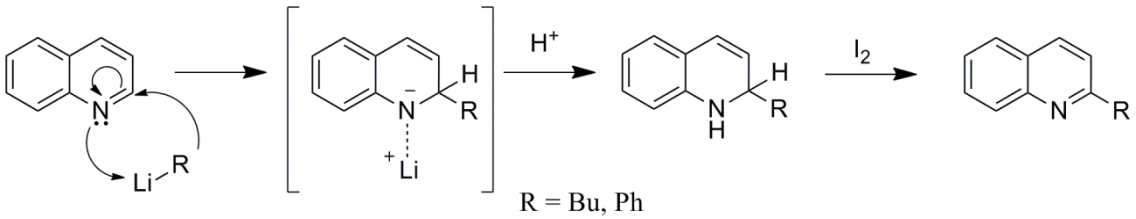


Scheme 52

Dioxide **148** was treated with carbon disulphide (6 equivalents) at 0 ºC in chloroform and refluxed for 16 h. However, only the dioxide **148** was obtained. Use of 12 equivalents of carbon disulphide also led to recovery of the dioxide **148** and no formation of the monoxide **164**.

4.2.2 Synthesis of substituted cinchona alkaloids using organolithium reagents

Due to the complexity in forming compound **148** (Section 4.2.1) and therefore, further generating 2-substituted cinchona analogs, an alternate route for the synthesis of 2-substituted cinchona alkaloids was required. A recent report identified that nucleophilic addition of organometallic reagents to cinchona alkaloids was viable.[172] These reactions proceed via nucleophilic attack of the nitrogen to generate a lithium amide, protonation to give the corresponding amine and followed by oxidation with iodine to restore aromaticity (Scheme 53).



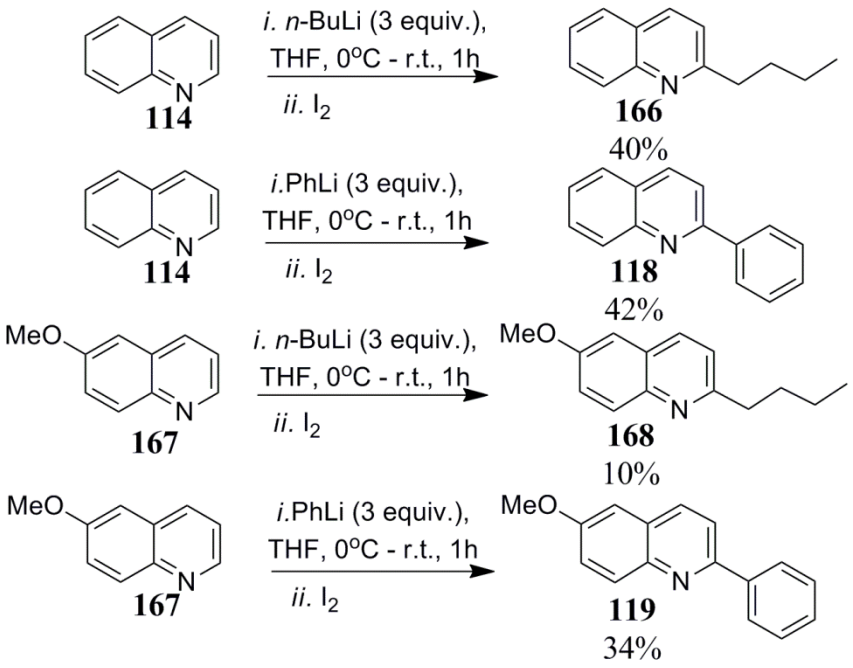
Scheme 53

In this section, the nucleophilic addition of an organolithium reagent to quinoline and cinchona alkaloids was investigated along with a comparative assessment of observed dynamic NMR spectroscopy profiles. ^{1}H NMR spectrum of quinoline and cinchona analogs are recorded in pyridine-d_5, unless otherwise stated and complete ^{1}H NMR

assignment was achieved by analysis of the 2D spectra (correlation spectroscopy (COSY), Heteronuclear Multiple Quantum Coherence (HMQC), and HMBC) obtained at 70 ºC.

4.2.2.1 *Formation of quinoline analogs via nucleophilic attack of organolithium reagents*

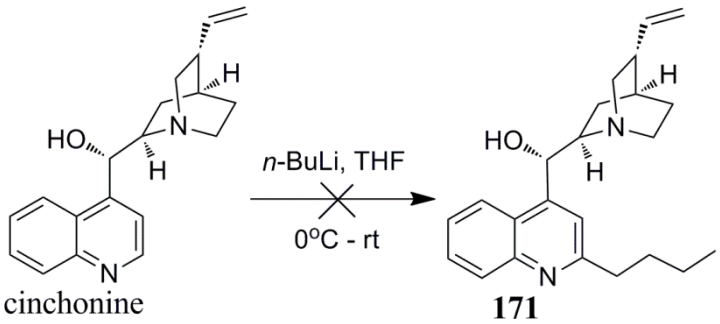
Initially, a model study investigation was undertaken using the more readily available quinoline **114** rather than the cinchona alkaloid **165**. The choice of solvent is important when using organolithium reagents. Organolithium reagents require an ether based solvent to reduce the formation of aggregates. The addition of THF or *tert*-butylmethoxy ether (TBME) chelate prevents the formation of aggregates making organolithium reagents more soluble and reactive. Reaction of quinoline **114** with *n*-butyllithium in THF or TBME followed by oxidation with iodine gave quinoline **166** in yields of 40% and 38% respectively. Reaction of quinoline (1 equiv.) with phenyllithium (3 equiv.) in anhydrous THF gave **118** (34%). Similarly reaction of 6-methoxy quinoline **167** (1 equiv.) with n-butyllithium or phenyllithium in THF gave **168** (10%) and **119** (34%) respectively (Scheme 54).



Scheme 54

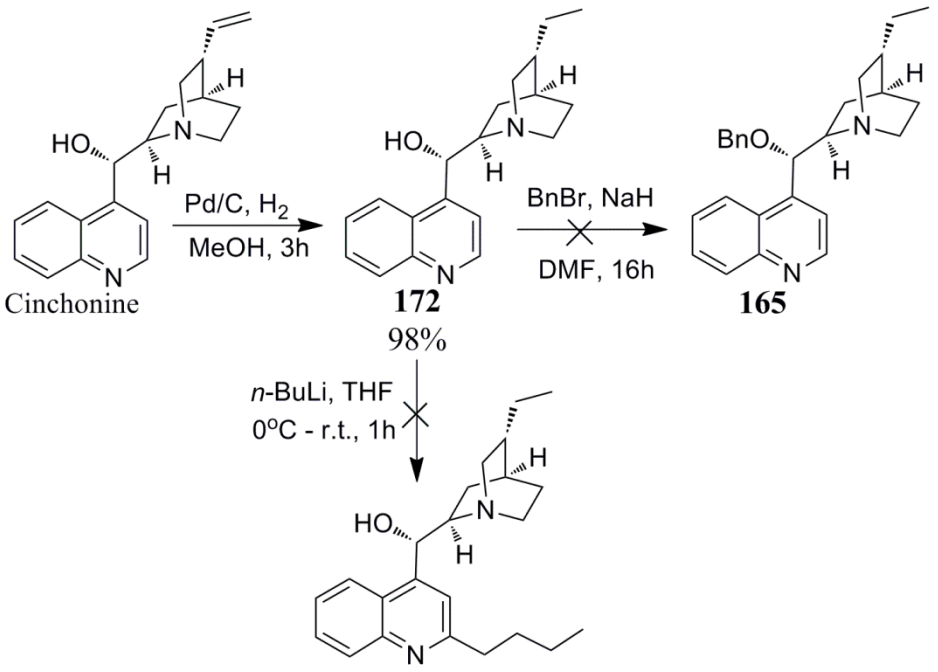
4.2.2.2 *Synthesis of cinchona alkaloids **169** and **170***

The next case examined was the reaction of cinchonine with *n*-butyllithium. *n*-Butyllithium in hexanes (3 equiv. 1.1 M) was added to a solution of cinchonine suspended in dry THF and stirred under inert atmosphere at 0 ºC for 20 minutes before warming to room temperature for 1 h. The reaction mixture was quenched (acetic acid) and reduced with excess iodine before the reaction mixture was neutralised (Scheme 55). ^{1}H NMR spectroscopic analysis of the crude material identified only recovered cinchonine. The lack of formation of **171** is presumably due to the vinyl group on the quinuclidine ring and the unprotected hydroxyl group.



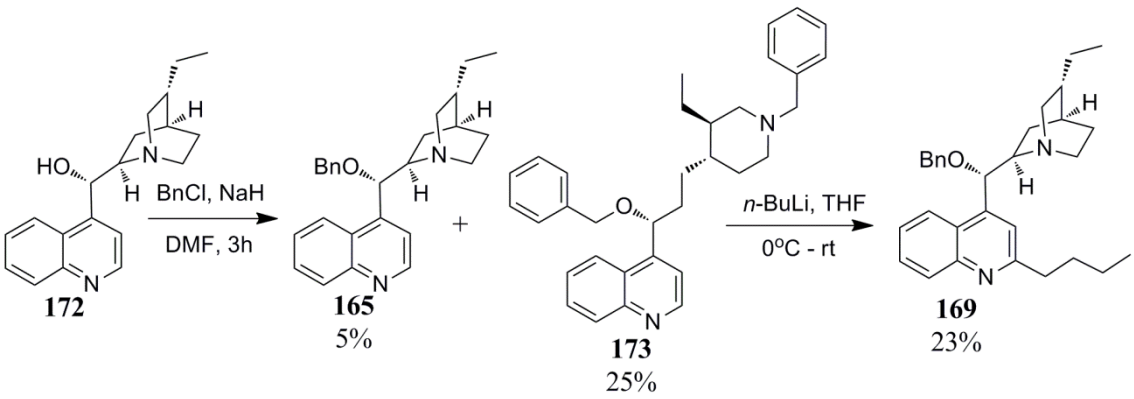
Scheme 55

Work reported by another research group working on similar cinchona alkaloids deduced the need to reduce the vinyl group and protect the hydroxyl group prior to addition of the organolithium reaction.[190] Hydrogenation of cinchonine using palladium-on-carbon in methanol under an atmosphere of hydrogen afforded alkaloid **172** in 98% yield. With **172** in hand treatment of alkaloid **172** with *n*-butyllithium was still unsuccessful, confirming the requirement for protection of the hydroxyl group. Instead, sodium hydride (2.5 equiv. 57% suspension) was added to a solution of alkaloid **171** (1 equiv.) in dry DMF and reacted for 2 h at room temperature under an inert atmosphere. The intermediate anion was reacted with benzyl bromide (1.1 equiv.) for 16 h. Purification of the crude mixture by silica gel column chromatography gave a complex mixture (Scheme 56).



Scheme 56

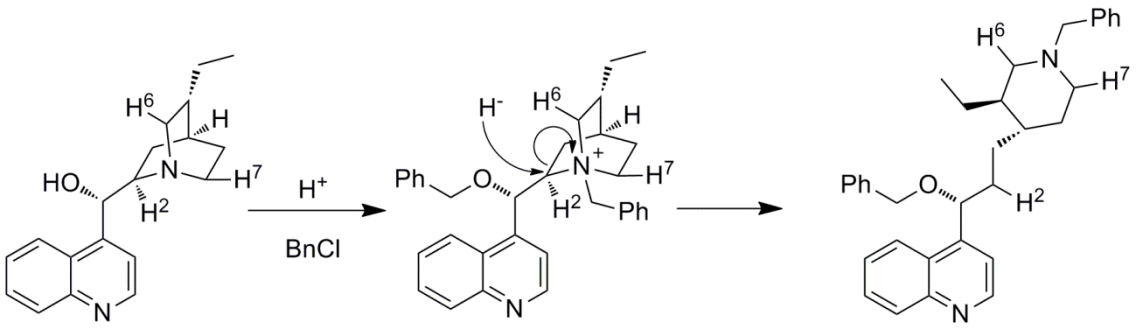
The conditions for the attempted protection of the hydroxyl group on hydrocinchonine (**172**) were changed.[191,192] The initial reaction time of sodium hydride (2 equiv., 98%) with alkaloid **172** (1 equiv.) in DMF was increased to 4 h. Purification of the crude product mixture identified two products, alkaloid **165** (5%) and alkaloid **173** (25%) (Scheme 57). Reducing the equivalents of sodium hydride (from 2 to 1 equiv.) and the reaction time (from 4 h to 2 h) allowed for a decrease in the production of alkaloid **173** and an increase in the isolated yield of alkaloid **165** to 37% (Scheme 57).



Scheme 57

Treatment of **172** with hydride and benzyl chloride probably gave a mixture of monoalkylated **165** and the unexpected dialkylated **173** *in situ*. Over the time of the reaction alkylation of the hydroxyl group is likely to occur first, followed by the slower

alkylation of the amine. An amine will usually react with benzyl chloride in the presence of a base.[193] The use of excess sodium hydride allowed for nucleophilic attack of hydride to the C2 of the quinuclidine ring system breaking the N1-C2 bond (Scheme 58). This bond is most susceptible to cleavage due to the electronic effects of the adjacent nitrogen atom and proximal inductive influence of the oxygen atom.[194] Nucleophilic attack is supported by the lack of COSY correlations between H2 and H6/H7 (Figure 13).



Scheme 58

The structural characterisation by NMR spectroscopy of **165** was obtained in acetone-d_6. The stereochemistry of alkaloid **165** is inferred from **172**. The structure of quinoline **173** was probed using high temperature ^{1}H NMR spectroscopy. Key correlations in the COSY spectrum of alkaloid **173** were identified to establish the connectivity of the ring system (Figure 13). A correlation between H2 and H3 (δ 2.48 and δ 1.72 ppm respectively) and H6/H7 (δ 2.81 and δ 2.73 ppm respectively) were observed. However, no correlations were observed between H2 (δ 2.48 ppm) and H6/H7 (δ 2.81 and δ 2.73 ppm respectively) confirming breakage of the C2-N1 bond of alkaloid **165**.

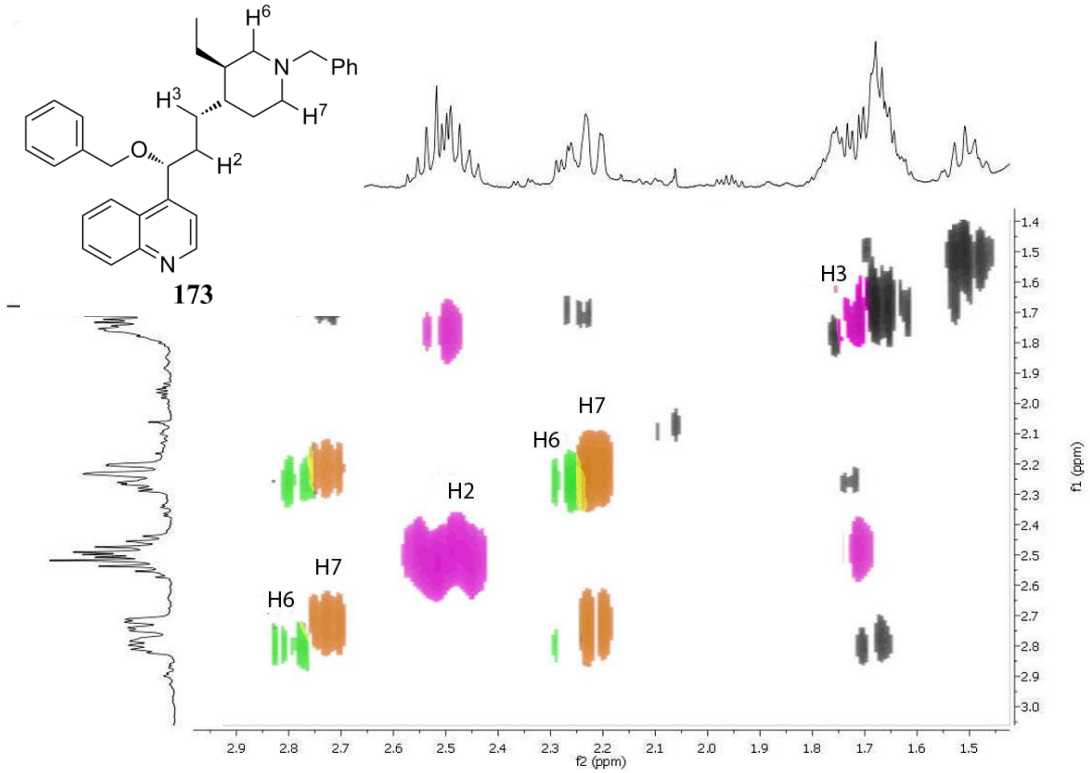


Figure 13 COSY correlations (400 MHz, 70 ºC, pyridine-d_5) showing no correlation between H2 (purple) and H6/H7 (green/orange) of alkaloid **172**.

The resonance attributed to H2 of quinoline **173** showed a significant downfield shift (δ 2.51 ppm) compared to H2 resonances seen in alkaloid **172** (δ 3.31 ppm) (Figure 14). The downfield shift identified was due to the change in proximity of the H2 and alkaloid nitrogen atom.

Obtaining the protected alkaloid **165** enabled its use to synthesise product **169**. *n*-Butyllithium (3 equiv.) was added to a solution of alkaloid **165** (1 equiv.) suspended in anhydrous THF and following conditions outlined in Scheme 57. Purification of the crude mixture by column chromatography provided alkaloid **169** (23%). Characterisation of alkaloid **169** by NMR spectroscopy proved quite difficult. The spectra of 2-substituted cinchona alkaloids showed generally poor dispersion in all solvents examined. The ^{1}H NMR spectrum $CDCl_3$ at ambient temperature was both poorly dispersed and exchange broadened (Figure 15a).

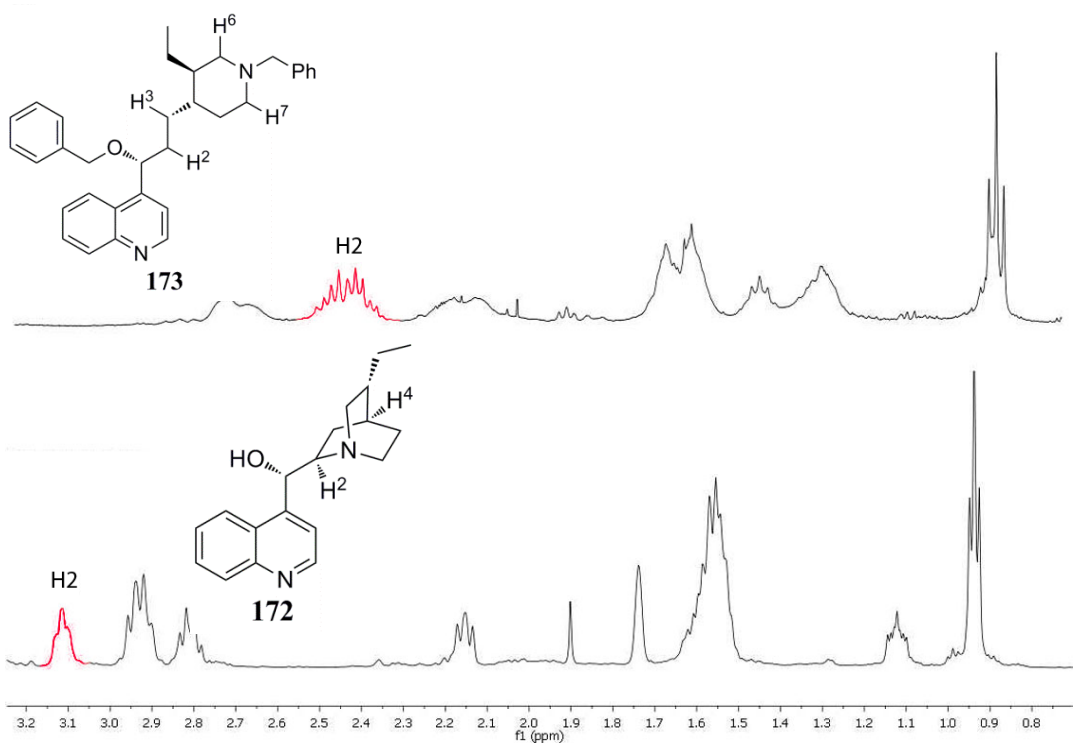


Figure 14 Expansion of (a) ^{1}H NMR spectrum (400 MHz, 70 ºC, pyridine-d_5) of alkaloid **173** and (b) ^{1}H NMR spectrum (400 MHz, 70 ºC, methanol-d_4) alkaloid **172**. Showing shift of H2 after quinuclidine ring opens at the C2 position.

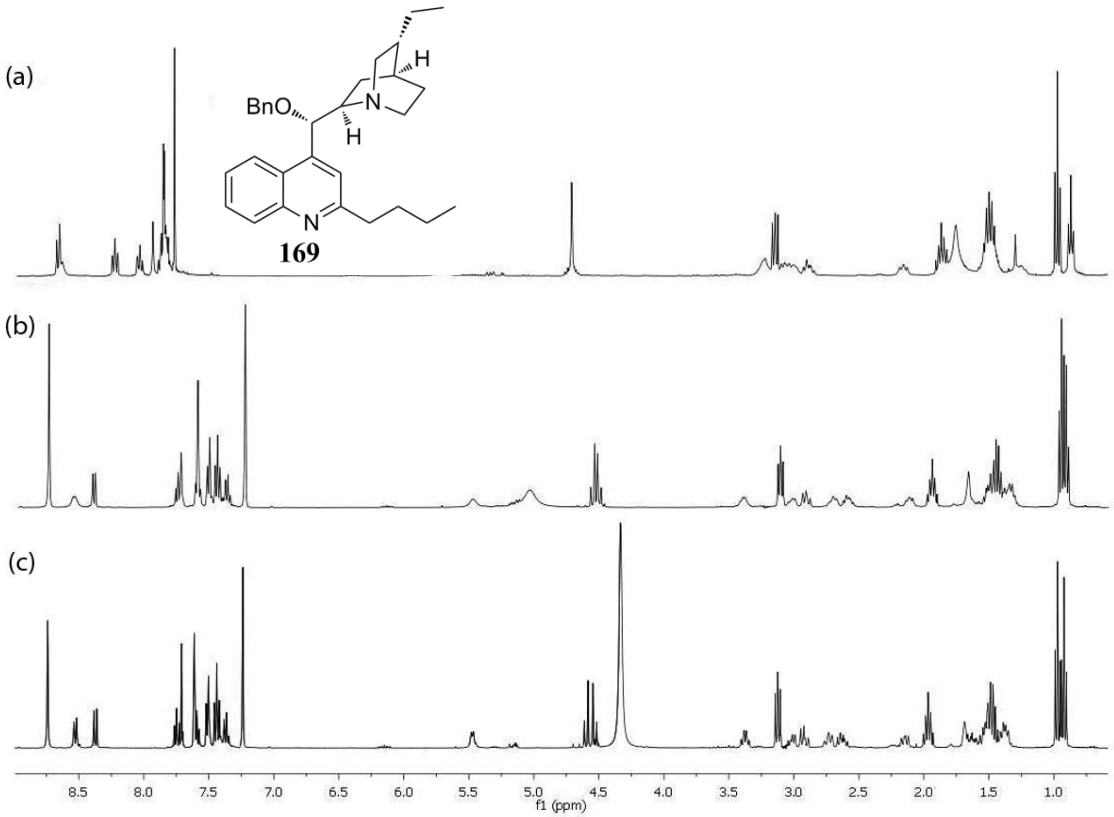


Figure 15 Comparison of ^{1}H NMR spectra and the broadening associated with alkaloid **169** (400 MHz) (a) $CDCl_3$ at 25 ºC, (b) pyridine-d_5 at 25 ºC and (c) pyridine-d_5 at 70 ºC.

Initial characterisation of the alkaloids proved difficult presumably due to the slow interconversion of rotamers. To avoid these difficulties a number of solvent systems were examined e.g. acetone, toluene and pyridine. Significant dispersion was identified when using pyridine-d_5 as the solvent. However, broadening of many resonances was still observed and unable to be resolved which hampered assignment (Figure 15b). Alkaloid **169** was probed using high temperature (70 ºC) ^{1}H NMR spectroscopy to facilitate characterisation. A well resolved, fast exchange ^{1}H NMR spectrum of alkaloid **169** was able to be obtained (Figure 15c). Broadening of ^{1}H NMR spectrum for all alkaloid derivatives was identified at ambient temperature. Therefore, all ^{1}H NMR studies were undertaken at the elevated temperature, 70 ºC, in pyridine-d_5 unless otherwise stated.

The broadening of ^{1}H NMR spectra at ambient temperature may be due to restricted rotation around the adjoining bond of the quinoline (4”-position) and hydroxy-quinuclidine substituent (Figure 16). It is hypothesised that the fast exchange at elevated temperatures presumably overcomes the interaction between the tertiary amine and acidic protons (H3” and / or H5”) of the quinoline ring system. The intermolecular interaction between such groups has been previously discussed, however not within the cinchona alkaloid ring system.[195] The potential H-bonding between the tertiary amine and protons of the quinoline ring merits further analysis. Dynamic ^{1}H NMR spectroscopy between the freezing and boiling points of pyridine was unable to achieve slow exchange of the required region. Future work would be to quantify the H-bond interaction between the tertiary amine and quinoline protons. In order to do so, an appropriate solvent, other than pyridine-d_5, would need to be used.

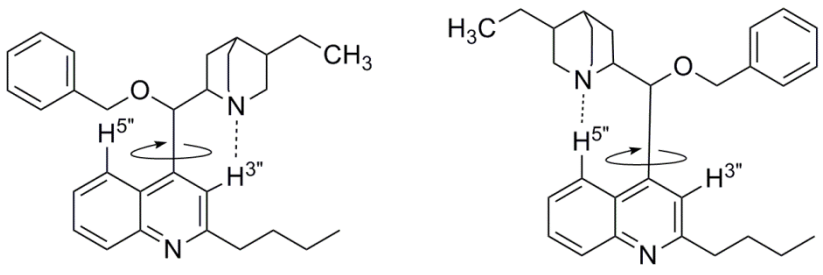


Figure 16 Presumed rotation and H-bonding between tertiary amine and H3” and H5” of the quinoline ring system.

The ^{1}H NMR spectrum of the substituted quinoline precursor **166** was generally well resolved at ambient temperature (Figure 17). Comparison of the resonances prior to the

addition of the extended chain at the 4”-position of the quinoline ring identified downfield shifts of H3 and H5 of **166** (δ H3 = 7.29, H5 = 7.84 ppm; *cf.* alkaloid **169** δ H3” = 7.71, H5” = 8.35 ppm). Introduction of the extended chain at the 4”-position identifies broadening of the ^{1}H NMR spectrum and the need for elevated temperatures to discern the resonances associated within the quinuclidine ring system (Figure 17).

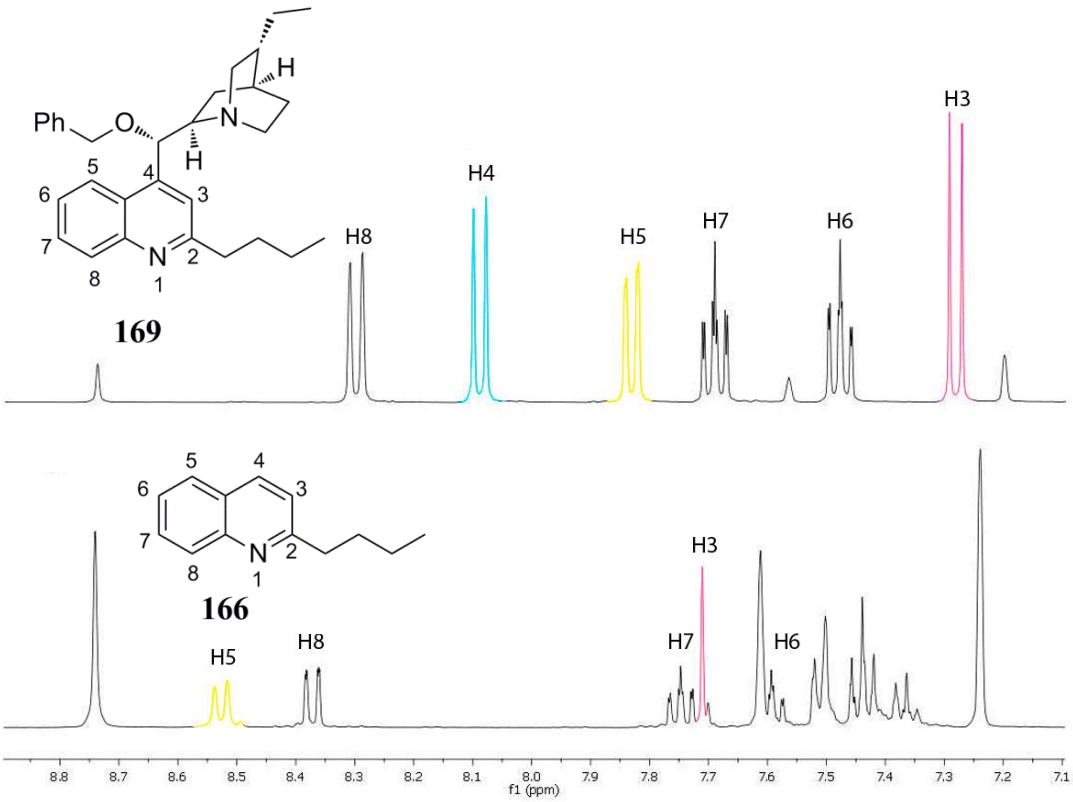


Figure 17 Expansion of (a) the ^{1}H NMR spectrum (400 MHz, 70 ºC, pyridine-d_5) of alkaloid **169** and (b) the ^{1}H NMR spectrum (400 MHz, 25 ºC, pyridine-d_5) of quinoline **166**.

The synthetic study was then extended to include an alternative organolithium reagent, phenyllithium. Phenyllithium (3 equiv.) was added to a solution of alkaloid **165** (1 equiv.) in anhydrous THF under conditions identical to those previously described (Scheme 57). Phenyl alkaloid **170** was isolated in poor yield (3%). ^{1}H NMR spectroscopic analysis of alkaloid **170** was completed at elevated temperature to assist with characterisation. Expansions of the aryl region of the ^{1}H NMR spectra obtained for alkaloids **169** and **170** are provided in Figure 18. Differences in the shift of H3” are due to effects of substitution at the 2-position of the quinoline ring. A marked upfield shift (to δ 8.40 ppm) of the H3” resonance is consistant with the substitution change to the 2-aryl substituent.

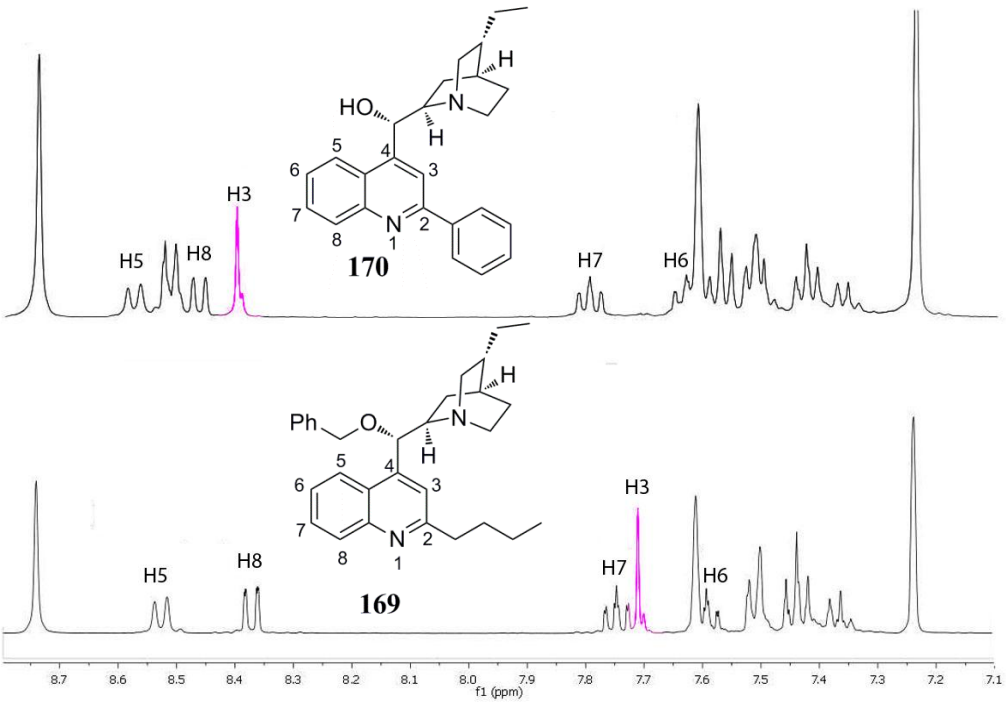
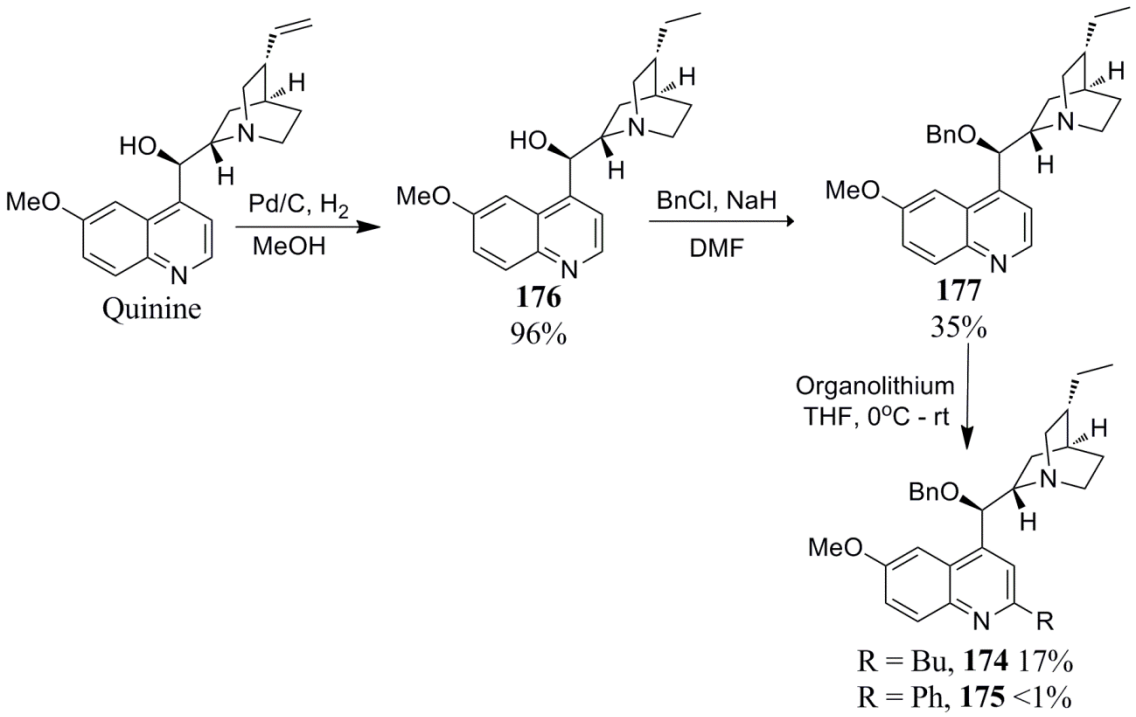


Figure 18 Expansion of (a) the ^{1}H NMR spectrum (400 MHz, 70 ºC, pyridine-d_5) of alkaloid **170** and (b) the ^{1}H NMR spectrum (400 MHz, 70 ºC, pyridine-d_5) of alkaloid **169**.

4.2.2.3 *Synthesis of alkaloid derivatives **174** and **175***

The study was further extended to include an electron-donating substituent on the quinoline ring. Hydrogenation of quinine using palladium-on-carbon in methanol gave hydroquinine **176** (96%). Protection of the hydroxyl group of **176** with sodium hydride (1 equiv. 98%) and benzyl chloride (1.1 equiv.) in DMF gave alkaloid **177** in moderate yield (35%). Alkaloid **177** (1 equiv.) was then treated with either *n*-butyllithium (3 equiv.) or phenyllithium (3 equiv.) in THF to generate the final series of cinchona alkaloids **174** (17%) and **175** (<1%) in poor yields (Scheme 59).

The ^{1}H NMR spectra of the 2",6"-substituted alkaloids **174-177** were aquired at elevated temperature due to exchange broadening. The shielding of ^{1}H and ^{13}C NMR signals of H5" and H7" of alkaloid **174** (δ H5" = 7.92, H7" = 7.53, C5" = 103.6, C7" = 121.4 ppm; *cf.* alkaloid **169** δ H5" = 8.53, H7" = 7.75, C5" = 124.3, C7" = 129.3 ppm) is consistent with location of the 6"-methoxy group. The upfield shift of C6" (δ 157.5 ppm; *cf.* 126.1 ppm) is also consistent with 6"-methoxy substitution (Figure 19).



Scheme 59

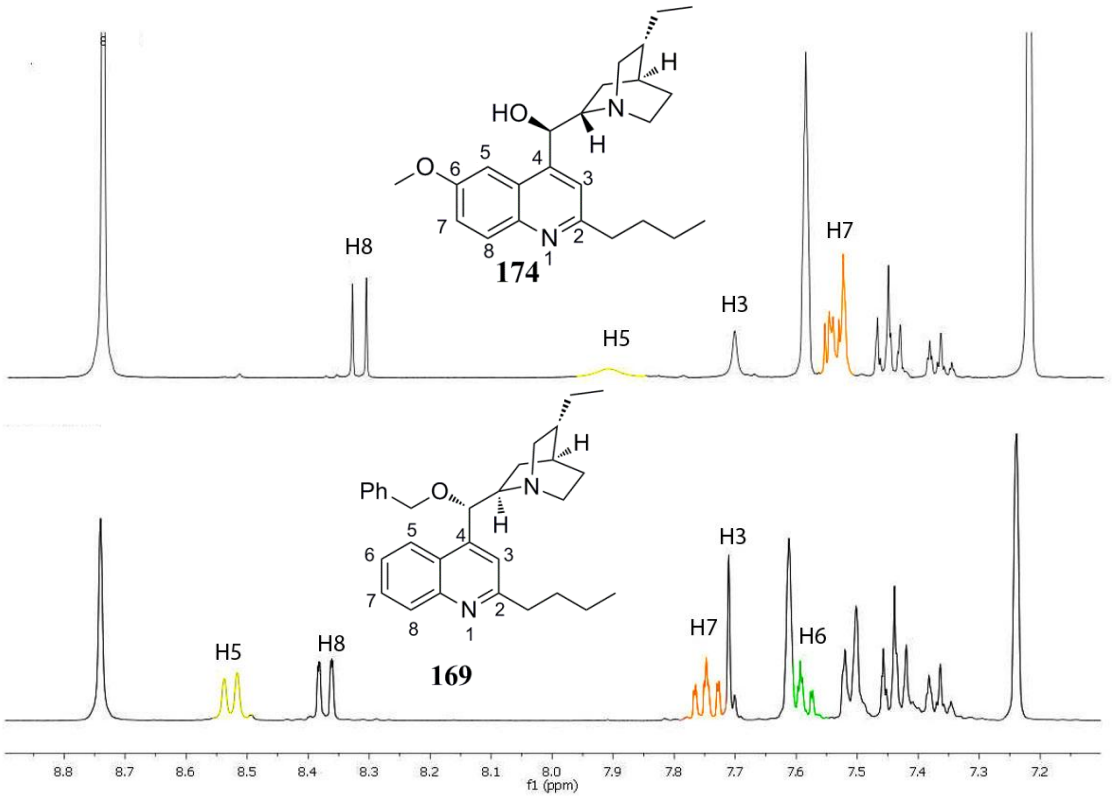


Figure 19 Expansion of (a) the ^{1}H NMR spectrum (400 MHz, 70 ºC, pyridine-d_5) of alkaloid **174** and (b) the ^{1}H NMR spectrum (400 MHz, 70 ºC, pyridine-d_5) of alkaloid **169**.

4.3 Conclusion

Substituted quinolines **118**, **119**, **166**, **168** and cinchona alkaloids **169**, **170**, **174**, **175** were successfully synthesised by reaction of quinoline **114**, 6-methoxy quinoline **167**, hydrocinchonine **172** or hydroquinine **176** with *n*-butyllithium and phenyllithium. While reaction of *N*-oxide **147** yielded the corresponding 2-substituted quinolines, *N*-oxide **164** was an ineffective starting material and an alternative synthesis was required. The use of organolithium reagents produced four cinchona alkaloid derivatives (**169**, **170**, **174**, **175**) and 4 quinoline derivatives (**118**, **119**, **166** and **168**), in poor to moderate yields (<1-40%). The alkaloid derivatives were analysed by NMR spectroscopy and broadening of ^{1}H NMR peaks due to bond rotation in cinchona alkaloids **169**, **170**, **174**, **175**. Activity of these quinoline derivatives against *Leishmania* parasites is outlined in Chapter Five.

Chapter Five

Antiparasitic Studies

A number of substituted quinolines have been reported to have a variety of activities against a diverse range of *Leishmania* species (see Chapter One). Current leishmanial treatments suffer from unwanted toxicity, expense, parasite resistance and can be difficult to administer. Simple quinoline and related compounds could potentially solve some of these problems.

5.1 Structure Activity Relationship

The most frequently used analysis for determining new potential therapies is to compare the functional group structure of analogs with their pharmacological activity. Identification of structure or compounds with a range of activities against the same target give rise to structure activity relationships. Effective identification of SAR in a lead compound is an essential step in drug design.[196]

SAR studies are generally accomplished by making minor changes to the structure of a lead compound to produce analogs and to then assess the resulting changes in biological activity. This information may then be used to develop new compounds with increased activity, fewer side effects and potentially improved bioavailability.[196]

5.1.1 Altering the carbon chain length

The addition of methylene groups increases the lipophilicity of analogs and may also improve membrane diffusion. Increasing chain length, however, may also cause micelle formation, producing large aggregates which can inhibit binding to active sites or receptors.[196]

5.1.2 Degree of saturation

Increasing the saturation of a chain affects its flexibility, making it easier for the analog to fit into an active site or receptor. Decreasing the saturation within the chain increases rigidity of the substituent. The introduction of *E* or *Z* isomers may vary activities and thus change the degree of potency of a lead. Carbon double bonds are also more sensitive to metabolic oxidation and may decrease the toxicity of a lead compound.[196]

5.1.3 Introducing or removing ring systems

The addition of ring systems to a lead compound changes the shape and overall size of

the resultant analog. The effects of potency or activity of leads are not generally predictable. However, the size may help fill a hydrophobic pocket within a target site. The introduction of an aryl ring system can be used to shrink and simplify an analog which might be too big for a target site.[196]

5.1.4 Bioavailability

Bioavailability is fundamental for the development of bioactive therapeutic agents. In order to obtain good oral bioavailability including membrane permeability and good intestinal absorption, it is helpful to use predictive algorithms to determine the choice of functional groups.[196] These predictors include the degree of molecular flexibility (measured by the number of rotational bonds (ROTB)), low polar surface area and / or total hydrogen bond count (sum of donors and acceptors).[197] The total polar surface area (TPSA) is used to establish transport across membranes and is a useful parameter to determine the sum of surface polar atoms (<120 Å) within a molecule.[197,198]

Molecular descriptors such as LogP (the logarithm of the octanol / water partition coefficient), LogS (compound solubility), molecular weight, or hydrogen bond acceptor (ON) and donor (OHNH) counts are important for establishing membrane permeability and bioavailability and Lipinski's 'rule of five' is well known.[199] The 'rule of five' parameters suggest a novel compound should have a molecular mass of <500, LogP values less than 5, no greater than 10 H-bond acceptors and <5 H-bond donors. However there are limitations to this rule. Some drugs will lie outside the parameter cut-offs and the rule specifically excludes natural products and substrates for biological transporters.[199]

5.1.5 SAR of Active Literature Quinolines

In the present project, a generalised SAR analysis of previously synthesised and biologically active quinoline analogs was established. This analysis included a correlation of substituted patterns on the quinoline ring and a comparison of functional groups at each position with reported bioactivities. Literature analogs[58,86,88,67,74,87,89,90,96,200,201] include mono-, or multi-substituted structures allowing for a generalised SAR to be established. [64,91,100,106,114,202-205] Unfortunately due to the unknown mechanism of action of quinoline analogs it is difficult to assume that all

quinoline analogs act in the same way. Therefore, this SAR has been generated to show potentially useful synthetic substitutions (Figure 20).

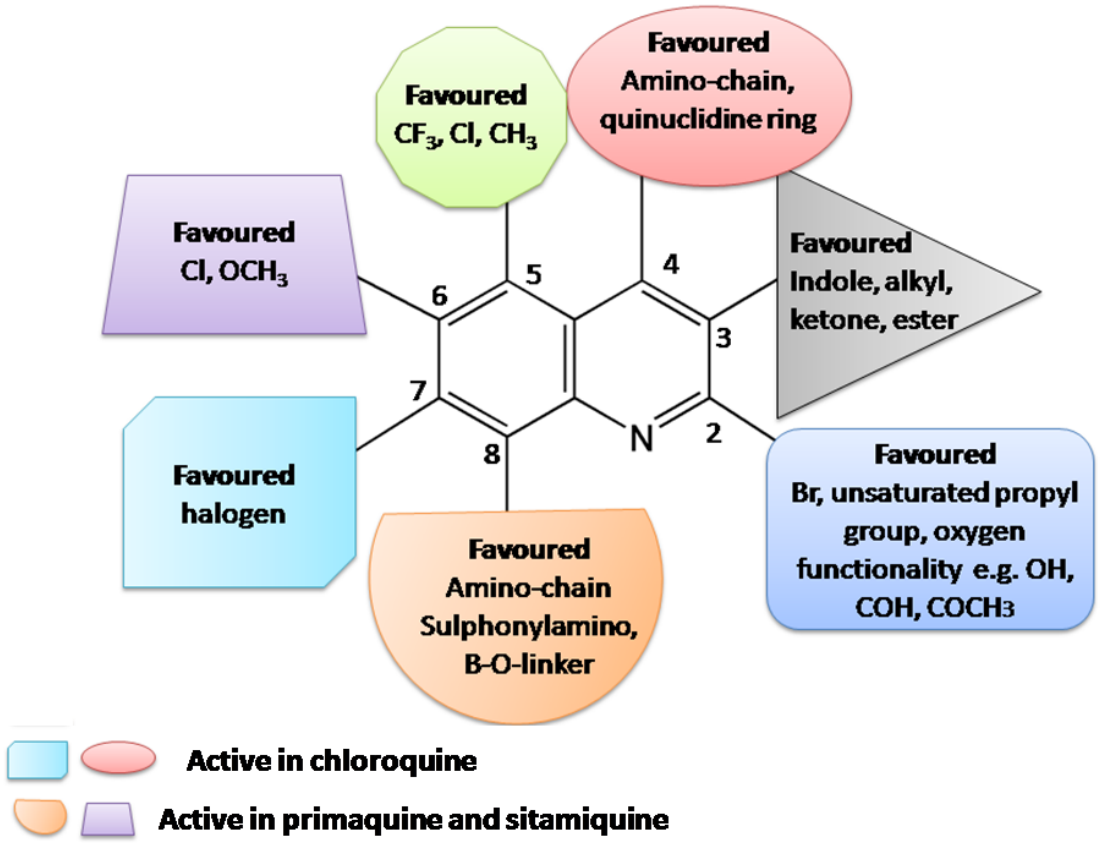


Figure 20 SAR of bioactive literature quinoline analogs

Quinoline ring substitution is favoured at all positions (Figure 20), however activity is generally seen when more than one substitution is used. In summary, 2-substituted analogs are more active if they contain an unsaturated propyl group or contain an oxygen, for example, an alcohol or ester. Extending the chain beyond three carbons or adding a bulky substituent to the end of the propyl chain decreases activity. 2-Aryl substitution shows partial activity, although less than an alkyl chain. So far, few studies have synthesised quinolines with substitution only at the 3 position. Substitution with an indole, alkyl, ketone or ester at the 3-position in combination with substitution of a methyl or extended chain at the 4-position indicated activity.[87,200] The 5-, 6-, and 7-positions generally contain methoxy, halogen or methyl functionalities in combination with other substitutions throughout the ring system. Long chain substituents at any of these position decreases activity dramatically. Finally, the 4- and 8-positions are similarly substituted with amino-chains, complex ring systems, or aryl substitutions joined via sulphonylamino or boronic acid linkers, all showing high potency against

various *Leishmania* species. Examplars of some potent quinoline analogs are shown in Figure 21.

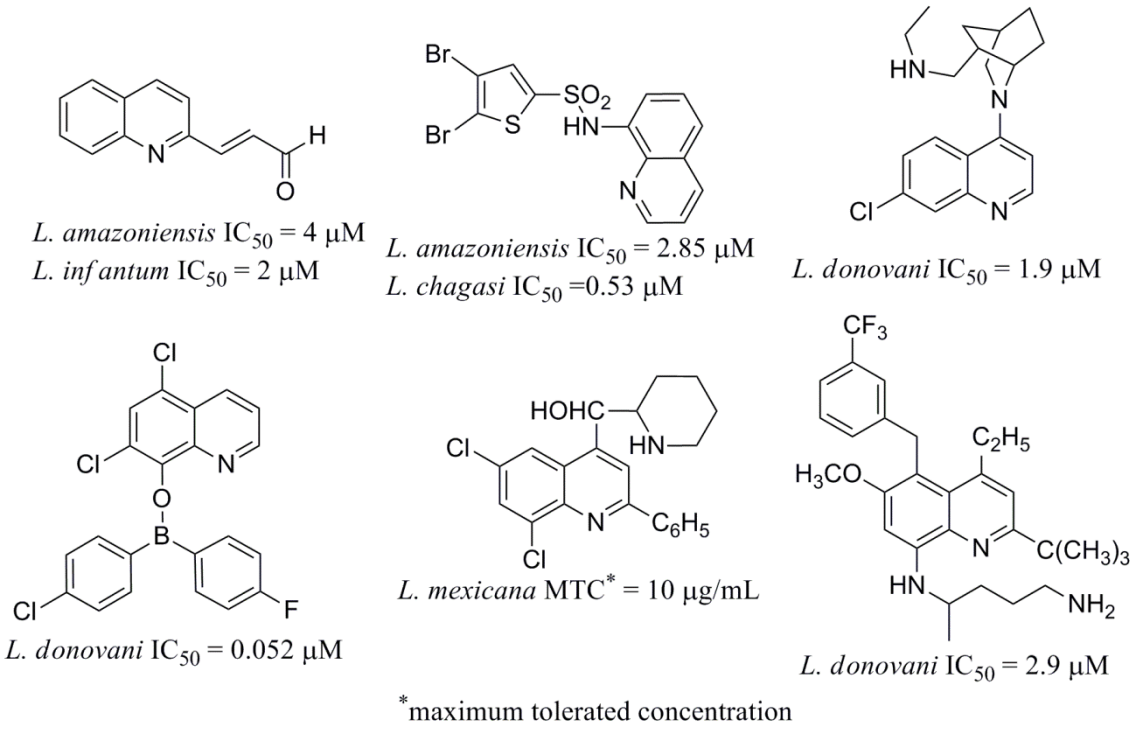


Figure 21 Examplars of literature compounds active against *Leishmaniasis*, and used to generate the generalised SAR.

In this project, the generalised SAR of quinoline analogs was used to design a new library of simple quinolines that have not been previously tested. A number of novel quinoline analogs were tested against leishmaniasis to determine their activity and potency. Any activity identified was compared against the generalised SAR model established from the literature (Figure 20). Furthermore, this chapter reports on the screening of five potential TryR inhibitors (results in Chapter Two), 22 variously substituted quinolines (4 of which were obtained from Associate Professor Naresh Kumar at the School of Chemistry, UNSW) and 11 cinchona alkaloids, against *L. major* MHOM/IL/81/FE/BNI amastigotes. This chapter presents the results of all the biologically relevant studies performed on the quinoline and cinchona alkaloids. Testing was undertaken by the candidate (K. Reynolds) under the supervision of Associate Proffesor Heinrick Korner at James Cook University, Townsville.

5.2 Cytotoxicity and antiparasitic activity

In vitro cytotoxicity of a chemical agent on mouse bone marrow-derived macrophages is typically investigated by means of a cellular assay. The major assay used in this

project to determine *in vitro* cytotoxicity involved development of mouse macrophage cells and addition of functionalised quinolines at high concentrations. Initially, compound cytotoxicity was established followed by cellular assays against *Leishmania* parasites. Cells were identified as cytotoxic if they were rigid or apoptotic. Ideally, it is desirable that the screened compounds exhibit low / no cytotoxicity combined with potency against *Leishmania*. In this project the biological activity of derivatives was assessed against *L. major* amastigotes and the results were used to further identify generalised SAR patterns.

5.3 Results and Discussion

5.3.1 Antiparasitic Assays

The assays used to screen the compounds synthesised in this project included determination of IC_{50} values against *L. major*[86] and the development of NO produced by macrophage cells.[206] NO plays an important role in neurotransmission, vascular regulation, immune responses and apoptosis. Since most of NO is oxidised to nitrite (NO_2^-) and nitrate (NO_3^-), the concentrations of these anions have been used as a quantitative measure of NO production. Preliminary compound activity was assessed against *L. major* to determine rough outer concentration limits and to allow for the final 10 point concentrations to be established for IC_{50} value determination. Infected cells were evaluated and counted by fixing and staining the cells with Kwik-Diff stain solution before being viewed under a microscope. The three step stain initially fixes the cells followed by eosin stain (cytoplasmic) and a methylene blue stain (nuclear). Biological activity was assessed and performed in duplicate and replicated (x2). Dose-response curves were generated from the averages of test results and were used to calculate IC_{50} values. Where IC_{50} values could not be determined a maximum percentage of inhibition is reported. The results of these assays are summarised in Tables 8-10 (page 103-105) and Table 11 (page 108) below, with compounds grouped according to chemical structure features.

5.3.1.1 *Mono-substituted quinoline derivatives*

The activities of mono-substituted quinoline derivatives were measured against *L. major* parasites *in vitro*. Phenyl quinoline **118** (Lit. IC_{50} 100μM)[86] was used as a control compound. Phenyl quinoline **118** showed a maximal inhibition of 65% at 125 μM, thus validating the assay used in the present studies (*L. major*) for the determination of active

compounds.[86] All compounds were found to be inactive against the parasites at either 100 μM (**178**) or 200 μM (**116**, **160**, **162**, **166**) or cytotoxic at 150-200 μM (**135**, **136**, **152**) (Figure 22).

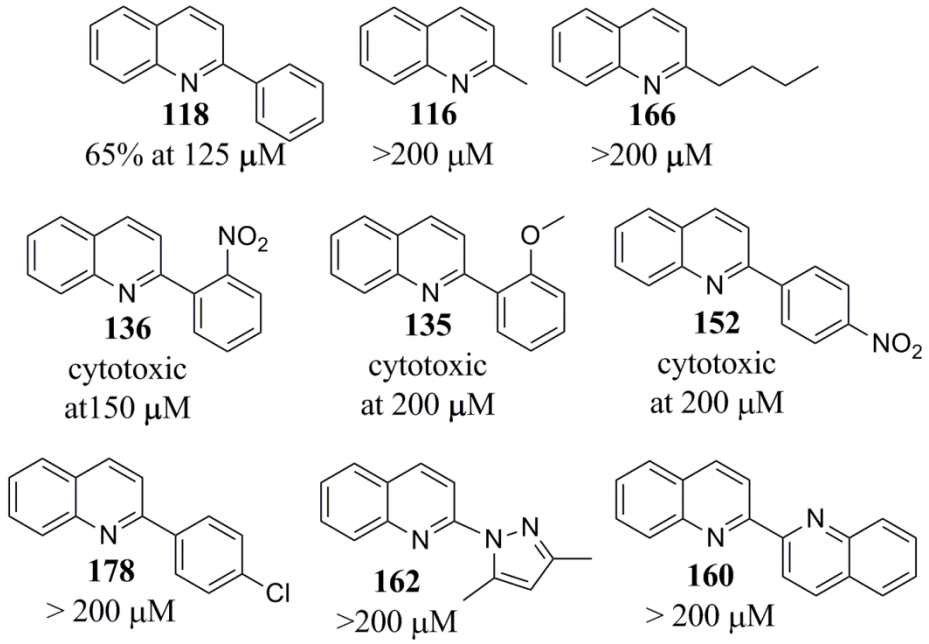


Figure 22 Quinoline derivatives screened against *L. major*

Variation in substitution at the 2-position is similar to the literature results obtained for 2-substituted quinoline analogs. The lack of activity seen for both **116** and **166** is consistent with the previous SAR discussion, and lends confirmation to the statement that the ideal chain length for 2-substituted analogs is a propyl group and that unsaturation is required. The lack of activity identified with aryl-substituted compounds **135**, **136**, **152**, **160**, **162** and **178** follows the SAR discussed previously; that few 2-aryl substituted quinolines are active. Compounds **116**, **118**, **135**, **136**, **152**, **160**, **162**, **166** and **178**, and follow Lipinski's rule of five and have a TPSA within the required limit (Table 8).

5.3.1.2 *Multi-substituted quinoline derivatives*

There are no reports of screening of di-substituted quinolines **117**, **119**, **125-127**, **168**, **179, 180** and **181** against *Leishmaia* strains. In this study di-substituted quinolines **117**, **119**, **125-127**, **168**, **179, 180** and **181** were screened against *L. major* (Figure 23; Table 9). Comparison of the activities of the di- and tri- derivatives to the monosubstituted derivatives (Table 8) revealed interesting observations. 2-Methyl quinoline **116** was

inactive (>200 μM), however, inclusion of a nitro or methoxy group at the 6-position, or a methyl group at the 8-position led to an increase in potency against *L. major*; 6-nitroquinoline **126** (50% inhibition at 50 μM), 6-methoxyquinoline **117** (IC_{50} = 71.4 μM), and 2,8-dimethyl quinoline **127** (80% inhibition at 12.5 μM) (Table 9).

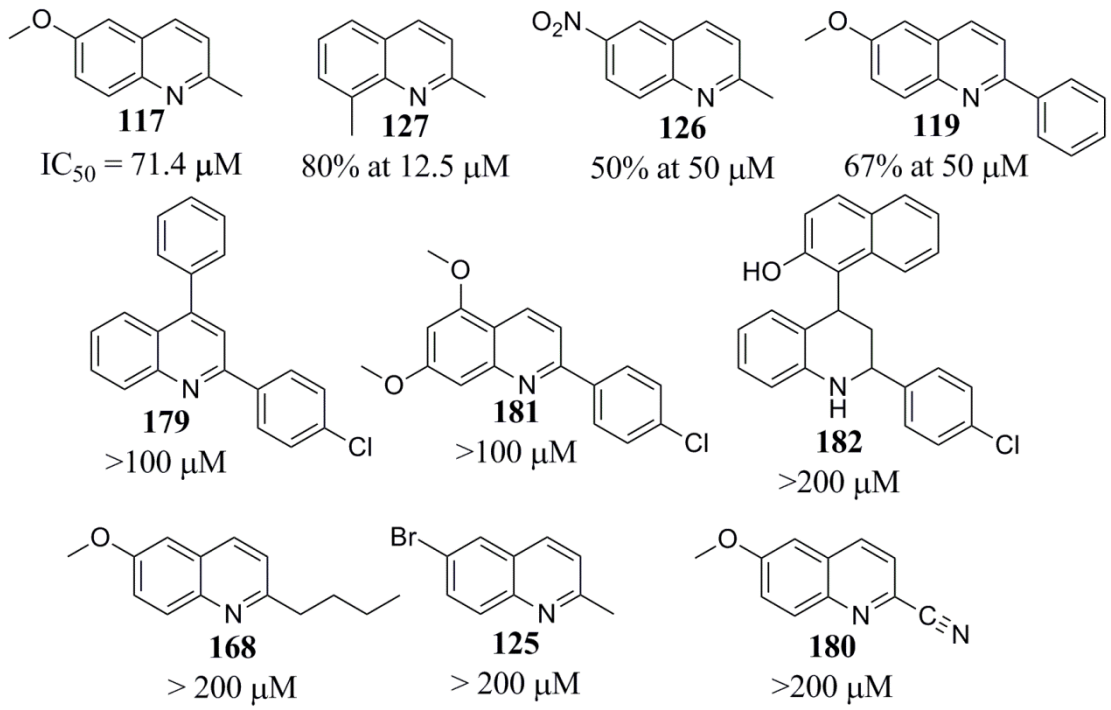


Figure 23 Quinoline derivatives screened against *L. major*

A similar effect in activity was identified in the comparison between phenyl quinoline **118** (65% inhibition at 125 μM) (Table 8) and 6-methoxy quinoline **119** (67% inhibition at 50μM) (Table 9). Quinolines **168** and **181** follow the general trend of 2-substituted quinoline being inactive, even though quinolines **168** and **181** are substituted at the 5-, 6- and 7-position with methoxy groups. Compounds **179** and **181** (>100 μM) as well as **125**, **168** and **180** (>200 μM) were all inactive against *L. major* amastigotes.

Compound **182** was donated by Associate Professor Naresh Kumar from the University of NSW, and was added to the study to identify if a hydroquinoline was active against *L. major* amastigotes. To the best of our knowledge, hydroquinoline compounds have never before been tested against *Leishmania* species. In the current study no comparison can be drawn between the activity of hydroquinoline **182** and the quinolines screened. However, compound **182** provides a degree of structural diversity. Compound **182** was inactive (>200 μM) against *L. major* amastigotes.

5.3.1.3 *N-Oxide Quinoline Derivatives*

N-Oxide quinolines **147**-**149** and **151** were all inactive against *L. major* parasites (Figure 24; Table 10). Both compounds **147** and **149** were inactive (>200 μM) compared to the control compound 2-phenylquinoline (**118**) which showed moderate activity against the parasites (65% inhibition at 125 μM). Both the non-oxidised and oxidised compounds **135** and **151** were cytotoxic against *L. major*. These results suggested that both quinoline-*N*-oxides and complex 2-aryl substituted quinolines are inactive towards *L. major* parasites. No activity was observed in the case of the dioxide **148**. These results add information to the SAR identifying that oxidised quinoline species are inactive against *L. major*.

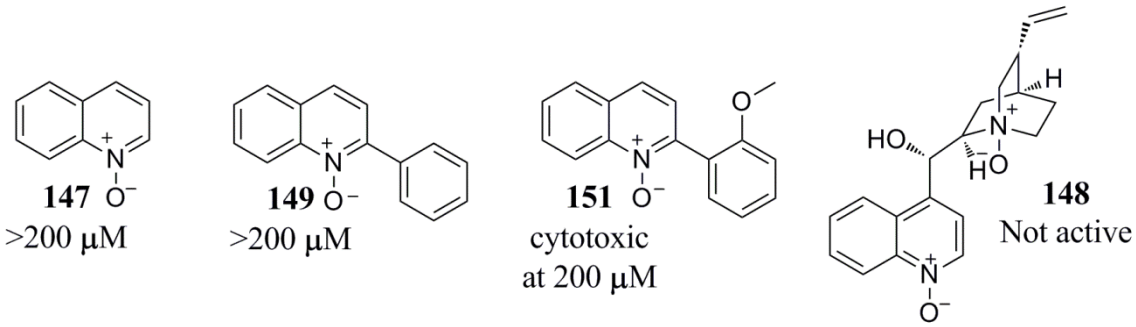


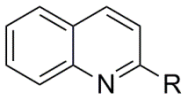
Figure 24 *N*-Oxide quinoline derivatives screened against *L. major*

5.3.2 Cinchona alkaloid derviatives

The cinchona alkaloids consist of 4 major alkaloids; quinine, quinidine, cinchonine and cinchonidine (Figure 25). Quinine and quinidine are substituted at the 6-position with a methoxy group. All four derivatives are isolated from the bark of the cinchona tree. Quinine has been shown to have an effect on choline inhibition within *L. major* promastigotes at low concentrations, and thus is a potential lead compound.[85]

The various IC_{50} activities and inhibition values of the cinchona alkaloid derivatives of cinchonine and quinine are highlighted below (Figure 26; Table 11). Of the alkaloids tested **169**, **170**, **174**, **175** and **177** were cytotoxic to cells at 10 μM, while compounds **164** and **172** were cytotoxic above 20 μM. Notably compounds **165**, **169**, **170**, **172**, **174**, **175** and **177** showed parasite inhibition in low micromolar concentrations. In particular, benzyl protection of the hydroxyl group gave an increase in parasite

Table 8 Inhibition values and Lipinski calculations of mono-substituted quinoline derivatives **116**, **118**, **127**, **135**, **152**, **160**, **162**, **166** and **178** tested against *L. major*

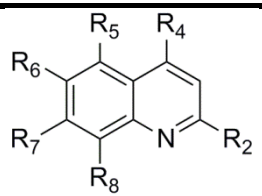


Compound	*Inhibition value (μM)*	*MW*	*LogP*	*LogS*	*# ROTB*	*# ON*	*# OHNH*	*TPSA (Å²)*	*# Lipinski Violations*
R = CH_3 (**116**)	>200	143.19	2.66	2.3	0	1	0	12.892	0
R = C_6H_5 (**118**)	65% at 125	205.25	4.25	-4.09	1	1	0	12.892	0
R = (2'-OCH_3)C_6H_5 (**135**)	200[§]	235.28	4.12	3.95	2	2	0	12.892	0
R = (2'-NO_2)C_6H_5 (**136**)	150[§]	250.25	3.88	-4.67	2	4	0	58.716	0
R = (4'-NO_2)C_6H_5 (**152**)	200[§]	250.28	3.79	-2.75	2	4	0	58.716	0
Bisquinoline (**160**)	>200	256.30	4.31	-5.04	1	2	0	25.784	0
R = (3',5'-CH_3)$_2N_2C_3H$ (**162**)	>200	223.27	2.874	-3.05	1	3	0	30.718	0
R = $CH_2CH_2CH_2CH_3$ (**166**)	>200	185.26	4.31	-5.04	1	2	0	12.892	0
R = (4'-Cl)C_6H_5 (**178**)[†]	>100	239.70	4.51	-4.80	1	1	0	12.892	0

[§]Cytotoxic at specified concentration

[†]Donated by A/Prof. Naresh Kumar

Table 9 Inhibition values and Lipinski calculations of di- and tri-substituted quinoline derivatives **117**, **119**, **125**-**127**, **168**, **179**- **180** and hydroquinoline **182** against *L. major*

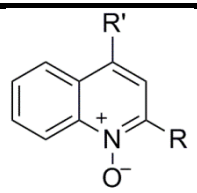


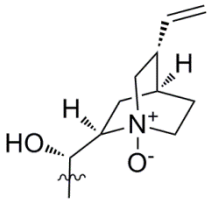
Compound	*Inhibition value (μM)*	*MW*	*LogP*	*LogS*	*# ROTB*	*# ON*	*# OHNH*	*TPSA ($Å^2$)*	*# Lipinski Violations*
$R_2 = CH_3$, $R_6 = OCH_3$ (**117**)	$IC_{50} = 71.4 \pm 0.6$	173.21	2.96	-2.32	0	1	0	22.126	0
$R_2 = C_6H_5$, $R_6 = OCH_3$(**119**)	67% at 50‡	235.28	4.18	3.95	2	2	0	22.126	0
$R_2 = CH_3$, R_6 = Br (**125**)	>200	222.09	3.39	-3.67	0	1	0	12.892	0
$R_2 = CH_3$, $R_6 = NO_2$ (**126**)	50% at 50	188.18	2.61	-2.63	1	4	0	58.716	0
$R_{2,8} = CH_3$ (**127**)	80% at 12.5‡	157.21	2.96	-2.32	0	1	0	12.892	0
$R_2 = CH_2CH_2CH_2CH_3$, $R_6 = OCH_3$ (**168**)	>200	215.29	3.36	-3.76	4	2	0	22.126	0
R_2 = (4-Cl)C_6H_5, $R_4 = C_6H_5$ (**179**)*	>100	315.80	6.561	-6.99	2	1	0	12.892	1
R_2 = (4-Cl)C_6H_5, $R_{5,7} = OCH_3$ (**181**)*	>100	229.75	4.832	-4.73	3	3	0	31.36	0
$R_2 = CN$, $R_6 = OCH_3$ (**180**)	>200	184.19	1.801	-2.87	1	3	0	45.918	0
Hydroquinoline (**182**)*	>200	385.89	6.803	-6.88	2	2	2	32.255	1

‡Hillslope >5.0 suggesting compound insolubility

* Donated by Associate Proffesor Naresh Kumar

Table 10 Inhibition values and Lipinski calculations of *N*-oxide quinoline derivatives **147**-**149** and **151** tested against *L. major*



Compound	*Inhibition value (μM)*	*MW*	*LogP*	*LogS*	*# ROTB*	*# ON*	*# OHNH*	*TPSA (Å^2)*	*# Lipinski Violations*
R, R' = H (**147**)	>200	145.16	0.15	-2.37	0	2	0	25.459	0
R = C_5H_6 R' = H (**149**)	>200	221.26	1.64	-5.09	2	3	0	34.693	0
R' =  (**148**)	NA[†]	326.39	0.15	-4.62	3	5	1	62.758	0
R = $(OCH_3)C_5H_6$, R' = H (**151**)	200[§]	251.28	1.65	-5.09	2	3	0	34.693	0

[§]Cytotoxic at specified concentration

[†]NA – not active

inhibition; **165** (74% at 4 μM); *cf.* **172** (10% inhibition of parasites at 24 μM) and **177** (IC_{50} = 0.49 μM) and **176** (98% inhibition at 125 μM). Further bioassays would need to be completed on other unprotected 2 or 2,6-substituted alkaloids to confirm if the benzyl group is an important functionality for activity.

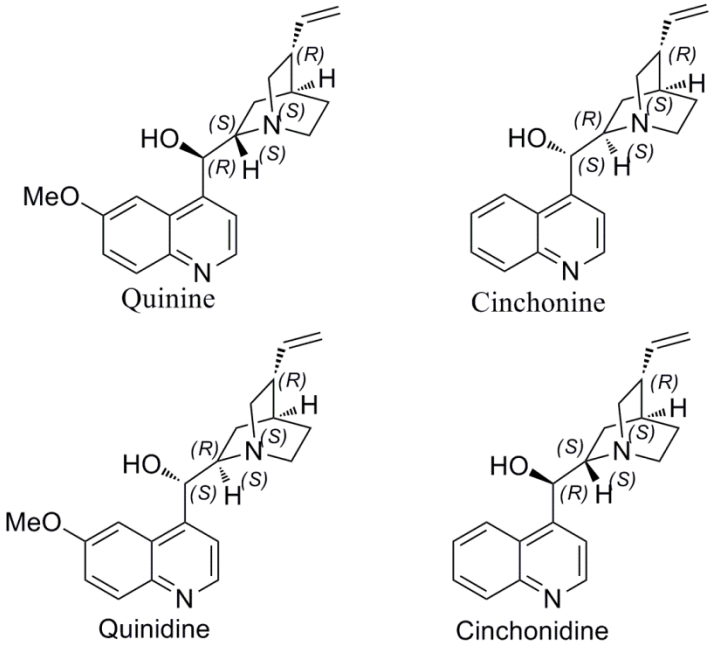


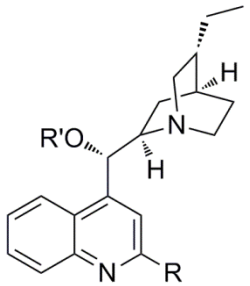
Figure 25 Cinchona alkaloids

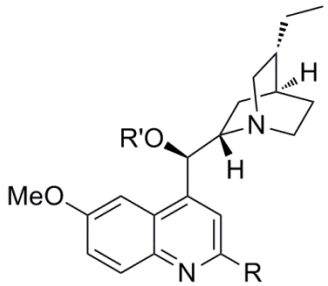
Quinine and cinchonine alkaloids **169**, **170**, **174** and **175** all displayed a level of activity ranging from an IC_{50} of 1.83 μM for **170** to 82% inhibition at 1.6 μM for both **174** and **175**. Compounds **165**, **169**, **170**, **174**, **175** and **176** have Lipinski violations which are expected because of the natural product component of the compounds.

As these bioassays were completed against *L. major* amastigotes, alkaloids **166**, **169**, **170**, **172** and **174-177** may have a similar effect on choline inhibition within the *Leishmania* parasite however, further studies are required before these assumptions can be made. This may also suggest why the cellular cytotoxicity for compounds **165**, **169**, **170**, **172**, **174**, **175**, and **177** was identified at such low concentrations (Table 11, page 110; cytotoxicity percentage 10-20 μM).

Table 11 Inhibition values and Lipinski calculations of Cinchona alkaloid derivatives **165**, **169**, **171** and **172-177** tested against *L. major*

Cinchonine derivative



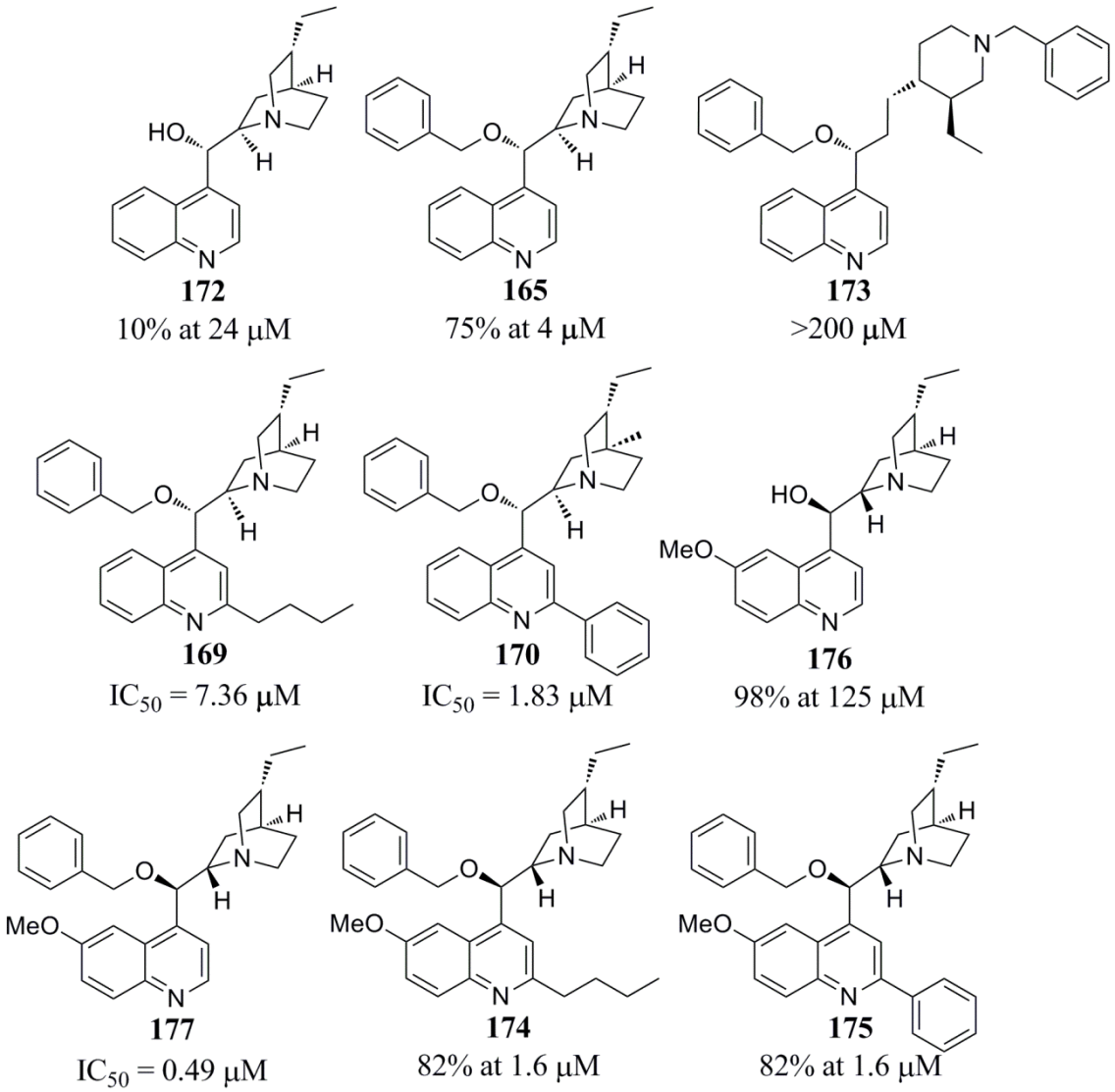


Quinine derivative

Compound	*Inhibition value (μM)*	*MW*	*LogP*	*LogS*	*# ROTB*	*# ON*	*# OHNH*	*TPSA (Å^2)*	*# Lipinski Violations*
Cinchonine									
R = H, R' = benzyl (**165**)	75% at 4	386.53	5.35	-5.71	6	3	0	25.364	1
R = butyl, R' = benzyl (**169**)	7.36 ± 2.6‡	442.64	6.92	-6.69	9	3	0	25.364	1
R = phenyl, R' = benzyl (**170**)	1.83 ± 1.7‡	462.64	6.96	-6.75	7	3	0	25.364	1
R, R' = H (**172**)	10% at 24	296.19	3.26	-2.96	3	3	1	36.358	0
Quinine									
Quinoline **173**	>200	478.67	7.43	-7.24	10	3	0	25.364	0
R = butyl, R' = benzyl (**174**)	82% at 1.6‡	472.66	6.97	-6.5	10	4	0	34.598	1
R = phenyl, R' = benzyl (**175**)	82% at 1.6‡	492.65	6.97	-6.63	8	4	0	34.598	1
R, R' = H (**176**)	98% at 125*	326.43	3.36	-3.02	4	4	1	45.592	0
R = H, R' = benzyl (**177**)	0.49 ± 1.0‡	416.56	5.35	-5.75	7	4	0	34.598	1

‡Determined on two separate occasions and averaged

*Compound partially cytotoxic (cells beginning to look granular and rigid)



169, **170**, **174** and **177** cytotoxic to cells at 10 μM,
165 and **172** cytotoxic above 20 μM

Figure 26 Cinchona alkaloid derivatives screened against *L. major*

5.3.3 Nitrite Assays

After the assays against *L. major* were completed, the media was taken and used to determine cellular nitrite production. Formation of nitrite from macrophage cells is indicative of cell activation against microbial diseases, as described in Chapter One. If nitrite production 5-days after compound introduction is observed (detection limit 550 nm), in combination with the death of parasites, activation of an oxidative immune response can be identified (Figure 27). Therefore, the compound potentially activates glutathione reductase leading to parasite death.

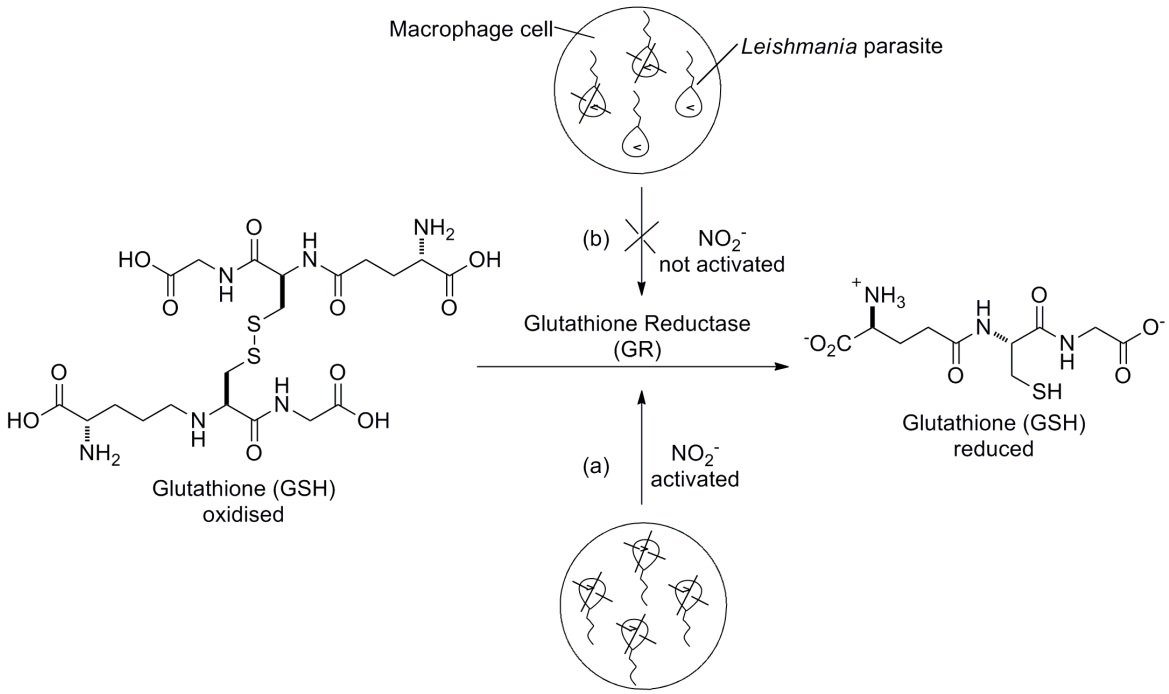


Figure 27 Diagramatic representation of glutathione reductase activation. (a) Parasites die, glutathione reductase is activated, nitrite is produced. (b) Parasites live and / or die, glutathione reductase is not activated, nitrite not produced.

Unfortunately, no nitrite formation was observed with any of the compounds (**116-119**, **125-127**, **135**, **136**, **147-149**, **151**, **152**, **160**, **162**, **165-166**, **168**, **169**, **170**, **172-177**, **178-182**) tested. This suggests that the MOA for the quinoline and cinchona alkaloid analogs does not proceed via the oxidative pathway. However, this result helps to rule out one of the possible MOA for these compounds. It is also possible that many of these quinoline compounds perform differently depending on the species and nature of disease type of *Leishmania*.

5.4 Conclusion

A total of 39 quinoline and cinchona alkaloid derivatives have been evaluated against *L. major*. The main variations focused around mono- or multi-substituted derivatives substituted at various arrangements around the quinoline ring system. 6-Methoxy-2-methyl quinoline **117** (IC_{50} = 71.4 μM) was the most active compound, within the quinoline library. Biological activity observed with cinchona alkaloid derivatives **165**, **169**, **170**, **172** and **174-177** proved promising, with all but quinoline **173** inhibiting *L. major* amastigotes at low μM concentrations. Cinchona alkaloid **177** (IC_{50} = 0.49 μM) was the most active compound, within the cinchona alkaloid library. *N*-oxide quinolines **147-149** and **151** were all inactive against *L. major* parasites. Testing against other

Leishmania species, such as those of the mucocutaneous or visceral forms would need to be carried out before *N*-oxide quinolines could be completely discounted. Quinine derivatives, **172** and **175**, were more active than the cinchonine derivatives, **169** and **170**, suggesting the importance of a 6-methoxy group (assuming a similar MOA). Previously conducted studies on quinine, suggest that cinchona alkaloids play a role in inhibiting choline transport. This establishes a potential MOA associated with these alkaloid derivatives.

As a final SAR assessment, the quinoline and cinchona alkaloid derivative libraries need to be considered separately as they may possess different sites of action. Of the quinoline derivatives, the addition of the methoxy group to the 6-position promotes activity, yet only in cases where the 2-substitution is a short chain carbon or an unsubstituted aryl group, such as phenyl. This relationship was identified previously. The addition of a carbon chain to the 8-position is similarly beneficial. When a methyl group was attached to the 2- and 8-positions, an increase in activity was observed compared to the 2-substituted quinoline **116**. Combined substitution at the 2- and 4-position must be considered as only 2-alkyl (rather than aryl) and 4-aryl substitution promotes biological activity. The cinchona alkaloids require a methoxy functional group at the 6-position to increase activity. Yet substitution at the 2-position with an aryl or alky chain improves activity relative to the non-substituted analogs. Protection of the hydroxyl group of alkaloids **165** and **177** also increased activity compared to the non-protected derivatives **172** and **176**. Unprotected derivatives of compounds **169**, **170**, **174** and **175** should also be tested to fully evaluate the effects of the benzyl group.

Future long term directions for developing bioactive quinoline or cinchona alkaloids might involve:

1. Testing the bioactivity of all analogs against other *Leishmania* species, including those that belong to the mucocutaneous or visceral disease types, such as *L. braziliensis* and *L. donovani*. It may also prove beneficial to test these compounds against other disease types such as malaria.
2. Further studies investigating the site of action and the general nature of the drug-cell interaction through cell mediated processes so that a specific MOA can be established for both quinoline and cinchona alkaloids.
3. The design and development of cinchona alkaloid derivatives also requires attention. These analogs have not previously been tested against the *Leishmania*

parasites, even though activity had been identified with quinine. A valuable extension of this work would be to compare protected and deprotected hydroxyl analogs as well as further substitution at the 2-position to identify the ideal chain length.

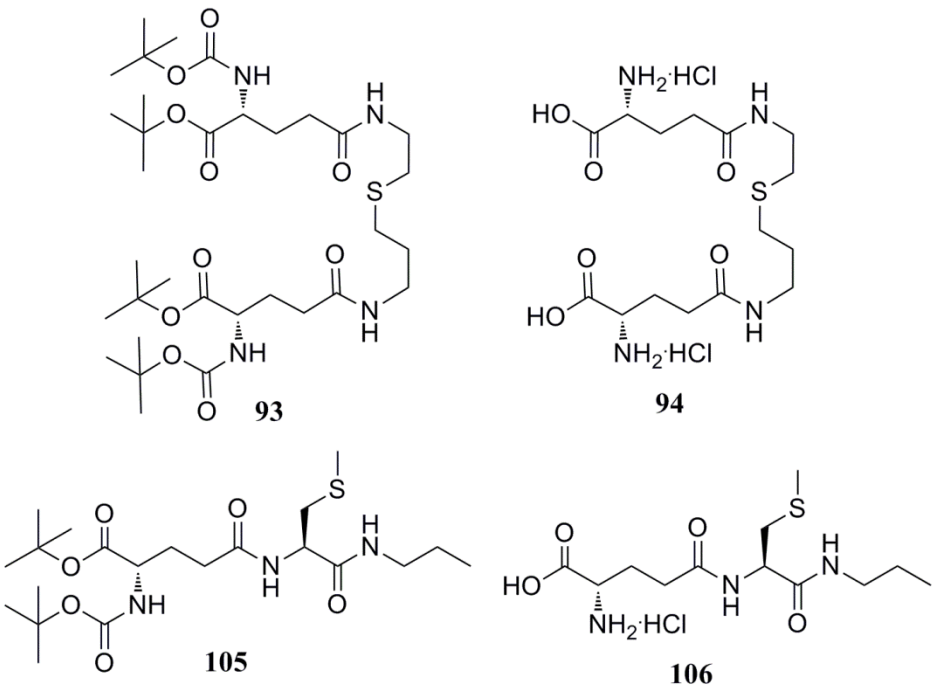
Chapter Six

Conclusions and Future Work

In the quest for novel inhibitors of *Leishmania* parasites, two approaches were explored. The first approach involved the synthesis and evaluation of TryR inhibitors via a structure-guided design, while the second approach involved the synthesis and development of structurally diverse quinoline and cinchona alkaloid derivatives

6.1 Trypanothione Reductase (TryR) inhibitors

TryR is responsible for the survival of *Leishmania* parasites in host macrophage cells. Left-hand side (**93** and **94**) and other side chain (**105** and **106**) TryR mimics were generated via solution phase peptide synthesis. The left-hand side chain mimics **93**/**94** were achieved from thioamine **85** and bromopropylamine **89** in six steps and in an overall yield of 2.3%. Side chain mimics **105**/**106** were obtained from cysteine methyl ester **103** in five steps and in an overall yield of 3.5%.



Preliminary screening of compounds **94** and **106** were undertaken against *T. brucei* TryS. Compound **106** was inactive and partial activity (inhibition at >45 μM) was observed with compound **94**. All five mimics **93**, **94**, **98**, **105** and **106** were screened against *L. major* but were inactive. It may be that some conformation constraints and fewer amide bonds would yield more bioactive compounds capable of inhibiting enzymes in the trypanothione biosynthesis pathway.

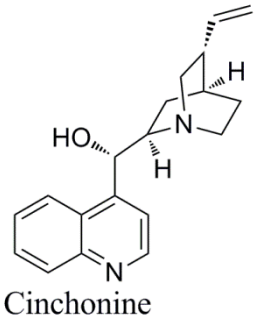
6.2 Quinoline derivatives

Many of the methods described in Chapter Three for the synthesis of quinolines suffer from harsh reaction conditions and low yields. This is especially true for quinoline synthesis involving non-heterocyclic precursors, such as the Doebner-Miller reaction. In this project the Doebner-Miller reaction was chosen because of its simplicity and literature reports acceptable yields. However, it was soon discovered that the reaction formed polymerised products with most substrates, particularly bulky starting materials. Only sterically accessable, γ-substituted α,β-unsaturated aldehydes yield quinoline derivatives using this method.

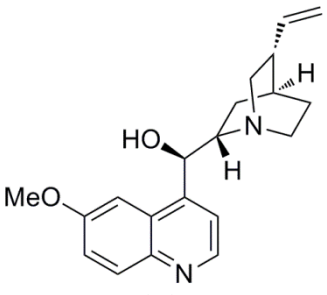
Chapter Four describes the addition of organometallic reagents to quinoline derived starting materials which proved to be a more successful method for making the desired 2-substituted quinolines. The first approach involved the reaction of quinoline-*N*-oxide with Grignard reagents and generated a variety of oxidised and reduced quinoline compounds in low to moderate yields (2-60%). The second approach utilised the reaction of quinoline or 6-methoxy quinoline with an organolithium reagent to generate 2-substituted aryl or alkyl quinoline derivatives in moderate yields (10-40%). A library of 23 quinoline derivatives was screened against *L. major*.

6.3 Cinchona alkaloid derivatives

Cinchona alkaloids have proven effective in the treatment of malaria quinine actively inhibits choline uptake in *Leishmania* parasites. Derivatives of cinchonine and quinine were obtained in this study by reducing the alkene, protecting the alcohol and finally substituting the 2-position (phenyl / butyl group) of the quinoline ring system (**165**, **169-170**, **172-173**, **176** and **177**, Chapter Four).

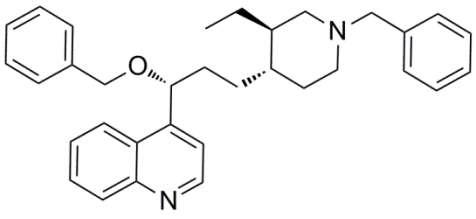


Cinchonine



Quinine

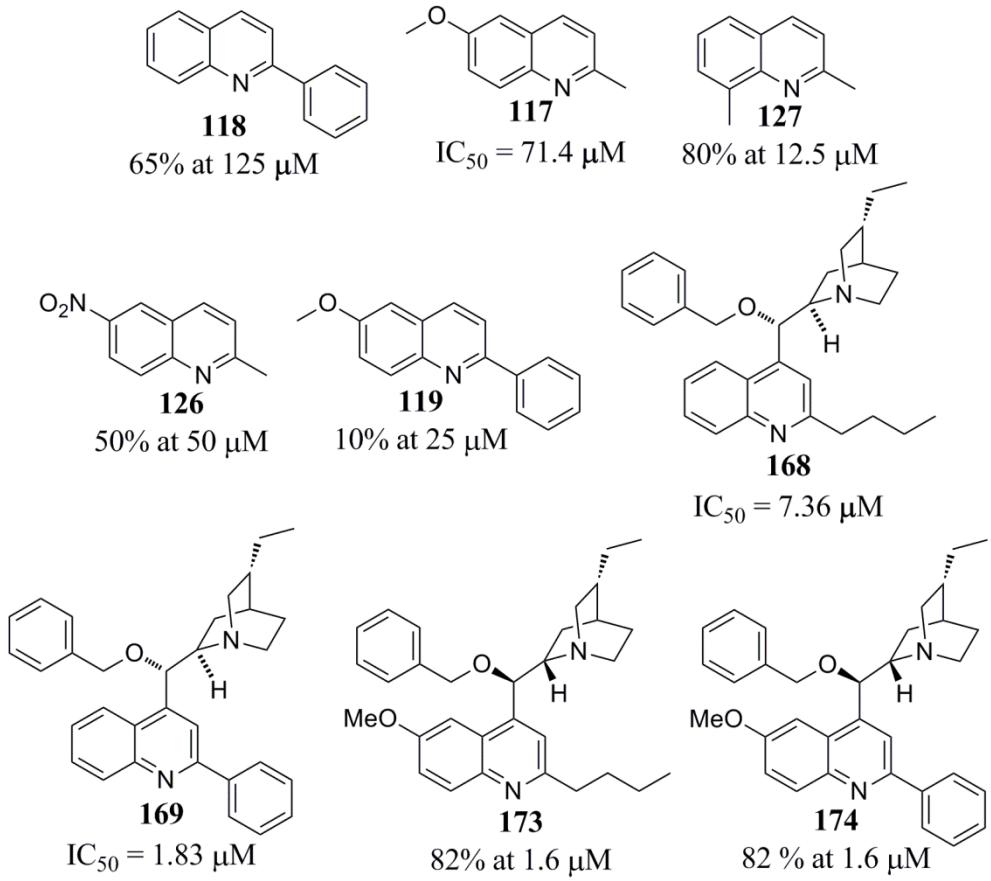
During these studies an unexpected ring opening reaction was observed. Increased equivalents of sodium hydride caused the tertiary amine to react with benzyl chloride leading to cleavage of the C2-N1 bond and formation of unknown compound **173**.



173

6.4 Summary of the quinoline and cinchona alkaloid derivatives, and TryR mimics in the antiparasitic study

This work has identified a number of biologically active quinoline and cinchona alkaloid derivatives. From the selection of quinoline compounds screened (**116-119**, **125-127**, **135**, **136**, **147-149**, **151**, **152**, **160**, **162**, **166**, **168** and **178-180**) only compounds **117-119**, **126** and **127** displayed a level of inhibition.



118
65% at 125 μM

117
IC_{50} = 71.4 μM

127
80% at 12.5 μM

126
50% at 50 μM

119
10% at 25 μM

168
IC_{50} = 7.36 μM

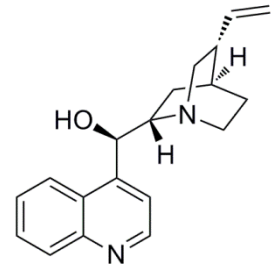
169
IC_{50} = 1.83 μM

173
82% at 1.6 μM

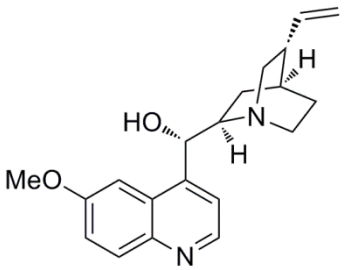
174
82 % at 1.6 μM

By comparison all of the cinchona alkaloid derivatives displayed inhibition of *L. major* in low to moderate micromolar ranges. Quinine derivatives **174** and **175** (82% at 1.6 μM and 82% at 1.6 μM respectively) were less active than the Cinchonine derivatives **169** and **170** (IC_{50} = 7.36 μM and IC_{50} = 1.83 μM, respectively).

This study confirmed that quinoline derivatives possessing 6-methoxy group exhibited improved activity (*c.f.* **117** IC_{50} = 71.4 μM, **116** >200 μM), as did alkyl, rather than aryl, substituents at the 2-position (*c.f.* **117** IC_{50} = 71.4 μM, **118** 65% at 125 μM). The cinchona alkaloid SAR indicated increased activity for derivatives possessing a 6-methoxy group (*c.f.* **177** IC_{50} = 0.49 μM, **172** 10% at 24 μM) and notably protection of the 4'- hydroxyl group (*c.f.***169** IC_{50} = 1.83 μM, **118** 65% at 125 μM). Further development of the cinchona alkaloids to improve activity against *Leishmania* parasites could include quinidine and cinchonidine derivatives. Overall, further studies into the mode of action of these derivatives are required to better develop bioactive treatments for parasitic diseases such as *Leishmania.*



Cinchonidine



Quinidine

Chapter Seven

Experimental

7.1 General

^{1}H NMR and ^{13}C NMR spectra were obtained using a 300 MHz (Varian Gemini 300) or a 400 MHz (Varian Unity 400) NMR spectrometer in solutions of $CDCl_3$, acetone-d_6, DMSO-d_6, methanol-d_4, pyridine-d_5 or deuterium oxide as indicated. ^{1}H NMR and ^{13}C NMR spectra were recorded in chemical shift (δ in ppm) referenced to solvent DMSO-d_6 at ^{1}H, 2.49 ppm and ^{13}C, 39.51 ppm respectively. I wish to acknowledge that MestReNova was used in the development of all NMR spectra used throughout this thesis.

Mass spectra were recorded on a Fisions VG-Platform II spectrometer, using electrospray as the ionisation technique with 40% formic acid and 60% acetonitrile as the solvents. Mass Lynx Version II (IBM) software was used to acquire and process the data. High Resolution Mass Spectrometry (HRMS) was performed by Griffith University FTMS High Resolution Mass Spectrometry service. Compounds with HRMS values only have ^{1}H NMR spectra (with <5% impurities) on file at Griffith University. Fourier transform infrared (FTIR) spectra were recorded in the range 4000-400 cm^{-1} on a Perkin-Elmer FTIR 1725X spectrophotometer. Spectra were recorded using KBr discs. Analytical HPLC was carried out using a Waters 600 pump equipped with a Waters 996 PDA detector and a Waters 717 autosampler. A ThermoElectron C_{18} Betasil 5 μm 143 Å column (21.2 mm, 150 mm) was used for semi-preparative HPLC separations. All solvents used for chromatography, ultraviolet (UV) spectroscopy, circular dichomism, $[\alpha]_D$, mass spectroscopy were Lab-Scan HPLC grade. The water used was Millipore Milli-Q PF filtered.

Analytical thin-layer chromatography (TLC) was carried out on Merck precoated aluminium TLC plates coated with silica gel 60 F_{254} (0.2 mm). TLC fractions were visualised by means of ultra-violet light or using the TLC stains cerium-ammonium-molybdate (CAM) dip (5.0 g ammonium molybdate, 1.0 g cerium sulphate, 10 mL H_2SO_4), vanillin dip (6.0 g vanillin / 250 ml ethanol, 2.5 ml H_2SO_4) or phosphomolybdic acid dip (10 g of phosphomolybdic acid in 100 mL of ethanol) followed by heating of the plate. Reactions under anhydrous conditions were performed in oven-dried glassware and under a flow of nitrogen gas. THF was pre-dried over sodium and distilled from sodium using benzophenone as indicator. Solvent was dispensed from the still under nitrogen immediately prior to use. Reagents and starting

materials were bought from Sigma-Aldrich or Acros Organics and used as received. Some literature compounds have incomplete data, thus full spectral data is provided for these cases herein.

7.2 Nomenclature and Numbering

The nomenclature and numbering used in the following experimental section conforms with the IUPAC nomenclature of organic compounds (Figure 28). Selected examples of compounds referred to in this section are displayed below with their corresponding numbering.

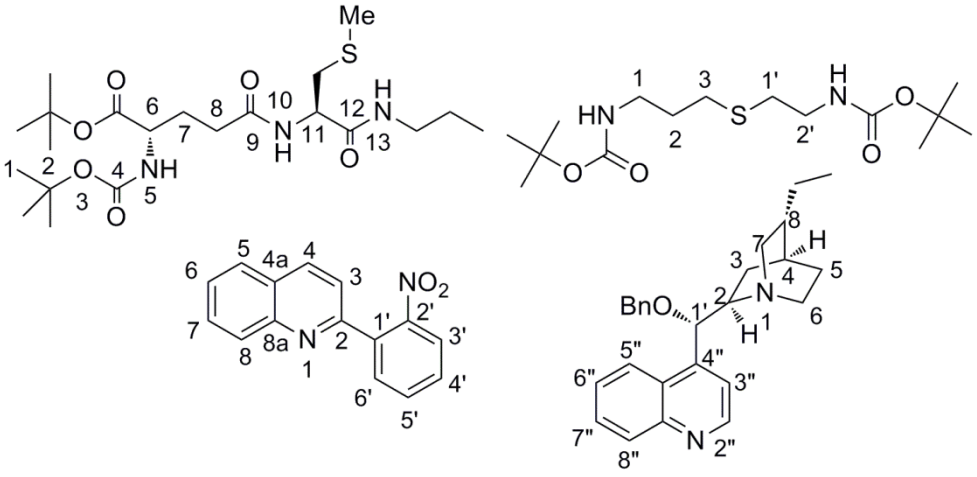
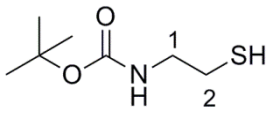


Figure 28 Examples of IUPAC numbering.

7.3 Chapter Two Experimental Procedures

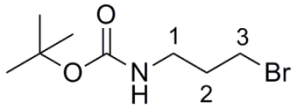
7.3.1 Synthesis of *tert*-butyl 2-mercaptoethylcarbamate (86)



86

Cysteamine hydrochloride (5.0 g, 44.01 mmol), triethylamine (Et_3N) (9.42 g, 92.42 mmol) and DCM (80 mL) were stirred for 5 min at room temperature. A solution of di-*tert*-butyl dicarbonate (Boc_2O) (9.61 g, 44.01 mmol) in DCM (20 mL) and dithiotheitol (DTT) (0.068 g, 0.44 mmol) was added. The reaction mixture was stirred at room temperature for 2.5 h, and quenched with aqueous hydrochloric acid (HCl) (1 M, 100 mL). The organic layer was washed with water (100 mL), brine (100 mL), dried (anhydrous $MgSO_4$), filtered and concentrated *in vacuo*. Purification of the crude residue by silica gel column chromatography (chloroform) gave compound **86**[123,207] (6.27 g, 82%) as a colourless oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.45 (s, 9 H, 3xCH_3), 1.65 (s, 1H, -SH), 2.61-2.69 (m, 2H, H2), 3.31 (dt, *J* = 5.8, 5.4Hz, 2H, H1), 4.91 (brs, $W_{h/2}$ = 38 Hz, NH); MS (ESI) m/z (%) 200.08 ($[M+Na]^+$, 100). The ^{1}H NMR spectrum of **86** was consistant with reported values.[123,207]

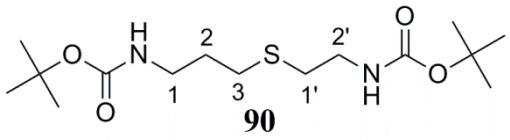
7.3.2 Synthesis of *tert*-butyl 3-bromopropylcarbamate (88)



88

3-Bromopropylamine hydrobromide (5.0 g, 22.84 mmol), Et_3N (5.55 g, 54.82 mmol), and DCM (50 mL) were stirred for five min at room temperature. Boc_2O (5.99 g, 27.42 mmol) was dissolved in DCM (5 mL) and added to the reaction. The reaction mixture was stirred at room temperature for 21 h, and then quenched with aqueous HCl (1 M, 20 mL). The organic layer was washed with water (20 mL), brine (20 mL), dried (anhydrous $MgSO_4$), filtered and concentrated *in vacuo*. Purification of the crude residue by silica gel column chromatography (chloroform) gave compound **88**[208,209] (5.09 g, 92%) as a yellow oil. ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.44 (s, 9 H, 3xCH_3), 2.05 (tt, *J* = 6.3, 6.6 Hz, 2H, H2), 3.27 (dt, *J* = 6.3, 6.6 Hz, 2H, H1), 3.44 (t, *J* = 6.6 Hz, 2H, H3), 4.64 (brs, $W_{h/2}$ = 25 Hz, NH); MS (ESI) m/z (%) 261.91 ($[M+Na]^+$, 100). The ^{1}H NMR spectrum of **88** was consistant with reported values.[208,209]

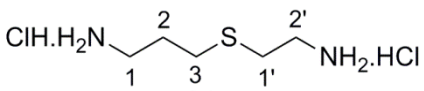
7.3.3 Synthesis of *tert*-butyl-[3-({2'-[(*tert*-butoxycarbonyl)amino]ethyl}sulfanyl) propyl] carbamate (90)



90

Compound **88** (4.84 g, 20.31 mmol) and potassium carbonate (2.57 g, 18.62 mmol) were added to a solution of compound **86** (3.0 g, 16.92 mmol) in DMF (20 mL) at 0 ^{o}C. The reaction was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (5 x 50 mL). The combined organic layers were washed with brine (100 mL), dried (anhydrous $MgSO_4$), filtered and the solvent removed *in vacuo*. Recrystallisation (EtOAc) gave compound **90** (2.5 g, 44%) as a white solid. m.p. 69-71 ºC; FTIR (KBr, cm^{-1}) 3355, 1681; ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.43 (s, 18H, 6xCH_3), 1.75 (tt, *J* = 6.9, 7.2 Hz, 2H, H2), 2.54 (t, *J* = 7.2 Hz, 2H, H3), 2.62 (t, *J* = 6.6 Hz, 2H, H1'), 3.20 (t, *J* = 6.6 Hz, 2H, H1), 3.28 (t, *J* = 6.6 Hz, 2H, H2´), NH not observed; ^{13}C NMR (400 MHz, $CDCl_3$) δ 28.4 (6 x CH_3), 28.9 (C2), 29.7 (C3), 32.2 (C1'), 39.5 (C1, C2'), 79.2 (*C*(CH_3)), 155.8 (2 x C=O); MS (ESI) m/z (%) 357.15 ([M+Na]$^{+}$, 100); HRMS Calcd for $C_{15}H_{30}N_2O_4S$.Na 357.1824; found 357.1802.

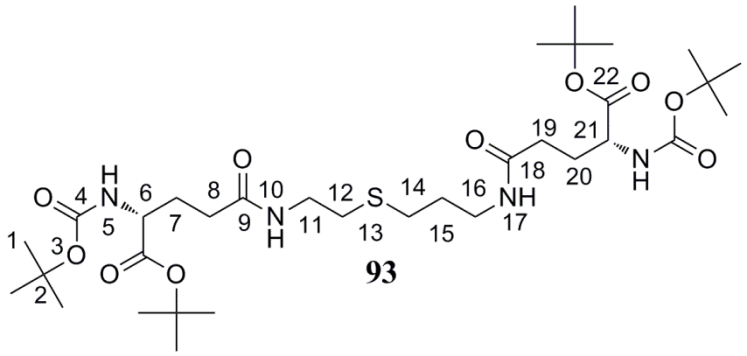
7.3.4 Synthesis of 3-(2'-aminoethylthio)propan-1-amine dihydrochloride (91)



91

Compound **90** (2.5 g, 7.47 mmol) was added to a solution of anhydrous HCl/ether (1 M, 20 mL) under a nitrogen atmosphere at 0 ^{o}C. The mixture was allowed to warm to room temperature and stirred for 30 min. The solvent was concentrated *in vacuo*. The product was washed with cold ethyl ether, and dried *in vacuo*. Compound **91**[210,211] (1.54 g, 98%) was obtained pure as a white solid. m.p. 185-187 ºC (Lit.[211] m.p. 186-189 ºC); ^{1}H NMR (300 MHz, D_2O) δ (ppm) 1.83 (tt, *J* = 7.2, 7.5 Hz, 2H, H2), 2.54 (t, *J* = 7.2 Hz, 2H, H3), 2.71 (t, *J* = 6.6 Hz, 2H, H2'), 2.96 (t, *J* = 7.5 Hz, 2H, H1), 3.05 (t, *J* = 6 Hz, 2H, H1'); MS (ESI) m/z (%) 135.67 (100).

7.3.5 Attempted synthesis of (6R,21*R*)-*tert*-butyl-6-(*tert*-butoxycarbonyl)-21-(*tert*-butoxycarbonylamino)-2,2-dimethyl-4,9,18-trioxo-3-oxa-13-thia-5,10,17-triazadocosan-22-oate (93)



Procedure 1

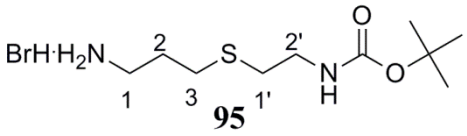
Boc-L-glutamic acid 1-*tert*-butylester (**92**) (0.234 g, 0.771 mmol) in DCM (3.5 mL) was added to a solution of **91** (0.079 g, 0.385 mmol) in DCM (3.5 mL) and reaction cooled to 0 ºC. HOBt (0.125 g, 0.927 mmol), DIPEA (0.699 g, 5.404 mmol) and DIC (0.107 g, 0.850 mmol) were added successively to the reaction mixture. The reaction was allowed to warm to room temperature and stirred for 16 h. The reaction solution was washed with a mixture of saturated brine and saturated sodium bicarbonate solution (1:1, 30mL) and the aqueous layer extracted with DCM (3 x 30 mL). The combined organic layers were washed with a mixture of brine and aqueous sodium bicarbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), filtered and concentrated *in vacuo*. Purification of the crude residue (219 mg) by silica gel column chromatography (DCM:MeOH; 0.5-1.5%) gave a complex mixture, which was then used in the next reaction. HCl/dioxane (4 M, 6 equiv.) and TIPS (0.5%) were added to the complex mixture (201 mg) and reacted under an atmosphere of nitrogen at room temperature for 1 h. Analysis of the crude mixture by ^{1}H NMR spectroscopy identified a complex mixture.

Procedure 2

Compound **91** (0.079 g, 0.385 mmol) was dissolved in sodium hydroxide (NaOH) (1 M, pH 12) and extracted with anhydrous DCM. The organic layers were dried (anhydrous $MgSO_4$) and the solvent removed *in vacuo*. Boc-L-Glutamic acid 1-*tert*-butylester (**92**) (0.234 g, 0.771 mmol) in anhydrous DCM (3.5 mL) was cooled to 0 ºC and added to a cooled (0 ºC) solution of **91** redissolved in anhydrous DCM (3.5 mL). HOBt (0.125 g, 0.927 mmol), DIPEA (0.699 g, 5.404 mmol) and DIC (0.107 g, 0.850 mmol) were added successively to the reaction mixture. The reaction was allowed to warm to room temperature and stirred for 16 h. The reaction solution was washed with a mixture of

saturated brine and aqueous sodium bicarbonate solution (1:1, 30 mL). The aqueous solution was extracted with DCM (3 x 30 mL). The combined organic layers were washed with a mixture of brine and aqueous sodium bicarbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), filtered and concentrated *in vacuo*. Purification of the crude residue by silica gel column chromatography (DCM:MeOH; 0.5-1.5%) gave a yellow solid (27 mg) as a complex mixture.

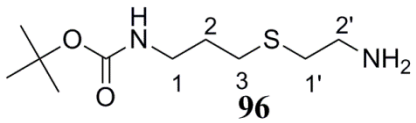
7.3.6 Attempted synthesis of *tert*-butyl (2-((3-aminopropyl)thio)ethyl)carbamate hydrobromide (95)



95

3-bromopropan-1-amine hydrobromide (**89**) (1.25 g, 5.281 mmol) and potassium carbonate (0.67 g, 4.841 mmol) were added to a solution of compound **86** (0.5 g, 4.401 mmol) in DMF (6 mL) at 0 ^oC. The reaction was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (5 x 50 mL). The combined organic layers were washed with brine (100 mL), dried (anhydrous $MgSO_4$), filtered and the solvent removed *in vacuo*. Analysis of the crude mixture by ^1H NMR spectroscopy identified a complex mixture.

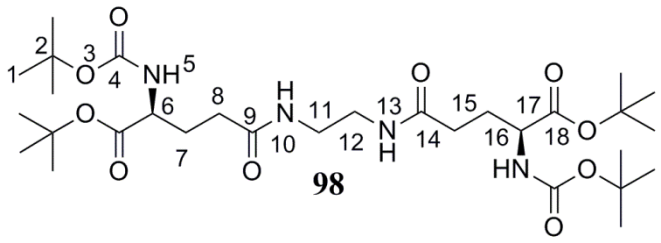
7.3.7 Attempted synthesis of *tert*-butyl (3-((2-aminoethyl)thio)propyl)carbamate (96)



96

Compound **88** (1.25 g, 5.281 mmol) and potassium carbonate (0.67 g, 4.841 mmol) were added to a solution of 2-aminoethanethiol **95** (0.5 g, 4.401 mmol) in DMF (6 mL) at 0 ^oC. The reaction was warmed to room temperature and stirred for 16 h. The reaction mixture was diluted with water (100 mL) and extracted with EtOAc (5 x 50 mL). The combined organic layers were washed with brine (100 mL), dried (anhydrous $MgSO_4$), filtered and the solvent removed *in vacuo*. Analysis of the crude mixture by ^1H NMR spectroscopy identified a complex mixture.

7.3.8 Synthesis of (6R,17*R*)-*tert*-butyl-6-(*tert*-butoxycarbonyl)-17-(*tert*-butoxy carbonyl amino)-2,2-dimethyl-4,9,14-trioxo-3-oxa-5,10,13-triazaoctadecan-18-oate (98)



98

Procedure 1

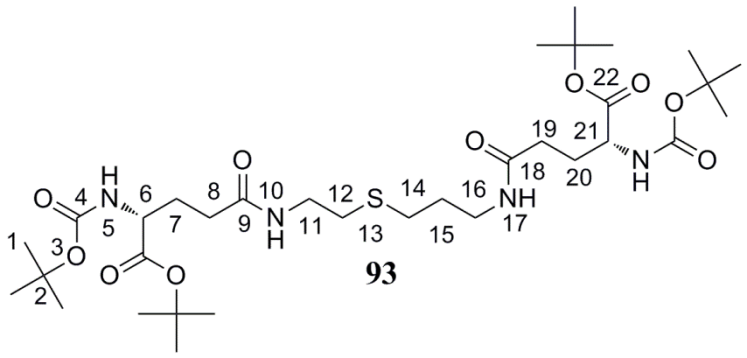
Boc-L-Glutamic acid 1-*tert*-butylester (**92**) (0.5 g, 1.64 mmol) in anhydrous DCM (5 mL) was added to a solution of ethane-1,2-diamine (0.05 g, 0.824 mmol) in anhydrous DCM (2 mL) at 0 ºC. HOBt (0.267 g, 1.978 mmol), DIPEA (1.49 g, 11.536 mmol) and DIC (0.228 g, 1.813 mmol) were added successively. The reaction mixture was allowed to warm to room temperature and stirred for 16 h. Any formed precipitate was removed by filtration. The filtrate was extracted with a mixture of brine and aqueous sodium bicarbonate (1:1, 30 mL) and the aqueous phase extracted with DCM (3 x 30 mL). The combined organic layers were washed with a mixture of brine and aqueous sodium bicarbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), filtered, evaporated. The residue was purified by silica gel column chromatography (DCM:MeOH; 0.5-2%). A complex mixture was obtained.

Procedure 2

Boc-L-Glutamic acid 1-*tert*-butylester (**92**) (0.5 g, 1.64 mmol), DIPEA (1.49 g, 11.536 mmol), anhydrous DCM (5 mL), were stirred at 0 ºC under an atmosphere of nitrogen. HOBt (0.267 g, 1.978 mmol), and PyBOP (0.428 g, 0.824 mmol) were added successively. Ethane-1,2-diamine (0.05 g, 0.824 mmol) was added to the reaction mixture and was warmed to room temperature and stirred for 16 h. The solution was washed with a mixture of brine and aqueous sodium bicarbonate (1:1, 30 mL) and the aqueous phase extracted with DCM (3 x 30 mL). The combined organic layers were washed with a mixture of brine and aqueous sodium bicarbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), filtered and evaporated. Purification of the residue by silica gel column chromatography (DCM:MeOH, 0.5-2%) gave compound **98** (146 mg, 28%) as a white solid. An analytically pure sample of **98** was obtained by semi-preparative (H_2O:MeOH, 90:10 to 0:100; rf = 28.5min) from the major chromatography fraction. m.p. 97-99 ºC; FTIR (KBr, cm^{-1}) 3322, 2980, 1693, 1392, 1157; ^1H NMR (300 MHz,

MeOH-d_4) δ (ppm) 1.25-1.6 (m, 36H, 3 x 2-CH_3, 6-$CO_2C(CH_3)_3$, 17-$NHCO_2C(CH_3)_3$, 18-$OC(CH_3)_3$), 1.8-1.95 (m, 4H, H8, H15), 2.0-2.2 (m, 2H, H7, H16), 2.23-2.39 (m, 2H, H7, H16), 3.2-3.4 (m, 4H, H11, H12), 3.86-4.03 (m, 2H, H6, H17) 6.78 (brd, J = 8 Hz, 2H, 5-NH, 17-NH), 7.89 (brs, $W_{h/2}$= 14 Hz, 2H, 10-NH, 13-NH); ^{13}C NMR (300 MHz, MeOH-d_4) δ 25.2 (C7, C16), 28.4 (C1, 2 x 2-CH_3, 6-$CO_2C(\mathbf{C}H_3)_3$, 17-$NHCO_2C(\mathbf{C}H_3)_3$, 18-$OC(\mathbf{C}H_3)_3$), 33.1 (C8, C15), 39.9 (C11, C12), 55.2 (C6, C17) 80.5 (C-2, 17-$NHCO_2\mathbf{C}(CH_3)_3$), 82.2 (6-$CO_2\mathbf{C}(CH_3)_3$, 18-$CO_2\mathbf{C}(CH_3)_3$), 157.9 (C-14, 17-$NH\mathbf{C}O_2C(CH_3)_3$), 173.1 (6-$\mathbf{C}O_2C(CH_3)_3$, C18), 175.0 (C9, C14); MS (ESI) m/z (%) 653.09 ($[M+Na]^+$, 100); HRMS Calcd for $C_{30}H_{54}N_4O_{10}Na$ 653.3732; found 653.3729.

7.3.9 Synthesis of (6*R*,21*R*)-*tert*-butyl-6-(*tert*-butoxycarbonyl)-21-(*tert*-butoxy carbonylamino)-2,2-dimethyl-4,9,18-trioxo-3-oxa-13-thia-5,10,17-triazadocosan-22-oate (93)

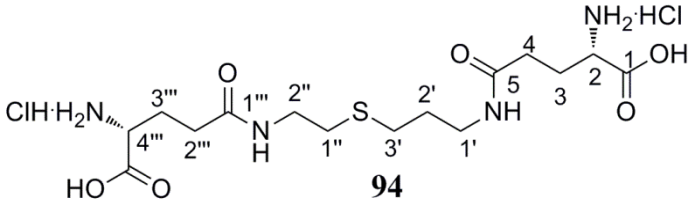


Procedure 3

Compound **91** (0.44 g, 3.229 mmol) was added to DIPEA (3.94 mL, 7 eq) in anhydrous DMF (15 mL). The mixture was stirred at room temperature for 2 h under an atmosphere of nitrogen. Boc-L-Glutamic acid 1-*tert*-butylester (**92**) (1.96 g, 6.458 mmol) in DMF (15 mL) was cooled to 0 ºC. HOBt (1.407 g, 7.749 mmol), DIPEA (3.94 mL, 7 eq) and benzotriazol-1-yl-oxytripyrrolidinophosphonium hexafluorophosphate (PyBOP) (3.345 g, 6.428 mmol) were added successively to the second mixture which was stirred for 30 min at room temperature under nitrogen. The solution of compound **91** was added to the Boc-L-glutamic acid 1-*tert*-butylester solution. The reaction mixture solution was stirred overnight at room temperature for 16 h under a flow of nitrogen. The solution was extracted with a mixture of brine and aqueous sodium carbonate (1:1, 30 mL) and the aqueous phase extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with a mixture of brine and aqueous sodium carbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), and the solvent removed *in vacuo*. Purification of the residue was achieved by silica gel column chromatography

(EtOAc:hexane; 4:1). Compound **93** (706 mg, 31%) was obtained as a viscous oil. An analytically pure sample of **93** was obtained by semi-preparative HPLC (H_2O:MeOH, 90:10 to 0:100; rf = 28.5min) from the major chromatography fraction. FTIR (KBr, cm^{-1}) 2978, 1715, 1651, 1525; ^1H NMR (400 MHz, $CDCl_3$) δ (ppm) 1.42 (s, 36H, H1, 2 x 2-CH_3, 6-$CO_2C(CH_3)_3$, 21-$NHCO_2C(CH_3)_3$, 22-$OC(CH_3)_3$,), 1.52-1.82 (m, 2H, H15), 1.82-1.93 (m, 2H, H20), 2.04-2.16 (m, 4H, H7, H19), 2.27 (t, *J* = 7.2 Hz, 2H, H8), 2.56 (t, *J* = 6.8 Hz, 2H, H12), 2.63 (t, *J* = 6.6 Hz, 2H, H14), 3.24-3.51 (m, 4H, H11, H16), 4.00-4.20 (m, 2H, H6, H21), 5.34 (brs, $W_{h/2}$ = 5 Hz, 2H, H10, H17), 6.76 (brs, 2H, $W_{h/2}$ = 2.5 Hz, H5, 21-N**H**$CO_2C(CH_3)_3$); ^{13}C NMR (400 MHz, $CDCl_3$) δ 27.7 (C7, C20), 27.9 (C1, 2 x 2-CH_3, 6-CO_2C(**C**$H_3)_3$, 21-$NHCO_2C$(**C**$H_3)_3$, 22-CO_2C(**C**$H_3)_3$), 28.6 (C14), 28.8 (C15), 29.3 (C19), 31.5 (C12), 32.4 (C8), 38.3 (C11, C16), 53.4 (C6, C21), 79.9 (C2, 21-$NHCO_2$**C**$(CH_3)_3$,) 82.2 (6-CO_2**C**$(CH_3)_3$, 22-CO_2**C**$(CH_3)_3$), 155.9 (21-NH**C**$O_2C(CH_3)_3$, C4), 171.3 (6-CO_2, C22), 172.8 (C9, C18); MS (ESI) m/z (%) 727.11 ($[M+Na]^+$, 100); HRMS Calcd for $C_{33}H_{60}N_4O_{10}S.Na$ 727.3922; found 727.3952.

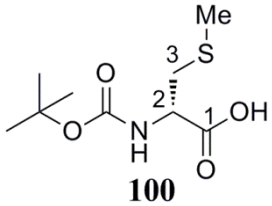
7.3.10 Synthesis of (R)-2-amino-5-{3'-[2"-((R)-4'"-amino-4'"-carboxybutanamido) ethylthio]propylamino}-5-oxopentanoic acid dihydrochloride (94)



Compound **93** (0.09 g, 0.127 mmol) was added to a cooled solution of HCl/dioxane (4 M, 5.0 mL) and triisopropyl silane (TIPS) (1 mL) under an atmosphere of nitrogen. The ice bath was removed. The mixture was allowed to warm to room temperature and stirred for 60 min. The solvent was removed *in vacuo.* The product was collected by filtration and washed with cold ethyl ether. An analytically pure sample of **94** was obtained from the residue by semi-preparative HPLC (H_2O:MeOH, 90:10 to 0:100; rf = 6.5min). Compound **94** (57 mg, 20%) was obtained as an oil. FTIR (KBr, cm^{-1}) 2978, 1647, 1199; ^1H NMR (300 MHz, D_2O) δ (ppm) 1.646 (tt, *J* = 6.6, 7.2 Hz, 2H, H2'), 1.95-2.10 (m, 4H, H4, H2"'), 2.20-2.40 (m, 4H, H3, H3"'), 2.449 (t, *J* = 7.3 Hz, 2H, H3'), 2.561 (t, *J* = 6.6 Hz, 2H, H1"), 3.134 (t, *J* = 6.6 Hz, 2H, H1'), 3.259 (t, *J* = 6.4 Hz, 2H, H2"), 4.62-4.74 (m, 2H, H2, H4"); ^{13}C NMR (300 MHz, D_2O) δ 26.2 (C3, C3"'), 28.0 (C3'), 28.1 (C2'), 30.2 (C1"), 31.3 (C4), 31.4 (C2"'), 38.1 (C1'), 38.4 (C2"), 53.6 (C2, C4"'), 173.3 (C5, C1"'), 174.3 (C1), 174.4 (C4"'-CO_2H); MS (ESI) m/z (%)

392.93 ($[M+Na]^+$, 100); HRMS Calcd for $C_{15}H_{29}N_4O_6S$, (M+H): 393.1802; found 393.1812.

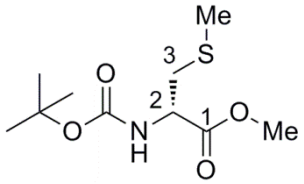
7.3.11 Synthesis of (S)-2-((*tert*-butoxycarbonyl)amino)-3-(methylthio)propanoic acid (100)



100

Boc_2O (8.074 g, 36.98 mmol) was added to a stirred solution of S-methyl-L-cysteine (15.0 g, 36.98 mmol), NaOH (1.479 g, 36.98 mmol), water (10 mL) and *tert*-butanol (8 mL). The reaction mixture was stirred overnight at room temperature and water (4 mL) was added. The aqueous phase was extracted with hexane (3 x 20 mL), acidified with H_2SO_4 (pH 2) and extracted with EtOAc (4 x 20 mL). The EtOAc organic layers were combined, dried (anhydrous $MgSO_4$), and filtered and the solvent was removed *in vacuo*. Recrystalisation (EtOAc) of the solid residue gave compound **100**[212] (8.324 g, 96%) as a white solid. m.p. 72-75 ºC (Lit.[212] m.p. 73-75 ºC); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 1.41 (s, 9H, 3xCH_3), 2.16 (s, 3H, -SCH_3), 2.98 (d, J = 5.4 Hz, 2H, 2 x H3), 4.45-4.57 (m, 1H, H2), 5.30-5.42 (m, 1H, NH), OH not observed; MS (ESI) m/z (%) 258.08 ($[M+Na]^+$, 100).

7.3.12 Synthesis of methyl (S)-2-(*tert*-butoxycarbonylamino)-3-(methylthio) propanoate (101)



101

Procedure 1

N-Boc-S-methyl-L-cysteine (0.235 g, 1.0 mol), sodium bicarbonate (0.168 g, 2.0 mol) and DMF (5 mL) were stirred for 5 min. Methyl iodide (0.709 g, 5.0 mol) was added at room temperature and the reaction stirred for 24 h. Water (10 mL) was added and the mixture extracted with EtOAc (3 x 30 mL). The water layer was further partitioned with EtOAc (50 mL). The combined organic layers were washed with brine (50 mL), dried (anhydrous $MgSO_4$), and the solvent was evaporated *in vacuo*. Purification of the

residue by silica gel column chromatography (hexane:chloroform; 65:35) gave compound **101** (0.075 g, 30%) as an orange oil.

Procedure 2

N-Boc-S-methyl-L-cysteine (0.235 g, 1.0 mol), sodium bicarbonate (0.212 g, 2.0 mol), DMF (5 mL) and methyl iodide (0.35 g, 2.5 mol) were reacted accordingly to procedure 1, using an initial reaction time of 30 min. The mixture was worked up according and purified according to procedure 1. Compound **101** (93 mg, 37%) was obtained as a yellow oil.

Procedure 3

N-Boc-S-methyl-L-cysteine (5.0 g, 1.0 mol), sodium bicarbonate (4.51 g, 2.0 mol), DMF (25 mL) and methyl iodide (7.54 g, 2.5 mol) were reacted accordingly to procedure 1, using an initial reaction time of 30 min. The mixture was worked up according and purified according to procedure 1. Compound **101** (1.1 g, 20%) was obtained as a yellow oil.

Procedure 4

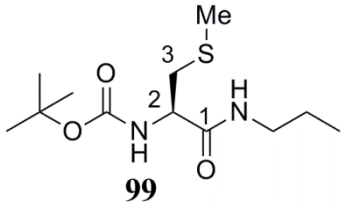
N-Boc-S-methyl-L-cysteine (1.0 g, 4.255 mmol), sodium bicarbonate (0.90 g, 8.541 mmol) and DMF (14 mL) were stirred for 30 min at 65 °C. Methyl iodide (1.51 g, 10.63 mmol) was and the reaction stirred for 16 h a 65 °C. Water (10 mL) was added and the mixture extracted with EtOAc (3 x 30 mL). The mixture was worked up according and purified according to procedure 1. Compound **101** (0.22 g, 20%) was obtained as a yellow oil.

Procedure 5

N-Boc-L-cysteine methyl ester (5.0 g, 21.25 mmol), methanol (20 mL), Et_3N (5.85 g, 57.38 mmol) and methyl iodide (3.64 g, 27.63 mmol) were stirred for 15 h at 25 °C. The solvent was removed *in vacuo*. Water (20 mL) was added until the residue dissolved and the aqueous layer was extracted with ether (4 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and the solvent removed *in vacuo* without further purification. Compound **101**[213,214] (4.598 g, 87%) was obtained pure as a yellow oil in >98% purity. ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm) 1.44 (s, 9H, 3xCH_3), 2.11 (s, 3H, S-CH_3), 2.93 (m, 2H, H3), 3.76 (s, 3H, OCH_3), 4.53 (dt, J = 5.4 Hz, 1H, H2), 5.33 (brs,

$W_{h/2}$ = 9 Hz, 1H, NH); MS (ESI) m/z (%) 272.93 ($[M+Na]^+$, 100). The ^1H NMR spectrum of **101** was consistant with reported values.[213,214]

7.3.13 Synthesis of (R)-*tert*-butyl 3-(methylthio)-1-oxo-1-(propylamino)propan-2-yl carbamate (99)



99

Procedure 1

Propylamine (0.126 g, 2.14 mmol) in DCM (10 mL) was added to a solution of compound **100** (0.5 g, 2.14 mmol) in DCM (4 mL) cooled to 0 ^oC. HOBt (0.346 g, 2.56 mmol), DIPEA (1.932 g, 14.95 mmol) and DIC (0.296 g, 2.349 mmol) were added successively. The reaction mixture was allowed to warm to room temperature and stirred for 20 h. The solution was extracted with a mixture of brine and aqueous sodium bicarbonate (1:1, 30 mL). The aqueous phase was extracted with DCM (3 x 30 mL). The combined organic layers were washed with a mixture of brine and sodium bicarbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), and the solvent removed *in vacuo*. Purification of the residue by silica gel column chromatography (DCM:MeOH; 2-3 % in), did not obtain compound **99**.

Procedure 2

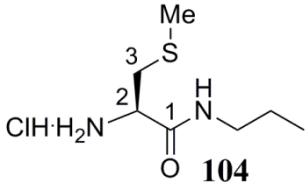
Compound **101** (3.0 g, 11.84 mmol), propylamine (0.69 mL, 11.84 mmol) and methanol (0.48 mL, 11.84 mmol) were stirred for 1 hour at room temperature. Ether (2.3 mL) was added and the solvent removed *in vacuo*. Purification of the residue by silica gel column chromatography (chloroform:MeOH; 1-2%), gave **99** (0.6 g, 18%) as an orange oil.

Procedure 3

Compound **101** (1.0 g, 4.065 mmol) and propylamine (0.97 mL, 4.06 mmol) were reacted at room temperature for 16 h. Propylamine was removed *in vacuo* for 4 h. DCM was added, then removed *in vacuo* and the residue recrystallised (DCM). Compound **99** (586 mg, 52%) was obtained as a white solid. m.p. 95-98 ºC; FTIR (KBr, cm^{-1}) 3326, 3265, 1682, 1646; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 0.91 (t, *J* = 5.7 Hz, 3H, H3´), 1.509 (s, 9H, 3xCH_3), 1.47-1.60 (m, 2H, H2´), 2.13 (s, 3H, S-CH_3), 2.73-2.95 (m, 2H,

H3), 3.22 (dt, *J* = 6.4, 6.8 Hz, 2H, H1´), 4.18 (dt, *J* = 6.8, 7.2 Hz, 1H, H2), 5.34 (brs, $W_{h/2}$ = 6 Hz, 1H, NH), 6.35 (brs, $W_{h/2}$ = 7.5 Hz, 1H, NH); ^{13}C NMR (400 MHz, $CDCl_3$) δ 11.2 (C3'), 15.8 (SMe), 22.6 (C2'), 28.2 ($C(CH_3)_3$), 36.5 (C3), 41.3 (C1'), 53.5 (C2), 80.3 ($C(CH_3)_3$), 155.4 ($NHCO_2$), 178.5 (C1); MS (ESI) m/z (%) 299.15 ($[M+Na]^+$, 100); HRMS Calcd for $C_{12}H_{24}N_2O_3SNa$ 299.1405; found 299.1405.

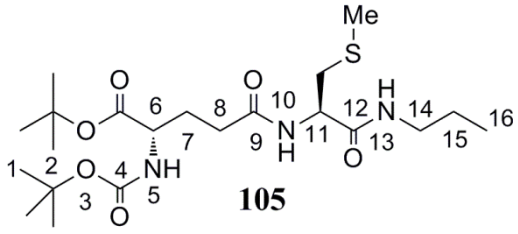
7.3.14 Synthesis of (R)-2-amino-3-(methylthio)-N-propylpropanamide hydrochloride (104)



104

Compound **99** (0.043 g, 0.15 mmol) was added to a solution of HCl/dioxane (4 M, 10 mL) at 0 ^{o}C under an atmosphere of nitrogen. The ice bath was removed and the mixture was allowed to warm to room temperature and stirred for 60 min. The solvent was removed *in vacuo* and the residue washed by decanting with cold diethyl ether without further purification. Title compound **104** (26 mg, 94%) was obtained as a yellow oil. FTIR (KBr, cm^{-1}) 2360, 1652, 1588; ^{1}H NMR (300 MHz, D_2O) δ (ppm) 0.95 (t, *J* = 5.4 Hz, 3H, H3'), 1.36-1.51 (dq, *J* = 4.0, 6.8 Hz, 2H, H2'), 1.96 (s, 2H, NH_2), 2.10 (s, 3H, SMe), 2.80-2.96 (m, 2H, H3), 2.99-3.15 (m, 2H, H1'), 4.00 (dt, *J* = 6.0, 6.0 Hz, 1H, H2), 8.31 (brs, $W_{h/2}$ = 71 Hz, 3H, 1-NH, 2-NH_2); ^{13}C NMR (400 MHz, $CDCl_3$) δ 13.3 (C3'), 17.4 (SMe), 24.3 (C2'), 37.2 (C3), 44.2 (C1'), 54.7 (C2), 170.9 (C1); MS (ESI) m/z (%) 199.85 ($[M+Na]^+$, 100), 176.85 (80); HRMS Calcd for $C_7H_{17}N_2O_1S$, (M+H): 177.1056; found 177.1048.

7.3.15 Synthesis of (S)-*tert*-butyl 2-((*tert*-butoxycarbonyl)amino)-5-(((R)-3-(methyl thio)-1-oxo-1-(propylamino)propan-2-yl)amino)-5-oxopentanoate (105)

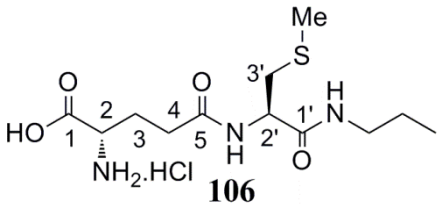


105

Compound **104** (0.44 g, 2.508 mmol) was added to DIPEA (1.53 mL, 3.5 eq) in anhydrous DMF (10 mL) and stirred at room temperature for 2 h under an atmosphere of nitrogen. Boc-L-Glutamic acid 1-*tert*-butylester (0.761 g, 2.508 mmol) in DMF (10

mL) was cooled to 0 ºC. HOBt (0.406 g, 3.009 mmol), DIPEA (1.53 mL, 3.5 eq) and PyBOP (1.305 g, 2.508 mmol) were added successively to the solution which was stirred for 30 min at room temperature under an atmosphere of nitrogen. The solution of compound **104** was added to the Boc-L-glutamic acid 1-*tert*-butylester solution and the reaction mixture stirred overnight at room temperature for 16 h under a flow of nitrogen. The solution was extracted with a mixture of brine and aqueous sodium bicarbonate (1:1, 30 mL) and the aqueous phase extracted with EtOAc (3 x 30 mL). The combined organic layers were washed with a mixture of brine and aqueous sodium bicarbonate (1:1, 10 mL), dried (anhydrous $MgSO_4$), filtered, evaporated *in vacuo*. The residue was purified by silica gel column chromatography (EtOAc:hexane; 4:1). Compound **105** (179 mg, 68%) was obtained as a white solid. An analytically pure sample of **105** was obtained by semi-preparative HPLC (H_2O:MeOH, 90:10 to 0:100; rf = 26.5min) from the major chromatography fraction. m.p. 153-154 ºC; FTIR (KBr, cm^{-1}) 3305, 2979, 1641, 1283; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.92 (t, *J* = 5.4 Hz, 3H, H16), 1.45 (s, 18H, 2-$(CH_3)_3$, 6-$CO_2C(C\mathbf{H}_3)_3$), 1.50-1.62 (m, 2H, H15), 1.82-2.02 (m, 2H, H7), 2.12 (s, 3H, SMe), 2.28-2.49 (m, 2H, H8), 2.73-2.94 (m, 2H, 11-CH_2), 3.10-3.31 (m, 2H, H14) 4.08-4.23 (m, 1H, H6), 4.69 (dt, *J* = 7.2, 6.8 Hz, 1H, H11), 5.63 (d, *J* = 7.6 Hz, 1H, H5), 7.37-7.65 (m, 2H, H5, H13); ^{13}C NMR (400 MHz, $CDCl_3$) δ 10.9 (C16), 15.3 (SMe), 22.1 (C15), 27.5 (2-$(CH_3)_3$, 6-$CO_2C(\mathbf{C}H_3)_3$), 27.9 (C7), 31.7 (C8), 36.0 (11-CH_2), 41.0 (C14), 51.9 (C11), 53.2 (C6), 79.3 (6-$CO_2\mathbf{C}(CH_3)_3$), 81.6 (C2), 155.4 (C4), 170.3 (6-$\mathbf{C}O_2C(CH_3)_3$), 171.1 (C12), 172.1 (C9); MS (ESI) m/z (%) 483.96 ($[M+Na]^+$, 100); HRMS Calcd for $C_{21}H_{39}N_3O_6S$, (M+H): 484.2451; found 484.2439.

7.3.16 Synthesis of (S)-2-amino-5-(((R)-3-(methylthio)-1-oxo-1-(propylamino) propan-2-yl)amino)-5-oxopentanoic acid hydrochloride (106)

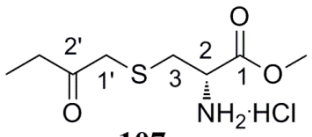


106

Compound **105** (0.33 g, 0.714 mmol) was added to a cooled solution of HCl/dioxane (4 M, 10 mL) and TIPS (1 mL) at 0 °C under an atmosphere of nitrogen. The mixture was allowed to warm to room temperature and stirred for 60 min. The solvent was removed *in vacuo* and the product collected by filtration and washed with cold ethyl ether without further purification to give compound **106** (27 mg, 12%) as a gum. An analytically pure sample of **106** was obtained by semi-preparative HPLC (H_2O:MeOH,

90:10 to 0:100; rf = 21 min). FTIR (KBr, cm^{-1}) 3436, 1265, 739; ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm) 0.914 (t, *J* = 7.3 Hz, 3H, $CH_2CH_2C\mathbf{H}_3$), 1.524 (dt, *J* = 7.2, 7.4 Hz, 2H, $CH_2C\mathbf{H}_2CH_3$), 2.112 (s, 3H, SMe), 2.0-2.25 (m, 2H, H3), 2.41-2.58 (m, 2H, H4), 2.6-2.9 (m, 2H, H3'), 3.05-3.2 (m, 2H, $C\mathbf{H}_2CH_2CH_3$), 2.65-2.85 (m, 1H, H2), 4.477 (dd, *J* = 5.7, 6 Hz, 1H, H2'), 1'-NH, CO_2H, $NH_2{\cdot}HCl$ not observed; ^{13}C NMR (300 MHz, MeOH-d_4) δ 11.6 ($CH_2CH_2C\mathbf{H}_3$), 15.6 (SMe), 23.5 ($CH_2C\mathbf{H}_2CH_3$), 27.4 (C3), 32.6 (C3') 37.0 (C4), 42.3 ($C\mathbf{H}_2CH_2CH_3$), 54.1 (C2, C2'), 172.8 (C1, C5), 174.6 (C1'); MS (ESI) m/z (%) 305.89 (100); HRMS Calcd for $C_{12}H_{23}N_3O_4S.Na$: 328.1301; found 328.1305.

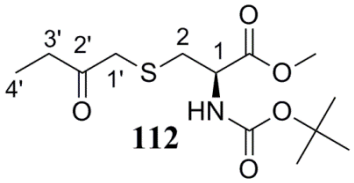
7.3.17 Attempted synthesis of (S)-methyl 2-amino-3-((2-oxobutyl)thio)propanoate hydrochloride (107)



107

1-Bromobutan-2-one (0.5 g, 3.31 mmol) and anhydrous potassium carbonate (0.48 g, 3.64 mmol) were added to a solution of cysteine methyl ester hydrochloric acid (0.68 g, 3.97 mmol) in DMF (6 mL) at 0 ^{o}C. The reaction was warmed to room temperature and stirred overnight. The reaction mixture was diluted with water (50 mL) and extracted with EtOAc (5 x 50 mL). The combined organic layers were washed with brine (50 mL), dried (anhydrous $MgSO_4$) and the solvent removed *in vacuo*. Purification of the crude residue by silica gel column chromatography (hexane:EtOAc; 3:1) gave an orange oil as a complex mixture.

7.3.18 Synthesis of (R)-methyl 2-((*tert*-butoxycarbonyl)amino)-3-((2-oxobutyl) thio)propanoate (112)



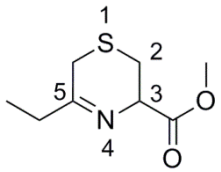
112

Procedure 1

1-Bromobutan-2-one (**108**) (2.025 g, 8.609 mmol) and Et_3N (1.809 g, 17.748 mmol) were added to a solution of N-(*tert*-butoxycarbonyl)-L-cysteine methyl ester (**CQ**) (1 g, 6.622 mmol), methanol (5 mL) and stirred at room temperature for 16 h. The solvent was removed *in vacuo*. The residue was dissolved in HCl (1 M, 10 mL) and the aqueous

layer was extracted with ether (3 x 20 mL). The combined organic layers were washed with water (10 mL), dried (anhydrous $MgSO_4$), and concentrated *in vacuo*. Purification of the residue by column chromatography (hexane:EtOAc; 3:1) gave compound **112** (236 mg, 9%) as an orange oil. FTIR (KBr, cm^{-1}) 3420, 1716, 1683, 1652; ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm) 1.043 (t, J = 7.5 Hz, 3H, H4'), 1.438 (s, 9H, $(CH_3)_3$), 2.654 (q, J = 7.5 Hz, 2H, H3'), 2.81-3.14 (m, 2H, H1, H2), 3.385 (s, 2H, H1'), 3.721 (s, 3H, OCH_3), 4.514 (brs, $W_{h/2}$ = 19.5 Hz, 1H, NH); ^{13}C NMR (300 MHz, MeOH-d_4) δ (ppm) 8.24 (C4'), 28.67 ($(CH_3)_3$), 34.55 (C3'), 41.53 (C2), 52.88 (OCH_3), 54.42 (C1), 80.614 (**C**$(CH_3)_3$), 157.40 (NH**C**$O_2C(CH_3)_3$), 172.836 (**C**O_2CH_3), 208.26 (C2'); MS (ESI) m/z (%) 328.07 ($[M+Na]^+$, 100); HRMS Calcd for $C_{13}H_{23}N_1O_5S_1Na$ 328.1189; found 328.1177.

7.3.19 Attempted synthesis of methyl 5-ethyl-3,6-dihydro-2H-1,4-thiazine-3-carboxylate (110)



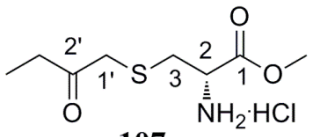
110

Procedure 1

Compound **112** (176 mg, 0.5763 mmol) was added to a solution of potassium carbonate (318 mg, 2.301 mmol) and methanol (10 mL). The reaction mixture was warmed to 50 ºC and stirred for 8 h. The reaction mixture was cooled to room temperature and filtered through celite, rinsed with methanol and concentrated *in vacuo*. Analysis of the crude mixture by ^{1}H NMR spectroscopy identified recovered compound **112** (170 mg) as an orange oil.

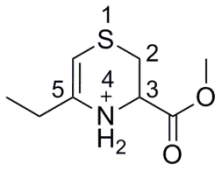
Procedure 2

Compound **112** (170 mg, 0.556 mmol) was added to a cooled solution of HCl/dioxane (4 M, 10 mL) at 0 °C under an atmosphere of nitrogen. The mixture was allowed to warm to room temperature and stirred for 2.5 h. The solvent was removed *in vacuo* and the product collected by filtration and washed with cold ethyl ether. Purification by column chromatography gave a crude residue (86 mg). Analysis of the mixture by ^{1}H NMR spectroscopy and mass spectrometry suggested the presence of **107** and **113** in the ration of 1:2.



107

(S)-methyl 2-amino-3-((2-oxobutyl)thio)propanoate hydrochloride ***(107)*** ^{1}H NMR component (300 MHz, MeOH-d_4) δ (ppm) 1.107 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.04-2.15 (m, 2H, CH_2CH_3), 2.87-3.01 (m. 2H, H2), 3.334 (s, H3, OCH_3), 3.72-3.80 (m, 1H, H3),; MS (ESI) m/z (%) 205.83 ($[M+Na]^+$, 20).



113

5-ethyl-3-(methoxycarbonyl)-3,4-dihydro-2H-1,4-thiazin-4-ium ***(113)*** ^{1}H NMR component (300 MHz, MeOH-d_4) δ (ppm) 1.249 (t, J = 7.5 Hz, 3H, CH_2CH_3), 2.3-2.5(m, 2H, CH_2CH_3), 2.61-2.72 (m, 1H, H3), 3.04-4.12 (m, 1H, H3), 3.329 (s, 2H, H1'), 3.334 (s, 3H, OCH_3), 4.28-4.35 (m, 1H, H2); MS (ESI) m/z (%) 187.80 ($[M+Na]^+$, 100).

Procedure 3

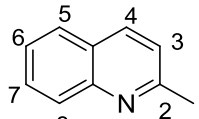
Compound **112** (284 mg, 0.974 mmol), potassium carbonate (539 mg, 3.898 mmol) and methanol (2 mL) were at 110 °C, 200 W for 10 mins using a microwave vessel. The reaction mixture was filtered through celite, washed with methanol and suspended in diethyl ether for 1 h. The crude mixture was concentrated *in vacuo*. Analysis of the crude mixture by ^{1}H NMR spectroscopy identified a complex mixture.

7.4 Chapter Three Experimental Procedures

7.4.1 General Procedure for synthesis of Quinolines 118-135

Toluene (10 mL) was added to a stirred solution of substituted aniline (1.0 equiv), tetrahexylammonium bromide (5%) and concentrated HCl (10 M, 40 mL) at 80-90 ºC. α,β-Unsaturated aldehyde (2.0 equivalents) was added slowly and the mixture stirred for 1.5 h at 80-90 ºC. The reaction mixture was cooled to room temperature. Sodium hydroxide (2 M) was added. The resulting mixture was extracted with chloroform (3 x 20 mL), washed with brine (30 mL), and dried (anhydrous Mg_2SO_4). The solvent was removed *in vacuo* to afford the crude product or a complex mixture. The crude product was either recrystallised (EtOAc/hexane) (compound **126**) or purified by silica gel column chromatography (hexane:EtOAc; 5:1) (compounds **116**, **118**, **119**, **125**, **127**, **136**), and in some cases recrystallised (EtOAc) following chromatography (compound **125**).

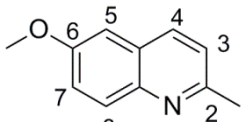
7.4.2 Synthesis of 2-methylquinoline (116)



116

Aniline (0.98 mL, 10.74 mmol) and crotonaldehyde (1.78 mL, 21.48 mmol) were reacted according to the general procedure (section 7.4.3). Purification gave compound **116**[215,216] (600 mg, 40%) as a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 3.233 (s, 3H, 2-CH_3), 7.625 (d, *J* = 8.4 Hz, 1H, H3), 7.832 (dd, *J* = 8.4, 8.2 Hz, 1H, H6), 8.029 (dd, *J* = 8.0, 7.6 Hz, 1H, H7), 8.038 (d, *J* = 7.6 Hz, 1H, H5), 8.605 (d, *J* = 8.8 Hz, 1H, H8), 9.041 (d, *J* = 8.4 Hz, 1H, H4); MS (ESI) m/z (%) 143.77 (100). The ^{1}H NMR spectrum of **116** was consistent with reported values. [215,216]

7.4.3 Synthesis of 6-methoxy-2-methylquinoline (117)

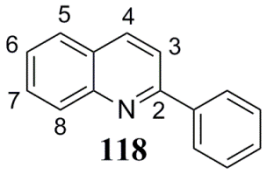


117

p-Methoxyaniline (0.94 mL, 8.12 mmol) and crotonaldehyde (1.35 mL, 16.24 mmol) were reacted according to the general procedure (section 7.4.3). Purification gave compound **117**[215] (106 mg, 20%) as a brown oil. m.p. 68-69 ºC (Lit.[215] m.p. 67-68 ºC);

^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.742 (s, 3H, 2-CH_3), 3.915 (s, 3H, OCH_3), 7.054 (d, *J* = 2.8 Hz, 1H, H5), 7.263 (d, *J* = 7.2 Hz, 1H, H3), 7.353 (dd, *J* = 6.4, 2.8 Hz, 1H, H7), 7.990 (d, *J* = 8.0 Hz, 1H, H8), 8.010 (d, *J* = 8.4 Hz, 1H, H4); MS (ESI) m/z (%) 173.84 (100).

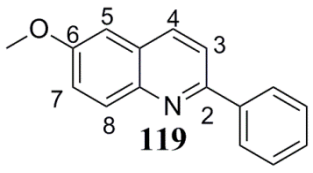
7.4.4 Synthesis of 2-phenylquinoline (118)



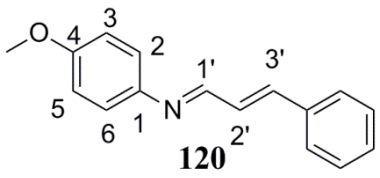
118

Aniline (0.49 mL, 5.37 mmol) and cinnamaldehyde (1.35 mL, 10.74 mmol) were reacted according to the general procedure (section 7.4.3). Purification gave compound **118**[20,21,22,23] (2 mg, <1%) as an orange oil. m.p. 68-70 ºC (Lit.[217] m.p. 67-69 ºC); ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 7.470 (dd, *J* = 8, 2 Hz, H1, *p*-C_6H_5), 7.522 (dd, *J* = 7.2, 8 Hz, 1H, H6), 7.540 (dd, *J* = 9.2, 8 Hz, 2H, *m*-C_6H_5), 7.716 (ddd, *J* = 6.8, 7.2, 2 Hz, 1H, H7), 7.840 (d, *J* = 8 Hz, 1H, H5), 7.988 (d, *J* = 8.8 Hz, 1H, H3), 8.221 (d, *J* = 8.8 Hz, 1H, H4), 8.362 (d, *J* = 8.4 Hz, 1H, H8), 8.430 (d, *J* = 7.2 Hz, 2H, *o*-C_6H_5); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 119.1 (C3), 126.7 (C6), 127.7 (C4a), 127.9 (*o*-C_6H_5), 128.1 (C5), 129.2 (*m*-C_6H_5), 129.8 (*p*-C_6H_5), 130.1 (C7, C8), 137.2 (C4), 139.9 (*i*-C_6H_5), 148.7 (C8a), 157.1 (C2); MS (ESI) m/z (%) 205.88 (100).

7.4.5 Synthesis of 6-methoxy-2-phenylquinoline (119)



119



120

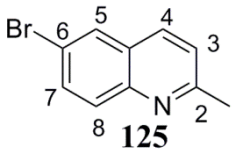
p-Methoxy aniline (5.0 g, 29.06 mmol) and cinnamaldehyde (4.83 mL, 58.12 mmol) were reacted according to the general procedure (section 7.4.3). Purification gave two major products compound **119** and compound **120**.

*6-Methoxy-2-phenylquinoline (**119**)*[162,218] (60 mg, 1.6 %) as an orange solid. m.p. 127-129 ºC (Lit.[218] m.p. 129-130 ºC); ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 3.783 (s, 3H, OCH_3), 7.235 (d, *J* = 2.4 Hz, 1H, H5), 7.439 (dd, *J* = 6.4, 2 Hz, 1H, *p*-C_6H_5), 7.350 (d, *J* = 2.8 Hz, 1H, H7), 7.524 (dd, *J* = 7.6, 6.4 Hz, 2H, *m*-C_6H_5), 7.973 (d, *J* = 8.8 Hz, 1H, H3), 8.171 (d, *J* = 8.8 Hz, 1H, H4), 8.283 (d, *J* = 9.2 Hz, 1H, H8), 8.423 (d, *J* = 8.4

Hz, 2H, *o*-C_6H_5); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 55.5 (OCH_3), 105.8 (C5), 119.3 (C3), 122.8 (C7), 127.6 (*o*-C_6H_5), 128.8 (C4a), 129.2 (*m*-C_6H_5), 129.4 (*p*-C_6H_5), 131.5 (C8), 136.0 (C4), 140.1 (*i*-C_6H_5), 144.8 (C8a), 154.7 (C2), 158.2 (C6); MS (ESI) m/z (%) 235.70 (100).

(E)-4-methoxy-N-((E)-3-phenylallylidene)aniline (***120***)[193] (0.37 mg, <1 %) as an orange solid. m.p. 196-198 ºC (Lit.[193] m.p. 195-197 ºC); FTIR (KBr, cm^{-1}) 3002, 3024, 3057, 3098, 3329; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 3.718 (s, 3H, OCH_3), 7.080 (ddd, *J* = 8.8, 6.8, 4.4 Hz, 2H, *m*-N-C_6H_5), 7.18-7.24 (m, 1H, H3'), 7.31-7.43 (m, 4H, H2', *m*-C_6H_5, *p*-C_6H_5), 7.461 (ddd, *J* = 8.8, 6.8, 4.4 Hz, 2H, *o*-N-C_6H_5), 7.626 (d, *J* = 7.2 Hz, 2H, *o*-C_6H_5), 8.462 (d, *J* = 8.8 Hz, 1H, H1'); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 55.0 (OCH_3), 114.9 (*m*-N-C_6H_5), 122.9 (*o*-N-C_6H_5), 127.8 (*o*-C_6H_5), 129.3 (*m*-C_6H_5), 129.6 (C2', *p*-C_6H_5) 136.5 (*i*-C_6H_5), 142.9 (C3'), 145.6 (*i*-N-C_6H_5), 158.9 (*p*-N-C_6H_5), 159.7 (C1'); MS (ESI) m/z (%) 235.70 (100); HRMS (ESI) m/z calcd for $C_{16}H_{15}NO$: 237.1154; found 237.1148.

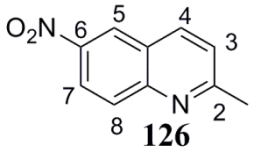
7.4.6 Synthesis of 6-bromo-2-methylquinoline (125)



125

p-Bromoaniline (0.5 g, 2.91 mmol) and crotonaldehyde (0.48 mL, 5.81 mmol) were reacted according to the general procedure (section 7.4.3). Purification of compound **125**[219] (48 mg, 17%) resulted in red brown crystals. m.p. 97-99 ºC (Lit.[219] m.p. 95-96 ºC); FTIR (KBr, cm^{-1}) 3048, 1488, 646; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.752 (s, 3H, 2-CH_3), 7.319 (d, *J* = 8.4 Hz, 1H, H3), 7.743 (d, *J* = 2.0 Hz, 1H, H7), 7.765 (d, *J* = 2.0 Hz, 1H, H8), 7.942 (d, *J* = 2 Hz, 1H, H5), 7.981 (d, *J* = 8.9 Hz, 1H, H4); ^{13}C NMR (400 MHz, $CDCl_3$) δ (ppm) 25.2 (2-CH_3), 119.4 (C3), 122.8 (C6), 127.5 (C4a), 129.4 (C5), 130.2 (C8), 132.8 (C7), 135.2 (C4), 146.2 (C8a), 159.4 (C2); MS (ESI) m/z (%) 223.73 ($[M+H]^+$, 100). HRMS (ESI) m/z calcd for $C_{10}H_9Br_1N_1$: 221.9912; found 221.9902.

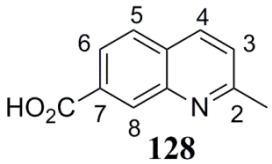
7.4.7 Synthesis of 2-methyl-6-nitroquinoline (126)



126

p-Nitroaniline (0.5 g, 3.62 mmol) and crotonaldehyde (0.60 mL, 7.24 mmol) were reacted according to the general procedure (section 7.4.3). Purification of compound **126**[219,220] (154 mg, 23%) gave green crystals. m.p. 162-164 ºC (Lit.[219] m.p. 165-166 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.816 (s, 3H, 2-CH_3), 7.453 (d, *J* = 8.4 Hz, 1H, H3), 8.162 (d, *J* = 8.8 Hz, 1H, H7), 8.235 (d, *J* = 8.8 Hz, 1H, H8), 8.449 (dd, *J* = 6.8, 8.2 Hz, 1H, H4), 8.745 (d, *J* = 2.4 Hz, 1H, H5); MS (ESI) m/z (%) 188.82 (100). The ^{1}H NMR spectrum of **125** was consistent with reported values.[219,220]

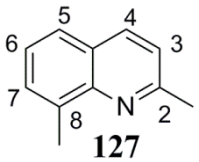
7.4.8 Attempted synthesis of 2-methylquinoline-7-carboxylic acid (128)



128

3-Aminobenzoic acid (5.0 g, 36.46 mmol) and crotonaldehyde (5.11 g, 72.92 mmol) were reacted and worked up according to the general procedure (section 7.4.3). Purification of the residue (8.73 g) by silica gel column chromatography (EtOAc:Hex; 4:1) gave a complex mixture.

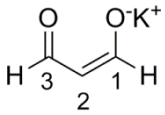
7.4.9 Synthesis of 2,8-dimethylquinoline (127)



127

o-Methylaniline (0.49 mL, 4.66 mmol) and crotonaldehyde (0.77 mL, 9.33 mmol) were reacted according to the general procedure (section 7.4.3). Purification of compound **127**[215] (54 mg, 7%) gave a yellow oil. ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 2.759 (s, 3H, 8-CH_3), 2.807 (s, 3H, 2-CH_3), 7.250 (d, *J* = 8.4 Hz, 1H, H3), 7.358 (d, *J* = 8.0 Hz, 1H, H6), 7.511 (d, *J* = 6.8 Hz, 1H, H7), 7.591 (d, *J* = 8.0 Hz, 1H, H5), 7.985 (d, *J* = 8.4 Hz, 1H, H4); MS (ESI) m/z (%) 157.80 (100). The ^{1}H NMR spectra of **127** was consistent with reported values.[215]

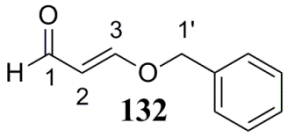
7.4.10 Synthesis of potassium (Z) 3-oxoprop-1-en-1-oate (131)



131

Tetraethoxy propane (5 g, 22.69 mmol), HCl (1 M, 2.5 mL), and water (2.5 mL) were stirred vigorously for 75 min at room temperature. The solution was cooled to 0 ºC and adjusted to pH 10 with potassium hydroxide (5 M) to give a red solution. Acetone (100 mL) was added at the first sign of a precipitate forming. The precipitate was isolated by filtration, washed with acetone and dried at room temperature for 3 h. The solid was dissolved in hot methanol (15 mL), treated with charcoal and filtered and the methanol was removed *in vacuo*. Compound **131** (1.94 g, 78%) was obtained as a unstable brown solid. m.p. >300 ^{o}C; FTIR (KBr, cm^{-1}) 3086, 2834, 2694; ^{1}H NMR (400 MHz, D_2O) δ ppm 5.234 (dd, *J* = 9.9, 10.2 Hz, 1H), 8.60-8.63 (m, 2H); ^{13}C NMR (400 MHz, MeOH-d_4) δ ppm 110.2 (C2), 170.4 (C1), 192.2 (C3); MS (ESI) m/z (%) 110.60 ([M+Na]$^{+}$, 100).

7.4.11 (E)-3-(Benzyloxy)acrylaldehyde (132)



132

Procedure 1

Benzyl bromide (4.66 g, 27.79 mmol) was added to a solution of **131** (1.939 g, 27.29 mmol) in DMF (10 mL) at 0 ºC. The reaction was warmed to room temperature and stirred for 2 h. Water (40 mL) was added and the mixture was extracted with ether (3 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. A brown oil was obtained as a complex mixture.

Procedure 2

Benzyl bromide (4.66 g, 27.79 mmol) was added to a solution of **131** (1.939 g, 27.29 mmol) in anhydrous THF (10 mL) at 0 ºC. The reaction was allowed to warm to room temperature and heated at 60 ºC for 16 h. Water (40 mL) was added and the mixture was extracted with ether (3 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. A brown oil was obtained as a complex mixture.

Procedure 3

Benzyl bromide (0.12 g, 0.704 mmol) and **131** (0.050 g, 0.704 mmol) was dissolved in MeOH-d_4 (1 mL) in a 5 mm NMR tube. The mixture was vortexed and left to stand for 24 h. A ^{1}H NMR spectrum was acquired.

Procedure 4

Benzyl bromide (0.12 g, 0.704 mmol) and **131** (0.050 g, 0.704 mmol) was dissolved in DMSO-d_6 (1 mL) in a 5 mm NMR tube. The mixture was vortexed and left to stand for 24 h. A ^{1}H NMR spectrum was acquired of compounds **132**. ^{1}H NMR (400 MHz, DMSO-d_6) δ = 5.080 (s, 1H, H1'), 5.633 (dd, J = 8.4, 8.4 Hz, 1H, H2), 7.2-7.5 (m, 5H, C_6H_5), 7.932 (d, J = 8.2 Hz, 1H, H3), 9.340 (d, J = 8 Hz, 1H, H1); MS (ESI) m/z (%) 157.88 ($[M+Na]^+$, 100).

Procedure 5

Benzyl bromide (1.17 g, 6.880 mmol) was added to a solution of **131** (0.5 g, 7.037 mmol) in anhydrous DMSO (10 or 5 mL). The reaction mixture was stirred at room temperature for 16 h. Water (40 mL) was added and the mixture was extracted with ether (3 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Analysis of the crude mixture by ^{1}H NMR spectroscopy identified a complex mixture.

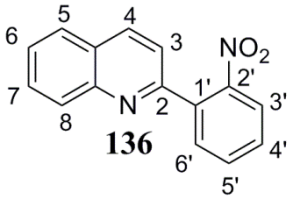
Procedure 6

Benzyl bromide (574 mg, 4.836 mmol) was added to a solution of **131** (378 mg, 5.320 mmol) in anhydrous DMSO (5 mL). The reaction mixture heated to 40 ºC for 16 h. Water (40 mL) was added and the mixture was extracted with ether (3 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Analysis of the crude mixture by ^{1}H NMR spectroscopy identified a complex mixture.

Procedure 7

Benzyl bromide (129 mg, 0.756 mmol) was added to a solution of **131** (100 mg, 0.908 mmol) in anhydrous DMSO (1.2 mL). The reaction mixture heated to 40 ºC for 48 h under oxygen or inert atmosphere. Water (40 mL) was added and the mixture was extracted with ether (3 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Analysis of the crude mixture by ^{1}H NMR spectroscopy identified a complex mixture.

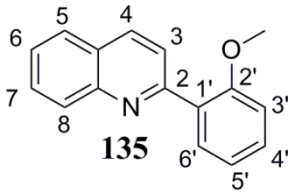
7.4.12 Synthesis of 2-(2'-nitrophenyl)quinoline (136)



136

Aniline (0.98 mL, 10.74 mmol) and trans-2-nitrocinnamaldehyde (3.80 g, 21.47 mmol) were reacted according to the general procedure (section 7.4.3). Analysis of the residue (50 mg) by ^{1}H NMR spectroscopy indicated a mixture of **DK** and **136**. The residue was treated with sodium borohydride (1.1 equiv.) in methanol (8 mL). Purification by column chromatography gave compound **136**[38] (3 mg, <1%) as a brown solid. m.p. 116-118 ^{o}C (Lit.[38] m.p. 118-119 ^{o}C); FTIR (KBr, cm^{-1}) 3076, 3005, 1524; ^{1}H NMR (400 MHz, $CDCl_3$) δ = 7.525 (d, *J* =8.4 Hz, 1H, H3), 7.55-7.61 (m, 2H, H6, H5'), 7.70-7.76 (m, 3H, H7, H4', H6'), 7.862 (d, *J* =6.8 Hz, 1H, H8), 7.985 (d, *J* = 9.2 Hz, 1H, H3'), 8.092 (d, *J* = 9.6 Hz, 1H, H5), 8.237 (d, *J* = 8.4 Hz, 1H, H4); ^{13}C NMR (500 MHz, $CDCl_3$) δ (ppm) 120.5 (C3), 124.5 (C3'), 127.0 (C6), 127.2 (C8), 127.5 (C4a), 129.3 (C5'), 129.7 (C5), 130.0 (C7), 131.6 (C6') 132.6 (C4'), 135.9 (C1'), 136.8 (C4), 147.9 (C8a, C2'), 155.6 (C2); MS (ESI) m/z (%) 204.62 (100), 250.70 (45); HRMS (ESI) m/z calcd for $C_{15}H_{11}N_2O_2$: $(M+H)^+$ 251.0815; found 251.0823.

7.4.13 Attempted synthesis of 2-(2-methoxyphenyl)quinoline (135)

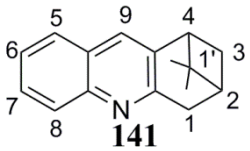


135

Procedure 1

Aniline (0.98 mL, 10.738 mmol) and 2-methoxycinnamaldehyde (3.48 g, 21.476 mmol) were reacted and worked up according to the general procedure (section 7.4.3). Purification of the residue (4.13 g) by silica gel column chromatography (hexane:EtOAc; 5:1) gave a complex mixture.

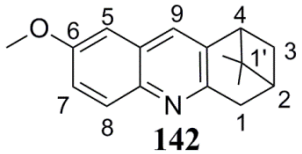
7.4.14 Attempted synthesis of 2,4-(1',1'-dimethylmethano)-1,2,3,4-tetrahydro acridine (141)



141

Aniline (0.49 mL, 3.33 mmol) and 2-myrtenal (0.72g, 6.66 mmol) were reacted and worked up according to the general procedure (section 7.4.3). Purification of the residue (1.35 g) by silica gel column chromatography (EtOAc:Hex; 4:1) gave a complex mixture.

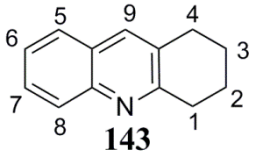
7.4.15 Attempted synthesis of 6-methoxy-2,4-(1',1'-dimethylmethano)-1,2,3,4-tetrahydroacridine (142)



142

p-Methoxy aniline (0.5 mg, 4.06 mmol) and 2-myrtenal (1.41 g, 8.12 mmol) were reacted and worked up according to the general procedure (section 7.4.3). Purification of the residue (0.83 g) by silica gel column chromatography (EtOAc:Hex; 4:1) gave a complex mixture.

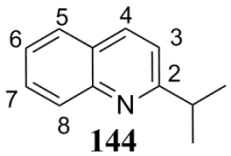
7.4.16 Attempted synthesis of 1,2,3,4-tetrahydroacridine (143)



143

p-Methoxy aniline (0.52 mL, 4.06 mmol) and cyclohex-1-enecarbaldehyde (0.245 g, 2.27 mmol) were reacted and worked up according to the general procedure (section 7.4.3). Purification of the residue (540 mg) by silica gel column chromatography (Hex:DCM:EtOAc; 55:25:20) gave a complex mixture.

7.4.17 Attempted synthesis of 2-isopropylquinoline (144)

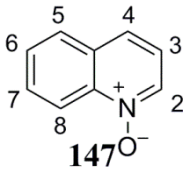


144

Aniline (0.49 mL, 5.37 mmol) and 4-methylpent-2-enal (1.24 g, 10.74 mmol) were reacted were reacted and worked up according to the general procedure (section 7.4.3). Purification of the residue (1.41 g) by silica gel column chromatography (Hex:EtOAc; 15:1) gave a complex mixture.

7.5 Chapter Four Experimental Procedures

7.5.1 Synthesis of quinoline 1-oxide (147)



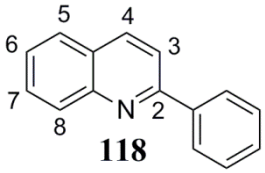
147

Hydrogen peroxide (8.8 M in water) (0.54 mL, 22.28 mmol) was added to phthalic anhydride (3.0 g, 20.25 mmol) and ether (60 mL) at 0 ^{o}C. The peracid solution was stirred for 10 min. Quinoline (1.0 g, 7.742 mmol) in methanol (10 mL) was added dropwise, and the solution was stirred for 16 h under an atmosphere of nitrogen. Aqueous sodium hydroxide (15 mL, 10%), was added and the solution stirred for 10 min. The mixture was extracted with chloroform (3 x 30 mL). The combined organic extracts were washed with brine (20 mL) and solvent removed *in vacuo*. Purification of the residue was achieved by silica gel column chromatography (EtOAc:MeOH; 95:5) gave **147**[77] (672 mg, 56%) and gave a brown solid. m.p. 59-61 ºC (Lit.[77] m.p. 58-60 ºC); ^{1}H NMR (400 MHz, MeOH-d_4) δ (ppm) 7.552 (dd, J = 6, 6 Hz, 1H, H3), 7.789 (dd, J = 7.6, 7.2 Hz, 1H, H6), 7.921 (dd, J = 8, 8.4 Hz, 1H, H7), 8.098 (d, J = 8.4 Hz, 1H, H4), 8.165 (d, J = 8.4 Hz, 1H, H5), 8.648 (d, J = 6.6 Hz, 1H, H2), 8.688 (d, J = 6 Hz, 2H, H8); MS (ESI) m/z (%) 145.72 (100).

7.5.2 General procedures for synthesis of Quinolines 118 & 135

Magnesium turnings (3 equivalents), and iodine (1 crystal, ~1 mg) were stirred for 16 h under an atmosphere of nitrogen. The organobromine (3 equivalents) was dissolved in freshly distilled THF (10 ml). An aliquot of the organobromide-THF solution (1 mL) was added to the magnesium turnings. Once the reaction commenced, the turnings were covered with THF (10 mL). The remainder of the organobromide-THF solution (~9 mL) was added dropwise over 30 min so as to maintain the solution at reflux. The N-oxide (1 equivalent) in dry THF (10 mL) was slowly added to the Grignard reagent over 15 min. The mixture was stirred for 1 hour, cooled to -15 °C and quenched with ice cold water. The solution was acidified with excess HCl (2 M). Chloroform (15 mL) was added and the mixture stirred for 15 min. The solution was filtered though celite and the filtrate was extracted with ether (3 x 30 mL). The combined organic layers were dried (anhydrous $MgSO_4$), filtered and concentrated *in vacuo*.

7.5.3 Synthesis of 2-phenylquinoline (118)

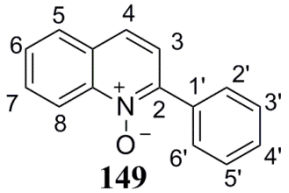


118

Procedure 2

Phenyl bromide (1.62 g, 10.332 mmol) and quinoline-N-oxide (328 mg, 3.444 mmol) were reacted according to the general procedure (section 7.5.2). Purification of the residue by silica gel column chromatography (DCM:2-propanol:Et_3N; 95:5:0.5%) yielded compound **118**[162] (287 mg, 41%) as an orange solid and **174** (38%).

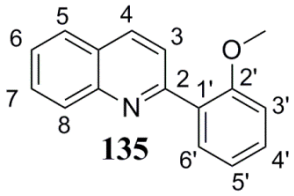
7.5.4 Synthesis of 2-phenylquinoline 1-oxide (149)



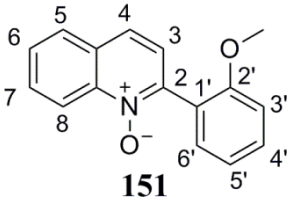
149

2-Phenyl quinoline (0.2 mg, 0.974 mmol) in MeOH (10 mL) was added dropwise to *m*-CPBA (0.84 g, 4.872 mmol) and ether at 0 ^oC. The solution was stirred for 16 h at room temperature under an atmosphere of nitrogen. The mixture was quenched with aqueous sodium hydroxide (10%, 15 mL), and was stirred for 10 min. The reaction mixture was extracted with chloroform (3 x 30 mL), and the combined organic layers were washed with brine (1 x 20 mL) and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (EtOAc:MeOH; 95:5) gave **149**[221] (34 mg, 16%) as light yellow crystals. m.p. 121-122 ºC (Lit.[215] m.p. 119-120 ºC); ^1H NMR (300 MHz, MeOH-d_4) δ (ppm) 7.116 (dd, *J* = 7.5, 7.5 Hz, 1H, H4'), 7.174 (d, *J* = 8.4 Hz, 1H, H3), 7.42-7.57 (m, 3H, H4, H3', H5'), 7.778 (dd, *J* = 7.2, 8.1 Hz, 1H, H6), 7.911 (dd, *J* = 7.2, 8.7 Hz, 1H, H7), 8.093 (d, *J* = 6.9 Hz, 2H, H2', H6'), 8.111 (d, *J* = 8.4 Hz, 1H, H5), 8.689 (d, *J* = 8.7 Hz, 1H, H8). MS (ESI) m/z (%) 221.86 (100).

7.5.5 Synthesis of 2-(2-methoxyphenyl)quinoline (135) and 2-(2'-methoxyphenyl) quinoline 1-oxide (151)



135



151

Procedure 2

2-Bromoanisole (1.074 g, 5.745 mmol) and quinoline-N-oxide (0.28 g, 1.915 mmol) were reacted according to the general procedure (section 7.5.2). Purification of the residue by silica gel column chromatography (hexane:EtOAc; 7:3) gave compound **135**[222] (13 mg, 3%) as an oil and compound **151**[223] (231 mg, 48%) as yellow crystals.

*2-(2-Methoxyphenyl)quinoline (**135**)*; ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 7.022 (d, *J* = 8.4 Hz, 1H, H3'), 7.115 (dd, *J* = 7.2, 8 Hz, 1H, H5'), 7.406 (dd, *J* = 6.4, 7.6 Hz, 1H, H4'), 7.508 (dd, *J* = 8, 7.2 Hz, 1H, H6), 7.690 (dd, *J* = 6.8, 8.4Hz, 1H, H7), 7.824 (dd, *J* = 4, 8 Hz, 2H, H3 , H5), 7.867 (d, *J* = 8.8 Hz,1H, H4), 8.139 (dd, *J* = 10.4, 10 Hz, 2H, H6', H8); MS (ESI) m/z (%) 235.79 (100). The ^{1}H NMR spectrum of **135** was consistent with reported values.[222]

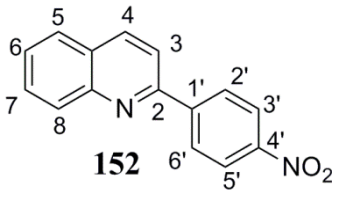
*2-(2'-Methoxyphenyl) quinoline 1-oxide (**151**)*; m.p. 184-186 ºC (Lit.[223] m.p. 185-187 ºC); FTIR (KBr, cm^{-1}) 3061, 1491, 1340, 780; ^{1}H NMR (400 MHz, MeOH-d_4) δ (ppm) 4.844 (s, 3H, OMe), 7.115 (dd, *J* = 7.5, 7.5 Hz, H5'), 7.172 (d, *J* = 8.4 Hz, 1H, H3'), 7.442 (d, *J* = 6 Hz, 1H, H6'), 7.510 (dd, *J* = 7.8, 8.2 Hz, 1H, H4'), 7.537 (d, *J* = 8.7 Hz, 1H, H4), 7.770 (dd, *J* = 6.9, 7.5 Hz, 1H, H6), 7.906 (dd, *J* = 7.2, 7.5 Hz, 1H, H7), 8.05-8.15 (m, 2H, H3, H5), 8.687 (d, *J* = 8.7, 1H, H8); ^{13}C NMR (400 MHz, MeOH-d_4) δ (ppm) 56.1 (OMe), 112.4 (C3'), 120.1 (C8), 121.6 (C1', C5'), 123.7 (C4a), 125.7 (C4), 129.5 (C5), 129.7 (C3), 130.0 (C6), 131.3 (C7), 131.4 (C6'), 132.5 (C4'), 142.3 (C8a), 147.4 (C2), 158.7 (C2'). MS (ESI) m/z (%) 251.76 (100); HRMS Calcd for $C_{16}H_{14}N_1O_2S$: $(M+H)^+$ 252.1019; found 252.1008.

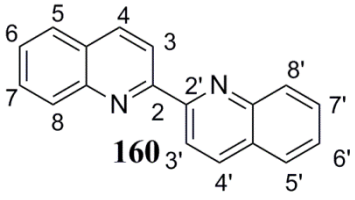
Procedure 3

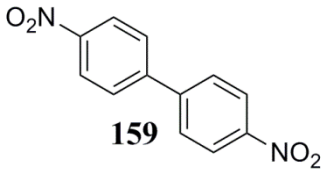
Ammonium formate (0.273 g, 4.421 mmol) was added to a stirred solution of compound **151** (0.111 g, 0.442 mmol) and palladium on carbon (10%) in methanol (0.15 mL) under an inert atmosphere at room temperature for 2 h. Purification of the

residue by silica gel column chromatography (DCM:acetone; 9:1) gave compound **135**[222] (46 mg, 48%) as an oil and compound **151**[223] (57 mg, 52%) as yellow crystals.

7.5.6 Synthesis of 2-(4'-nitrophenyl)quinoline (152) and 2,2-biquinoline (160)







A solution of 2-chloroquinoline (142 mg, 0.868 mmol, 0.3 M) was prepared in dry toluene (3 mL) and degassed with argon. Palladium (II) acetate (10 mg, 0.043 mmol, 5 mol %), potassium carbonate (239 mg, 1.932 mmol) and 4-nitrophenylboronic acid (289 mg, 1.736 mmol) were put under a vacuum, and then flushed with argon (~ 5 times). The aryl halide solution was added, and the reaction was heated to 110 °C for 16 h under an atmosphere of argon. The cooled reaction mixture was filtered through celite and the celite was washed with acetone and DCM. The solvent was removed *in vacuo*. Purification of the residue by subjected column chromatography (silica gel, Hexane: EtOAc; 4:1) gave compound **160**[214] (16 mg, 10%) as a yellow solid and a mixture of compounds **152** and **159**. Purification of the mixture by column chromatography (alumina gel, Hexane:DCM:EtOAc; 4:1:2%) gave compound **152** (5 mg, 2%) as a yellow solid and compound **159** (32 mg, 13%) as yellow crystals.

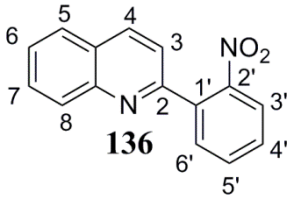
*2-(4'-Nitrophenyl)quinoline (**152**)*; m.p. 57-59 ºC; FTIR (KBr, cm^{-1}) 3082, 3067, 1598; ^{1}H NMR (300 MHz, acetone-d_6) δ (ppm) 7.651 (t, *J* = 7.2, 7.2 Hz, 1H, H6), 7.831 (t, *J* = 6.9, 6.9 Hz, 1H, H7), 8.021 (d, *J* = 8.1 Hz, 1H, H8), 8.071 (d, *J* = 9 Hz, 1H, H5'), 8.161 (d, *J* = 8.4 Hz, 1H, H5), 8.243 (d, *J* = 8.7 Hz, 1H, H3), 8.380 (d, *J* = 7.2 Hz, 1H, H3'), 8.407 (d, *J* = 9 Hz, 1H, H6'), 8.512 (d, *J* = 8.7 Hz, 1H, H4), 8.587 (d, *J* = 9 Hz, 1H, H2); ^{13}C NMR (300 MHz, acetone-d_6) δ (ppm) 119.7 (C3), 124.7 (C5'), 125.0 (C3'), 128.1 (C4a), 128.7 (C5), 129.3 (C2', 6'), 129.5 (C6), 130.6 (C8), 131.1 (C7), 138.4 (C4), 146.0 (C4'), 149.1 (C8a), 149.4 (C1'), 155.1 (C2); MS (ESI) m/z (%) 204.8 (100), 250.8 (50); HRMS Calcd for $C_{15}H_{11}N_2O_2$: $(M+H)^+$ 251.0815; found 251.0826.

*2,2-Biquinoline (**160**);* m.p. 191-194 ºC (Lit.[224] m.p. 192-194 ºC); ^{1}H NMR (300 MHz, MeOH-d_4) δ (ppm) 7.557 (dd, *J* = 7.8, 7.8 Hz, 2H, H6, H6'), 7.741 (dd, *J* = 8.4, 8.4 Hz, 2H, H7, H7'), 7.836 (d, *J* = 7.8 Hz, 2H, H5, H5'), 8.261 (d, *J* = 8.1 Hz, 2H, H8, H8')

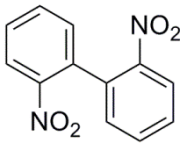
8.310 (d, *J* = 8.7 Hz, 2H, H3, H3'), 8.833 (d, *J* = 8.7 Hz, 2H, H4, H4'); MS (ESI) m/z (%) 256.96 (100).

*4,4'-dinitro-1,1'-biphenyl (**159**)*; m.p. 237-239 ºC (Lit.[225] m.p. 238-239 ºC); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.764 (d, *J* = 8.7 Hz, 4H), 8.363 (d, *J* = 8.4 Hz, 4H) MS (ESI) m/z (%) 244.2 (100).

7.5.7 Synthesis of 2-(2'-nitrophenyl)quinoline (136)



136

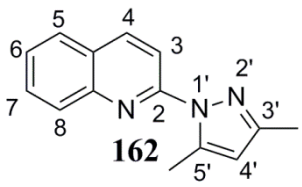


161

A solution of 2-chloroquinoline (100 mg, 6.112 mmol, 0.3 M) was prepared in dry toluene (2 mL) and degassed with argon. Palladium (II) acetate (6.9 mg, 0.306 mmol, 5 mol %), potassium carbonate (203 mg, 14.68 mmol) and 4-nitrophenylboronic acid (245 mg, 14.68 mmol) were put under a vacuum, and then flushed with argon (~ 5 times). The aryl halide solution was added, and the reaction was heated to 110 °C for 16 h under an atmosphere of argon. The cooled reaction mixture was filtered through celite and the celite was washed with acetone and DCM. The solvent was removed *in vacuo*. Purification of the residue by subjected column chromatography (silica gel, Hexane: EtOAc; 4:1) gave compounds **136**[38] (4.57 mg, 3%) as a brown solid and **161**[226] as a light brown solid.

*2,2'-dinitrobiphenyl (**161**)*; m.p. 123-124 ºC (Lit. m.p.[226] 120-122 ºC); ^{1}H NMR (300 MHz, $CDCl_3$) δ (ppm) 7.289 (dd, *J* = 8.4, 2.2 Hz, 1H), 7.583 (ddd, *J* = 8.4, 7.8, 2.2 Hz, 1H), 7.678 (ddd, *J* = 7.8, 7.6, 2.2 Hz, 1H), 8.205 (dd, *J* = 7.8, 2.2 Hz, 1H); MS (ESI) m/z (%) 244.25 (100).

7.5.8 Synthesis of 2-(3',5'-dimethyl-1H-pyrazol-1'-yl)quinoline (162)

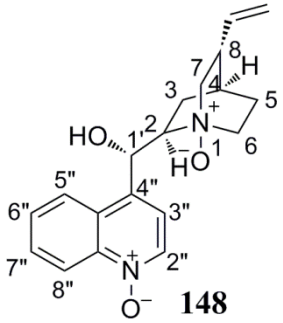


162

2-Chloroquinoline (1.0 g, 6.112 mmol), hydrazine (7.89 g, 60 % aq. solution, 40 eq.) were added to ethanol (50 mL). The mixture was heated at reflux under an atmosphere of nitrogen for 48 h, then cooled to room temperature. The solvent was removed under vacuum. EtOAc (50 mL) was added and the organic phase was successively washed with water (3 × 50 mL) and brine (50 mL). The organic phase was dried over anhydrous $MgSO_4$ and the solvent removed *in vacuo*. The crude product (1.15 g) was dried under vacuum and used without further purification.

A mixture of crude 2-hydrazine quinoline (1.15 g, 7.274 mmol) and acetylacetone (0.85 g, 8.307 mmol) were added to EtOH (50 mL). The mixture was heated at reflux under an atmosphere of nitrogen for 48 h. The solvent was removed to give an orange, oily product which was dried under vacuum without further purification. Compound **162**[227] (959 mg, 60%, over two steps) gave a yellow solid. m.p. 56-58 ºC (Lit.[217] m.p. 57 ºC); FTIR (KBr, cm^{-1}) 3029, 2946, 2824, 1644; ^1H NMR (300 MHz, $CDCl_3$) δ (ppm) 2.315 (s, 3H, 5'-CH_3), 2.801 (s, 3H, 3'-CH_3), 6.030 (s, 1H, H4'), 7.473 (dd, *J* = 7.1 Hz, 1H, H6), 7.672 (dd, *J* = 6.9, 6.9 Hz, 1H, H7), 7.788 (d, *J* = 6.6 Hz, 1H, H8), 7.960 (d, *J* = 7.8 Hz, H5), 8.083 (d, *J* = 9 Hz, 1H, H3), 8.194 (d, *J* = 9 Hz, 1H, H4); ^{13}C NMR (300 MHz, $CDCl_3$) δ (ppm) 13.7 (5'-CH_3), 15.1 (3'-CH_3), 109.5 (C4'), 115.1 (C3), 125.8 (C4a), 126.3 (C6), 127.5 (C8), 128.6 (C7), 128.2 (C4), 142.3 (C3'), 146.3 (C8a), 150.1 (C5'), 152.3 (C2'); MS (ESI) m/z (%) 223.87 (100); HRMS Calcd for $C_{14}H_{13}N_3Na$: 246.1002; found 246.1000.

7.5.9 Synthesis of (2S,4R,8S)-2-((R)-hydroxy(1"-oxidoquinolin-4"-yl)methyl)-8-vinyl quinuclidine 1-oxide (148)



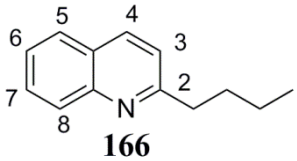
Phthalic anhydride (5.0 g, 0.033 mol) was added to diethylether (40 mL) at 0 ^oC. Hydrogen peroxide (8.8 M) (1.64 mL, 0.067 mol) was slowly added and the solution stirred for 10 min at 0 ^oC. Cinchonine (1 g, 3.396 mmol) in methanol (10 mL) was added dropwise to the ether solution. The mixture was stirred for 16 h under an

atmosphere of nitrogen. Aqueous sodium hydroxide (15 mL, 10%) was added and the solution stirred until cool. The reaction mixture was extracted with chloroform, and the combined organic extract layers were washed with brine (40 mL) and dried (anhydrous $MgSO_4$). 1H NMR analysis of the crude residue indicated the precense of **148** (85%) and cinchonine (15%). The residue was purified by silica gel column chromatography (hexane:EtOAc; 4:1). An analytically pure sample of **148** (166 mg, 15 %) was obtained as a yellow solid by semi-preparative HPLC (H_2O:MeOH, 90:10 to 0:100; rf = 8 min) from the major chromatography fraction. m.p. 132-134 ºC; FTIR (KBr, cm^{-1}) 3837, 3053, 2360, 1558, 1265, 731; ^{1}H NMR (400 MHz, MeOH-d_4) δ (ppm) 1.2-1.8 (m, 2H, H3, H5), 1.8-2.2 (m, 2H, H4, H5), 2.6-2.75 (m, 1H, H3), 2.916 (dt, *J* = 6.9, 6.3 Hz, 1H, H8), 3.35-3.65 (m, 4H, H6, H7), 4.3-4.4 (m, 1H, H2), 5.11-5.26 (m, 2H, 8-CH=C**H**$_3$), 6.10-6.25 (m, 1H, 8-C**H**=CH_3), 6.63-6.79 (m, 1H, H1'), 7.705 (dd, *J* = 3.6, 5.1 Hz, 1H, H6"), 7.792 (d, *J* = 4.8 Hz, 1H, H3"), 7.832 (dd, *J* = 5.4, 5.7 Hz, 1H, H7"), 8.326 (d, *J* = 6 Hz, 1H, H5"), 8.601 (d, *J* = 5.4 Hz, H8), 8.618 (d, *J* = 4.5 Hz, H2"); ^{13}C NMR (300 MHz, MeOH-d_4) δ (ppm) 21.1 (C3), 27.2 (C5), 28.9 (C4), 42.2 (C8), 64.4 (C7), 64.5 (C1'), 66.2 (C6), 73.6 (C2), 117.2 (8-CH=**C**H_3), 120.4 (C8"), 120.5 (C3"), 125.3 (C5"), 128.1 (C4a"), 130.6 (C6"), 132.3 (C7"), 137.6 (8-**C**H=CH_3), 138.9 (C2"), 141.0 (C8a"), 143.3 (C4"); MS (ESI) m/z (%) 326.87 (100); HRMS Calcd for $C_{19}H_{23}N_2O_3$: $(M+H)^+$ 327.1703; found 327.1698.

7.5.10 General procedures for synthesis of 2-substituted quinolines

Quinoline (1 equivalent) was suspended in anhydrous THF (24 mL) under an atmosphere of nitrogen and the mixture cooled to -10 ºC. The organolithium (3 equivalents) was added dropwise and the mixture stirred vigorously for 20 min at -10 ºC. The reaction was warmed to room temperature and stirred for 1 h. The reaction was monitored by TLC (EtOAc:MeOH:Et_3N; 10:1:1 to 30:1:1). The reaction mixture was cooled to 5 ºC and acetic acid (1 mL) was added. Water (30 mL) and EtOAc (30 mL) were added and the mixture stirred. Iodine (0.5-0.8 g) was added in portions until the iodine has dissolved and a strong brown colouration persisted. Sodium metabisulfate solution (10 mL, 1 g in 20 mL water) was added. The mixture was basified with aqueous ammonia (25%, 8 mL) and mixed thoroughly. The organic phase was separated and washed with brine (20 mL). The aqueous phase was extracted with DCM (30 mL). The combined organic phases were dried (anhydrous $MgSO_4$) and concentrated *in vacuo*.

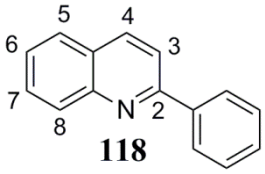
7.5.11 Synthesis of 2-butylquinoline (166)



166

Quinoline (0.46 ml, 3.871 mmol) and butyllithium (1.1 ml, 2 M, 11.61 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue by silica gel column chromatography (hexane:EtOAc; 15:1) gave compound **166**[228] (284 mg, 40%) as an oil. ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 0.939 (t, J = 7.2 Hz, 3H, H4'), 1.424 (tq, J = 7.2, 7.6 Hz, 2H, H3'), 1.885 (tt, J = 7.6, 7.6 Hz, 2H, H2'), 3.021 (t, J = 7.6 Hz, 2H, H1'), 7.287 (d, J = 8.4 Hz, 1H, H3), 7.468 (ddd, J = 7.8, 7.8, 2 Hz, 1H, H6), 7.677 (ddd, J = 7.8, 8.1, 2 Hz, 1H, H7), 7.807 (d, J = 8 Hz, 1H, H5), 7.067 (d, J = 8.4 Hz, 1H, H4), 8.252 (d, J = 8 Hz, 1H, H8); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 14.1 (C4'), 22.7 (C3'), 31.9 (C2'), 38.9 (C1'), 121.9 (C3), 125.9 (C6), 127.2 (C4a), 128.0 (C5), 129.5 (C7, C8), 136.2 (C4), 148.6 (C8a), 163.1 (C2); MS (ESI) m/z (%) 185.68 ([M+Na]$^+$, 100). The ^{1}H NMR spectrum of **166** was consistant with reported values.[228]

7.5.12 Synthesis of 2-phenylquinoline (118)

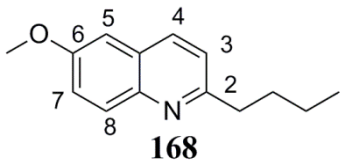


118

Procedure 3

Quinoline (1.0 g, 7.742 mmol) and phenyl lithium (2.30 mL, 2 M, 23.22 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue by silica gel column chromatography (EtOAc:MeOH:Et_3N; 10-30:1:1 or PhMe:MeOH:Et_3N; 10:1:1) gave compound **118** (0.66 g, 42%) as an orange solid.

7.5.13 Synthesis of 2-butyl-6-methoxyquinoline (168)

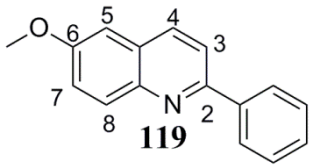


168

6-Methoxyquinoline (1 g, 6.282 mmol) and butyllithium (1.78 ml, 2 M, 18.846 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue

by silica gel column chromatography (hexane:EtOAc; 15:1) gave compound **168** (126 mg, 10%) as an orange oil. FTIR (KBr, cm^{-1}) 3096, 2982, 1618; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 0.944 (t, *J* = 7.6 Hz, 3H, H4'), 1.433 (tq, *J* = 7.2, 7.6 Hz, 2H, H3'), 1.887 (tt, *J* = 7.6, 7.6 Hz, 2H, H2'), 3.009 (t, *J* = 7.6 Hz, 2H, H1'), 3.825 (s, 3H, OCH_3), 7.226 (d, *J* = 2.4 Hz, 1H, H5), 7.278 (d, *J* = 8.4 Hz, 1H, H3), 7.459 (dd, *J* = 9.2, 9.2 Hz, 1H, H7), 8.028 (d, *J* = 8.4 Hz, 1H, H4), 8.164 (d, *J* = 9.2 Hz, 1H, H8); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 14.1 (C4'), 22.8 (C3'), 32.1 (C2'), 38.7 (C1'), 55.5 (OCH_3), 105.9 (C5), 122.1 (C3, C7), 128.1 (C4a), 130.9 (C8), 135.2 (C4), 144.7 (C8a), 157.6 (C6), 160.5 (C2); MS (ESI) m/z (%) 215.73 ($[M+Na]^+$, 100); HRMS Calcd for $C_{14}H_{18}N_1O_1$: $(M+H)^+$ 216.1383; found 216.1377.

7.5.14 Synthesis of 6-methoxy-2-phenylquinoline (119)

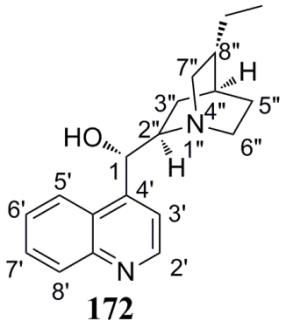


119

Procedure 2

6-Methoxy quinoline (1.0 g, 6.282 mmol) and phenyl lithium (1.86 mL, 2 M, 18.846 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue by silica gel column chromatography (EtOAc:MeOH:Et_3N; 10-30:1:1 or PhMe:MeOH:Et_3N; 10:1:1) gave compound **119** (495 g, 34%) as an orange solid.

7.5.15 Synthesis of (*R*)-((2"*S*,4"*R*,8"*S*)-8"-ethylquinuclidin-2"-yl)(quinolin-4'-yl)methanol (172)

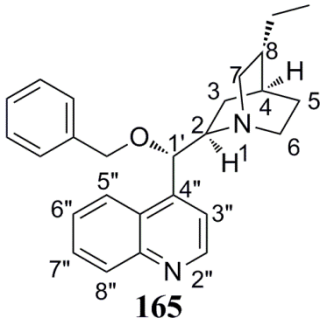


172

Cinchonine (3.0 g, 10.1 mmol) and palladium-on-carbon (106 mg, 10%, 1 equiv.) in MeOH (50 mL) were reacted under an atmosphere of hydrogen for 3 h at room temperature. The reaction mixture was filtered though celite, and the filtrate concentrated *in vacuo*. Title compound **172** (3.0 mg, 98%) was obtained as a white solid. m.p. 250-255 ºC; FTIR (KBr, cm^{-1}) 2941, 1508, 1112, 1061; ^{1}H NMR (300 MHz,

MeOH-d_4) δ (ppm) 0.927 (t, J = 9.6 Hz, 3H, 8”-$CH_2C\mathbf{H}_3$), 1.02-1.18 (m, 1H, H3”), 1.40-1.66 (m, 5H, H6”, H7”, H8”, 8”-$C\mathbf{H}_2CH_3$), 1.709 (brs, $W_{h/2}$ = 11.5 Hz, 1H, H4”), 2.05-2.22 (m, 2H, H3”, H6”), 2.67-2.97 (m, 3H, H5”, H7”), 2.99-3.13 (m, 1H, H2”), 5.675 (d, J = 5.2 Hz, 1H, H1), 7.656 (dd, J = 6.9, 7.5 Hz, 1H, H6’), 7.720 (d, J = 4.5 Hz, 1H, H3’), 7.768 (dd, J = 6.9, 6.9 Hz, 1H, H7’), 8.051 (d, J = 7.5 Hz, 1H, H8’), 8.182 (d, J = 8.1 Hz, 1H, H5’), 8.817 (d, J = 6.4 Hz, 1H, H2’); ^{13}C NMR (300 MHz, MeOH-d_4) δ (ppm) 12.3 (8”-$CH_2\mathbf{C}H_3$), 21.4 (C3”), 26.1 (8”-$\mathbf{C}H_2CH_3$), 27.4 (C5”), 27.6 (C4”), 38.4 (C8”), 50.9 (C7”), 51.7 (C6”), 61.4 (C2”), 72.0 (C1), 119.9 (C3’), 123.3 (C5’), 124.5 (C6’), 127.1 (C8’), 128.2 (C7’), 130.6 (C4a’), 148.8 (C2’), 150.9 (C4’), 151.9 (C8a’); MS (ESI) m/z (%) 296.94 (100); HRMS Calcd for $C_{19}H_{24}N_2O$: $(M+H)^+$ 297.1961; found 297.1950.

7.5.16 Synthesis of (2*S*,4*R*,8*S*)-2-((*R*)-benzyloxy(quinolin-4”-yl)methyl)-8-ethyl quinuclidine (165)



165

Procedure 1

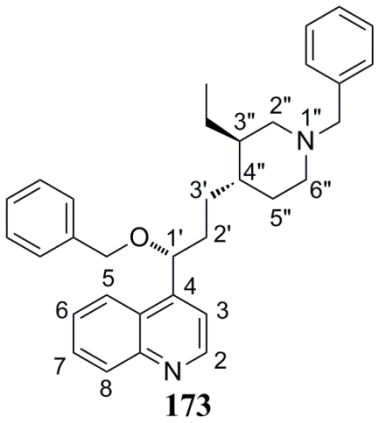
Sodium hydride (50%, 0.607 mg, 25.30 mmol) was added to a solution of **172** (3.0 g, 10.12 mmol) in DMF (13 mL) and the mixture stirred for 2 h at room temperature under an atmosphere of nitrogen. Benzyl bromide (1.1 mL, 11.133 mmol) was added dropwise over 10 min and the mixture stirred for 16 h. Brine (20 ml) was carefully added and the resulting mixture extracted with EtOAc (1 x 100 ml). The organic phase was washed with brine (3 x 50 mL), dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Purification of the residue was achieved by silica gel column chromatography (chloroform:EtOAc; 90:10) gave a complex mixture.

Procedure 2

Sodium hydride (95%, 0.162 mg, 6.746 mmol) was added to a solution of **172** (1.0 g, 3.373 mmol) in DMF (6.6 mL) and stirred at room temperature for 2 h under an atmosphere of nitrogen. Benzyl chloride (0.47 mL, 3.711 mmol) was added dropwise

over 10 min and the mixture was stirred for 16 h. Brine (20 ml) was carefully added and the resulting mixture extracted with EtOAc (1 x 100 ml). The organic phase was washed with brine (3 x 50 mL), dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Analysis of the residue (1.709 g, 130%) by ^{1}H NMR spectroscopy indicated a 60:40 mixture of compounds **165**:**173** (5%:25%) respectively. Purification by silica gel column chromatography (hexane:EtOAc; 7:3) gave two major fractions.

*(2S,4R,8S)-2-((R)-Benzyloxy(quinolin-4''-yl)methyl)-8-ethyl quinuclidine (**165**)*[191] was obtained as a viscous oil. ^{1}H NMR (400 MHz, acetone-d_6) δ (ppm) 0.837 (t, *J* = 7.2 Hz, 3H, 8-$CH_2C\mathbf{H}_3$), 1.3-1.6 (m, 4H, H4, H8, H5), 1.9-2.0 (m, 2H, 8-$C\mathbf{H}_2CH_3$), 1.9-2.0 (m, 3 Hz, 2H, H3), 2.5-2.7 (m, 2H, H7), 2.72-2.95 (m, 2H, H6), 3.184 (dt, *J* = 8.4, 7.6 Hz, 1H, H2), 4.427 (s, 2H, $OC\mathbf{H}_2$), 5.392 (d, *J* = 5.6 Hz, 1H, H1'), 7.35-7.4 (m, 5H, C_6H_5), 7.55-7.63 (m, 2H, H3'', H7''), 7.732 (dd, *J* = 8, 6.8 Hz, 1H, H6''), 8.082 (d, *J* = 7.6 Hz, 1H, H5''), 8.345 (d, *J* = 8.4 Hz, 1H, H-8''), 8.878 (d, *J* = 4.4 Hz, 1H, H2''); MS (ESI) m/z (%) 387.03 (100). The ^{1}H NMR spectrum of **165** was consistent with reported values.[191]



173

*4''-(3'-(1''-Benzyl-3''-ethylpiperidin-4''-yl)-(1'R)-(benzyloxy)propyl)quinoline (**173**)*

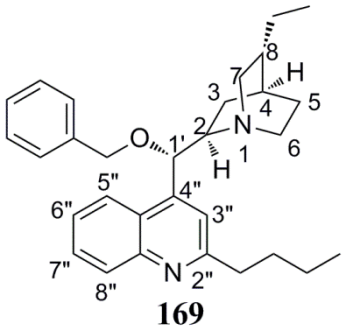
Compound **173** was obtained from fraction 2 as a brown oil. FTIR (KBr, cm^{-1}) 3029, 2930, 2871, 1653; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 0.901 (t, *J* = 7.2 Hz, 3H, 3''-$CH_2C\mathbf{H}_3$), 1.40-1.54 (m, 1H, 3''-$C\mathbf{H}_2CH_3$), 1.58-1.78 (m, 7H, 3''-$C\mathbf{H}_2CH_3$, H3', H4'', H3'', H6''), 2.15-2.28 (m, 2H, H2'', H5''), 2.40-2.56 (m, 2H, H2'), 2.65-2.75 (m, 1H, H5''), 2.75-2.80 (m, 1H, H2''), 3.516 (ABq, *J* = 13.2 Hz, 2H, 1''-NCH_2), 4.413 (s, 2H, 1'-OCH_2), 4.619 (dd, *J* = 7.6, 7.6 Hz, 1H, H1'), 7.28-7.44 (m, 8H, *m,p*-1''-$CH_2C_6H_5$, 1'-OCH_2-C_6H_5), 7.45-7.50 (m, 3H, H3, *o*-1''-N-$CH_2C_6H_5$), 7.616 (dd, *J* = 7.6, 8 Hz, 1H, H6), 7.771 (dd, *J* = 7.2, 7.2 Hz, 1H, H7), 8.398 (d, *J* = 8.4 Hz, 1H, H8), 8.516 (d, *J* = 8.4 Hz, 1H, H5), 9.080 (d, *J* = 4.4 Hz, 1H, H2); ^{13}C NMR (400 MHz, pyridine-d_5) δ

(ppm) 12.4 (3”-CH_2**C**H_3), 19.5 (3”-**C**H_2CH_3), 27.5 (C2’), 28.7 (C3’, C5”), 38.9 (C4”), 40.6 (C3”), 53.3 (C6”), 55.9 (C2”), 63.5 (1”-NCH_2), 71.7 (1’-OCH_2), 118.1 (C1’), 121.9 (C3), 126.0 (C5), 126.8 (C4a), 127.2 (*p*-1’-C_6H_5), 127.3 (C6), 128.3/128.4/128.5/128.7/128.74 (*m,p*-1”-N-$CH_2C_6H_5$ / *o,m*-1’-O-$CH_2C_6H_5$), 129.2 (*o*-1”-N-$CH_2C_6H_5$), 129.8 (C7), 130.5 (C8), 137.9 (O-*i*-C_6H_5), 139.9 (N-*i*-C_6H_5), 148.2 (C8a), 150.8 (C2), 151.9 (C4); MS (ESI) m/z (%) 476.99 (100); HRMS Calcd for $C_{33}H_{374}N_2O$: (M+1) 477.29; found 477.2903.

Procedure 3

Sodium hydride (95%, 0.291 mg, 12.145 mmol) was added to a solution of **172** (3.0 g, 10.121 mmol) in DMF (19.8 mL) and the mixture was stirred for 4 h at room temperature under an atmosphere of argon. Benzyl chloride (1.41 mL, 11.133 mmol) was added dropwise over 10 min and mixture stirred for 16 h. Brine (20 ml) was carefully added and resulting mixture extracted with EtOAc (1 x 100 ml). The organic phase was washed with brine (3 x 50 mL), dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Purification of the residue by silica gel column chromatography (hexane:EtOAc; 7:3) gave compound **165** (1.43 g, 37%)

7.5.17 Synthesis of (2*S*,4*R*,8*S*)-2-((*R*)-benzyloxy(2”-butylquinolin-4”-yl)methyl)-8-ethylquinuclidine (169)

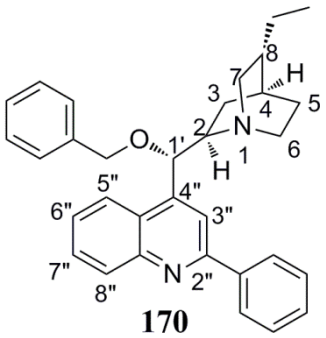


169

Compound **165** (256 mg, 0.663 mmol) and butyllithium (0.19 ml, 2 M, 1.989 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue was achieved by silica gel column chromatography (hexane:EtOAc; 15:1). Title compound **169** (68 mg, 23%) was obtained pure as a yellow oil. FTIR (KBr, cm^{-1}) 3097, 2830, 1635; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 0.893 (t, *J* = 7.6 Hz, 3H, 8-CH_2C**H**$_3$), 0.943 (t, *J* = 7.2 Hz, 3H, 2”-$CH_2CH_2CH_2C$**H**$_3$), 1.20-1.55 (m, 7H, H5, H8, 8-C**H**$_2CH_3$, 2”-CH_2CH_2C**H**$_2CH_3$), 1.56-1.60 (m, 2H, H3, H4), 1.939 (dt, *J* = 7.2, 7.6 Hz, 2H, 2”-CH_2C**H**$_2CH_2CH_3$), 2.08-2.18 (m, 1H, H3), 2.54-2.78 (m, 2H, H6), 2.85-3.04 (m,

2H, H7), 3.094 (t, *J* = 7.6 Hz, 2H, 2”-C**H**$_2$CH$_2$CH$_2$CH$_3$), 3.346 (dt, *J* = 7.2, 8.4 Hz, 1H, H2), 4.536 (ABq, 2H, 1’-OCH$_2$), 5.4-5.5 (m, 1H, H1’), 7.333 (dd, *J* = 7.2, 7.2 Hz, 1H, *p*-C$_6$H$_5$), 7.25-7.45 (m, 1H, *m*-C$_6$H$_5$), 7.40-7.51 (m, 2H, *o*-C$_6$H$_5$), 7.563 (ddd, *J* = 7.2, 7.6, 2 Hz, 1H, H6”), 7.680 (s, 1H, H3”), 7.716 (ddd, *J* = 8.4, 8.8, 2 Hz, 1H, H7”), 8.341 (d, *J* = 8.4 Hz, 1H, H8”), 8.499 (d, *J* = 8.4 Hz, 1H, H5”); ^{13}C NMR (400 MHz, pyridine-*d*$_5$) δ (ppm) 12.2 (8-CH$_2$**C**H$_3$), 14.2 (2”-CH$_2$CH$_2$CH$_2$**C**H$_3$), 22.8 (2”-CH$_2$CH$_2$**C**H$_2$CH$_3$), 24.3 (C3), 25.7 (8-**C**H$_2$CH$_3$), 26.8 (C4), 27.8 (C5), 32.1 (2”-CH$_2$**C**H$_2$CH$_2$CH$_3$), 37.8 (C8), 39.0 (2”-**C**H$_2$CH$_2$CH$_2$CH$_3$), 50.3 (C6), 50.9 (C7), 61.6 (C2), 71.5 (1’-OCH$_2$), 81.6 (C1’), 120.3 (C3”), 124.4 (C5”), 125.9 (C6”), 126.2 (C4a”), 128.1 (*p*-C$_6$H$_5$), 128.4 (*o*-C$_6$H$_5$5), 128.8 (*m*-C$_6$H$_5$), 129.3 (C7”), 130.5 (C8”), 138.9 (*i*-C$_6$H$_5$), 147.1 (C4”), 149.2 (C8a”), 162.9 (C2”); MS (ESI) m/z (%) 443.04 (100); HRMS Calcd for C$_{30}$H$_{39}$N$_2$O$_1$: (M+H)$^+$ 443.3157; found 443.3062.

7.5.18 Synthesis of (2*S*,4*R*,8*S*)-2-((*R*)-benzyloxy(2”-phenylquinolin-4”-yl)methyl)-8-ethylquinuclidine (170)

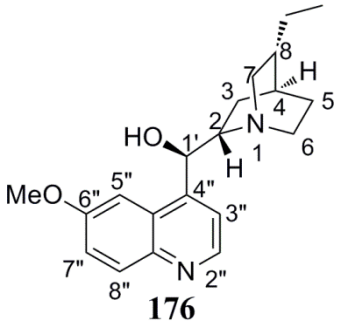


170

Compound **165** (417 mg, 1.080 mmol) and phenyl lithium (0.32 ml, 2 M, 11.61 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue by silica gel column chromatography (hexane:EtOAc; 15:1). Title compound **170** (14 mg, 3%) was obtained pure as a yellow oil. FTIR (KBr, cm^{-1}) 3106, 2876, 1682; ^{1}H NMR (400 MHz, pyridine-*d*$_5$) δ (ppm) 0.890 (t, *J* = 7.6 Hz, 3H, 8-CH$_2$C**H**$_3$), 1.3-1.5 (m, 5H, H5, H8, 8-C**H**$_2$CH$_3$), 1.6-1.7 (m, 2H, H4, H3), 2.1-2.3 (m, 1H, H3), 2.52-2.78 (m, 2H, H6), 2.85-3.08 (m, 2H, H7), 3.410 (dt, *J* = 7.2, 8 Hz, 1H, H2), 4.585 (ABq, 2H, 1’-OCH$_2$), 5.5-5.6 (m, 1H, H1’), 7.327 (brd, *J* = 7.2 Hz, 1H, *p*-C$_6$H$_5$), 7.32-7.43 (m, 2H, *m*-C$_6$H$_5$), 7.45-7.51 (m, 3H, *o*-C$_6$H$_5$, 2”-*p*-C$_6$H$_5$), 7.45-7.56 (m, 2H, 2”-*m*-C$_6$H$_5$), 7.54-7.64 (m, 1H, H6”), 7.768 (dd, *J* = 7.2, 7.2 Hz, 1H, H7”), 8.372 (s, 1H, H3”), 8.436 (d, *J* = 8.4 Hz, 1H, H8”), 8.486 (d, *J* = 7.2 Hz, 1H, 2”-*o*-C$_6$H$_5$), 8.548 (d, *J* = 8.4 Hz, 1H, H5”); ^{13}C NMR (400 MHz, pyridine-*d*$_5$) δ (ppm) 11.8 (8-CH$_2$**C**H$_3$), 24.4 (C3), 25.7 (8-**C**H$_2$CH$_3$), 26.8 (C4), 27.8 (C5), 37.9 (C8), 50.2 (C6), 50.9 (C7), 61.7 (C2), 71.6 (1’-OCH$_2$), 81.6

(C1'), 117.4 (C3"), 124.5 (C5"), 126.6 (C6"), 126.7 (C4a"), 128.1 (*p*-C_6H_5, 2"-*p*-C_6H_5), 128.4 (*m*-C_6H_5), 128.8 (*o*-C_6H_5), 129.2 (2"-*m*-C_6H_5), 129.8 (2"-*o*-C_6H_5, C7"), 131.5 (C8"), 138.9 (*i*-C_6H_5), 140.2 (2"-*i*-C_6H_5), 149.0 (C4"), 149.4 (C8a"), 156.8 (C2"); MS (ESI) m/z (%) 463.26 (100); HRMS Calcd for $C_{32}H_{35}N_2O_1$: $(M+H)^+$ 463.2744; found 463.2733.

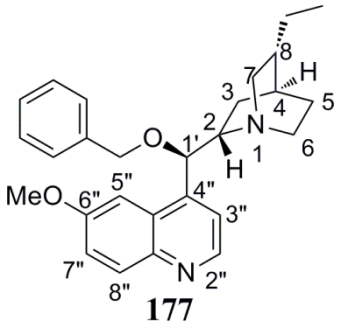
7.5.19 Synthesis of (*R*)-((2"*S*,4"*R*,8"*S*)-8"-ethylquinuclidin-2"-yl)(8"-methoxy quinolin-4'-yl)methanol (176)



176

Quinine (3.0 g, 9.247 mmol) and palladium-on-carbon (98 mg, 10%, 1 equiv.) in MeOH (50 mL) were reacted under an atmosphere of hydrogen for 3 h at room temperature. The reaction mixture was filtered though celite, and the filtrate concentrated *in vacuo*. Compound **176**[229,230] (2.89 g, 96%) was obtained as a white solid. m.p. 169-171 ºC (Lit.[230] m.p. 170 ºC); ^{1}H NMR (400 MHz, $CDCl_3$) δ (ppm) 0.806 (t, *J* = 7.2 Hz, 3H, 8"-$CH_2C\mathbf{H}_3$), 1.20-1.32 (m, 2H, 8"-C$\mathbf{H}_2CH_3$), 1.33-1.48 (m, 2H, H3", H5"), 1.48-1.8 (m, 4H, H3", H4", H5", H8"), 2.32-2.42 (m, 1H, H7"), 2.58-2.70 (m, 1H, H6"), 3.11-3.20 (m, 2H, H6", H7"), 3.29-3.41 (m, 1H, H2"), 3.914 (s, 3H, 1-OCH_3), 5.516 (s, 1H, H1), 7.258 (d, *J* = 9.2 Hz, 1H, H7'), 7.346 (d, *J* = 4.2 Hz, 1H, H3'), 7.510 (d, *J* = 3 Hz, 1H, H5'), 8.005 (d, *J* = 9 Hz, 1H, H8'), 8.731 (d, *J* = 4.2 Hz, 1H, H2'), OH not observed; MS (ESI) m/z (%) 326.98 (100).

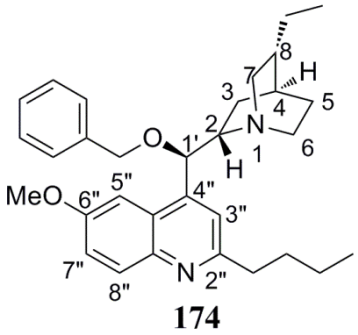
7.5.20 Synthesis of (2*S*,4*R*,8*S*)-2-((*R*)-benzyloxy(6”-methoxyquinolin-4”-yl) methyl)-8-ethylquinuclidine (177)



177

Sodium hydride (95%, 0.176 mg, 7.351 mmol) was added to a solution of **176** (2.0 g, 6.126 mmol) in DMF (12 mL) and the mixture was stirred for 1 h at room temperature under an atmosphere of argon. Benzyl chloride (0.77 mL, 6.738 mmol) was added dropwise over 10 min and mixture stirred for 16 h. Brine (20 ml) was carefully added and resulting mixture extracted with EtOAc (1 x 100 ml). The organic phase was washed with brine (3 x 50 mL), dried (anhydrous $MgSO_4$) and concentrated *in vacuo*. Purification by silica gel column chromatography (hexane:EtOAc; 7:3) gave compound **177** (897 mg, 35%) as an oil. FTIR (KBr, cm^{-1}) 3082, 3001, 2867, 1263; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm); 0.713 (t, J = 7.2 Hz, 3H, 8-$CH_2C\mathbf{H}_3$), 1.06-1.27 (m, 3H, H8, 8-$C\mathbf{H}_2CH_3$), 1.27-1.42 (m, 1H, H5), 1.62-1.74 (m, 2H, H3, H4), 1.74-1.92 (m, 2H, H3, H5), 2.20-2.32 (m, 1H, H7), 2.56-2.68 (m, 1H, H6), 2.84-2.98 (m, 1H, H7), 3.32-3.52 (m, 2H, H2, H6), 3.848 (s, 3H, 6”-OCH_3), 4.477 (ABq, J = 13.2 Hz, 2H, 1’-OCH_2), 5.370 (brs, $W_{h/2}$ = 19 Hz, 1H, H1’), 7.30-7.39 (m, 5H, C_6H_5), 7.408 (dd, J = 8.4, 2.8 Hz, 1H, H7”), 7.678 (d, J = 2.8 Hz, 1H, H3”), 7.878 (s, 1H, H5”), 8.318 (d, J = 9.2 Hz, 1H, H8”), 8.989 (d, J = 4.4 Hz, 1H, H2”); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 12.3 (8-$CH_2\mathbf{C}H_3$), 24.4 (C3), 25.9 (C4), 27.8 (8-$\mathbf{C}H_2CH_3$), 28.9 (C5), 37.9 (C8), 43.0 (C6), 55.6 (6”-OCH_3), 58.5 (C7), 61.2 (C2), 71.3 (1’-OCH_2), 82.3 (C1’), 102.8 (C5”), 120.2 (C3”), 121.8 (C7”), 128.1 (C4a”), 128.2 (*p*-C_6H_5), 128.4 (*m*-C_6H_5), 128.8 (*o*-C_6H_5), 132.5 (C8”), 138.9 (*i*-C_6H_5), 145.6 (C8a”), 148.2 (C4”), 158.1 (C6”), 160.2 (C2”); MS (ESI) m/z (%) 417.20 (100); HRMS Calcd for $C_{27}H_{33}N_2O_2$: $(M+H)^+$ 417.2536; found 417.2524.

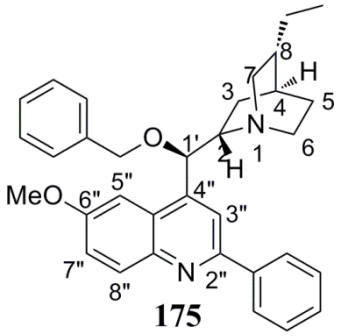
7.5.21 Synthesis of (*2S*,4*R*,8*S*)-2-((*R*)-benzyloxy(2”-butyl-6”-methoxyquinolin-4”-yl)methyl)-8-ethylquinuclidine (174)



174

Compound **177** (505 mg, 1.212 mmol) and butyllithium (2 M, 0.342 ml, 11.61 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue by silica gel column chromatography (hexane:EtOAc; 15:1) gave compound **174** (100 mg, 17%) as an oil. FTIR (KBr, cm^{-1}) 3028, 2956, 1654, 1153; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 0.785 (t, J = 7.2 Hz, 3H, 8-$CH_2C\mathbf{H}_3$), 0.941 (t, J = 7.6 Hz, 3H, 2”-$CH_2CH_2CH_2C\mathbf{H}_3$), 1.16-1.36 (m, 3H, H8, 8-$C\mathbf{H}_2CH_3$), 1.38-1.50 (m, 3H, H5, 2”-$CH_2CH_2C\mathbf{H}_2CH_3$), 1.70-1.82 (m, 2H, H4, H5), 1.62-2.08 (m, 4H, H3, 2”-$CH_2C\mathbf{H}_2CH_2CH_3$), 2.28-2.38 (m, 1H, H7), 2.60-2.72 (m, 1H, H6), 2.91-3.02 (m, 1H, H7), 3.071 (t, J = 7.6 Hz, 2H, 2”-$C\mathbf{H}_2CH_2CH_2CH_3$), 3.38-3.54 (m, 2H, H2, H6), 3.885 (s, 3H, 6”-OMe), 4.572 (ABq, J = 12.4 Hz, 2H, 1’-OCH_2), 5.344 (brs, $W_{h/2}$ = 38 Hz, 1H, H1’), 7.337 (brd, J = 7.6 Hz, 1H, p-C_6H_5), 7.38-7.47 (m, 2H, m-C_6H_5), 7.47-7.51 (m, 3H, H7”, o-C_6H_5), 7.659 (s, 1H, H3”), 7.886 (s, 1H, H5”), 8.258 (d, J = 9.2 Hz, 1H, H8”); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 12.2 (8-$CH_2\mathbf{C}H_3$), 14.1 (2”-$CH_2CH_2CH_2\mathbf{C}H_3$), 22.7 (2”-$CH_2CH_2\mathbf{C}H_2CH_3$), 25.6 (C3), 26.0 (C4), 27.8 (8-$\mathbf{C}H_2CH_3$), 29.2 (C5), 32.1 (2”-$CH_2\mathbf{C}H_2CH_2CH_3$), 37.8 (C8), 38.7 (2”-$\mathbf{C}H_2CH_2CH_2CH_3$), 43.0 (C6), 55.6 (6”-$OCH_3$), 58.6 (C7), 61.2 (C2), 71.4 (1’-OCH_2), 81.5 (C1’), 103.0 (C5”), 120.4 (C3”), 121.4 (C7”), 126.7 (C4a”), 128.1 (p-C_6H_5), 128.2 (m-C_6H_5), 128.8 (o-C_6H_5), 131.9 (C8”), 138.9 (i-C_6H_5), 145.2 (C8a”), 147.5 (C4”), 157.6 (C6”), 160.2 (C2”); MS (ESI) m/z (%) 473.34 (100); HRMS Calcd for $C_{31}H_{41}N_2O_2$: $(M+H)^+$ 473.3163; found 473.3172.

7.5.22 Synthesis of (2*S*,4*R*,8*S*)-2-((*R*)-benzyloxy(-6”-methoxy-2”-phenylquinolin-4”-yl)methyl)-8-ethylquinuclidine (175)



175

Compound **177** (392 mg, 0.941 mmol) and phenyl lithium (2 M, 0.28 ml, 2.823 mmol) were reacted according to general procedure (section 7.5.8). Purification of the residue by silica gel column chromatography (hexane:EtOAc; 15:1) gave compound **175** (4 mg, <1%) as an oil. FTIR (KBr, cm^{-1}) 3056, 2961, 2895, 1329; ^{1}H NMR (400 MHz, pyridine-d_5) δ (ppm) 0.776 (t, J = 7.2 Hz, 3H, 8-$CH_2C\mathbf{H}_3$), 1.15-1.46 (m, 4H, H5, H8, 8-$C\mathbf{H}_2CH_3$), 1.71-1.82 (m, 2H, H4, H5), 1.86-1.98 (m, 2H, H3), 2.30-2.38 (m, 1H, H7), 2.62-2.72 (m, 1H, H6), 2.92-3.02 (m, 1H, H7), 3.4-3.6 (m, 2H, H6, H2), 3.898 (s, 3H, 6”-OCH_3), 4.758 (ABq, J = 13.4 Hz, 2H, 1’-OCH_2), 5.4-5.5 (m, 1H, H1’), 7.320 (dd, J = 7.2, 2 Hz, 1H, 2”-p-C_6H_5), 7.396 (dd, J = 7.2, 7.6 Hz, 2H, o-C_6H_5), 7.451 (d, J = 7.2 Hz, 1H, p-C_6H_5) 7.47-7.58 (m, 5H, H7”, 2”-m-C_6H_5, m-C_6H_5), 7.910 (s, 1H, H5”), 8.343 (s, 1H, H3”), 8.344 (d, J = 9.2 Hz, 1H, H8”), 8.462 (d, J = 7.6 Hz, 2H, 2”-o-C_6H_5); ^{13}C NMR (400 MHz, pyridine-d_5) δ (ppm) 12.2 (8-$CH_2\mathbf{C}H_3$), 24.8 (C3), 26.0 (C4), 27.9 (8-$\mathbf{C}H_2CH_3$), 29.0 (C5), 37.9 (C8), 43.0 (C6), 55.6 (6”-OCH_3), 58.7 (C7), 61.3 (C2), 71.5 (1’-OCH_2), 82.9 (C1’), 102.9 (C5”), 117.6 (C3”), 122.2 (C7”), 127.5 (C4a”), 127.7 (2”-o-C_6H_5), 128.1 (2”-p-C_6H_5), 128.3 (o- C_6H_5), 128.9/129.2 (m-C_6H_5, 2”-m-C_6H_5) 129.4 (p-C_6H_5), 132.6 (C8”), 139.0 (1’-i-C_6H_5), 140.3 (2”-i-C_6H_5), 145.5 (C8a”), 147.3 (C4”), 154.5 (C2”), 158.1 (C6”); MS (ESI) m/z (%) 493.26 (100); HRMS Calcd for $C_{33}H_{37}N_2O_2$: $(M+H)^+$ 493.2850; found 493.2836.

7.6 Chapter Five Experimental Procedures

7.6.1 General

Slides were observed with a brightfield Ziess-axio microscope (20x and 40x objective) A1 fitted with an axiocam Kc3 at 20x or 40x objective and viewed with AxioVision Rel 4.7 Brightfield microscope (20x objective) Olympus Bx50 VPlanF1, RTSE spot camera with spot software 4.6. Nitrite analysis was recorded on a versamax microplate reader with softmax pro V5.2 software. All graphs were generated using GraphPad Prism 5.0. Compounds **94**, **116-119**, **125-127**, **135**, **147**, **148**, **151**, **160**, **162**, **166**, **167**, **170**, **172**, **175** and **177-182** were analytically pure. Compounds **98 (**97 %), **105** (97%), **106** (98%), **136** (98%), **148** (97%), **152** (95%), **169** (90%), **170** (90%), **173** (95%), **175** (95%), **177** (90%) and **180** (95%), were >90% pure at the time of testing. Assays were carried out by the candidate (K. Reynolds) in the laboratory of Associate Proffesor Heinrick Koerner at James Cook University, Townsville.

7.6.2 Parasites and Infection

The virulent *L. major* isolate MHOM/IL/81/FE/BNI was maintained through serial passage in BALB/c mice *in vivo* and cultured *in vitro* in Novy-Nicolle-MacNeal blood agar slants in RPMI (Roswell Park Memorial Institute) media containing 10% new born calf serum, Penicillin/Streptomycin, non essential amino acids and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES,[231] 10 mM), which were all supplied by Invitrogen. For infection, stationary phase *L. major* promastigotes were used between passage two and six and $3x10^6$ parasites were injected in a volume of 40 μL into one hind footpad. The infection site was monitored daily and the increase in lesion size was noted twice weekly by measuring the footpad thickness with a metric caliper (Kroeplin Schnelltaster, Schluechtern, Germany). The increase in footpad thickness (percentage) was determined by the formula:

Thickness of infected footpad / mean thickness of non-infected footpad x 100

Parasite burden was calculated as a proportion of tissue weight at day 28 after infection using a limiting dilution method and L-Calc software version 1.1 (Stem Cell Technologies; www.Stemcell.com) which performs a generalised Pearson Chi-squared test.[232]

7.6.3 Culture and infection of macrophages

Bone marrow (BM) cells were harvested from the femurs and tibias of B6.WT or gene-deficient mice using a 26 g needle. Red blood cells were lysed in 0.17 M sterile ammonium chloride and 20 mM HEPES buffer for 10 minutes at 4 °C. Cells ($1x10^5$ cells/ml) were seeded into Teflon bags containing 50 mL RPMI-1680 supplemented with 10% fetal bovine serum, 5% horse serum, L-glutamine, non-essential amino acids and sodium pyruvate (Invitrogen) in the absence of antibiotics as published.[233] L929 conditioned medium (a gift from Matt Sweet, IMB, Brisbane) was added as a source of macrophage colony stimulating factor (M-CSF) to a final concentration of 10% - 15% after titration on primary BM cells. Cells were incubated for 7-10 days at 37 °C at 5% CO_2. For cytokine assays, macrophages ($1x10^6$ cell/ml) were seeded into 8 or 16 well chamber slides (Labtek, Sigma or ProSciTech). After 2 h non-adhered cells were washed off and both interferon gamma (IFN-γ) (20 ng/ml, Invitrogen) and lipopolysaccharide (LPS) (1 μg/mL, *e. coli*, 0111.B4 strain, cell culture tested, Sigma) were added for a period of 3 h prior to infection with stationary phase *L. major* promastigotes at a multiplicity of infection (MOI) of 3. Media was harvested at 16 h and extracellular promastigotes were removed by extensive washing with phosphate buffer solution (PBS). Cells were then fixed and microscopic visualisation was performed following staining with Kwik Diff (Thermo Fischer Scientific) (fixing stain, cytoplasmic eosin stain and a nuclear methalene blue stain). For NO analysis superanatants were harvested at 72 h. Toxic effects in the macrophages were evidenced by the change in morphological features i.e. loss of refringency, vacuolation of cytoplasm or loss of cytoplasmic material.

7.6.4 Preparation of test compounds

Stock solutions were prepared in culture medium supplemented with DMSO with a maximum of 0.5% DMSO at the first test compound concentration. DMSO had no effect on control cultures. Freshly prepared compound solutions were added to the infected cultures in duplicate 24 h after infection. Leishmanicidal effects were detectable by quantifying the decrease in parasite number.

7.6.5 Nitrite test for oxidative stress by test compounds against L. major amasitgotes

Under physiological conditions, NO is readily oxidized to nitrite. The Griess reagent (1% sulfanilic acid in 5% phosphoric acid : 0.1% *N*-(1-naphthyl)-ethylenediamine dihydrochloride; 1:1) provides a simple colorimetric assay (detection limit ~100 nM) for nitrites.[234] Nitrites react with sulfanilic acid in acidic solution to form an intermediate diazonium salt that couples to *N*-(1-naphthyl) ethylenediamine to yield a pink azo derivative that can be monitored by absorbance at 540 nm (Figure 1).[235]

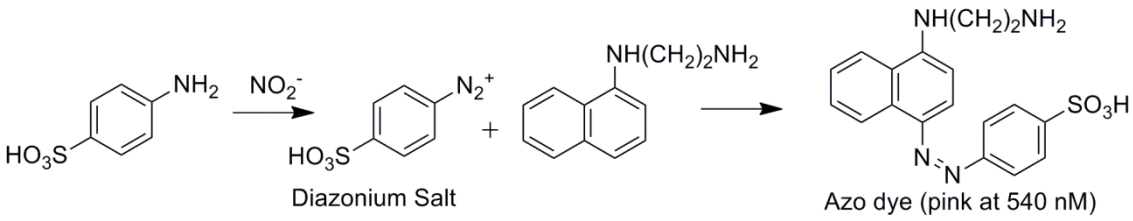


Figure 29 Principle of nitrite quantitation using the Griess Reagent. Formation of the azo dye is detected via its absorbance at 540 nm.

Culture medium from infected cultures (*L. major* amastigotes) was placed into flat bottom 96 well plates (Nunc, Invitro Technologies). Stock solutions of 100 µM were made from 1 mM sodium nitrite ($NaNO_2$) solution in 2-fold dilutions and 96-well plates were read on a Versamax microplate reader at 540 nm. A standard curve was obtained from 2-fold dilutions of sodium nitrite (Figure 29).

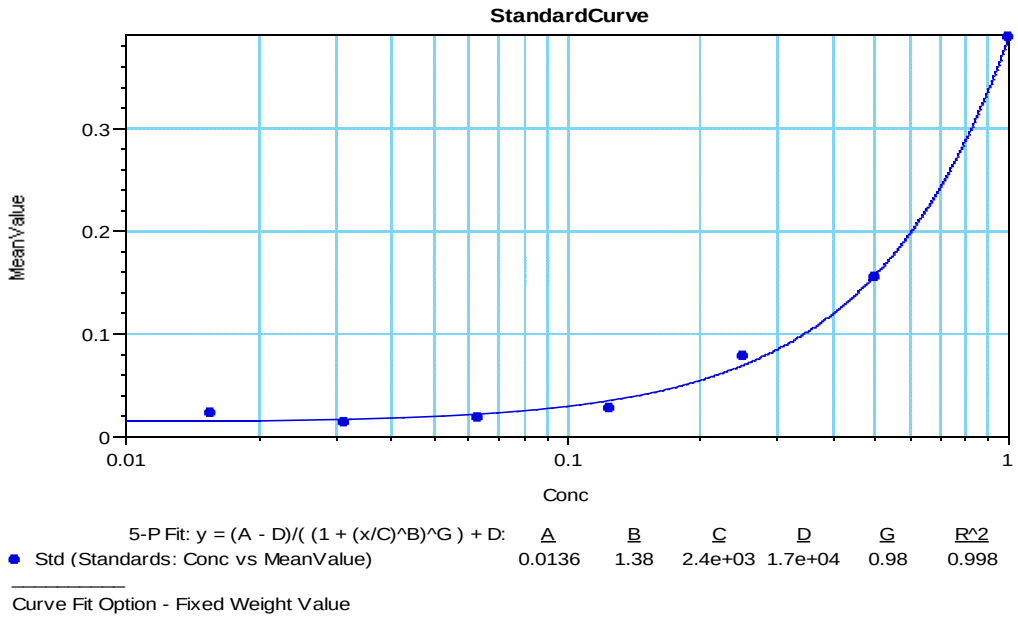


Figure 30 Standard curve of nitrite production at 540 nm.

The various concentrations of each compound were then compared to the standard to determine if any nitrite was produced (Table 12a, Table 12b). This test identified the test compounds that stimulate macrophages to produce nitrite – one of the metabolites formed when the cells undergo oxidative stress.

Table 12a Nitrite production from compound **171**

Concentration of compound 171 (μM)	**Nitrite Production (nm)**
100 μM	0.135
100 μM	1.536
50 μM	1.386
50 μM	0.124
25 μM	2.02
25 μM	0.746
12.5 μM	0.071
12.5 μM	0.126
6.25 μM	1.773
6.25 μM	1.203
3.13 μM	0.198
3.13 μM	0.064
15.6 μM	1.596
15.6 μM	1.299
0.78 μM	1.706
0.78 μM	0.505

Table 12b Average nitrite production of compounds **118-136**, **135-162**, **178-182**,

Compound	**Nitrite Production (nm)**	**Standard Deviation**
116	0.066	0
117	0.687	0
118	0.668	0
119	0.773	0
125	0.069	0
126	0.053	0
127	0.308	0

Table 12b Continued

Compound	Nitrite Production (nm)	Standard Deviation
135	0.073	0
136	0.032	0
147	0.071	0
148	0.062	0
149	-0.003	0.154
151	0.088	0
152	0.223	0
160	0.532	0
162	0.055	0
165	0.080	0.084
166	0.037	0
168	0.048	0
169	0.768	0
170	0.814	0
172	0.906	0.060
173	0.072	0.270
174	1.146	0.089
175	0.852	0.042
176	0.067	0
177	0.018	0
178	0.046	0
179	0.099	0
181	0.1	0
182	0.102	0

7.6.6 Infectivity of *L. major* amastigotes

Each test compound was tested for activity against *L. major* amastigotes against both positive and negative controls to determine the half maximal inhibitory concentration (IC_{50}). The positive control represents the comparison of the test compounds to a gold standard (INF-γ and LPS; 10 ng/mL and 10 μg/mL respectively). The negative control was the comparison of the test compound at 100% survival rate. The negative control only contained macrophage cells infected with *L. major* amastigotes (MOI of 3). Controls were set up for each experiment.

Tabulation of the data for a variety of compounds is displayed in Table 13a – Table 13e. The activity of compounds **169**, **170**, **174** and **175** were determined from 8 and 10 concentrations in two separate experiments. While the activity of compounds **118**-**119**, **127**, **165**, **176**, and **177** were determined from 8 concentrations at either 2-fold or 2.5-fold. Compounds were added 16 h post-infection, and left for 5 days.

Macrophages	=	total number of macrophages in viewed area.
Macrophages (infected)	=	total number of only infected macrophages in viewed area.
Infectivity rate	=	(# macrophages (infected) / # macrophages (total)) x 100
Number of Parasites	=	total number of parasites within infected macrophages in viewed area.
Average amastigotes /100 macrophages	=	(# parasites / # macrophages (infected)) x infectivity rate.

Table 13a Total parasite burden for test compound **165** at 2.5-fold concentration

Compound 165 (μM)	**Number of macrophages (total)**	**Number of macrophages (infected)**	**Infectivity rate**	**Number of parasites**	**Number of amastigotes / 100 macrophages**
Parasites only (-ve)	144	69	47.90	135	94
INF-γ / LPS (+ve)	132	2	1.52	4	3
10	110	8	7.27	15	14
	80	12	15.00	12	15
4	161	15	9.32	26	16
	138	9	6.52	18	13
1.6	137	14	10.22	35	26
	92	32	34.78	50	54
0.64	121	39	32.23	59	49
	186	43	23.12	58	31
0.102	175	54	30.86	77	44
	140	41	29.29	85	61
0.041	161	67	41.61	101	63
	150	52	34.67	71	47
0.00655	183	85	46.45	131	72
	130	52	40.00	79	61
0.00262	181	100	55.25	184	102
	161	86	53.42	139	86

Table 13b Averaged parasite burden for test compound **170** at 2.5-fold concentration

Compound 170 (μM)	Number of macrophages (total)	Number of macrophages (infected)	Infectivity rate	Number of parasites	Number of amastigotes / 100 macrophages
10	32	1	3.13	1	1
4	49	24	48.98	42	48
1.6	43	20	46.51	28	41
0.64	36	23	63.89	60	98
0.256	21	13	61.90	18	21
0.102	57	26	45.61	56	59
0.041	50	28	56.00	54	65
0.0164	15	14	93.34	46	75
0.00655	44	24	54.54	43	71
0.00262	50	33	66.00	58	75

Table 13c Average parasite burden for test compound **119** at 2-fold concentration

Compound 119 (μM)	Number of macrophages (total)	Number of macrophages (infected)	Infectivity rate	Number of parasites	Number of amastigotes / 100 macrophages
100	87	9	10.23	19	22
50	116	11	9.27	25	21
25	190	73	38.63	104	56
12.5	223	62	27.82	100	44
3.13	145	45	30.72	86	59
1.56	136	47	35.22	118	88
0.391	143	34	23.48	73	51
0.195	165	61	36.83	85	51

Table 13d Average parasite burden for test compound **117** at 2-fold concentration

Compound 117 (μM)	Number of macrophages (total)	Number of macrophages (infected)	Infectivity rate	Number of parasites	Number of amastigotes / 100 macrophages
100	21	1	4.76	0	0
50	28	4	14.29	8	19
25	231	92	39.83	135	57
12.5	175	71	40.57	120	69
3.13	192	87	45.31	132	68
1.56	154	77	50.00	126	81
0.391	142	77	54.23	135	98
0.195	194	94	48.45	145	75

Table 13e Average parasite burden for test compound **118** at 2-fold concentration

Compound 118 (μM)	Number of macrophages (total)	Number of macrophages (infected)	Infectivity rate	Number of parasites	Number of amastigotes / 100 macrophages
100	119	29	24.32	46	38
50	122	44	37.21	70	59
25	163	35	20.96	37	22
12.5	137	43	31.60	55	41
3.13	89	27	31.07	49	57
1.56	120	33	27.84	55	49
0.391	92	21	22.39	49	54
0.195	108	40	38.09	79	76

From these results % levels of inhibition or IC_{50} values were obtained by calculating the percentage of the test compound concentration against the negative control to determine the percent of parasite burden. The IC_{50} values were plotted using GraphPad Prism 5 with 8 and / or 10 CRC points. The IC_{50} graphs were determined as a percent of parasite survival, with 0% being no parasite burden and measured and 100% indicating parasite survival measured against the negative control. In some cases compounds were been assigned values >200 μM instead of IC_{50} values.

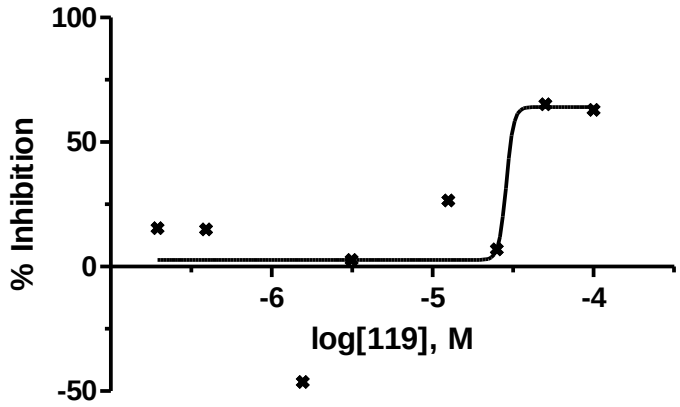


Figure 31 Percent inhibition of *L. major* amastigotes of compound **119** (67% at 50 μM)

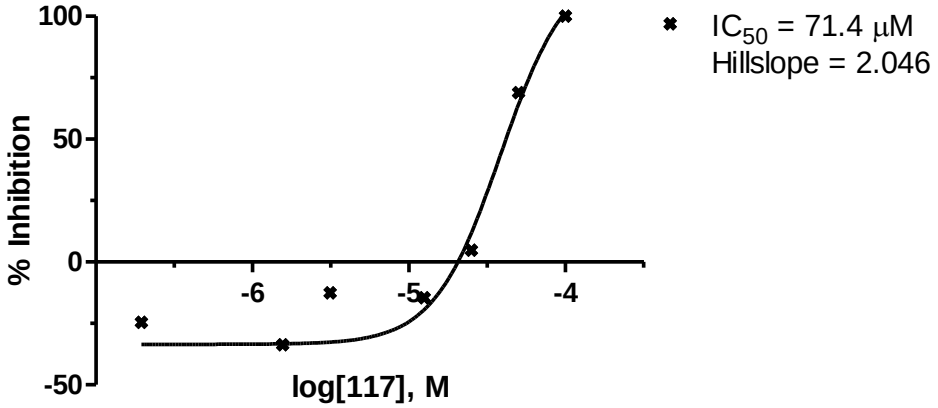


Figure 32 IC_{50} value of compound **117**

Table 14 Inhibition values of Compounds **93-94**, **98**, **105-106**, **116-119**, **125-127**, **135-136**, **147-149**, **151-152**, **160**, **162**, **165-166**, **168-170**, **172-182** against *L. major* amastigotes.

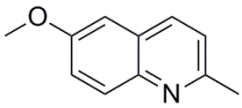
Compound	*Inhibition Level (μM)*	*MW*	*LogP*	*LogS*	*# ROTB*	*# ON*	*# OHNH*	*TPSA*	*# Lipinski Violations*
93	100[†]	704.41	3.41	-5.84	25	14	4	187.47	3
94	100[†]	394.47	2.73	-4.97	21	14	4	188.078	3
98	100	630.77	2.73	-4.97	21	14	4	187.47	3
105	60% at 100	461.25	2.25	-4.56	15	9	3	122.833	0
106	50% at 100	306.39	-4.16	-4.34	10	7	6	123.137	1
116	>200	143.19	2.66	2.30	0	1	0	12.892	0
117	IC_{50} = 71.4 ± 0.6	173.21	2.96	-2.32	0	1	0	22.126	0
118	65% at 125	205.25	4.25	-4.09	1	1	0	12.892	0
119	67% at 50[‡]	235.28	4.18	3.95	2	2	0	22.126	0
125	>200	222.09	3.39	-3.67	0	1	0	12.892	0
126	50% at 50	188.18	2.61	-2.63	1	4	0	58.716	0
127	80% at 12.5[‡]	157.21	2.96	-2.32	0	1	0	12.892	0
135	200[†]	235.28	4.12	3.95	2	2	0	12.892	0
136	200[†]	250.25	3.88	-4.67	2	4	0	58.716	0
147	>200	145.16	0.15	-2.37	0	2	0	25.459	0
148	NA[*]	326.39	0.15	-4.62	3	5	1	62.758	0
149	>200	221.26	1.64	-5.09	2	3	0	34.693	0

151	200[†]	251.28	1.65	-5.09	2	3	0	34.693	0
152	200[†]	250.28	3.79	-2.75	2	4	0	58.716	0
160	>200	256.30	4.31	-5.04	1	2	0	25.784	0
162	>200	223.27	2.874	-3.05	1	3	0	30.718	0
165	75% at 4	386.53	5.35	-5.71	6	3	0	25.364	1
166	>200	185.26	4.31	-5.04	1	2	0	12.892	0
168	>200	215.29	3.36	-3.76	4	2	0	22.126	0
169	IC_{50} = 7.36 ± 2.6[§]	442.64	6.92	-6.69	9	3	0	25.364	1
170	IC_{50} = 1.83 ± 1.7[§]	462.64	6.96	-6.75	7	3	0	25.364	1
172	10% at 24	296.19	3.26	-2.96	3	3	1	36.358	0
173	>200	478.67	7.43	-7.24	10	3	0	25.364	0
174	82% at 1.6[§]	472.66	6.97	-6.5	10	4	0	34.598	1
175	82% at 1.6[§]	492.65	6.97	-6.63	8	4	0	34.598	1
176	98% at 125[††]	326.43	3.36	-3.02	4	4	1	45.592	0
177	IC_{50} = 0.49 ± 1.0[§]	416.56	5.35	-5.75	7	4	0	34.598	1
178	>100	239.70	4.51	-4.80	1	1	0	12.892	0
179	>100	315.80	6.561	-6.99	2	1	0	12.892	1
180	>200	184.19	1.801	-2.87	1	3	0	45.918	0
181	>100	229.75	4.832	-4.73	3	3	0	31.36	0
182	>200	385.89	6.803	-6.88	2	2	2	32.255	1

[†]Cytotoxic; [‡]Hillslope >5.0 suggesting compound insolubility; [*]NA - Not Active; [§] Determined on 2 separate occasions and averaged; [††]Compound partly cytotoxic

7.6.7 Infectivity (picture)

Infectivity was determined from the number of parasites within macrophage cells. Each macrophage consists of a cytoplasm (light purple) and nucleus (dark blue). The parasites are seen as smaller spots (dark purple) within the cytoplasm as labelled in Figure 30. Each figure represents a small region of a well of a different concentration. Four pictures were taken per well to get an average count of parasite infectivity.



117

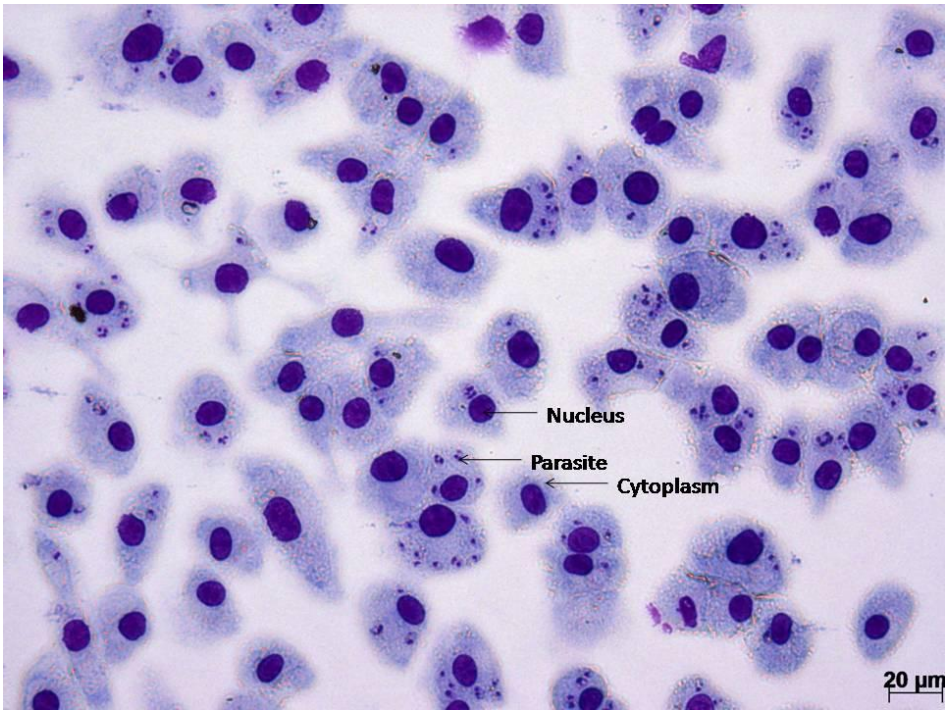


Figure 33 Infected macrophages of *L. major* inhibited by **117** at 12.5 µM.

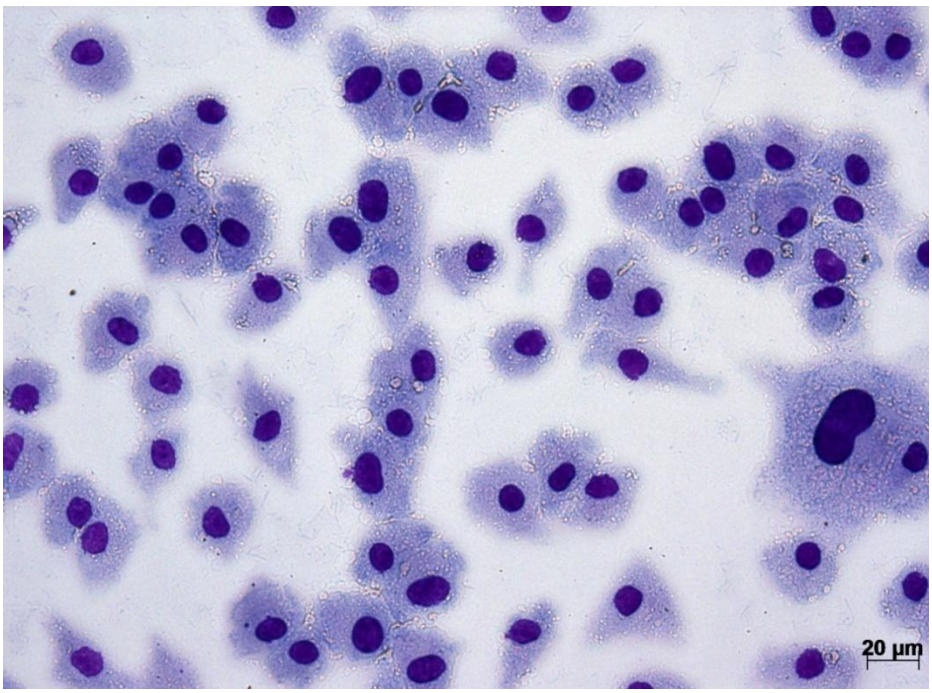
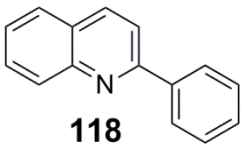


Figure 34 Infected macrophages of *L. major* inhibited by **117** at 100 µM.



118

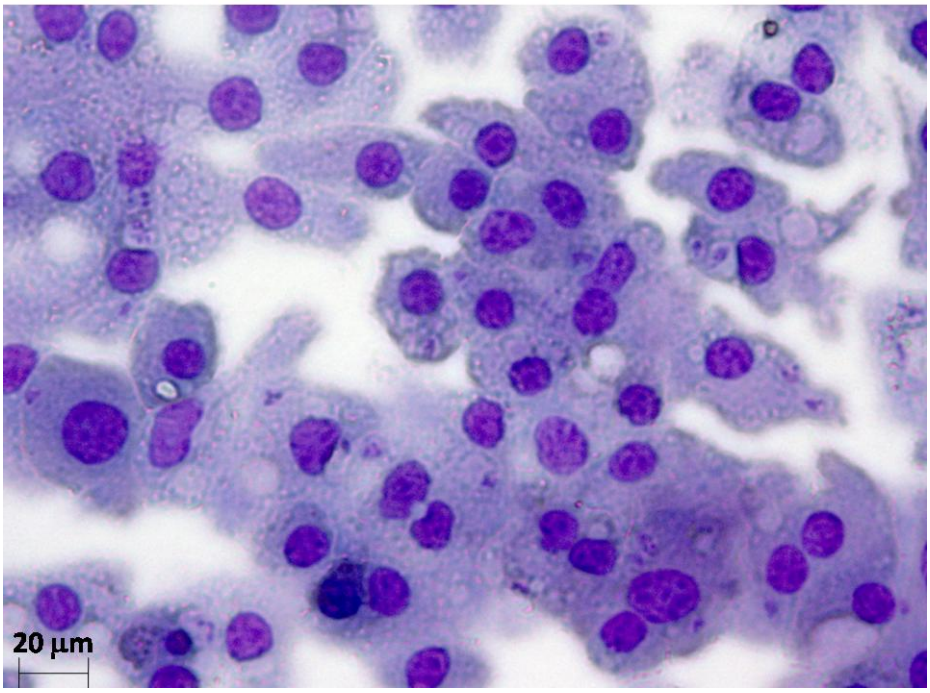


Figure 35 Infected macrophages of *L. major* inhibited by **118** at 0.195 µM.

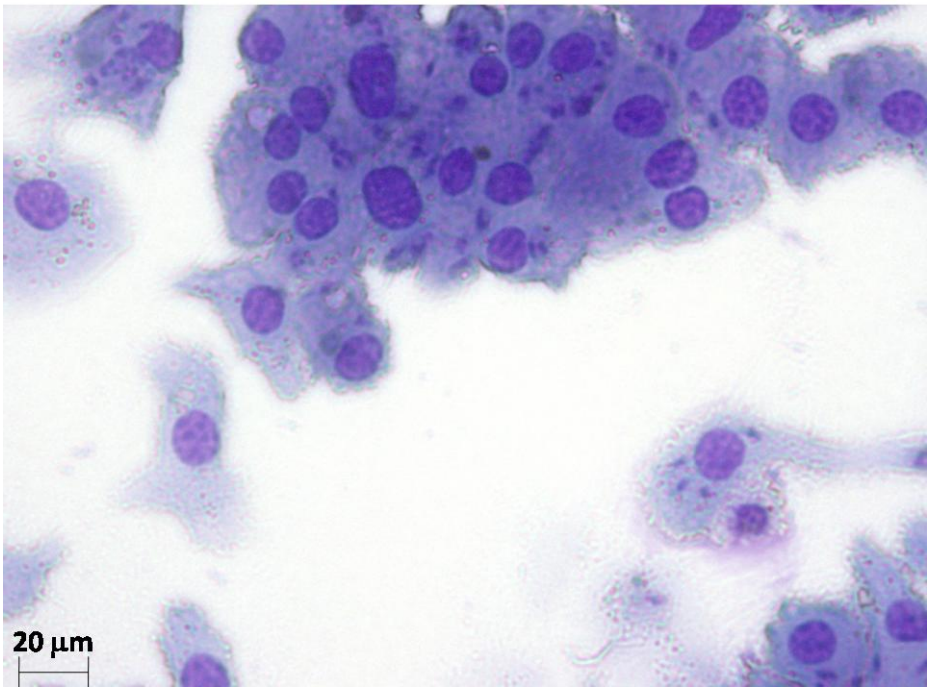
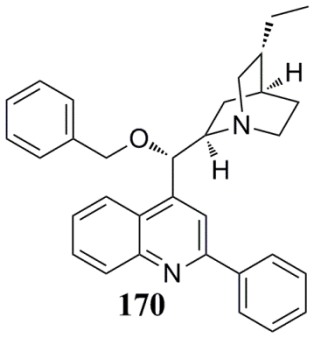


Figure 36 Infected macrophages of *L. major* inhibited by **118** at 50 µM.



170

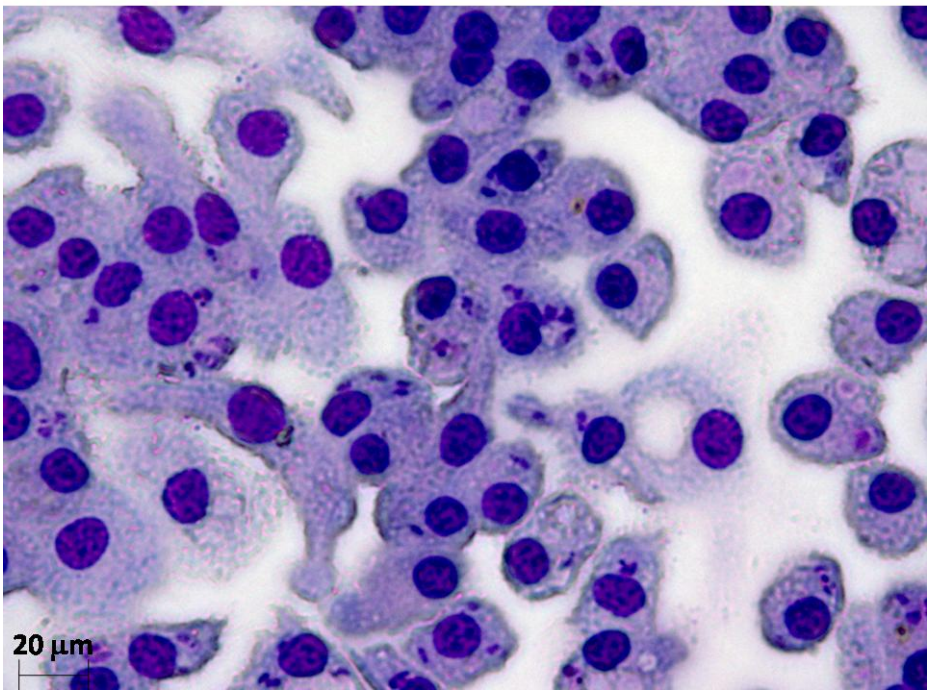


Figure 37 Infected macrophages of *L. major* inhibited by **170** at 0.102 µM.

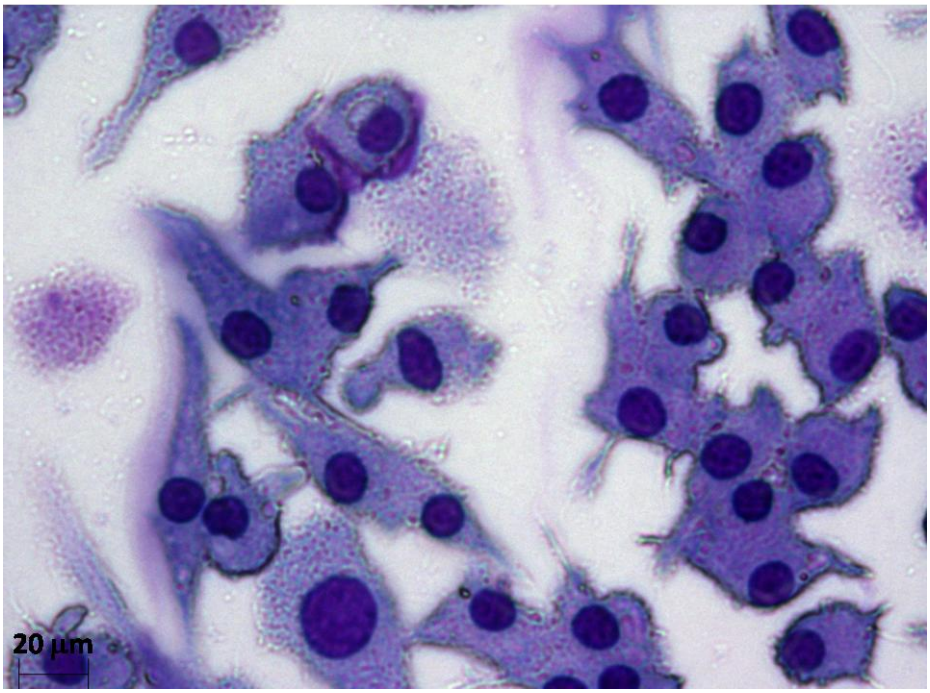
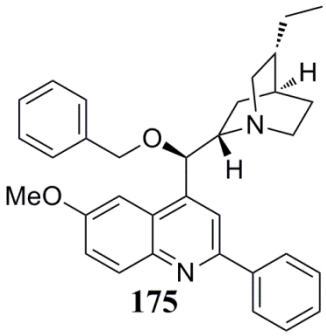


Figure 38 Infected macrophages of *L. major* inhibited by **170** at 10 µM.



175

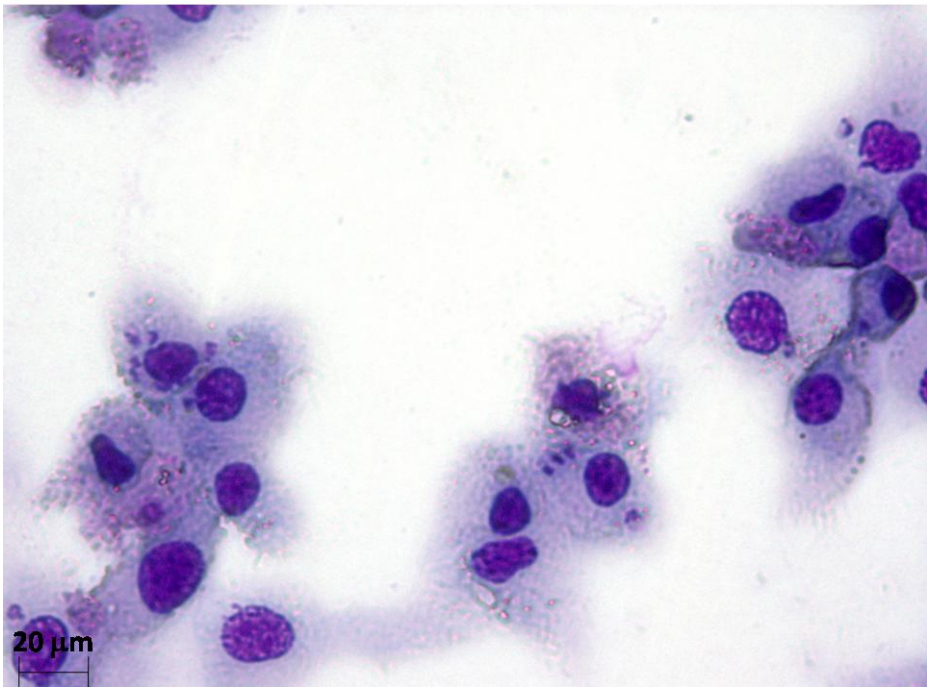


Figure 39 Infected macrophages of *L. major* inhibited by **175** at 0.0164 µM.

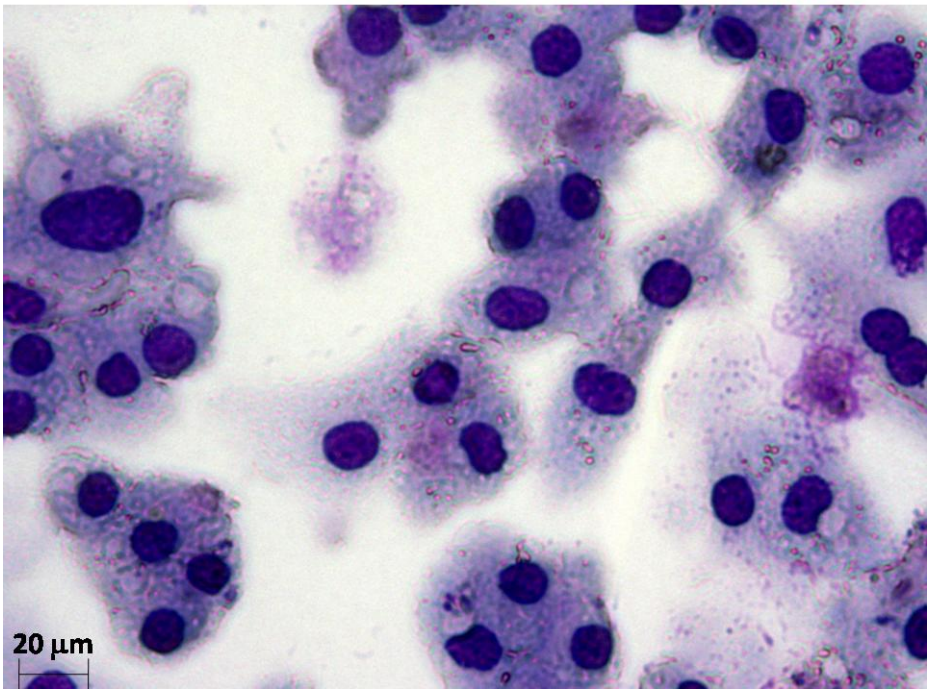


Figure 40 Infected macrophages of *L. major* inhibited by **175** at 0.64 µM.

7.7 Chapter Two Trypanothione Synthase Assays

Trypanothione synthase assays were carried out by Associate Professor David J. Young. The assay was performed as described in the literature[236] in the lab of Professor Maragaret Phillips, UT Southwestern Medical Center. Preliminary assays for compounds **94** and **106** were carried out to identify their inhibition against *T. brucei* TryS. The enzyme was isolated and purified as per Oza *et. al.*[236]

The Michaelis Menten plot (Figure 40) indicates that the enzyme was active, with a K_m (Michaelis constant) of 281µM and a K_{cat} (maximum number of substrate molecules that can be hydrolysed per active site per minute) of 0.24 s^{-1}.

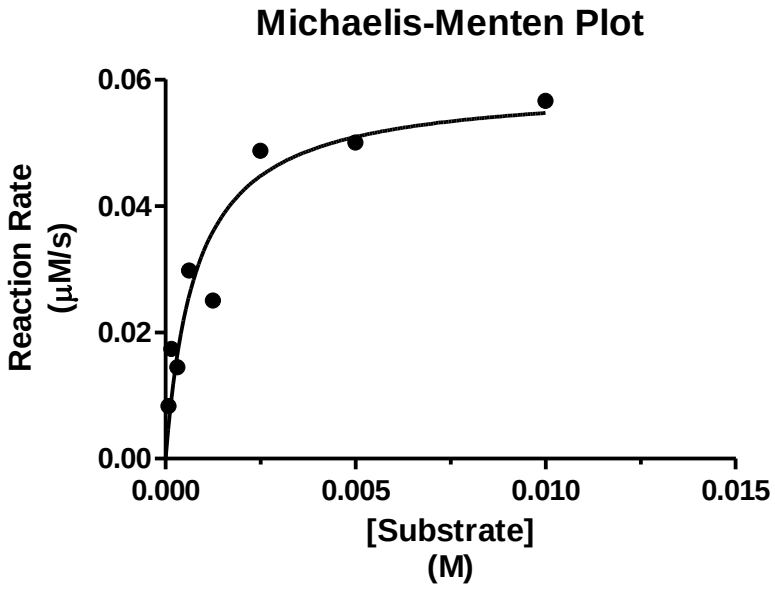


Figure 41 Michaelis-Menten Plot

Of the two compounds analysed only compound **94** inhibited TryS. Compound **94** was dissolved in pH 8 HEPPS (3-[4-(2-Hydroxyethyl)-1-piperazinyl]propanesulfonic acid) buffer (0.5 M) to a final concentration of 45 μM and 200 μM to determine if any inhibition could be identified at relatively high concentrations. The raw data is given in Figure 41, representing inhibition rates of the substrate and the enzyme. The decrease in absorbance coincides with substrate (NADH) depletion, which is linear with time until ~10% of NADH. On each plot, the top line (black squares) is a control containing no enzyme (TryS The slight decrease seen in the 200 μM concentration (Figure 41b) is likely due to a slight temperature change. The dark blue line contains no inhibitor.

The decrease in absorbance due to NADH is indicative of a reaction occurring at the active site. The four lines (blue, pink, red and green) are replicate runs containing inhibitor at either 45 μM (Figure 41a) or 200 μM (Figure 41b). Thus this reaction is slightly faster for the inhibitor than the two controls. At 200 μM the enzyme is being inhibited slightly more than at 45 μM. The low degree of inhibition did not merit an accurate measurement of a K_i value.

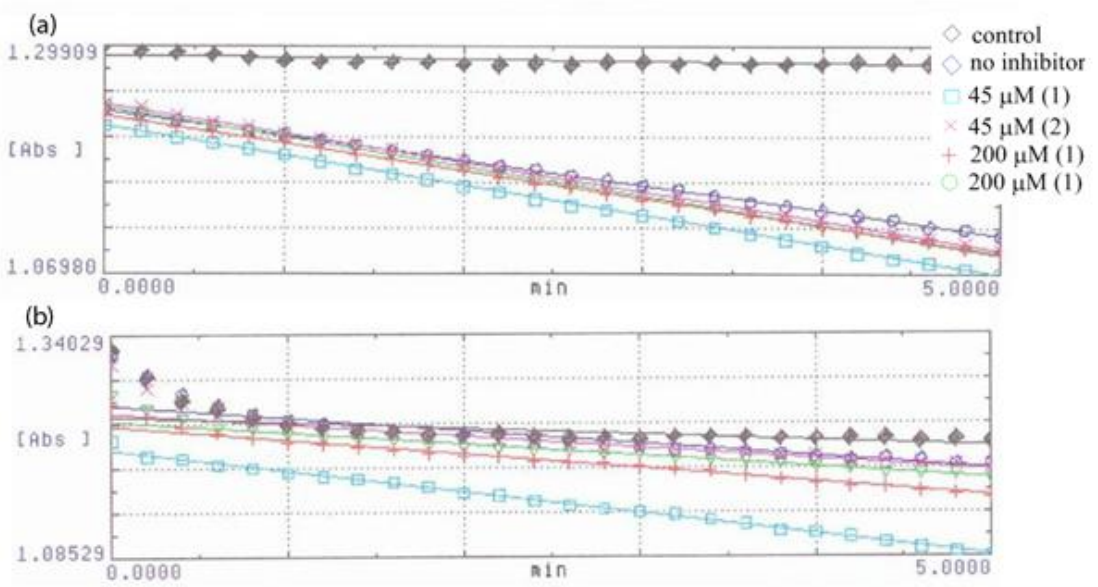


Figure 42 Inhibition of TryS at 45 µM (a) and 200 µM (b) by Compound **94**

Chapter Eight

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Chapter Nine

Appendix

Limitations of the 'Two-Phase' Doebner–Miller Reaction for the Synthesis of Quinolines

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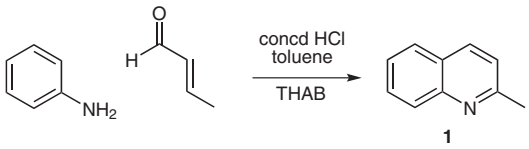
Abstract: The Doebner–Miller synthesis of quinolines under modified biphasic conditions was investigated. Crotonaldehyde, reacted readily with aniline to produce 2-methylquinoline. However, cinnamaldehyde and other γ-substituted α,β-unsaturated aldehydes yielded complex mixtures with substituted anilines to provide only trace quantities of quinolines. The Doebner–Miller reaction under these conditions is only suitable for sterically accessible α,β-unsaturated aldehydes.

Key words: α,β-unsaturated aldehyde, aniline, quinoline, Doebner–Miller reaction, heterocycle

Quinoline rings feature in a variety of naturally occurring and medicinally active compounds,[1,2] including metabolites derived from various flora and fauna.[3] These compounds exhibit a broad range of antimicrobial,[4] antitubercular,[5] antimalarial,[1,6] antiallergic,[7] and antiasthmatic activity.[8]

The Doebner–Miller reaction between aromatic amines and α,β-unsaturated carbonyl compounds is regarded as a relatively general method for synthesizing quinolines from readily available starting materials (Scheme 1).[8] The mechanism is complex[9] and dependent on reagents and reaction conditions, but generally appears to proceed via an initial conjugate attack by the aniline on the Michael acceptor when catalyzed by Brönsted acids under aqueous conditions. Synthetic[8] and mechanistic[9] studies of the Doebner–Miller reaction and the related Skraup reaction have been reviewed elsewhere.[8–10]



1

Scheme 1 Doebner–Miller reaction of aniline and crotonaldehyde (THAB = tetrahexylammonium bromide)

In recent years modification of the Doebner–Miller reaction conditions have included the use of two-phase systems with toluene,[11] other strong acids, such as trifluoroacetic acid,[8] and phase-transfer catalysts.[12] Although low yields are sometimes obtained, the easy formation of quinolines from inexpensive starting materials makes this reaction a potentially attractive route. The advantage of the two-phase solvent system is that it decreases polymerization of the aldehyde and makes products easier to isolate.[11] This is particularly true if the acid is a polyoxometallate[12] and the reaction is performed with microwave irradiation. However, the latter conditions are still not suitable for highly polymerizable substrates like acrolein and propenals and it is necessary to immobilize the aldehyde on silica. In a quest to develop more general, user-friendly conditions we have examined the two-phase Doebner–Miller reaction with aqueous acid and a range of aldehydes and anilines. This study revealed the narrow range of substrates suitable for this methodology.

The reaction between aniline and cinnamaldehyde (**2**) under modified Doebner–Miller conditions (toluene, 10 M HCl, THAB, 80–90 °C, 2 h) did not yield 2-phenylquinoline (**10**) (Figures 1 and 2).[13] The electron-rich 4-methoxyaniline provided some of quinoline **11** but in low conversion (1.6%). The more electrophilic crotonaldehyde did react with aniline in a moderate yield (40%) (Table 1, entry 1) and surprisingly, in a reduced yield with 4-methoxyaniline using this aldehyde (entry 2).

The effect of substitution at the γ-position was explored, with 4-methylpent-2-enal (**3**), cyclohex-1-ene-1-carbaldehyde (**4**), and 2-myrtenal (**5**) under the general conditions (Figure 1). These substrates gave complex mixtures with no identifiable products present; quinolines **12**,[14] **13**, and **14** were not isolated (Figure 2). These results are consistent with other examples reported in the literature, where only unsubstituted α,β-unsaturated aldehydes, such as **8** and **9**, gave quinolines with aniline[15] or substituted anilines[16] under similar conditions.

We attempted to obtain aldehyde **19** (Scheme 2), where a benzyloxy group would be introduced at the 8-position of the quinoline product. Hydrolysis of 1,1,3,3-tetraethoxypropane (**17**) followed by treatment with sodium hydroxide gave the sodium salt **18**. In an NMR-scale experiment, an equivalent each of **18** and benzyl bromide were shaken and left to react in DMSO-d_6. After 24 hours, conversion of **18** into aldehyde **19** was complete, as determined by ^{1}H NMR spectroscopic analysis. Attempts to carry out this on a preparative scale of > 1 gram were unsuccessful.

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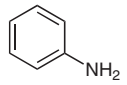
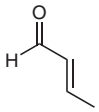
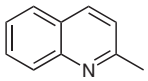
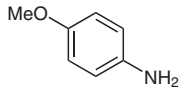
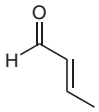
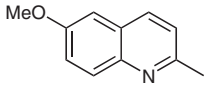
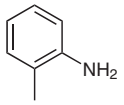
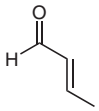
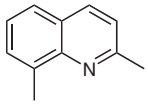
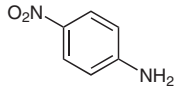
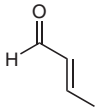
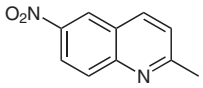
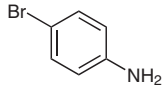
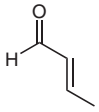
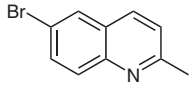
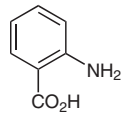
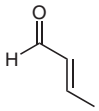
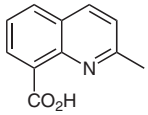
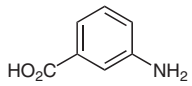
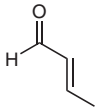
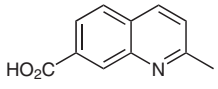
Table 1 Quinoline Derivatives **1**, **20**–**23** Produced Using Crotonaldehyde via Scheme 1

Entry	Aniline	Aldehyde	Conditions	Quinoline	Isolated yield (%)
1			10 M HCl, toluene, THAB,[a] 80–90°C, 90 min	 **1**	40
2			10 M HCl, toluene, THAB,[a] 80–90 °C, 90 min	 **20**	8[b]
3			10 M HCl, toluene, THAB,[a] 80–90 °C, 90 min	 **21**	7[b]
4			10 M HCl, toluene, THAB,[a] 80–90 °C, 90 min	 **22**	23
5			10 M HCl, toluene, THAB,[a] 80–90 °C, 90 min	 **23**	16
6			10 M HCl, toluene, THAB,[a] 80–90 °C, 90 min	 **24**	57[c]
7			10 M HCl, toluene, THAB,[a] 80–90 °C, 90 min	 **25**	–[d]

[a] THAB = tetrahexylammonium bromide.
[b] Yield after purification by chromatography.
[c] From ref. 13.
[d] Complex mixture.

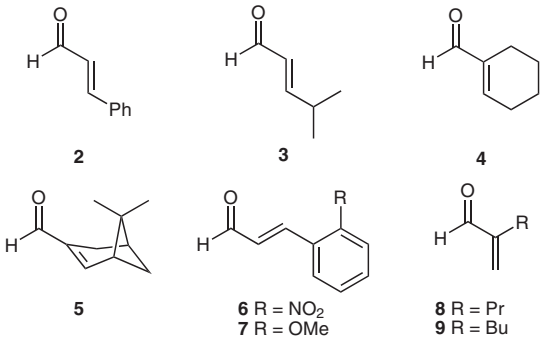


Figure 1

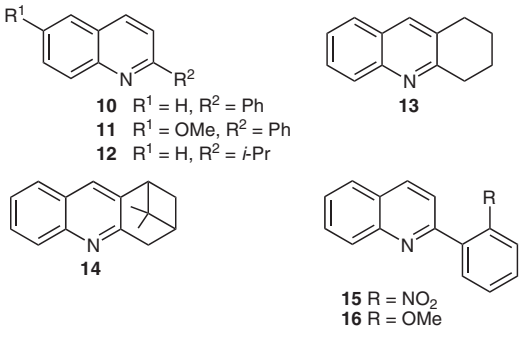
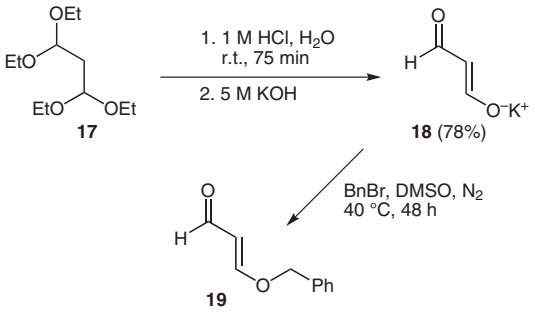


Figure 2

Instead, reaction of aniline with either 2-nitrocinnamaldehyde (**6**) or 2-methoxycinnamaldehyde (**7**) under the general conditions gave traces of 2-(2-nitrophenyl)quinoline (**15**) and a complex mixture not containing quinoline **16**, respectively.

The effect of substitution on the aromatic ring of aniline was examined (Table 1). Anilines with electron-donating (4-MeO, 2-Me), electron-withdrawing (4-NO_2, 2-CO_2H, or 4-CO_2H), or halogen (4-Br) groups were reacted with crotonaldehyde under the general conditions (entries 2–7). One literature example[13] (entry 6) is included in Table 1 for comparison. All anilines gave the corresponding substituted quinolines **20**–**24** in low to moderate yields; compound **25** was not obtained. The purification method provided variations in yield. Therefore, no corre-



Scheme 2 Formation of aldehyde **19**

lation between the potential influence of electron-donating or electron-withdrawing groups on the aniline and the isolated yield of the quinoline product was apparent.

In the crude ^{1}H NMR spectra and in the workups of reactions involving crotonaldehyde significant amounts of polymeric material were observed, and this both lowered product yields and hampered isolation. While some quinoline products could be readily purified by recrystallization (**22** and **23**), others required multiple chromatography (**20** and **21**) resulting in some decomposition and low yields.

Steric bulk at the γ-position of the α,β-unsaturated aldehyde prevented the formation of quinolines in the Doebner–Miller reaction. More than one mechanism for the Doebner–Miller reaction has been proposed.[8,9] Formation of products from a sterically accessible crotonaldehyde lend support to a mechanism invoking conjugate addition of the aniline on the α,β-unsaturated aldehyde Michael acceptor.[8,9]

In conclusion, the two-phase Doebner–Miller reaction catalyzed with aqueous hydrochloric acid is limited to sterically accessible aldehydes, such as crotonaldehyde. Other sterically hindered aldehyde examples studied resulted in predominant polymerization under these conditions.

All chemicals were commercially available and used without further purification. Substituted aldehyde and aniline derivatives were purchased from Sigma-Aldrich and used as received. The ^{1}H and ^{13}C NMR spectra were obtained using a 300 MHz (Varian Gemini 300) or a 400 MHz (Varian Unity 400) spectrometer in $CDCl_3$. ^{1}H and ^{13}C NMR are referenced to solvent, i.e. $CDCl_3$ (δ = 7.24 and δ = 77.0, respectively). Mass spectra were recorded on a Fisions VG-Platform II spectrometer, using electrospray as the ionization technique with HCO_2H–MeCN (4:6). Mass Lynx Version I (IBM) software was used to acquire and process the data. HRMS (ESI) were recorded on a Bruker Daltonix 4.7T FT ion cyclotron resonance mass spectrometer (FTICR MS) fitted with an Apollo ESI source in positive ion or negative ion as stated. All MS samples were prepared as solns in MeOH or MeCN. FTIR spectra were recorded in the range 4000–400cm^{-1} on a Perkin-Elmer FTIR 1725X spectrophotometer; spectra were recorded using a KBr disc. Analytical TLC was carried out on Merck precoated aluminum TLC plates coated with silica gel 60 F254 (0.2 mm).

Potassium 3-Oxoprop-1-en-1-olate (18)

1,1,3,3-Tetraethoxypropane (**17**, 5 g, 22.69 mmol), 1 M HCl (5 mL), and H_2O (5 mL) were stirred vigorously at r.t. for 75 min. The resulting homogenous soln was cooled to 0 °C and adjusted to pH 10 with 5 M KOH. Acetone (80 mL) was added slowly to the soln until crystals separated. The precipitate was filtered, washed with acetone, and dried at r.t. The salt was dissolved in boiling MeOH, treated with charcoal, and filtered. Solvent was removed in vacuo to give an orange gum; yield: 1.94 g (78%).[17]

^{1}H NMR (400 MHz, CD_3OD): δ = 5.23 (t, *J* = 10, 7.2 Hz, 1 H), 8.61 (d, *J* = 7.2 Hz, 2 H).

MS (ESI): *m*/*z* (%) = 110.50 (100) [M + 1].

3-(Benzyloxy)propenal (19)

Potassium 3-oxoprop-1-en-1-olate (**18**, 0.5 g, 7.04 mmol), BnBr (0.82 mL, 6.68 mmol), and anhyd DMSO (10 mL) were stirred and the mixture was heated to 40 °C for 48 h under an atmosphere of N_2. The mixture was quenched with H_2O (50 mL) and extracted with Et_2O (4 × 40 mL). The combined organic layers were washed with brine (40 mL), dried (anhyd $MgSO_4$), and concentrated in vacuo to give an orange oil as a complex mixture.

^{1}H NMR Spectroscopic Study of 3-(Benzyloxy)propenal (19)

Potassium 3-oxoprop-1-en-1-olate (50 mg, 0.704 mmol) and BnBr (83.6 μL, 0.704 mmol) were dissolved in DMSO-d_6 (1 mL) in a 5-mm NMR tube the reaction was mixed and left for 24 h. A ^{1}H NMR spectrum was obtained.

^{1}H NMR (400 MHz, DMSO-d_6): δ = 5.080 (s, 1 H, H1′), 5.633 (dd, *J* = 8.4, 4, 8.4 Hz, 1 H, H2′), 7.2–7.5 (m, 5 H, H3′, H7′), 7.932 (d, *J* = 12.4 Hz, 1 H, H3′), 9.340 [d, *J* = 8 Hz, 1 H, C(O)H].

MS (ESI): *m*/*z* (%) = 163.87 (10) [M + 1].

Quinolines 1, 10, 11, 15, 20–23; General Procedure

A soln of toluene (10 mL), substituted aniline, THAB (5%), and concd HCl (10 M, 40 mL) was heated to 80–90 °C. α,β-Unsaturated aldehyde was added slowly and the mixture stirred for 1.5 h. After cooling to r.t., the mixture was neutralized with 2 M NaOH, and the resulting mixture was extracted with $CHCl_3$. The organic layer was washed with brine and dried (anhyd $MgSO_4$), and the solvent was removed in vacuo to afford the crude product. The crude product was either recrystallized (EtOAc–hexane) (**22**) or purified by column chromatography (silica gel, hexane–EtOAc, 5:1) (**1**, **10**, **11**, **15**, **20**, **21**, and **23**), and in some cases recrystallized (EtOAc) again (**22**).

2-Methylquinoline (1)

Following the general procedure using aniline (0.98 mL, 10.74 mmol) and crotonaldehyde (1.78 mL, 21.48 mmol), with purification by chromatography gave a yellow oil; yield: 600 mg (40%).

The ^{1}H NMR spectrum was consistent with previously reported data.[12,18]

2-Phenylquinoline (10)

Following the general procedure using aniline (0.49 mL, 5.36 mmol) and cinnamaldehyde (1.35 mL, 10.73 mmol), with purification by chromatography and recrystallization gave an orange solid; yield: 2 mg (<1%); mp 77–80 °C (Lit.[16,19,20] 67–69 °C).

The ^{1}H NMR spectrum was consistent with previously reported data.[19,20]

6-Methoxy-2-phenylquinoline (11)

Following the general procedure using 4-methoxyaniline (0.5 g, 4.06 mmol) and cinnamaldehyde (1.03 mL, 8.119 mmol), with purification by chromatography and recrystallization gave an orange solid; yield: 8 mg (<1%); mp 125–126 °C (Lit.[20,21] 129–130 °C).

The ^{1}H NMR spectrum was consistent with previously reported data.[20,21]

2-(2-Nitrophenyl)quinoline (15)
Following the general procedure using aniline (0.98 mL, 10.74 mmol) and *trans*-2-nitrocinnamaldehyde (3.80 g, 21.47 mmol) gave a brown solid; yield: 3 mg (<1%); mp 116–117 °C (Lit.[22] 118–119 °C)

^{1}H NMR (400 MHz, $CDCl_3$): δ = 7.53 (d, *J* = 8.4 Hz, 1 H, H3), 7.55–7.61 (m, 2 H, H6, H5′), 7.70–7.76 (m, 3 H, H7, H4′, H6′), 7.86 (d, *J* = 6.8 Hz, 1 H, H8), 7.98 (d, *J* = 9.2 Hz, 1 H, H3′), 8.09 (d, *J* = 9.6 Hz, 1 H, H5), 8.23 (d, *J* = 8.4 Hz, 1 H, H4).

^{13}C NMR (125 MHz, $CDCl_3$): δ = 120.5, 124.5, 127.0, 127.2, 127.5, 129.3, 129.7, 130.0, 131.6, 132.6, 135.9, 136.8, 147.9, 155.6.

MS (ESI) *m*/*z* (%) = 205.68 (100); 250.73 (30) [M + 1].

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{15}H_{11}N_2O_2$: 251.0815; found: 251.0823.

6-Methoxy-2-methylquinoline (20)
Following the general procedure using 4-methoxyaniline (0.94 mL, 8.12 mmol) and crotonaldehyde (1.35 mL, 16.24 mmol) gave a brown solid; yield: 106 mg (8%); mp 60–62 °C (Lit.[23] 67–68 °C).

The ^{1}H NMR spectrum was consistent with previously reported data.[23,24]

2,8-Dimethylquinoline (21)
Following the general procedure using 2-methylaniline (0.49 mL, 4.66 mmol) and crotonaldehyde (0.77 mL, 9.33 mmol) gave a yellow oil; yield: 54 mg (7%).

The ^{1}H NMR spectrum was consistent with previously reported data.[23]

2-Methyl-6-nitroquinoline (22)
Following the general procedure using 4-nitroaniline (0.5 g, 3.62 mmol) and crotonaldehyde (0.60 mL, 7.24 mmol) gave dark-green crystals; yield: 154 mg (23%); mp 162–164 °C (Lit.[25] 165 °C).

The ^{1}H NMR spectrum was consistent with previously reported data.[25]

6-Bromo-2-methylquinoline (23)
Following the general procedure using 4-bromoaniline (0.5 g, 2.91 mmol) and crotonaldehyde (0.48 mL, 5.81 mmol) gave red-brown crystals; yield: 48 mg (16%); mp 97–99 °C (Lit.[26] 95–96 °C).

IR (KBr): 3048, 1488, 646 cm^{-1}

^{1}H NMR (400 MHz, $CDCl_3$): δ = 2.752 (s, 3 H, CH_3), 7.319 (d, *J* = 8.4 Hz, 1 H, H3), 7.754 (d, *J* = 2.0, 2.0 Hz, 1 H, H4), 7.917 (br s, w ~7.0 Hz, 1 H, H5), 7.942 (d, *J* = 2.2 Hz, 1 H, H7), 7.981 (d, *J* = 8.9 Hz, 1 H, H8)

^{13}C NMR (100 MHz, $CDCl_3$): δ = 25.2, 119.4, 122.8, 127.5, 129.4, 130.2, 132.8, 135.2, 146.2, 159.4.

MS (ESI) *m*/*z* (%) = 223.73 (100) [M + H]$^+$.

HRMS: *m*/*z* [M + H]$^+$ calcd for $C_{10}H_9Br_1N_1$: 221.9912; found: 221.9902.

Supporting Information for this article is available online at http://www.thieme-connect.com/ejournals/toc/synthesis.

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