2005

Development of new benzo[b]thiophene amide-based antimicrobial agents

Johana M. Mbere

University of Wollongong

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Recommended Citation

Development of New Benzo[b]thiophene Amide-Based Antimicrobial Agents

A thesis submitted in fulfilment of the requirements for the award of the degree of Doctor of Philosophy

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February 2005
Declaration

I, Johana M. Mbere, declare that the ideas and the materials presented in this thesis, submitted in fulfilment of the requirements for the award of Doctor of Philosophy, in the Department of Chemistry, University of Wollongong, are exclusively of my own work and to the best of my knowledge the material contained herein has not been submitted by other persons for qualifications at any other academic institution, unless otherwise referenced or acknowledged.

Johana Muchiri Mbere.

February, 2005
A sense of abundance arises with the phrase “I can afford to…..”

‘I can afford to dedicate this Thesis to the Muchiris’ family’.
The rules for kindness are simple, listen with your mind. Speak from your heart. Be open, direct, encouraging. Believe in your capacity to affect others positively - Stephanie Dowrick.

This thesis is a result of four years of work of which I have received tremendous support from many people. It is with great pleasure that I now have the opportunity to express my sincere gratitude to most of them.

Firstly, I would like to express my sincere gratitude to my direct supervisor Professor John B. Bremner for accepting me as his student and who made this project a success. It is with John’s enthusiasm and integral view on research combined with his willingness to provide quality chemistry and not less that kept me going and I wish to say thank you for showing me this way of research. Besides being a wonderful Supervisor, John is as close as family and a very good friend and I am deeply honoured to have known him in my life. I wish to say thank you so much again John for all the help you offered over the years both in and out of my academic life in Australia. It is with no doubt that without your help I would not be where I am now, God bless you John.

I am deeply indebted to the Head of the Chemistry Department Associate Professor Will Price and also Professor Steve Pyne for their suggestions, encouragement and kindness that made my stay in Australia possible.

Big thanks to the staff at the research office (UOW) and also to Professor Margaret Sheil for all their help that made my stay in Australia possible.

I wish to thank several people from the various research centres whose collaboration had an impact on this project, most notably Dr. Sumalee Kamchonwongpaisan at the National Centre for Genetic Engineering and Biotechnology (BIOTEC) and National Science and Technology Development
Agency (NSTDA) for undertaking in vitro antimalarial studies. Much thanks to Professor A.H. White of the University of Western Australia, for his assistance in providing X-ray crystallographic data and lastly thanks to Dr. J. Deadman and staff at Avexa Ltd, Melbourne (formally Amrad) for undertaking the in vitro antibacterial evaluations.

Self-reliance is admirable. Recognising what other people contribute to your happiness is priceless. -Stephanie Dowrick

This thesis is as a result of the work of many staff members at the IBS, who enriched me with their comments and encouragement, particular mention must be made of the cheerful collaboration provided by the following people: Dr. John Korth for his immense help and trust with the mass spectroscopy equipment, Dr. Wilford Lee and Sandra Chapman for their ever ready assistance with nuclear magnetic resonance spectroscopy, Roger Kanitz and Larry Hick for their assistance with high resolution mass spectroscopic analysis and their friendship which always made me feel homely each time they said “JAMBO” to me. Thanks for learning those few lines of ‘Swahili’ guys (you too Graham). Much appreciation is also directed to Simon whose calm hand on deck came in handy during sudden computer storms and crises that arose during the writing of this thesis ‘thanks heaps dude’ for taking your time to repair my laptop and recovering lost data. I am grateful to Chris Dixon and Karin Maxwell for their friendship and assistance while we worked together as laboratory technicians.

Hang around people who love life. Sniff their armpits. Repeat their jokes. Live in their skin for a while. Get it? -Stephanie Dowrick

I wish to render a heartfelt thank you to each and every member of the Bremner research group who created a conducive work environment during this project. I will always treasure the friendship and times that we had together. Times of many hours of discussion, laughter, cries, enthusiasm and positive attitude
towards science, and topics which strayed rather far from science but brought us together as a Bremner group family (we were simply the best). Waya and Sirirton, thanks for your friendship and immense help in and out of the laboratory, you are just great. Jane and Joey, you always made me happy and I will always treasure those moments. Thanks for everything you have done for me and it is too much to even mention but you know I mean it. Jane, I will miss the “trot” around the department, and Joey you are a true “brutha” keep it up. Much thanks to Neal, Tom, Patrick, Kara and Emilie for their friendship. For the people who graduated early: Hadi, Phurpa, Collette, Susan and Yasmine thanks too for your help and friendship during the start of the project.

*Never hold back a kind word* -**Stephanie Dowrick**

I am deeply indebted to Dr. David Perkins and his family for accepting me as their son. It is hard to say thank you enough to you David because you opened my third eye in many ways when it came to chemistry and general knowledge. Thanks for getting my thesis started and the constant monitoring and encouragement during writing up. Even when you were far, you were still near, and ready to give advice. I thank you for your fatherly care and if I never told you, the film you made and dedicated to dad really touched me and I do and always will take “view(s) from the hill”. Thanks also for letting me be your sons mentor and I will treasure all the moments we shared together -“Wushka”.

*Know how to close a door inside your mind when you go home from work. Visualise your work in a closed drawer where you can’t reach it until next day. Be present to the world that exists outside your work* -**Stephanie Dowrick**

Additional energy and vitality for this research was also provided externally through my involvement in social activities. Thanks to all my Taekwondo friends who played a substantial role in my life in Australia and provided me the opportunity to meet many good friends. Thanks a lot to master Sun yeoup Baek for
sharing his knowledge and skills on the sport, and his compassion, concerns and care during tough times. Much thanks to Grand master Hyung kyu Kang for his support and care. And much appreciation to the people who showed concern during the writing of this thesis- you know who you are.

*Living as a loving person does not mean loving everyone in the same way. It simply means being thoughtful and respectful whomever you are with. If you love someone, let love guide your behaviour* -Stephanie Dowrick

My deepest gratitude are reserved for the “apple of my eye”- Uta, whose patience and understanding I am very grateful for. I do not have strong enough words to say thanks to you for all the joys (not to mention blue times) that you have shown but have brought us much closer. Without your constant help, care, loving nature, support and nagging this thesis would not have been any easier. Thanks darling and much love for you -“Schmumpy”. I wish to express thanks to your family too, Binh (mum), Unity and also George for being there for me and supporting me in many ways and sharing mature views under vast topics, thanks family. Thank you again for allowing me to be a member of the family.

*See a bad day for what it is: a bad day. It is no more a predictor of the rest of your life than your last great day was. Good days. Bad days. They come. And they go* -Stephanie Dowrick

The chain of gratitude would definitely be incomplete if I would not express a deep sense of appreciation for my late father and to my ever loving mother who formed part of my dreams and taught me the good things that really matter in life. Dad, I am sure you still watch over me and happy memories of you provide me with the strength to carry on. Mum, you know you are the greatest mum ever and I love you so much. Thanks for your constant prayers and strength, even when we are miles apart. I am grateful to my sisters, Alice and Margaret- thanks heaps for being there when needed and for your care over the family and for your sisterly love. I love your kids”- Koi, Mwangi and Hans big time, thanks for letting me know I am
their best uncle. Credit to brother Edwin whom I know cares a lot too and for taking charge of family matters, I am proud of you small ‘bro’. For all the Muchiris’ and those who believed in me, I dedicate this thesis to you with much love.

*Listen to the teachings of your heart. At the end of each day find something to be thankful for. Give thanks. Sleep in peace* -Stephanie Dowrick. *Thank you God.*
### List of Abbreviations

<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Å</td>
<td>Angstrom</td>
</tr>
<tr>
<td>Abs</td>
<td>Absorbance (UV spectroscopy)</td>
</tr>
<tr>
<td>ACP</td>
<td>Acyl carrier protein</td>
</tr>
<tr>
<td>AD</td>
<td>Anno Domini</td>
</tr>
<tr>
<td>AIDS</td>
<td>Acquired Immune Deficiency Syndrome</td>
</tr>
<tr>
<td>AIBN</td>
<td>2,2′-azobis(isobutyronitrile)</td>
</tr>
<tr>
<td>AM1</td>
<td>Austin model 1 (molecular modelling)</td>
</tr>
<tr>
<td>AR</td>
<td>Analytical reagent</td>
</tr>
<tr>
<td>ArH</td>
<td>Aromatic protons</td>
</tr>
<tr>
<td>ATP</td>
<td>Adenosine triphosphate</td>
</tr>
<tr>
<td>ATPase</td>
<td>Adenosine triphosphatase</td>
</tr>
<tr>
<td>AMRAD</td>
<td>Australian Medical Research and Development</td>
</tr>
<tr>
<td>BC</td>
<td>Before Christ</td>
</tr>
<tr>
<td>BF₃·Et₂O</td>
<td>Boron trifluoride etherate</td>
</tr>
<tr>
<td>BIOTEC</td>
<td>National Centre for Genetic Engineering and Biotechnology</td>
</tr>
<tr>
<td>Boc</td>
<td>tert-butoxycarbonyl</td>
</tr>
<tr>
<td>bs</td>
<td>Broad singlet (spectroscopy)</td>
</tr>
<tr>
<td>BTP</td>
<td>Benzo[b]thiophene</td>
</tr>
<tr>
<td>Bu₃SnH</td>
<td>Tributyltin hydride</td>
</tr>
<tr>
<td>Bu₃SnBr</td>
<td>Tributyltin bromide</td>
</tr>
<tr>
<td>°C</td>
<td>Degrees centigrade</td>
</tr>
<tr>
<td>C-C</td>
<td>Carbon to carbon (bond)</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>CDCl₃</td>
<td>Deuterio chloroform</td>
</tr>
<tr>
<td>CHCl₃</td>
<td>Chloroform</td>
</tr>
<tr>
<td>(CH₃COO)₂O</td>
<td>Acetic anhydride</td>
</tr>
<tr>
<td>Cl⁺</td>
<td>Chemical ionisation (mass spectroscopy)</td>
</tr>
<tr>
<td>CM</td>
<td>Cross metathesis</td>
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<tr>
<td>C-N</td>
<td>Carbon-nitrogen bond</td>
</tr>
<tr>
<td>¹³C-NMR</td>
<td>Carbon 13 nuclear magnetic resonance (spectroscopy)</td>
</tr>
<tr>
<td>conc.</td>
<td>Concentration/ concentrated</td>
</tr>
<tr>
<td>Cu(II)</td>
<td>Copper(II) ions</td>
</tr>
<tr>
<td>Cu(OAc)₂</td>
<td>Copper acetate</td>
</tr>
<tr>
<td>δ</td>
<td>Delta-chemical shift downfield relative to TMS (spectroscopy)</td>
</tr>
<tr>
<td>d</td>
<td>Doublet (spectroscopy)</td>
</tr>
<tr>
<td>D1</td>
<td>Daughter plate</td>
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<tr>
<td>DCC</td>
<td>Dicyclohexylcarbodiimide</td>
</tr>
<tr>
<td>DCM</td>
<td>Dichloromethane</td>
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<tr>
<td>DDT</td>
<td>1,1-bis(p-chlorophenyl)-2,2,2-trichloroethane</td>
</tr>
<tr>
<td>DEPT</td>
<td>Distortionless Enhancement by Polarization Transfer</td>
</tr>
<tr>
<td>DHFR</td>
<td>Dihydrofolate reductase</td>
</tr>
<tr>
<td>DHPS</td>
<td>Dihydropteroate synthase</td>
</tr>
<tr>
<td>DMAP</td>
<td>4-Dimethylaminopyridine (base catalyst)</td>
</tr>
<tr>
<td>DMF</td>
<td>N,N-Dimethylformamide (solvent)</td>
</tr>
<tr>
<td>DMSO</td>
<td>Dimethyl sulfoxide (solvent)</td>
</tr>
<tr>
<td>DNA</td>
<td>Deoxyribonucleic acid</td>
</tr>
<tr>
<td>EDCI</td>
<td>1-(3-dimethylaminopropyl)-3-ethylcarbodiimide hydrochloride</td>
</tr>
</tbody>
</table>
\( E.\ coli \quad Escherichia\ coli \)
\( E.\ faecium \quad Enterococcus\ faecium \)
\( \text{EI}^1 \quad \text{Electron impact (mass spectroscopy)} \)
\( \text{Et}_3\text{N} \quad \text{Triethylamine} \)
\( \text{FAB} \quad \text{Fatty acid biosynthesis} \)
\( \text{FAS} \quad \text{Fatty acid synthesis} \)
\( \text{FMOC} \quad 9\text{-fluorenylmethoxycarbonyl} \)
\( g \quad \text{Gram(s)} \)
\( \text{gla.} \quad \text{Glacial (acetic acid)} \)
\( \text{GCMS} \quad \text{Gas Chromatography Mass Spectrometer} \)
\( h \quad \text{Hour} \)
\( \text{HIV} \quad \text{Human Immunodeficiency Virus} \)
\( ^1\text{H-NMR} \quad \text{Proton Nuclear Magnetic Resonance (spectroscopy)} \)
\( \text{HOAc} \quad \text{Acetic acid} \)
\( \text{HOBt} \quad 1\text{-Hydroxybenzotriazole} \)
\( \text{HRMS} \quad \text{High Resolution Mass Spectrometry} \)
\( \text{HRCIMS} \quad \text{High Resolution Chemical Ionisation Mass Spectrometry} \)
\( \text{HTS} \quad \text{High Throughput Screening} \)
\( \text{Hz} \quad \text{Hertz} \)
\( \text{IC}_{50} \quad \text{Inhibitory Concentration with 50} \% \text{ inhibition} \)
\( \text{IR} \quad \text{Infrared (spectroscopy)} \)
\( \text{ITBN} \quad \text{Insecticide treated bed nets} \)
\( J \quad \text{Coupling constant (spectroscopy)} \)
\( K \quad \text{Kelvin} \)
K$_2$OsO$_4$  Potassium osmate
K$_2$CO$_3$  Potassium carbonate
kcal  Kilocalorie
LiOH  Lithium hydroxide
Lit.  Literature
m  Multiplet (Spectroscopy)
M  Molar (moles per litre)
mbar  Millibar
MeOH  Methanol
MeSO$_2$NH$_2$  Methylsulfonamide
mg/ mL  Milligrams per millilitre
MIC  Minimum Inhibitory Concentration
mL  Millilitre
mmol  Millimole
Mo  Molybdenum
mol  Mole
Mp/ mp  Melting point
MRSA  Methicillin resistant staphylococcus aureus
mRNA  Messenger ribonucleic acid
MS  Mass Spectrometer
MHA  Mueller-Hinton Agar
MHC  Mueller-Hinton Broth
m/z  Mass to charge ratio (mass spectrometry)
NaH  Sodium hydride
<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Full Form</th>
</tr>
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<tr>
<td>NBS</td>
<td>N-Bromosuccinimide</td>
</tr>
<tr>
<td>nm</td>
<td>Nanometre</td>
</tr>
<tr>
<td>NMO</td>
<td>N-Methylmorpholine-N-oxide</td>
</tr>
<tr>
<td>NMR</td>
<td>Nuclear Magnetic Resonance</td>
</tr>
<tr>
<td>NSTDA</td>
<td>National Science and Technology Development Agency</td>
</tr>
<tr>
<td>-OH</td>
<td>Hydroxyl</td>
</tr>
<tr>
<td>-OMe</td>
<td>Methoxy</td>
</tr>
<tr>
<td>P1</td>
<td>Parent plate</td>
</tr>
<tr>
<td>PABA</td>
<td>Para amino benzoic acid</td>
</tr>
<tr>
<td>pet.</td>
<td>Petroleum</td>
</tr>
<tr>
<td>Pd(II)</td>
<td>Palladium(II)</td>
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<tr>
<td>Pd(OAc)2</td>
<td>Palladium acetate</td>
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<tr>
<td>Ph₃P</td>
<td>Triphenylphosphine</td>
</tr>
<tr>
<td>P. bras</td>
<td>Plasmodium brasilianum</td>
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<td>P. vivax</td>
<td>Plasmodium vivax</td>
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<tr>
<td>P. malariae</td>
<td>Plasmodium malariae</td>
</tr>
<tr>
<td>P. falc</td>
<td>Plasmodium falciparum</td>
</tr>
<tr>
<td>ppm</td>
<td>Parts per million</td>
</tr>
<tr>
<td>pyd</td>
<td>Pyridine</td>
</tr>
<tr>
<td>q</td>
<td>Quartet (spectroscopy)</td>
</tr>
<tr>
<td>Q</td>
<td>Quaternary</td>
</tr>
<tr>
<td>®</td>
<td>Registered trademark</td>
</tr>
<tr>
<td>RCM</td>
<td>Ring Closing Metathesis</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Definition</td>
</tr>
<tr>
<td>--------------</td>
<td>------------</td>
</tr>
<tr>
<td>ROMP</td>
<td>Ring Opening Metathesis Polymerization</td>
</tr>
<tr>
<td>R&lt;sub&gt;f&lt;/sub&gt;</td>
<td>Retention factor</td>
</tr>
<tr>
<td>r.t</td>
<td>Room temperature</td>
</tr>
<tr>
<td>rpm</td>
<td>Rotations per minute</td>
</tr>
<tr>
<td>s</td>
<td>Singlet (spectroscopy)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>Staphylococcus aureus</td>
</tr>
<tr>
<td>30S</td>
<td>Ribosomal unit</td>
</tr>
<tr>
<td>TFA</td>
<td>Trifluoroacetic acid</td>
</tr>
<tr>
<td>THF</td>
<td>Tetrahydrofuran</td>
</tr>
<tr>
<td>THIQ</td>
<td>Tetrahydroisoquinoline</td>
</tr>
<tr>
<td>TLC</td>
<td>Thin Layer Chromatography</td>
</tr>
<tr>
<td>TMS</td>
<td>Trimethylsilane</td>
</tr>
<tr>
<td>TOC</td>
<td>Table of contents</td>
</tr>
<tr>
<td>tRNA</td>
<td>Transfer ribonucleic acid</td>
</tr>
<tr>
<td>U.S.</td>
<td>United States</td>
</tr>
<tr>
<td>UV</td>
<td>Ultraviolet (spectroscopy)</td>
</tr>
<tr>
<td>VRE</td>
<td>Vancomycin resistant enterococci</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
</tr>
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</table>
Abstract

The overall aim of this project was to investigate the synthesis and activity of a range of compounds based on the benzo[b]thiophene-2-carboxamide structural motif as potential new antimalarial agents, and to a lesser extent as antibacterial agents.

In order to subsequently explore any structure-biological activity relationships, the first part of the project involved the systematic synthesis of some 39 non-fused substituted benzo[b]thiophene amide derivatives including tetrahydroisoquinolines, tetrahydro-β-carbolines, dihydropyrroles, piperazines, piperidines and other bridged ring systems as part of the amide-nitrogen component. Methods for the synthesis of the new amide derivatives were developed based on benzo[b]thiophene acid chloride and amine reactions, or on dicyclohexylcarbodiimide-mediated carboxylic acid amine coupling reactions.

Further substitution reactions were also undertaken on tetrahydro-β-carboline amides (55, 56, and 57) with the introduction of an N-benzyl group and an N-o-nitrobenzyl group in the case of 55. The N-boc protected piperazine amide 63 also served as a precursor for other N-substituted piperazine amide derivatives. A single crystal X-ray structure on amides 63 and 73 confirmed the amide rotamer geometry in the solid state with these compounds.
The second part of the project incorporated the synthesis of fused analogues which were more conformationally restricted while still retaining the benzo[\(b\)]thiophene amide structural motif. A new free radical cyclisation approach to the benzo[\(b\)]thieno[2,3-\(c\)]pyridin-1-one system in compound 92 was developed, together with the corresponding model isoquinolinone analogue 95. The reaction was based on the use of tributyltin hydride and AIBN to produce the required free radical intermediate from an arylbromide precursor.

The free radical cyclisation reaction was extended to synthesise \(N\)-benzyl and substituted \(N\)-benzyl analogues of 92. The dihydroxylation of the \(N\)-allyl substituent in 92 was achieved using potassium osmate and \(N\)-methylmorpholine-\(N\)-oxide (NMO). A further free radical cyclisation route to the \(N\)-benzo[\(b\)]thien-2-oyl derivatives 112 was also achieved.

The synthesis of the novel 9-membered ring containing fused derivatives 120 and 121 was also achieved. This synthesis involved ring closing metathesis methodology using the bis-allyl amides 118 and 119 and Grubbs’ I ruthenium catalyst. The polymer supported version of this catalyst gave better yields of the cyclisation products. A single crystal X-ray structure of 120 confirmed the \textit{cis} geometry of the double bond in the 9-membered ring. In the course of preparing the required precursor 119 for the 9-membered ring synthesis, a new imine allylation reagent combination was discovered involving zinc, allyltrIBUTYLtin and boron trifluoride etherate. This reaction is worthy of further investigation to determine its wider synthetic utility.
Ring closing metathesis reactions also afforded the dihydropyrrole amides 113 and 114 in good yields.

A palladium-mediated cyclisation approach to the new benzo[b]thieno[2,3-c]pyrrolo[2,3-a]indol-11-one system in 126 was also accomplished based on the N-acyl indoles 124 and 125.

A number of the compounds were tested for their possible in vitro antimalarial activity against two strains of Plasmodium falciparum (K1 CB1 and TM4/ 8.2) and two active leads, the benzo[b]thienoquinolinone derivatives 95 and 102, were discovered. Some potential structure-activity trends for the tested benzo[b]thiophene derivatives were observed (Chapter 4).

Antibacterial testing of a few benzo[b]thiophene compounds against Staphylococcus aureus and vancomycin-resistant Enterococcus faecium strains was also done, and the benzo[b]thienoquinolinone derivative 92 exhibited promising activity against Staphylococcus aureus.
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Chapter 1 Introduction

1.1. General

Malaria is the single most serious and widespread parasitic disease affecting humans. There are an estimated two billion people at risk from malaria in tropical and subtropical regions of the world. Around 300-500 million cases of malaria occur annually, causing the death of roughly 2.3 million people. Almost all of these deaths occur in Sub-Saharan Africa where a lack of development can be directly attributable to the prevalence of the disease.\textsuperscript{1,2} It drains limited health care infrastructures and depleted financial resources. The remainder of malaria cases is largely restricted geographically to South and Southeast Asia and some parts of South America.\textsuperscript{3} The distribution of the disease is depicted in Figure 1.1. The gravity of the malaria problem has been significantly aggravated in recent years due to several reasons. The disease has extended into new regions as a result of changing land usage. This has caused epidemics in areas previously known to be free from the disease.\textsuperscript{3} The failure of appropriate control measures in regions of conflict in Africa and Southeast Asia has also contributed to an increase of malaria cases in areas where it was previously considered to be under control.\textsuperscript{3}

1.2. Background

Hippocrates described the symptoms of malaria as early as the 5\textsuperscript{th} Century BC.\textsuperscript{4} Plato and Aristotle both identified outbreaks of a disease with the exact symptoms of malaria during their lifetimes (427 to 347 BC and 384 to 322 BC respectively).\textsuperscript{5} In the 2\textsuperscript{nd} Century AD, malaria was first associated with swamps and marshes, in fact a Spanish scholar of that era, Lucius Junius Moderatus alluded to insects being the disease transmitters e.g. malaria.
Figure 1.1 The Distribution of malaria worldwide indicating drug resistant areas.

Chloroquine resistant *P. falciparum*.

Chloroquine sensitive malaria.

Areas of chloroquine/mefloquine resistant *P. falc.

Malaria either not endemic or is eradicated.
“Buildings however, should not be built near a swamp because it exudes harmful poison and gives birth to creatures which are armed with menacing stings and fly in dense swarms against human beings … From their stings one often acquires occult diseases whose origin is not even known to doctors.”

For the next fifteen centuries no new advances in the knowledge of malaria occurred. In the middle ages, it was thought that malaria was spread by stale air; in fact the word ‘malaria’ is derived from the Italian *mala aria* meaning bad or evil air. As a means of combating the distribution of the disease, carers installed pots of sweet smelling flowers by the bedside of the patients, unaware that in doing so they were providing the very breeding site for the mosquitoes responsible for the propagation of the disease bringing together the vector and the host.

The discovery of cinchona bark (the bitter bark of *Cinchona ledgeriana*) as a useful therapeutic agent in the treatment of malaria in the 17th Century became the next key step forward in the study of malaria.

In the early 19th Century, post mortem examinations of the bodies of malaria victims revealed a characteristic brownish malarial colouration of some organs, which was referred to as melanemia.

It was not until 1880 that the French surgeon, Alphonse Laveran, working in Algeria, discovered malarial parasites in the blood of a malaria patient. This was a significant discovery in light of the fact that at the time the bacterium *Bacillus malariae* was considered to be the cause of malaria.

Six years later, the Roman physician Camillo Golgi described in detail two human malarial protozoal parasites, *Plasmodium vivax* and *Plasmodium malariae*. A further four years after this discovery, Marchiava and Celli described *Plasmodium*
Later a fourth malarial parasite, *Plasmodium ovale*, was discovered.\textsuperscript{11,12} A discussion of the mode and severity of infection of these species is deferred to later in the chapter.

Solving the mystery of *Plasmodium* transmission was the next key focus in malaria research. The popular long-held opinion towards the end of the 19th Century was that malaria was either airborne or waterborne. This belief was finally refuted with the discovery by Ronald Ross in 1897 of the role of the mosquito as a vector in the spread of malaria.\textsuperscript{13}

### 1.3. Treatment

#### 1.3.1. Traditional remedies

Throughout history, many societies have used traditional methods to treat and control the spread of malaria. One prominent example is the infusion of an herbal preparation,\textsuperscript{14} *qinghaosu* (*Artemesia annua*), used in China for at least 2000 years. As mentioned above, cinchona bark had been used in the treatment of malaria as early as the 17th Century. Furthermore, the antifebrile properties of cinchona bark were known in Peru at least before the 15th Century AD.\textsuperscript{8,9,15,16}

#### 1.3.2. Controlling the spread

It is believed by some researchers that many of the greatest advances in combating malaria have come through the development of methods of mosquito control, rather than in techniques which target the *Plasmodium* parasite itself.\textsuperscript{17} Although unaware of the origin of malaria and its mode of transmission, people have been using protective measures against the mosquito for many centuries. For example, Herodotus noted in 485 BC that inhabitants of swampy areas in Egypt slept in tall, tower-like structures beyond the reach of mosquitoes, while others slept in the same region under
This method of constructing houses on stilts has been independently adopted even in recent times to control malaria in countries such as North Vietnam, Sao Tome and some regions in Africa. More recently in other regions of the world, techniques for mosquito control have focused on eradicating the larvae by such means as minimizing breeding grounds, clearing of bush land, covering still water with oil or netting and the introduction of larvae eating fish such as *Gambusia affinis*.

Insecticides became prevalent during the 1940s when DDT was widely used in attempts to eliminate mosquitoes and their larvae from malaria infected regions. Repellents have also been employed to aid in preventing the carrying mosquito from coming into contact with humans.

Another recent advance is the development of insecticide-treated bed nets (ITBN) as a measure in controlling the spread of malaria. In a series of independent trials carried out in three countries with high infant mortality rates, the percentage reduction of total infant mortality with the usage of ITBN decreased significantly. The results of these studies are outlined in Table 1.1.

**Table 1.1 – Percentage reduction in total child mortality with the use of ITBNs**

<table>
<thead>
<tr>
<th>Country</th>
<th>% Reduction in Total Child Mortality</th>
<th>Project Organizer</th>
</tr>
</thead>
<tbody>
<tr>
<td>The Gambia</td>
<td>63</td>
<td>Medical Research Council Laboratories, The Gambia</td>
</tr>
<tr>
<td>The Gambia</td>
<td>25</td>
<td>Gambian National Bed net Impregnation Programme</td>
</tr>
<tr>
<td>Kenya</td>
<td>33</td>
<td>WHO organized trials</td>
</tr>
<tr>
<td>Ghana</td>
<td>17</td>
<td>WHO organized trials</td>
</tr>
</tbody>
</table>
Clearly, ITBNs are highly effective in bringing about a decline in the mortality of children in malaria stricken regions. They are, however, costly and uncomfortable in the hot and humid tropics so their practicality in warmer climates where malaria is prevalent and where poverty rates are high must also be evaluated. Alternatives such as insecticide-impregnated curtains have been considered. However while this proposal may alleviate the comfort issues it does little to reduce economic considerations so vital to any strategy affecting the spread of the disease in the developing world. There are however growing concerns that the mosquitoes may eventually develop resistance towards the insecticides used to impregnate the nets hence making the nets less effective in managing the disease.

1.3.3. The Plasmodium parasite

As mentioned previously, there are four known species of human malarial parasite of worldwide medical significance. However, modern molecular methods have recently aided the discovery of the existence of other potential species or morphological and geographical variants. Sequencing of the gene for the circumsporozoite surface protein, for example, showed that some individuals apparently infected with \( P. vivax \) were, in fact, infected with a distinct species more closely related to a malaria parasite known to infect apes. Also, molecular analysis indicate that some morphological variants are distinct parasites related to \( P. malariae \) and \( P. brasillianum \) the latter being another simian parasite.

Although each of the four major human \( Plasmodium \) parasites is known to cause malaria, they each cause symptoms of differing severity and duration. Table 1.2 summarizes the disease severity and duration for the four major human malarial parasites.
Table 1.2 - Disease severity and duration from each of the four major human malarial parasites\textsuperscript{25}

<table>
<thead>
<tr>
<th>Disease Severity and Duration</th>
<th>Vivax</th>
<th>Ovale</th>
<th>Malariae</th>
<th>Falciparum</th>
</tr>
</thead>
<tbody>
<tr>
<td>Initial Paraoxysm Severity</td>
<td>Moderate to severe</td>
<td>Mild</td>
<td>Moderate to severe</td>
<td>Severe</td>
</tr>
<tr>
<td>Average Parasitemia (mm\textsuperscript{3})</td>
<td>20,000</td>
<td>9,000</td>
<td>6,000</td>
<td>50,000-500,000</td>
</tr>
<tr>
<td>Maximum Parasitemia (mm\textsuperscript{3})</td>
<td>50,000</td>
<td>30,000</td>
<td>20,000</td>
<td>2,500,000</td>
</tr>
<tr>
<td>Symptom Duration (untreated)</td>
<td>3-8+ weeks</td>
<td>2-3 weeks</td>
<td>3-24 weeks</td>
<td>2-3 weeks</td>
</tr>
<tr>
<td>Maximum Infection Duration (untreated)</td>
<td>5-8 years</td>
<td>12-20 months</td>
<td>20-50+ years</td>
<td>6-17 months</td>
</tr>
<tr>
<td>Anaemia</td>
<td>++</td>
<td>+</td>
<td>++</td>
<td>++++</td>
</tr>
<tr>
<td>Complications</td>
<td></td>
<td>Renal</td>
<td></td>
<td>Cerebral</td>
</tr>
</tbody>
</table>

Modified from Markell and Voge's Medical Parasitology\textsuperscript{25}

Significantly, infections by \textit{P. falciparum} are known to be the most severe and consequently give rise to the highest mortality and morbidity rates. As a result of this observation, \textit{P. falciparum} is the focus of the majority of antimalarial research around the world and forms the basis for the current discussion.

\subsection*{1.3.4. Lifecycle}

In order to understand the mechanism by which mosquitoes spread \textit{Plasmodium} species, it is important to understand the lifecycle of this mosquito-borne parasite. In Figure 1.2 the complete lifecycle of \textit{Plasmodium falciparum} is represented, but other \textit{Plasmodium} species have the same lifecycle. The parasites lifecycle begins as male and female gametocytes in the female anopheles mosquito where they combine to develop into a zygote in the salivary gland.\textsuperscript{1} They then migrate into the gut where they form...
oocytes in which sporozoites develop. Once the sporozoites have matured the oocytes rupture releasing them into the system of the mosquito, which then transfers them into a human host by biting the victim during a blood meal (step 5).

Figure 1.2- The life-cycle of the *Plasmodium falciparum* parasite.\textsuperscript{26}
Once introduced into the blood stream by the *Anopheles* mosquito, the newly released sporozoites rapidly invade the liver where they adopt the form of liver schizonts (step 6). During the next two weeks, the intracellular parasite multiplies within a liver cell reaching numbers as high as 200,000. These schizonts invade red blood cells forming trophozoites on maturation. It is at this stage that the first clinical symptoms of malaria manifest themselves as chills, fever, prostration and anaemia. Severe disease can include delirium, metabolic acidosis, cerebral malaria and multi-organ system failure; coma and death may follow.\(^1,2,7\) On maturation, the trophozoites form schizonts (step 8), which rupture to release merozoites into the blood where they invade more human erythrocytes. In these red blood cells the parasite matures asexually to produce another cycle of merozoites which are in turn released into the blood to invade still more red cells (step 9a). The rupture of schizonts leads to the formation of male and female gametocytes, which are then taken up by another female anopheles mosquito during a blood meal to begin the cycle again\(^1,2,7\) (step 9b).

### 1.3.5. Modern approaches

Although traditional medicines often form the foundation for modern drugs, a sound knowledge of the lifecycle of the infecting organism allows the scientific community a greater scope for a suitable methodology. For example, cinchona bark had been used for the treatment of malaria for centuries in Peru and, until recently, its main active component quinine, and the related semi-synthetic derivatives chloroquine and mefloquine, held a large share of the worldwide antimalarial agent market.\(^8,9\) However, their mode of action has remained problematic. Quinine and the various semi synthetic quinoline-based antimalarial agents were once thought to achieve their effect by plasmodial DNA intercalation after being concentrated in parasite-affected erythrocytes. However, these effects could occur only at concentrations greater than that achieved *in*
vivo; moreover, a non-specific effect on plasmodial DNA could not explain the action of these compounds at such precise points in the lifecycle of the parasite. The knowledge and understanding of the biochemistry of malaria-causing parasites has led to identification of many potential targets for newer drugs and to the identification of possible mode(s) of action of the drugs already in use to manage malaria\textsuperscript{27,28} (Figure 1.3).

Clarification of the mode of action of these compounds, in the past, proved difficult, but current thinking suggests that chloroquine and related compounds act primarily by inhibiting haem polymerase, and hence prevent detoxification of ferriprotoporphyrin IX, which is produced by erythrocytic haemoglobin in the food vacuole of the parasite. Ferriprotoporphyrin IX, a metabolite toxic to the parasite, is normally rendered harmless by being polymerised. In fact, malarial pigmentation is caused by granules of this polymer.
Figure 1.3- Illustration of a red blood cell *P. falciparum* trophozoite denoting key parasite cell organelles and the sites of action of some of the major drugs for malaria.\(^{27}\)

Because they are weak bases, quinine, chloroquine and mefloquine are concentrated to very high levels in the food vacuole of the malarial parasite greatly enhancing their activity.\(^{29}\) However, quinine and mefloquine do not achieve the same levels of concentration as chloroquine, and consequently have significantly less effect on haem polymerisation, giving rise to the possibility that these compounds act on
It has been documented that although quinine itself acts as a blood schizonticide, it also has gametocidal activity against \textit{P. vivax} and \textit{P. malariae}.

Pyrimethamine and primaquine are tissue schizonticides. These drugs act on the primary tissue forms of the plasmodia which, after growth within the liver, initiate the erythrocytic stage. By blocking this stage, further development of the infection might be preventable. However given that it is impossible to predict infections before clinical symptoms begin, this mode of therapy is more exploratory. Pyrimethamine and primaquine can act however, in preventing relapse, as tissue schizonticides during the hypnozoite stage of \textit{P. vivax} and \textit{P. ovale} in the liver which can cause a recurrence of the disease.

Chloroquine, quinine, mefloquine, halofantrine, pyrimethamine, sulfadoxine, sulfones, and tetracyclines among others, also act as blood schizonticides. These drugs primarily focus on the blood forms of the parasite thereby terminating clinical attacks of malaria, and are useful in the treatment of malaria.

Chloroquine and quinine act also during the gametocyte stage of the lifecycles of \textit{P. vivax} and \textit{P. malariae}. These drugs destroy the sexual forms of the parasite in the blood thereby preventing transmission of the infection to the mosquito and helping to control its spread. It is perhaps interesting to note that these drugs do not have this mode of action against \textit{P. falciparum}. Primaquine, however, has gametocidal activity against all plasmodia, including \textit{P. falciparum}. Primaquine and chloroguanide also act as sporontocides. They prevent the development of oocysts in the mosquito and thus inhibit transmission.

Given each of the above antimalarial agents has one or more specific stages of the parasite’s life-cycle with a particular mode of action, and these stages of life-cycle
and modes of action are so varied, it is reasonable to assume that the drugs would represent a range of structures.

Quinine (1) and quinidine, both alkaloids extracted from cinchona bark, along with mefloquine (2) and halofantrine (3), are all aryl amino alcohols.
Chloroquine (4) and amodiaquine (5) are 4-aminoquinoline derivatives.

There are two types of folate synthesis inhibitors, those that competitively inhibit dihydropteroate synthase including the sulphones and sulphonamides, and those that inhibit DHFR including the biguanides like proguanil (6), chlorproguanil (7) and diaminopyrimidines like pyrimethamine. This whole class represents the antifolics and antifolinics.

Primaquine (8) and the potentially radical curative and causal prophylactic drug, WR238 605 (Tafenoquine) (9), belong to the class of 8-aminoquinolines.
Artemisinin (10) derived from the *Artemisia annua* and its derivatives and analogues: artemether, (11) arteether, (12) artesunate, and artelinic acid are all cyclic peroxides. Detailed studies\textsuperscript{33-35} on artemisinin and related peroxidic antimalarial agents have suggested that these compounds exert their parasiticidal activity subsequent to reductive activation by haem, which is released as a result of haemoglobin digestion by the *Plasmodium* parasite.

This irreversible redox reaction produces carbon-centred free radicals, that lead to alkylation of haem\textsuperscript{36} and proteins (enzymes)\textsuperscript{37}. One such enzyme is the sarcoplasmic-endoplasmic reticulum ATPase PfATP6\textsuperscript{38}, a cell organelle that is thought to be crucial for the survival of the *Plasmodium* parasite.\textsuperscript{39}
Atovaquone (13) is an example of a naphthoquinone, a highly lipophilic molecule that supposedly interferes with the mitochondrial electron transport and thereby ATP and pyrimidine biosynthesis and in \textit{Plasmodia}, it is found to target cytochrome bc\textsubscript{1} complex.

![Image of Atovaquone molecule](image13.png)

The compound, desferrioxamine (14) acts as an iron-chelating agent.

![Image of Desferrioxamine molecule](image14.png)

### 1.3.6. Antibiotics and malaria

Antibiotics also play a major role in the treatment of malaria. Tetracycline and chloramphenicol are well tolerated and effective as antimalarial drugs.\textsuperscript{40,41} Tetracycline (17) and its analogues doxycycline (18), azithromycin (15) and clindamycin (16) are known to block bacterial translation by binding reversibly to the 30S subunit and distorting it in such a way that the anticodons of the charged tRNAs cannot align properly with the codons of the mRNA.
At present, combination treatment of severe cases of malaria can be made with antibiotics such as clindamycin (16), lincomycin, minocycline, doxycycline (18) and azithromycin that are known to act as tissue schizonticides. Minocycline and 18 have been successfully used as potent antimalarials and are both used for management and
prophylaxis especially on the liver stages of the parasites life cycle, but parasitological response is slow to 16 and recovery rates are reported as higher. Cure rates have been reported to improve with the use of quinine (1) and 17 combinations in areas where response to 1 has declined.

The use of quinolone antibiotics in malaria chemotherapy, has also been reported

Combinations of 19 and 17 have been shown to have a modest additive effect in *P. falciparum* cultures. While clinical trials in India have been reported to be successful with the use of norfloxacin, it has been shown otherwise in Thailand where no benefits to patients were apparent with multidrug resistant malaria. This finding may be linked to the decreased sensitivity that is known to exist with chloroquine resistant strains.

1.4. Resistance

1.4.1. General resistance

The problem of drug resistance in the treatment of infectious diseases such as respiratory infections, HIV/AIDS, dysentery, tuberculosis and malaria, the leading killers among the infectious diseases, has proven to be a major dilemma in the community. In fact, resistance to front-line drugs has been observed in all of these infections, and in some cases the level of drug resistance has enforced a change to more expensive second or third-line chemotherapeutic agents. Resistance to drugs can be considered as a natural reaction to the selective pressure of the drug in use. Nevertheless, drug resistance has been worsened by several factors which include abuse of the antimicrobial drugs such as, their under use and misuse, poor patient compliance and the poor quality of readily available drugs.
In 1929, Sir Alexander Fleming observed the inhibition of *Staphylococcal* growth on an agar plate, which was contaminated by a *Penicillium* mould and this marked the discovery of the first antibiotic, penicillin. This discovery was particularly significant because of its application to wound infections resulting from conflict during World War II. Penicillin, at the time, had an unprecedented ability to kill these Gram-positive bacterial pathogens without causing any observable harm to their host. It was also widely available, and economically viable. Significantly, a considerable fraction of all human infections are caused by these two bacteria (e.g., strep throat, pneumonia, septicaemia, dermal related infections, wound infections, scarlet fever, toxic shock syndrome etc). Interestingly, penicillin has never been shown to be effective against most Gram-negative pathogens (e.g. *Salmonella*, *Shigella*, *Bordetella pertussis*, *Yersinia pestis*, *Pseudomonas*) with the notable exception of *Neisseria gonorrhoeae*. This resistance to penicillin by the Gram-negative bacteria can be attributed to the cell wall protection of the bacteria by an outer membrane that prevents permeation of the penicillin molecule.

The age of antibiotic chemotherapy came into dominance in the late 1940s and the early 1950s, marked by the discovery and introduction of streptomycin, chloramphenicol and tetracycline as new antibiotics. These new, broad-spectrum antibacterial agents were effective against both Gram-positive and Gram-negative bacteria, intracellular parasites, and the tuberculosis bacillus.

However, by 1953, during a *Shigella* outbreak in Japan, a strain of the dysentery *Bacillus* was isolated which was shown to exhibit multiple drug resistance, including resistance to chloramphenicol, tetracycline, streptomycin, and the sulfanilamides. There was also evidence suggesting that bacteria could pass genes for multiple drug resistance between strains and even between species. It was also apparent that *Mycobacterium*
*tuberculosis* was capable of rapidly developing resistance to streptomycin, a drug which had become a core in tuberculosis therapy.

Antibiotic resistance exists to the present day and even with the introduction of new and better antibacterial drugs there is constant apprehension that new strains will emerge, which exhibit even greater resistance to currently available antibiotics. New semi-synthetic penicillin derivatives, which act by inhibiting bacterial cell wall synthesis, and are also known to be unaffected by β-lactamase (penicillase), came into use: examples of such antibiotics included vancomycin, nafcillin and oxacillin.

In 1975, cases were reported describing methicillin resistant *Staphylococcus aureus* (MRSA). During this time, most of the strains of *Staphylococcus aureus* were now showing complete resistance to the drug methicillin especially to patients who were immuno-compromised.

In 1988, the first case of vancomycin resistant *enterococci* (VRE) was recorded. Vancomycin, a glycopeptide antibiotic acts by preventing transglycosylation and the cross linking of peptidoglycan layer in Gram-positive bacteria, is generally considered to be the last line of antibiotic defence.
1.4.2. Resistance and malaria

In the majority of the world where malaria is either emerging or is already endemic, resistance to the already available drugs that are recommended for the treatment of the disease is proving to be a concern. Since the 1960s the sensitivity of the *Plasmodium* parasites to chloroquine (16), which had been the most widely used drug of choice for the treatment of malaria, has been on the decline (Figure 1.4 shows the declining response to antimalarial drugs since 1976). To tackle this problem of antimalarial resistance, newer drugs have been developed and tested. However, difficulties such as unwanted side effects, prohibitive costs and the tendency for the
parasites, particularly, *P. falciparum*, to show resistance to these drugs as well, have diminished the efficacy of these new agents.

### 1.4.3. Definition of antimalarial drug resistance

Antimalarial drug resistance has been defined as “the ability of a parasite strain to survive and/or multiply even with the appropriate administration and absorption of a drug given in equal to or higher doses than that usually recommended but usually within the tolerance limit of the parasite.”\(^{42}\) The definition has been broadened to include the provision that the antimalarial drug in question must “gain access to the parasite or the erythrocytes infected by the *Plasmodium* parasites for the time that is necessary for its normal mode of action”\(^{49-51}\)

Several factors have been implicated with the emergence of resistance to antimalarial drugs. These factors include longer half-life, single mutation causing resistance, poor patient compliance, host immunity and the number of patients using these drugs. So far, resistance to antimalarial drugs has been more evident and pronounced in *P. falciparum* with sporadic cases of resistance also reported for the *P. vivax* strain. This resistance is more widely reported for the antimalarial drug chloroquine compared to other drugs in which resistance has also been reported.

### 1.4.4. Classification of the mechanism of drug resistance

The study of the mechanism of drug resistance can be approached from two main perspectives: where the drug target is unchanged or where there is an alteration in drug targets. From the former perspective, two drug resistance mechanisms are possible, firstly resistance by exclusion, where there is loss of the drug accumulation (decreased import) or, on the other hand, an increased elimination of a drug such that it has little or no effect. Secondly, resistance can result from the metabolism of an active drug into an
inactive compound, for example when penicillin is converted by penicillases to an inactive metabolite. The metabolic inactivation of prodrugs is also classified under this resistance mechanism. This is where, under ideal conditions, the drug is administered in the form of an inactive prodrug which is then converted by the pathogen into an active metabolite; an example of this form of resistance is the resistance to purine analogues in cancer cells.

In the second major approach to the study of the mechanism of drug resistance where there is considered to be an alteration of the drug targets, several changes may occur in a particular drug target which offer an explanation for drug resistance; these include cases where there is complete elimination of a drug target which the organism makes redundant by developing an alternative pathway. There can also be an alteration of the target in question such that the drug in use has a reduced affinity.

In other reported cases, overproduction of a drug target, for example by gene amplification, may render drugs being useless during chemotherapy and finally an accumulation of metabolite antagonistic to the drug being used may occur with para-aminobenzoic acid (PABA) overproduction by *pneumococci* bacteria.

The development of antimalarial drug resistance can thus be perceived to take place gradually or in a number of separate steps. One or more of the mechanisms mentioned above can be used to explain the origin of drug resistance which results in an adaptation of organisms or cells when continually stressed by exposure to sub-optimal and thus sub-lethal levels of drugs.

The exposure to sub-optimal drug levels through self-prescription of medication, in an effort to manage the early symptoms of malaria in developing countries where malaria is a problem is seen to be one of the most important reasons for the presence of (multiple) drug resistant malaria.
Single malaria isolates have been found to be made up of heterogeneous populations of parasites that can have widely varying antimalarial drug response characteristics, which vary from highly resistant to completely sensitive.\textsuperscript{52} Similarly, within a geographical area, malaria infections show a range of antimalarial drug susceptibility and over time, drug resistance becomes well established in the population and can be very stable, persisting long after specific drug pressure has been removed.

The biochemical mechanism of resistance has been well detailed for the antimalarial drugs chloroquine (4), and with antifolate combination drugs and atovaquone.

\textbf{1.4.5. Resistance to Chloroquine}

The treatment of malaria was revolutionized with the discovery of the drug chloroquine (4), but it was not until the early 1960s that resistance emerged from two epicenters, Columbia and Thailand, and since then the resistance to 4 has spread worldwide. Resistance to 4 has since been found to be conferred by a stable mutation which is carried forward to the progeny and involves multiple genetic transformations suggesting that this form of resistance need not be complete but can also be a partial resistance.

The resistance of \textit{P. falciparum} to 4 has been associated with an increased capacity of the \textit{Plasmodium} parasite to expel the drug at an unusual rate preventing 4 being concentrated at levels required to inhibit haem polymerization.\textsuperscript{53} The process is characterized by the digestion of haemoglobin by the malarial parasite, which ends with the formation of toxic by-products that are then polymerized in the \textit{Plasmodium} food vacuole to produce non-toxic haemozoin. The drug efflux from the \textit{Plasmodium} is seen
to take place at a rate of 40 to 50 times faster among the chloroquine resistant *P. falciparum* than the sensitive parasites.\textsuperscript{54}

### 1.4.6. Anti-folate resistance

Antifolate drug combinations such as sulfadoxine plus pyrimethamine are known to act *via* sequential and synergistic blockade of the enzymes involved with the folate synthesis within the parasite. Pyrimethamine and its analogues inhibit the step that is mediated by dihydrofolate reductase (DHFR) while sulfones and the sulfonamides inhibit the step mediated by the enzyme dihydropteroate synthase (DHPS). It is with these two key enzymes that specific gene mutations have been identified which can explain the resistance to this class of antimalarial drugs. The varying degrees of drug resistance known to occur among the antifolates can further be attributed to specific combinations of the above-mentioned types of mutations.\textsuperscript{42,55}

### 1.4.7. Atovaquone

The antimalarial drug, atovaquone (13), acts through the inhibition of electron transport at the cytochrome *bc1* complex.\textsuperscript{42,56} It has been observed that when used alone during the treatment of malaria, resistance develops more rapidly, but when it is used in combination with a second drug, such as proguanil or a tetracycline antibiotic, resistance to it develops gradually. Resistance to 13 is conferred by a single point transformation at the cytochrome-*b* gene.\textsuperscript{42}

### 1.4.8. Preventing drug resistance

Contributions which aim at preventing resistance to drugs generally, are more concerned with reducing the drug pressure through more selective drug use for the treatment and management of a disease. Measures aimed at stopping or reducing drug resistance and its spread can be controlled by observing sound medical practices such
as; improving the manner in which the drug(s) in question is used, by monitoring and improving the prescription of the drug; follow up practices and patient compliance; the use of drug combination therapy which could then reduce the rate or eliminate the conditions in which resistance of the drug could develop or could facilitate the spread of resistant parasites.

Concerning the reduction in the overall drug pressure, the treatment and management of a disease follows a more limited drug use and prescribing practice. This routine between patient and physician limits the introduction, spread, and intensification of resistance to the drug being used.

Direct observation therapy has gained recognition as a means of ensuring a high degree of compliance in patients. Even though this practice has not received much attention in relation to malaria treatment and management, the use of drugs with single-dose therapies such as sulfadoxine/pyrimethamine and mefloquine could potentially make direct observation therapy feasible.42 Another approach is to closely follow up and re-treat patients. The success of this method would rely on the availability of diagnostic tools to initially identify illnesses and confirm the failure of the treatment in question. Even though this method is not widely adopted, it could help minimize the development and spread of drug resistance by simply identifying the patients who fail to comply with treatment for any given reason. The identified patients can then be re-treated until they are free from the parasite.

A strategy that has recently received much attention is the multi-drug combination therapy in the treatment and management of malaria. Specific antimalarial drugs have been used in combination with an artemisinin57 derivative to offer a considerable degree of success in malaria chemotherapy.27,42,58 (for example, amodiaquine/artesunate59, mefloquine/artesunate60, chloroproguanil/dapsone/artesunate61
or lumefantrine/artemether. The success of this drug combination therapy emanates from the artemisinin drugs, which have been proven to be highly effective, and rapidly acting, their modes of action also operate against a wider range of the Plasmodium parasites at particular developmental stages in their life cycle. Some good results have been observed with the multi-drug therapy involving the use of artemisinin compounds. When used in combination with longer acting antimalarial drugs, there is a reported rapid reduction of the Plasmodium parasite density to levels low enough to allow the concentration of the longer acting antimalarial drug to be at its maximum efficacy. This in turn greatly reduces both the possibility of the malarial parasite surviving the initial treatment on exposure to the drug and the chances that the parasite will be exposed to sub-optimal levels of the longer acting drug.

The use of artemisinins has been shown to reduce gametocytogenesis by 8 to 18 fold suggesting that there are fewer chances of the plasmodial gametocytes which carry the gene responsible for drug resistance being passed onwards, and this may potentially reduce the rate at which malaria is transmitted. In countries where malaria is endemic, the use of combination therapy to treat malaria has been associated with the slowing down of the development of resistance towards the drug mefloquine. In malaria endemic areas, especially in Africa where the need for antimalarial drugs is greatest but resources are limited, other courses of therapy, offering combinations of inexpensive and available drugs (for example, chloroproguanil/ dapsone or amodiaquine plus sulfadoxine/ pyrimethamine), might be appropriate therapy approaches in managing malaria.

Future antimalarial therapies may be expanded greatly by combining drug treatment with vaccines (or other drugs) designed to specifically inhibit the transmission of malaria.
These “transmission-blocking” vaccines or combinations of drugs could significantly diminish the onward transmission of malaria parasites in particular gametocytes that have been identified as carriers for the genes encoding for antimalarial drug resistance, even if a large number of these parasites survive the early treatment. This form of chemotherapy would be successful if the drug(s) or vaccine(s) employed for the management of malaria have a high degree of specificity as anti-gametocides (such as primaquine and its analogues), or drugs that non-specifically reduce the possibility of gametocytes developing (as seen with artemisinin and its analogues). The use of antimalarial drugs showing similar modes of action to the combination of atovaquone and proguanil, which is known to interfere with the sexual reproduction and infection of the parasite within the mosquito when taken up during a blood meal, can be used for the treatment and management of malaria, hence minimizing the occurrence of drug resistance.

1.4.9. Target selection and malaria

Antimalarial drugs that are currently in use are known not to have been initially discovered based on any rationally identified targets. Ideally an appropriate target is one that is essential for the survival of the pathogen and is located at a critical step in a particular metabolic pathway making the pathogen vulnerable. Moreover it should be exclusively expressed by the pathogen to minimise the health risk to the host. Researchers and health planners expect their best chance lies in a multifaceted attack, drawing upon a variety of weapons suited to the local environment to then combine several approaches, both long-standing and the most up-to-date, which would give the hope of possibly outmanoeuvring the persistent and deadly malarial parasite.
Biological drug targets that have been identified to be common to both the parasite and the human host offer a good approach for drug therapy for malaria, especially if structural variations involving the two can be exploited. A good approach to this sort of target validation would be the proper identification of targets within enzymes or pathways present within the parasite but not known to exist within the human host. In some cases, parasite drug targets might be mutual with other microbial organisms for which classes of drug inhibitors have been generated and can hence be screened for possible similarity in their mode of action. A classic example is the use of the antibiotics 17 and 16, which are well known to inhibit prokaryotic protein synthesis and can also be used in the management and treatment of malaria. These compounds, in all probability, selectively act against the Plasmodium malarial parasite because of their action against remnants of plant and bacterial organisms known as the apicoplasts. (Figure 1.3). This essential organelle is thought to have arisen through the endosymbiosis of an algal cell which had previously incorporated a cyanobacterium. It is due to its origin that the apicoplast is important to researchers given that it contains numerous metabolic pathways that differ radically from those found in human hosts hence offering a wide range of possible targets for drug therapy using antimicrobial drugs.

Recently identified possible selective targets for antimalarial drugs include the components of type II fatty acid biosynthesis and mevalonate-independent isoprenoid synthesis. The two pathways are well known targets for the existing antibacterial drugs hence could be useful leads to novel antimalarial drugs for a similar target.

The discovery that the apicoplast was the site of fatty acid biosynthesis completely altered the initial view that the Plasmodium parasites were incapable of synthesizing their own fatty acids. The first indication of fatty acid synthesis (FAS) in
the apicoplast was the finding of nucleus-encoded FAS genes whose products were then targeted to the apicoplast. These included acyl carrier proteins (ACP), β-ketoacyl-ACP synthases III (FabH) and I/II (FabB/F), and enoyl ACP reductase (FabI). In 1996 a global programme was launched aimed at sequencing the *P. falciparum* genome with the hope that it would open new channels for malaria research.

The genome sequence is now complete for the *P. falciparum* parasite and a number of other plasmodial species, and this should assist significantly the drug discovery process by allowing rapid recognition of crucial plasmodial targets which are related to already known target proteins from other research systems.

Other recent technologies that have contributed to validating drug targets include genomics and proteomics. Genomic technology offers the opportunity to assess transcription processes in the plasmodial life cycle, and could shed light onto the transcriptional impact of target inhibition and if functionally related genes have a common transcription profile other pathways of interest may be identified. Proteomic knowledge on the other hand requires the separation of thousands of proteins and permits direct study of the biochemical impact of known drugs as potential antimalarials. It can also help researchers to discover the mode of action of older drugs, confirm mechanisms that were not known for new drugs and finally, suggest new drug use approaches.

1.4.10. Malaria vaccine development

The development of a malaria vaccine has so far proven to be difficult and challenging due to several factors. Firstly, the size and complexity of the malaria parasite means that each infection introduces millions of antigens into the human immune system and identifying which type of antigen for drug targeting has been
difficult.65 Secondly, in infected humans, the *Plasmodium* parasite undergoes maturation at different stages which introduces a different subset of antigen molecules for the immune system to attack. Thirdly, the parasite has developed strategies that mislead the host immune system and finally, it is possible to have multiple malarial infections from different species and strains of mosquito at the same time.

Much of the current malaria vaccine research is focused on methods of reproducing immunity in a safe and successful way. An effective malaria vaccine must offer protective immune responses equal to, or better than, those provided by natural immunity or immunization. Due to the severity of the malaria caused by *P. falciparum*, particular attention has been turned to this species with an aim of producing a single vaccine against it. The vaccine would have to be tailored for different risk groups living in *P. falciparum* endemic areas. The vaccine requirement has generated at least four approaches for the species: An anti-infection vaccine aimed at giving protection to travellers or residents from low endemic areas from acquiring malaria; an anti-disease/anti-mortality vaccine targeting children, pregnant women and migrants occupying endemic areas and finally an anti-mosquito stage vaccine which will aim at preventing malaria transmission amongst humans65,78 or a toxin neutralizing vaccine designed to counteract toxins produced by the parasite and hence masking the malaria symptoms. However, a vaccine such as RTS S/AS02A, which is currently under phase IIb *in vivo* clinical, trials provides the first real hope for protection against malaria.79,80

To date, about thirty *P. falciparum* antigens are being evaluated for use in vaccines, and several have been tested in clinical trials. Partial protection with one vaccine has been reported in a field setting.81 The successful completion of genome sequencing for *P. falciparum*70,72 should also stimulate vaccine progress by the identification of potential antigens that could be scanned for desired properties such as
surface expression or limited antigenic diversity. This could be combined with data on stage-specific expression obtained by micro-array and proteomics\textsuperscript{75,76} analyses to identify potential antigens that are expressed in one or more stages of the parasite’s life cycle.

\textbf{1.5. Project aims}

\textbf{1.5.1 Background and significance}

In spite of the availability of currently effective drugs, the continuing battle against infectious diseases is far from over. Harmful microbes are exclusively responsible for a large number of infections and death, particularly in developing countries. The emergence and spread of resistance is now threatening to challenge the human capability to treat infections and save lives. This challenge has forced the scientific community to go to greater lengths to improve antimalarial drug design and development. In particular structurally different compounds are needed, hopefully with new modes of action.

\textbf{1.5.2 Aims}

The overall aim of this project was to investigate possible new antimalarial agents\textsuperscript{82} based on benzo[\emph{b}]thiophene amide derivatives. Preliminary studies\textsuperscript{83} on compounds of type (A) carried out at the Institute for Biomolecular Science and Department of Chemistry (University of Wollongong) have shown that they possess moderate antibacterial activity.\textsuperscript{83-85} There have been no previous reports of structures of this type having any such activity. In view of the fact that some antibacterial agents also show antimalarial activity, it was thus decided to explore the potential antimalarial activity of compounds of this general type.
The more specific aims of this project were thus:

1. To prepare analogues of the benzo[b]thiophene based compounds (B) and (C) in which R, and R₁ were systematically varied as substituents to include hydrophobic, aromatic, H-bond donor/ acceptor and ionisable groups. Enantiomers were also to be studied where possible. Conformationally restricted analogues, for example (D) and (E), were also to be investigated. Ring fused derivatives of type (D) and (E) would still preserve the benzo[b]thiophene amide motif. Approaches to such systems were to include free radical and ring closing metathesis procedures, exemplified by the synthesis of compounds of type (E) from precursors of type (F).
2. To assess the antimalarial potency, spectrum of activity and human cell toxicity of all the new heterocyclic derivatives synthesised. The assessment of some antibacterial activity was also to be undertaken.

3. To develop structure-activity relationships with a view to optimising activity.

4. To identify the mode of action of these compounds.

5. To identify possible compounds for pre-clinical development as novel antimalarial agents.

In this thesis, Chapter 2 primarily focusses on the synthesis of non-fused benzo[b]thiophene amides using specific coupling methods while Chapter 3 covers the synthesis of novel fused benzo[b]thiophene derivatives mainly through free radical
cyclisation and ring closing metathesis methodologies. Chapter 4 reports on the biological activities of various benzo[b]thiophene compounds covered in Chapter 2 and Chapter 3 against cultured malarial parasites, as well as antibacterial activity for some specific benzo[b]thiophene amides. The experimental data is covered in Chapter 6.
Chapter 2 Synthesis of Non-Fused Benzo[b]thiophene Amides

Introduction

For the purposes of this discussion benzo[b]thiophene amide derivatives will be divided into two classes of compounds: fused derivatives, and non-fused derivatives. The basis for the differentiation between the two groups is whether or not a linking group exists between the C3 position on the benzo[b]thiophene ring and the carbon α to the nitrogen of the amide group attached to the C2 carbon of the same ring.

A detailed discussion of the synthesis of the fused class of compounds is deferred until Chapter 3. This chapter, however, focusses on benzo[b]thiophene amide derivatives containing a variable substituent in the C3 position, and with the amide nitrogen incorporated within a ring (Figure 2.1).

![Figure 2.1 Generic benzo[b]thiophene amide structure.](image)

2.1.1. Target amides

The synthetic procedures developed during this study enabled the synthesis of a wide range of compounds within the above framework. However, this section will be concerned primarily with molecules incorporating a Cl, Br, H or allyl group in the C3 position (X in Figure 2.1) and the nitrogen-containing ring structures are limited to
tetrahydrossoquinolines, tetrahydro-\(\beta\)-carbolines, dihydropyrroles, piperazines, piperidines and other bridged ring systems.

**General Approach to Synthesis**

The overall synthetic strategy adopted towards the C3-substituted non-fused benzo[\(b\)]thiophene amide derivatives involved the conversion, in cases requiring chloro substitution in the C3 position, of the commercially available (\(E\))-cinnamic acid into the corresponding 3-chloro-benzo[\(b\)]thiophene-2-carbonyl chloride (Scheme 2.1; Step 2.1b). The 3-bromo substituted target (Step 2.1a) was prepared by the direct bromination at the C3 position of the commercially available benzo[\(b\)]thiophene-2-carboxylic acid (20).

\[
\begin{align*}
\text{20} & \quad R = H, R_1 = Cl, X = Cl \\
\text{21} & \quad R = H \\
\text{22} & \quad R = OMe \\
\text{23} & \quad R = H, R_1 = Cl, X = Cl \\
\text{24} & \quad R = OMe, R_1 = Cl, X = Cl \\
\text{25} & \quad R = H, R_1 = OH, X = Br
\end{align*}
\]

**Scheme 2.1** General procedure for generating target amides from precursors with \(R_1 = OH\) or Cl.
In the above approach (Scheme 2.1), Step 2.2 was carried out differently depending on the nature of the R₁ group. In the case where the R₁ group was chloro, the desired amide derivatives were synthesized by the direct acylation of a secondary amine with the acid chloride with the appropriate substituent at the C3 position. Where the carboxylic acid (R₁ = OH) was the precursor a coupling agent was used, namely, dicyclohexylcarbodiimide (DCC). In the case of the acid chloride, the basic nitrogen of the amine acts as a nucleophile, attacking the carbonyl carbon on the acid chloride, and displacing the chloro group as a chloride ion. The resulting hydrogen chloride is neutralized with a suitable base. The choice of base here was preferably potassium carbonate. The neutralization reaction generates CO₂ gas increasing entropy and engendering irreversibility. The discussion of the preparation of each individual amide derivative synthesised in this manner is deferred until later in this chapter.

Amide derivatives where the halogen present in the benzo[b]thiophene ring was a bromine (3-bromo-benzo[b]thiophene derivatives) were synthesized via a different pathway, which involved amide coupling between the carboxylic acid and the appropriate amine facilitated by dicyclohexylcarbodiimide (DCC)\textsuperscript{86,87} in the presence of 1-hydroxybenzotriazole (HOBt)\textsuperscript{88,89,90} (Step 2.2). The acid-amine coupling reactions giving rise to the 3-bromo benzo[b]thiophene amide derivatives are referred to as DCC-coupling reactions in the following chapters. DCC is often used to mediate coupling reactions between carboxylic acids and appropriate amines to form amide linkages. The HOBt assists adduct formation between the carboxylic acid and DCC. The highly reactive ester intermediate is then able to couple with the amine, leading to the cleavage of the ester bond and the formation of the desired amide. The other product of this procedure is dicyclohexyl urea, which is precipitated during the reaction in dimethylformamide (DMF) (Scheme 2.2).
Scheme 2.2 Proposed mechanism for the HOBr/DCC facilitated Acid/Amine coupling reaction.
The formation of dicyclohexylurea in this reaction is potentially problematic as it leads to extra purification steps required to isolate the pure amides. An alternative coupling method would be the use of 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI)\textsuperscript{91} and 4-dimethylaminopyridine (DMAP)\textsuperscript{92,93}, however, in this case, as discussed below, the DCC / HOBT system gave higher yields.

2.2.1. Generation of acid chlorides

*Synthesis of 3-chlorobenzo[\textit{b}]thiophene acid chloride*

The commercially available (\textit{E})-cinnamic acid (21) and (\textit{E})-3-methoxycinnamic acid (22) were used to prepare the starting 3-chloro- substituted acid chlorides 23 and 24, respectively, for subsequent direct coupling with the appropriate amines.

Although the preparation of acid chlorides from carboxylic acids with thionyl chlorides is a well-known and commonly used method, this reaction is of particular interest as it includes the sulfur from the thionyl chloride in the final benzo[\textit{b}]thiophene product\textsuperscript{83}. This reaction, although taking place through a multi-step pathway, is a single-pot, relatively high-yielding reaction, which proceeds smoothly to the final cyclized product within twelve hours (Scheme 2.3). Although the acid chloride may be considered susceptible to hydrolysis, it was stable enough to be flash chromatographed on silica gel, recrystallised from dichloromethane and kept in a desiccator for long periods of time without hydrolysis occurring. The 3-chloro-5-methoxybenzo[\textit{b}]thiophene-2-carbonyl chloride 24 was the only acid chloride substituted at the C5 position used for the synthesis of some of the benzo[\textit{b}]thiophene amide derivatives described below.
Scheme 2.3 The proposed steps involving the generation of the acid chlorides from (E)-cinnamic acids.

During the synthesis of 24 the formation of trace amounts of 3-chloro-5-methoxybenzo[b]thiophene-2-carboxylic acid were detected by mass spectrometry. However, the acid could be removed by saturated sodium bicarbonate solution.

**Other acid chlorides**

Benzo[b]thiophene-2-carbonyl chloride (26), and 3-bromobenzo[b]thiophene-2-carbonyl chloride (27), required for the synthesis of benzo[b]thiophene derivatives described later in Chapter 3 were synthesised via their corresponding benzo[b]thiophene carboxylic acids 20 and 25 on reaction with thionyl chloride and a catalytic amount of pyridine in refluxing toluene (Scheme 2.4). The synthesis of the acid chloride 26 was achieved in 62 % yield and it was used immediately to avoid hydrolysis. The 3-bromo-benzo[b]thiophene substituted acid chloride 27 was also prepared in good yield (67 %) by this method.
Scheme 2.4  Conversion of benzo[b]thiophene carboxylic acids 20 and 25 to their corresponding acid chlorides 26 and 27.

The synthesis of a benzo[b]thiophene acid derivative with a bromo- substituent at the C3 position was crucial for this project as it formed the basis for the synthesis of other benzo[b]thiophene amide derivatives including fused derivatives. Given the success in the synthesis of the acid chlorides 23 and 24, the possibility that an analogous bromo substituted acid bromide derivative might be achieved in a corresponding manner was investigated. A similar reaction to that used for the synthesis of 23 was attempted in which (E)-cinnamic acid was reacted with thionyl bromide in the presence of a catalytic amount of pyridine and the mixture heated at reflux in dry toluene for two and half days.

Scheme 2.5  The attempted synthesis 3-bromobenzo[b]thiophene-2-carbonyl bromide (28) from (E)-cinnamic acid (21).

However none of the expected 3-bromobenzo[b]thiophene-2-carbonyl bromide (28) was obtained from this reaction (Scheme 2.5).
Although pure product was isolated, its spectroscopic characterisation did not correspond to the data expected for the desired product. The identity of the isolated product was not ascertained within the time constraints of the current project. The failure of this reaction to give 28 prompted the search for an alternative method for accessing the required compound. The carboxylic acid 20 was seen as a means of acquiring the desired compound, and a method based on its bromination was followed.

The synthesis of the precursor acid, 3-bromobenzo[b]thiophene-2-carboxylic acid (25) was achieved through the direct bromination of the commercially available acid 20 (Scheme 2.6). The bromination technique was adopted from literature methods for the bromination of 2-methylbenzo[b]thiophene-5-carboxylic acid methyl ester to 3-bromo-2-methylbenzo[b]thiophene-5-carboxylic acid methyl ester and also the conversion of 2-methylbenzo[b]furan-5-carbonitrile to 3-bromo-2-methylbenzo[b]furan-5-carbonitrile\textsuperscript{94}. In the bromination reaction of 20, despite using three equivalents of bromine only one brominated product was isolated in good yield. This observation can be explained by the fact that benzo[b]thiophene is known to preferentially substitute at the C3 position during electrophilic aromatic substitution reactions even though the acid substituent might be expected to deactivate the C3 position in this case.

\[ \text{Scheme 2.6} \quad \text{Direct bromination of benzo[b]thiophene-2-carboxylic acid.} \]
The $^1$H-NMR spectrum of 25 confirmed the non-appearance of a signal for the H3 peak, which was present at 8.27 ppm in the case of 20. The $^{13}$C-NMR spectra showed the same number of carbons for both reactant and product with the carbonyl carbon peaks at 161.9. The mass spectrum of the product confirmed the molecular mass for 25 with the characteristic isotopic mass distribution for the presence of single bromine being observed.

2.2.2. Amine bases

_Tetrahydroisoquinolines_

It had been shown previously that the isoquinoline amide 29 and its derivatives showed mild activity against cultures of the Gram-negative bacterium _Escherichia coli_ and the Gram-positive bacterium _Staphylococcus aureus_. The analogous amide derivative with methoxy groups at the C6 and C7 positions of the tetrahydroisoquinoline ring and another methoxy at the C6 position of the benzo[b]thiophene ring, together with compound 29, were reported to be active against _E. coli_ at 100 µg/mL, although no MIC values were determined.

Amines having a methyl group at the C1 position, and methoxy groups at the C1, C6 and C7 positions of the tetrahydroisoquinoline ring coupled to the acid chloride substituted with a methoxy group or fluorine at the benzo[b]thiophene C6 position gave amide derivatives that had very weak bacteriostatic activity towards _S. aureus_ (1,000 µg/mL).
A search of the literature was undertaken to determine the potential for tetrahydroisoquinoline derivatives of benzo[b]thiophene to act as antimalarial agents. This investigation revealed that no previous work had been carried out in the area of synthetic tetrahydroisoquinoline amide derivatives of benzo[b]thiophene acting as antimalarial agents. In the light of this, and in consideration of their observed antibacterial properties, it was thought that a series of compounds in this class might also potentially provide some new antimalarial lead compounds. Benzo[b]thiophene amides based on the framework 29 and 30 were thus prepared. Substituents were varied in both rings of the tetrahydroisoquinoline moiety to give a range of compounds which were to be screened for biological activity. Since the synthetic strategy towards the appropriate acid or acid chloride has been mentioned previously, this discussion will concentrate on the synthesis of the tetrahydroisoquinoline bases used in the coupling reaction.

Bases 31 and 33 were purchased from Sigma Aldrich®. In cases where \( R = \) allyl, and \( R_1 = H \) (32) or \(-\text{OMe} \) (34), synthetic strategies were devised to prepare them as starting materials. The 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (33) was purchased as the hydrochloride salt and was subsequently converted to the free base before being used in the amide coupling reaction. The syntheses of the 1-allyl-1,2,3,4-
tetrahydroisoquinoline (32) and 6,7-dimethoxy-1-allyl-1,2,3,4-tetrahydroisoquinoline (34) intermediates are outlined in Scheme 2.7.

Scheme 2.7 The zinc/allyl bromide approach to 1-allyl-1,2,3,4-tetrahydroisoquinolines.

The amine, 32, and its 6,7-dimethoxy analogue 34, were synthesized from the commercially available tetrahydroisoquinolines 31 and 33 which were oxidized to their respective 3,4-dihydroisoquinolines with N-bromosuccinimide (NBS)\(^95\). These reactions produced product yields of around 80%. Due to their instability, these imine intermediates were reacted immediately with allyl bromide in the presence of activated zinc to synthesize both the 1-allyl-1,2,3,4-tetrahydroisoquinolines. This process involved the initial formation of an allylzinc bromide complex followed by the addition of this reagent to the imine (Scheme 2.7). The reaction involving 32 proceeded smoothly with good yields achieved in a relatively short time; however the synthesis of
34 did not proceed as smoothly as with 32. This reaction took more time to complete and in some cases there was little product isolated. A possible explanation is the electron donating effect of the methoxy groups reducing the susceptibility of the cyclic imine to nucleophilic attack.

**Other bases**

Other bases used to generate the target amides mentioned in this chapter were purchased from commercial suppliers. These included isoquinolines, such as the 1,2,3,4-tetrahydroisoquinoline (31) and the (S)-(−)-N-tert-butyl-1,2,3,4-tetrahydro-3-isoquinolinecarboxamide (45), carbazoles such as 1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole (40), piperazines such as 4-chloropiperazine (42), 1-Boc-piperazine (44), 1-(2-pyridyl)piperazine (38), 1-benzylpiperazine (48), 1-(2-pyrimidyl) piperazine (39) and bridged piperazine such as (1S,4S)-(−)-4-(chlorophenyl)-2,5-diazabicycloheptane (43), and finally the piperidine 4-(4-chlorophenyl)-4-hydroxypiperidine (41). Some of these bases, for example the carbazoles and the piperazines, also had the capacity for further functionalisation allowing for an even wider range of target compounds.
29  \( R = R_1 = H, X = Cl \)  
30  \( R = R_1 = H, X = Br \)  
49  \( R = \text{Benzyl}, R_1 = H, X = Br \)  
50  \( R = H, R_1 = \text{OMe}, X = Cl \)  
51  \( R = H, R_1 = \text{OMe}, X = Br \)  
52  \( R = \text{ Allyl}, R_1 = H, X = Cl \)  
53  \( R = \text{ Allyl}, R_1 = H, X = Br \)  
54  \( R = \text{ Allyl}, R_1 = X = H \)  
55  \( R = R_1 = H, X = Cl \)  
56  \( R = R_1 = H, X = Br \)  
57  \( R = H, R_1 = \text{OMe}, X = Cl \)  
58  \( R = \text{Benzyl}, R_1 = \text{OMe}, X = Cl \)  
59  \( R = \text{Benzyl}, R_1 = H, X = Cl \)  
60  \( R = \text{Benzyl}, R_1 = H, X = Br \)  
61  \( R = 2\text{-Nitrobenzyl}, R_1 = H, X = Cl \)
62 R = Boc, X = Cl
63 R = Boc, X = Br
64 R = H, X = Cl
65 R = H, X = Br
66 R = Benzyl, X = Cl
67 R = Benzyl, X = Br
68 R = α-Phenacyl, X = Cl
69 R = α-Phenacyl, X = Br
70 R = 4-Chlorophenyl, X = Cl
71 R = 4-Chlorophenyl, X = Br
72 R = 2-Pyridyl, X = Cl
73 R = 2-Pyridyl, X = Br
74 R = 2-Pyridyl, X = H
75 R = 2-Pyrimidyl, X = Br
76 R = Fmoc, X = Cl
77 R = Fmoc, X = Br

80 X = Cl
81 X = Br

82 X = Cl
83 X = Br

84 X = Cl
85 X = Br
2.2.3 Coupling Methods

Two methods were used for the generation of the target amides from the secondary amines outlined above: direct coupling with an acyl chloride and the use of a diimide coupling reagent with a carboxylic acid. The use of the reagent 1-(3-dimethylaminopropyl)-3-ethylcarbodiimide (EDCI) in the presence of a base catalyst such as 4-dimethylaminopyridine (DMAP) has been widely used as an alternative approach to coupling reactions as it offers some advantages over the DCC method, notably the production of a water-soluble urea by-product. The use of DCC as a coupling reagent in the current study provided better reaction yields and hence was preferred over the EDCI/DMAP approach. Compounds 77 and 78 were synthesised as precursors for further cyclisation chemistry, and as such they are discussed in detail in Chapter 3; they are, however, included here for the discussion involving the coupling chemistry.

Direct coupling with acid chloride

Reactions involving the direct coupling of the acid chloride 23 with amine bases gave optimized isolated amide yields ranging from 33–97 %. The results are summarized in Table 2.1, the data for which is also represented graphically in Figure 2.2. In the case where the amine was the parent 31, the yield was moderate (58 %) whereas with two methoxy groups in the C6 and C7 positions of 33 the yield was 85 %. This increase may be due to the electron donation by resonance of the two methoxy groups causing a subsequent increase inductively in nucleophilicity of the basic nitrogen. There is very little regularity in the pattern in yields for the acid chloride reaction with each of the bases listed in Table 2.1. Figure 2.2 shows that, while there is a large range of isolated yields, there are minimal differences structurally that could
account for the wide range in isolated yields. After the 3-chlorobenzo[b]thiophene amides were synthesized and purified they were fully characterised using spectroscopic techniques.

**Table 2.1 -** Amide reaction yields for secondary bases coupled with 23.

<table>
<thead>
<tr>
<th>Free Base</th>
<th>Amide % Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>31</td>
<td>59</td>
</tr>
<tr>
<td>33</td>
<td>86</td>
</tr>
<tr>
<td>32</td>
<td>47</td>
</tr>
<tr>
<td>45</td>
<td>90</td>
</tr>
<tr>
<td>41</td>
<td>93</td>
</tr>
<tr>
<td>43</td>
<td>33</td>
</tr>
<tr>
<td>44</td>
<td>97</td>
</tr>
<tr>
<td>47</td>
<td>95</td>
</tr>
<tr>
<td>42</td>
<td>52</td>
</tr>
<tr>
<td>38</td>
<td>63</td>
</tr>
<tr>
<td>39</td>
<td>50</td>
</tr>
</tbody>
</table>

![Diagram of 23](attachment:image.png)
Figure 2.2- Graphical representation of isolated amide yields for the reaction of the acid 23 with the specified secondary amines.
When an allyl group was present in the starting tetrahydroisoquinoline base at the C1 position, adjacent to the nitrogen, the yield diminished to 47 %, probably as a result of steric interference. It was expected that the presence of the allyl group might also have some bearing on the relative energies of the two most probable amide rotamers (Figure 2.3). An AM1\textsuperscript{96} model study of the both isomers showed only a slight energy advantage of 2.1 kcal mol\textsuperscript{-1} for the rotamer with the allyl group on the same side of the amide bond as the carbonyl oxygen. \textsuperscript{1}H-NMR spectra of 52 at 298 K showed the existence of two amide rotamers (Figure 2.3).

![3D model structures (AM1) showing the two possible rotamers of benzo[b]thiophene derivative 52.](image)

The representation of the preferred conformation of the non-fused benzo[b]thiophene amides was confirmed by an X-ray crystallographic study of amide 73 (Figure 2.4). This confirmed the chair conformation of the piperazine based amide. The carbonyl group was observed to orient itself away from the plane of the benzo[b]thiophene ring to reduce steric hindrance (Figure 2.4).
Figure 2.4- X-ray structure of amide 73 representing the non-fused benzo[b]thiophene amides and confirming their preferred conformation.
Apart from the direct coupling of 23 and 32 to give the C1 allylated amide 52, other methods reported in the literature that would lead to 52 were also investigated.

Scheme 2.8 The attempted one-pot synthesis of (1-benzyl-3,4-dihydroisoquinolin-2(1H)-yl)(3-bromobenzo[b]thiophen-2-yl)methanone (52) and the isolated by-products
One such method was the coupling of 3-chlorobenzo[b]thiophene-2-carbonyl chloride (23) with the imine 36 which would hopefully give an activated intermediate 87 which could react further with allylzinc bromide generated in situ to afford 52 (Scheme 2.8).

However, in the event, only a small amount (5 %) of the desired product 52 was obtained with the majority of the mass balance of the reaction being found in two major by-products; a TLC analysis of the reaction mixture showed these by-products had Rf values of 0.5 and 0.75, while 52 had an Rf of 0.6. From the low resolution mass spectrometric data, the major product (Rf 0.5) had an M+ peak at m/z 344, while the second M+ peak at m/z 326 was consistent with 52, and finally an M+ peak at m/z 241 was assigned to the other major product (Rf 0.75). The m/z values of the peaks for the two major by-products, m/z 344 and m/z 241, were identified as those belonging to an aldehyde and an ethyl ester respectively. However, the mechanism of the formation of the ethyl ester of the initial acid chloride, in this case ethyl 3-chlorobenzo[b]thiophene-2-carboxylate (86), remains unclear. Compound 86 may have been formed during chromatography when the compound came into contact with the solvent system which in this case was a mixture of ethyl acetate and hexane or most probably due to the compound coming into contact with chloroform which contains traces of ethanol normally added to stabilise the solvent.

To confirm the structure of the ethyl ester by-product, ethyl 3-chlorobenzo[b]thiophene-2-carboxylate (86) was synthesised from 23 and the 1H-NMR data for this authentic product was shown to be the same as that for the suspected ethyl ester. An analysis of the two using thin layer chromatography was also consistent with this finding. Prolonging the one-pot coupling reaction (Scheme 2.8) from three hours to twelve hours, gave the aldehyde compound as the dominant major product and only traces of the ethyl ester by-product was formed. The aldehyde was identified as N-(2-
formylphenethyl)-3-chlorobenzo[\textit{b}]thiophene-2-carboxamide (88). The formation of the product 88 may take place as a result of adventitious moisture being present which would hydrolyse the iminium ion intermediate 87 to the aldehyde 88 through the mechanism proposed in Figure 2.3.

![Figure 2.5](image)

**Figure 2.5**  Possible mechanism for the formation of the aldehydic by-product 88 during the attempted synthesis of 52.

Evidence of metal mediated allylation of carbonyl compounds in appropriate media to achieve the corresponding homoallylic alcohols have been reported using a variety of metals, particularly indium\textsuperscript{97}. However the indium-mediated allylation of aldimes to give the corresponding homoallylic amines has not been reported in detail\textsuperscript{97}. Such reactions are performed in aqueous media where imines are subject to hydrolysis even before the allylation can take place. Furthermore aldimes are generally less electrophilic than the corresponding carbonyl compounds. In organic solvents, however, such indium-mediated additions to imines are feasible\textsuperscript{97}.
The synthesis of 52 was thus attempted via a one pot system from 23, allyl bromide and the imine 36. The reactants were stirred together in tetrahydrofuran into which, at low temperature, indium powder was added and the mixture then stirred overnight (Scheme 2.9).

Scheme 2.9 The indium-mediated one pot approach to 52 also showing the by-products identified in the reaction.

After workup, a preliminary TLC analysis of the reaction showed the presence of the desired product 52 as a minor component.
A mass spectrum (Cl′) of the crude residue showed peak signals corresponding to 52 at $m/z$ 368 as well as peaks at $m/z$ 344, $m/z$ 326 and $m/z$ 386 which did not correspond to any possible fragments of 52. The $m/z$ 326 peak was assigned to the 2-(3-chlorobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydroisoquinoline (29); this product may have resulted from reduction of 36 to 31, and then the reaction of the latter with 23. The peak at $m/z$ 344 was consistent with $N$-(2-(hydroxymethyl)phenethyl)-3-chlorobenzo[b]thiophene-2-carboxamide (89) formed by the indium-mediated reduction of the aldehyde 88. Other spectroscopic evidence confirmed the structure of 89.

Scheme 2.10 The synthesis of a 5-methoxy substituted benzo[b]thiophene derivative (57) and its subsequent derivatisation to (58).
A methoxy-substituted benzo[b]thiophene derivative 57 was also synthesised (92 \%) by the reaction of 3-chloro-5-methoxybenzo[b]thiophene-2-carbonylchloride (24) (Scheme 2.3) and the amine 40 in boiling THF under basic conditions, (Scheme 2.10). Further functionalisation of 57 was then investigated by substituting the indolic proton with a benzyl group. This was achieved by reacting 57 with benzyl bromide in the presence of sodium hydride at low temperature in dimethylformamide. The desired compound 5-methoxy-2-(3-chlorobenzo[b]thiophene-2-oyl)-1,2,3,4-tetrahydro-9-benzyl-pyrido[3,4-b]indole (58) was then isolated and the crude residue was purified by flash column chromatography (Scheme 2.10). The spectroscopic data for 58 was consistent with N-benzylation, and the molecular formula was determined by HRMS analysis.

**DCC/HOBt assisted coupling**

The carbodiimide DCC was also used in the presence of HOBt to synthesize the desired 3-bromobenzo[b]thiophene amide derivatives from the acid 25. The target products (see pg 49-50) were obtained in good yields after a series of purification steps designed to eliminate the precipitated by-product, dicyclohexyl urea (Table 2.2). The 3-bromo- substituted derivatives were generally obtained in higher yields than their 3-chloro- substituted analogues prepared via the direct acylation of the desired amine by the acid chloride.

The prime motivation for synthesising the bromo- substituted derivatives was that they were predicted to be more reactive towards further derivatisation than their chloro- substituted counterparts, thus offering the opportunity for a wider range of potential new derivatives to be acquired via free radical or palladium-mediated reactions. A comparison between the chloro- substituted amide series and the bromo-substituted series across a range of secondary amines (Figure 2.6) indicates that with some exceptions, in most cases relatively comparable yields were achieved. Where a
low yield existed for the acid chloride synthetic route, a similarly low yield was observed if the DCC coupling reaction was used to carry out the reaction. Where steric or electronic considerations might affect the yield in a direct acylation reaction, they would also play the same role in a DCC coupling reaction.

Table 2.2 - Amide reaction yields for secondary bases coupled with 25 in the presence of DCC, HOBT

<table>
<thead>
<tr>
<th>Free Base</th>
<th>Amide % Yield</th>
</tr>
</thead>
<tbody>
<tr>
<td>30</td>
<td>81</td>
</tr>
<tr>
<td>33</td>
<td>30</td>
</tr>
<tr>
<td>32</td>
<td>43</td>
</tr>
<tr>
<td>45</td>
<td>60</td>
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<tr>
<td>41</td>
<td>93</td>
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<td>43</td>
<td>29</td>
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<td>44</td>
<td>85</td>
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<td>47</td>
<td>95</td>
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<td>42</td>
<td>96</td>
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<tr>
<td>38</td>
<td>48</td>
</tr>
<tr>
<td>39</td>
<td>80</td>
</tr>
<tr>
<td>40</td>
<td>36</td>
</tr>
</tbody>
</table>

25
For comparison purposes two cases where the acid to be coupled was not halogenated were included, and they gave the amides 54 and 74.

Significant differences in yields between the two methods might be explained by a number of different rationales. The different workup may affect the overall yield. For example, the direct acylation reactions gave cleaner products, which required less workup than the bromo- substituted series, which required steps specifically to remove the urea by-product associated with the DCC coupling method. The choice of solvent may also have a marked effect; for example, the preparation of the series of 3-chlorobenzo[b]thiophene amide derivatives required the use of tetrahydrofuran which upon workup was easy to evaporate under reduced pressure whereas dimethylformamide was used in the DCC coupling reactions and was difficult to eliminate hence requiring extra water washings to remove. This additional purification process coupled with that required to remove dicyclohexyl urea may have led to lower yields of some amide products. In cases where the yield of the directly acylated product was significantly lower than that of the DCC coupled product differences in yields may have been a result of product stability under the reaction conditions.

With 37 as the base, the presence of a halogen on the starting acid or acid chloride had little effect on the amide yield from the coupling reaction, whereas with the amine 32 there was a significant increase in the yield when there was a halogen in the C3 position of the benzo[b]thiophene moiety.
Figure 2.6- A comparison of the yields of the amide reactions involving direct acylation of (chloro acid chloride) 23 and DCC coupling of 25 (bromo-acid) to various amine bases as specified. Two results for benzo[b]thiophene-2-carboxylic acid (20) are also included.
**Preparation of amide 49**

The amide 49 was made from the amine 35 which was synthesised in turn by converting 31 to 36 with NBS in dry dichloromethane as shown in Scheme 2.7 followed by the reaction of the 3,4-dihydroisoquinoline (36) with benzyl bromide in the presence of activated zinc powder (Scheme 2.11). This reaction was expected to follow a similar mechanism to that outlined for the synthesis of 32 (Scheme 2.7), in which a benzylzinc bromide complex is generated and subsequently attacks the imine carbon, thereby adding the benzyl group.

![Scheme 2.11](image)

**Scheme 2.11** The synthesis of 2-(3-bromobenzo[b]thiophene-1-benzyl-1,2,3,4-tetrahydroisoquinoline (49).

The preparation of the amine 35 through this method gave the desired product in a low yield of 29 %, much lower than the yield obtained with the 1-allyl equivalent (32). A possible reason for this might be the faster rate of allylzinc bromide formation.
2.2.4. Further amide derivatisation

Some of the bases studied allowed for further derivatisation of the amides synthesized. The boc-protected amides 62 and 63 were deprotected allowing further chemistry to be performed on the resulting secondary amino group. The indolic proton on the amides with a tetrahydro-β-carboline moiety (55 and 56) could be deprotonated with a strong base permitting further functionalisation, for example by introduction of a benzyl or o-nitrobenzyl group to give the amides 59 and 60.

Scheme 2.12 Pathway to benzo[h]thiophene derivatives with substituted piperazine rings.
The diallylamine group on 47 affords access to ring closing metathesis reactions as well as the potential for addition across the double bonds. Furthermore the 3-bromo-substituted amide containing 1-allyl-tetrahydroisoquinoline functionality could undergo radical initiated ring fusion chemistry.

Probably the most important amides synthesized in terms of allowing further chemistry were the N-boc protected amides 62 and 63 which were precursors to several compounds prepared using the resulting secondary nitrogen upon deprotection. For example, removal of the boc group in 63 was carried out using trifluoroacetic acid to afford 65 in high yield (Scheme 2.12). Compound 65 was then converted by standard methods into its N-benzyl (67), N-phenacyl (69), and N-fmoc (77) derivatives for biological testing (Scheme 2.12).

A single crystal X-ray crystallographic analysis of the amide 63 was also carried out. This analysis revealed the presence of major and minor rotamers disordered in a 70:30 ratio over two sets of site, in the crystal lattice (Figure 2.7). This work was undertaken by Professor A.H White, University of Western Australia.
Figure 2.7- The X-ray crystallographic data of amide 63 showing the major (top) and minor (middle) rotamers in a 70:30 ratio as well as both components together (bottom).
Chapter 3 Synthesis of Fused Benzo[b]thiophene Amides

Introduction

The previous chapter focussed primarily on target amides prepared by coupling methods and their derivatisation by substitution reactions. This chapter will concentrate on amide derivatives which were further transformed by using free radical cyclisation reactions, ring closing metathesis and palladium-mediated cyclisation to produce fused-ring systems. The rationale behind the synthesis of such amide derivatives was that they could offer greater conformational restriction, and then afford better specificity in their biological activity. The molecules were still based on the benzo[b]thiophene amide structural motif (Figure 3.1)

3.1.1. Current project

In order to increase the breadth of benzo[b]thiophene amide derivatives synthesised, it was proposed that a C3 bromo- substituent in the benzo[b]thiophene would have the potential to form a free radical, enabling a free radical initiated ring closure to form a lactam (Figure 3.1).

![Figure 3.1](image-url)  
**Figure 3.1** Generic fused benzo[b]thiophene amide structure.
Compounds with multiple olefinic groups such as 118 and 119 were also prepared as precursors for ring-closing metathesis reactions as a means to create larger, conformationally restricted ring systems. Furthermore, a pyrrolidine derivative was synthesised from a diallylamine-based parent molecule 79 using ring closing metathesis methodology.

![Chemical structures](image)

118 \( R = H \)

119 \( R = OMe \)

### 3.1.2. Free radical reactions

A free radical is formed when a covalent bond between atoms is broken leaving an electron with each atom. Free radicals are initiated via homolytic bond cleavage within molecules with relatively weak covalent bonds. Chemical processes where a free radical species is generated from non-radical reactants are known as initiator reactions. A good example of a widely used initiator for free radical reactions is azobis(isobutyronitrile) (AIBN).

A free radical species can also be propagated; the radical can either undergo abstraction (as in cyclisation reactions) (Scheme 3.1) or can undergo addition (as in the case of polymerisation processes) and in both cases a new atom in the molecule being formed becomes the radical centre, thereby relocating the radical electron.
Finally, there is a termination process, which is in competition with propagation reactions.

**Scheme 3.1** - Cyclisation involving the reaction of tributyltin hydride (Bu$_3$SnH) with unsaturated alkyl halide in the presence of a radical initiator (AIBN).

Free radical reactions have been widely used in heterocyclic synthesis$^{98-100}$, and commonly involve the use of tributyltin hydride (Bu$_3$SnH). Other triorganostannanes have been used, but owing to the cheap availability, preparation, purification and ease of storage, tributyltin hydride is more widely employed, the only major drawbacks being handling and toxicity. Reaction conditions for these radical cyclisation are generally similar, using a small excess of tributyltin hydride with a smaller equivalent (10-25 %) of a suitable radical initiator, usually azobis(isobutyronitrile) (AIBN). The cyclisation reactions are normally carried out in dry refluxing benzene or toluene for periods ranging between 1-10 hours and require an inert atmosphere.
**Synthetic Studies**

3.2.1 Ring formation via free radical cyclisation

To further extend the range of benzo[b]thiophene-based compounds, a new set of derivatives were prepared using free radical cyclisation methods to incorporate a conformationally restricted lactam motif. Some of these new compounds also had the potential for further functionalisation. The chemistry of the cyclisation involved free radical formation at C3 of the benzo[b]thiophene moiety followed by the addition to an allyl group on the amide nitrogen to give cyclised compounds with a skeleton as shown in the general structure in Figure 3.1. The synthesis of the starting amide 79, required for the free radical cyclisation reaction, is discussed in Chapter 2.

The proposed radical cyclisation of 79 (Scheme 3.2) was predicted to proceed smoothly because of the existence of an allyl group on either side of the C-N amide bond, negating any steric difficulties associated with any inflexibility of this bond.

![Scheme 3.2](image)

**Scheme 3.2** - Radical cyclisation of 79 to the corresponding conformationally restricted lactam 92.

The radical cyclisation reaction for the amide was carried out using standard radical reaction conditions involving tributyltin hydride, and a radical initiator, AIBN. An anhydrous toluene solution of tributyltin hydride and AIBN was slowly added to a toluene solution at reflux of the starting material. This slow addition of the reagents
ensured that there were small amounts of the hydride being continually used up generating more Bu$_3$SnBr in a cycle. The reaction mechanism proceeds via the steps depicted in Scheme 3.1.

Before subjecting the amide 79 to radical cyclisation, however, a model reaction was carried out in order to gauge the synthetic viability of the process (Scheme 3.3).

Scheme 3.3- Free radical cyclisation of N,N-diallyl-2-bromobenzamide (94).

The model reaction involved the free radical cyclisation of the structurally analogous N,N-diallyl-2-bromobenzamide (94) to give the new isoquinolinone derivative 95 in good yield (Scheme 3.3). This represents a new approach to this ring system, although related fused systems have been made in this way$^{98}$. 
Scheme 3.4— Radical cyclisation of \(N,N\)-diallyl-3-bromobenzo[\(b\)]thiophene-2-carboxamide (79) giving 2-allyl-4-methyl-1,2,3,4-tetrahydrobenzo[\(b\)]thieno[2,3-\(c\)]pyridin-1-one (92).

Following this model study, the radical cyclisation of \(N,N\)-diallyl-3-bromobenzo[\(b\)]thiophene-2-carboxamide (79) was accomplished under similar conditions in toluene at reflux (Scheme 3.4). The required fused product 92 was obtained in moderate yield (48%).

The \(^1\)H-NMR spectrum of 92 confirmed the presence of the methyl protons with a doublet signal at 1.33 ppm while the allylic protons were ascribed to the peaks at 5.81-5.90 (m, 1H, CH), 5.19-5.31 (m, 2H, = CH\(_2\)) and 3.98 ppm (d, \(J = 6.6\) Hz, 2H, -CH\(_2\)). A multiplet peak at 4.36- 4.38 ppm was ascribed to the methine proton in the
six-membered ring. This radical cyclisation route represents a new approach to the benzo[b]thieno[2,3-c]pyridin-1-one system\textsuperscript{101}.

As an adjunct to this particular study, focus was directed towards the functionalisation of the allylic double bond in \textbf{92} in order to introduce hydrogen bond donor/ acceptor groups. Dihydroxylation was thus investigated using the Sharpless reagent (AD-mix\textsuperscript{®}), and osmium tetroxide\textsuperscript{102}. Several attempts to model the preparation of the asymmetric diol compound \textbf{96} using the appropriate AD-mix\textsuperscript{®} $\alpha$ reagent with \textbf{95} proved unsuccessful. In fact, even an attempt to model the dihydroxylation on a far simpler analogue, allyl bromide (\textbf{97}) to the corresponding 3-bromopropane-1,2-diol (\textbf{98}) failed (Scheme 3.5). No explanation for the lack of success with this reaction with \textbf{95} was apparent, as all prescribed steps for the use of the Sharpless reagent were followed\textsuperscript{102,103}. In this case only AD-mix-$\alpha$ was used to attempt the dihydroxylation\textsuperscript{103,104}.

However, an effort to synthesise the diol \textbf{99} from \textbf{92} using osmium tetroxide was successful with an overall yield of 80 %. This reaction though is not stereospecific (Scheme 3.6). The dihydroxylation reactions were carried out using standard literature methods\textsuperscript{105}, with potassium osmate (10 %), 2.5 equivalents of $N$-methylmorpholine-$N$-oxide (NMO), acetone and water (Scheme 3.6).
Scheme 3.5- Attempted dihydroxylation of the allylic compounds 95 and 97 using AD-mix® α.

When the osmium tetroxide dihydroxylation reaction was attempted using the model compound 95, dihydroxylation also proceeded smoothly to give the expected product dihydroisoquinoline 96.

Scheme 3.6- The dihydroxylation of the model compound 95 and the benzo[b]thiophene derivative 92 using the potassium osmate/ NMO method.
However, it was noted that even with a high crude yield (74%), upon using silica gel column chromatography for compound purification the yield dropped to 30%. Furthermore, the decomposition of the potassium osmate in air caused the crude product to turn black, possibly also breaking down the desired product in the mixture thus affecting yields and necessitating further purification.

Another variation on 92 which was undertaken was the replacement of the allyl group with a benzyl group. The number and variety of substituents possible on a benzyl substituent make it an excellent candidate for any biological SAR studies.

Scheme 3.7- Synthesis of the new benzo[b]thiophene compounds 92, 104, 105 and 106.
The removal of the allyl group in 92 could also afford opportunities for further functionalisation. Other electrophilic groups could then be attached easily after deprotonation of the secondary amide. The removal of an allyl group from an amide had some precedent in the literature. Two approaches for replacing the allyl group with a benzyl moiety in the cyclised molecule 92 were proposed. The first approach involved the removal of the allyl group from 92, which would leave the lactam nitrogen ring amenable to subsequent alkylation.

The second approach proposed was the synthesis of a 3-bromo-benzo[\(b\)]thiophene amide bearing a benzyl group and an allyl group about the nitrogen which could then be cyclised via the free radical methodology. The second method involved three synthetic steps starting with the bromination of 20 and subsequent DCC/ HOBt coupling with either benzylamine followed by alkylation with allyl bromide to give the cyclisation precursor, or the reverse sequence involving DCC/ HOBt coupling with allyl amine followed by N-alkylation with benzyl bromide. The proposed synthetic pathways are illustrated in (Scheme 3.7).

Rhodium(III) chloride has been noted to remove an allyl group from an amide\(^{106}\), however with this method, secondary amide yields were low. The specific reaction is shown in Scheme 3.8, where the non-cyclic de-allylated product was obtained in only 34 % overall yield.

Two methods were attempted for the removal of the allyl group from the lactam 92. Firstly, the reaction of 92 with rhodium(III) chloride\(^{106}\), at reflux in ethanol. However, none of the expected deallylated lactam 106 (Scheme 3.7) was obtained.
Scheme 3.8- The reported use of rhodium chloride in the deprotection of the allyl group in amides, \((R = CH_3, C_2H_5, i-C_3H_7)^{106}\).

In the second method, the lactam \(92\) was heated in formic acid in the presence of triphenylphosphine and palladium(II) acetate, following the general reported methods\(^{107}\) for the deallylation of \(N\)-allylethers\(^{108}\), and \(N\)-allylamides\(^{109}\) (pg-63). The reaction mixture was difficult to workup but ultimately the required lactam \(106\) was obtained in low yield (25 %). The \(^1\text{H}-\text{NMR}\) spectrum of \(106\) confirmed the absence of signals for the allyl group protons, and the retention of other signals which could be ascribed to the ring protons and the methyl substituent group.

At this point, approaches to the synthesis of \(N\)-benzyl-1-allyl amine (107) were then considered. The first involved the reaction of benzyl bromide with allylamine and the second, of allyl bromide with benzylamine. In both cases the amine needed to be in excess to minimise tertiary amine 108 formation and the reactions needed to be performed in the presence of a base to act as a proton sink. For the purposes of the current project the second allylation method with allyl bromide was chosen and gave 107 in good yield (76 %); an excess of benzylamine was used in this reaction. After coupling of 107 with 25 using DCC/ HOBt, the amide 101 was isolated in 16 % yield along with small amounts of the undesired tertiary amine 108.
as a side product carried over from the synthesis of the secondary amine (Scheme 3.9).

Scheme 3.9- The synthesis of 101 from 107 and 25, from coupled benzyl amine and allylbromide.

An alternative synthesis of 101 was also investigated. This involved the DCC/HOBt coupling of 25 with benzylamine which gave 100 in high yield (97 %) (Scheme 3.10). This compound was easy to work with and purify, the only issue being its relative insolubility in chromatographic solvents resulting in a tendency to crystallise in the chromatographic column. To overcome this problem a shorter column was used and flash column chromatography performed, which then gave 100 as a pure solid. The next step involved reaction of 100 with allyl bromide. This was done by first deprotonating the secondary lactam in 100 with sodium hydride at low
temperature, and subsequent nucleophilic substitution on nitrogen by reaction of the amide anion with allyl bromide to give 101 (Scheme 3.10).

![Scheme 3.10- The synthetic pathway for benzo[b]thiophene cyclisation precursors](image)

The same general approach was then used to make the p-methoxy substituted compound 103. The reactions involving coupling with DCC/ HOBt and N-allylation were again high yielding (97 % and 72 % respectively) (Scheme 3.10). The final step was the radical cyclisation of the amide precursors 101 and 103 using Bu3SnH/ AIBN in boiling toluene. This afforded the benzylated cyclised products 104 and 105 in moderate yield (Scheme 3.7).
Free radical cyclisation involving the tetrahydroisoquinoline moiety

Work was also undertaken directed towards the synthesis of radically cyclised amide compounds with conformationally restricted isoquinoline groups but lacking fusion with the benzo[b]thiophene moiety. The cyclisation reaction was based on the \( o \)-bromobenzoyl model reaction (Scheme 3.11) previously developed in the Bremner laboratory\(^{110,109}\). This reaction was repeated to refine reaction conditions.

**Scheme 3.11** - \( N \)-benzyl-7-methyl-6,7,8,9-tetrahydro-5\( H \)-benzocycloheptane-5,8-imine (110) synthesis via free radical cyclisation of 2-(\( o \)-bromobenzoyl)-1-(2-propenyl)-1,2,3,4-tetrahydroisoquinoline (109)\(^{110}\).
The amide 2-(o-bromobenzoyl)-1-(2-propenyl)-1,2,3,4-tetrahydroisoquinoline (109) was reacted with tributyltin hydride and AIBN in toluene at reflux to afford 110 and a trace amount of the reduced component 111 (Scheme 3.11).

In pursuit of the bridged amide N-benzo[b]thienoyl-7-methyl-6,7,8,9-tetrahydro-5H-benzocycloheptene-5,8-imine (112), free radical cyclisation of compound 53 was then undertaken. The precursor 53 was obtained from the amine 32 and the acid 25 (Scheme 3.12). The cyclisation of 53 with tributyltinhydride/ AIBN at reflux in toluene gave exclusively the 5-exo-trig product 112 in relatively high yield (89 %) as a mixture of diastereomers. The reduced product was also detected but in trace amounts. The structure of 112 was confirmed using spectroscopic techniques. The NMR of the compound showed the expected peak signals for the methyl protons at 1.02 ppm and the H3 proton signal of the benzo[b]thiophene ring system at 7.81 ppm.

The methylene and methyl groups in 112 were confirmed by DEPT experiments while the $^{13}$C-NMR confirmed the expected peaks for the characteristic
carbonyl carbon (168 ppm) and the other carbons. High-resolution mass spectroscopy gave a molecular formula for the MH$^+$ ion peak (C$_{21}$H$_{19}$NOS) consistent with that expected for 112.

3.2.2. Ring closing metathesis reactions

The term metathesis comes from the Greek words *meta* meaning change and *tithemi* meaning place. From a chemical perspective, it refers to the exchange of atoms between two molecules. In olefin chemistry, it expresses the formation of a carbon-carbon double bond from what were initially two double bonds. This type of metathesis is of particular interest because no additional reagents are employed with the exception of a small amount of a metal carbene complex catalyst. The by-product of the reaction is an easily removed, highly volatile olefin.

Scheme 3.13 - The four categories comprising the metathesis process$^{111}$. 

```plaintext
Scheme 3.13 - The four categories comprising the metathesis process$^{111}$. 
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This form of metathesis can be broadly categorised into four groups comprising exchange reactions, cross metathesis (CM), ring opening metathesis polymerization (ROMP) and ring closing metathesis (RCM) as depicted in Scheme 3.13. Of these four groups, ring-closing metathesis is of particular interest in terms of the current project.

3.2.3. Discovery of olefin metathesis

Olefin metathesis was first reported in the 1950s. In 1956 Herbert S. Eleutrio, synthesized a propylene-ethylene copolymer from propylene passed over a molybdenum-on-alumina catalyst. The output gas was analysed and found to be composed of propylene, ethylene and 1-butene, an observation that was also reported by other chemists working in other petrochemical companies. Later in 1960, a U.S patent was assigned to The Standard Oil Company of Indiana (USA), which recorded that propylene in presence of molybdenum oxide on alumina, treated with tri-isobutyl alumina, gave ethylene and butanes as products. In 1964, Phillips Petroleum reported the breakdown of propylene to ethylene and butanes using molybdenum hexacarbonyl on alumina (Scheme 3.14)

![Scheme 3.14- Breakdown of propylene using molybdenum hexacarbonyl on alumina](image)

In 1967, Nissim Calderon and others at Goodyear Tyre and Rubber sought an explanation for the unexpected by-products from the reactions mentioned above. They
suggested that the by products were due to the cleavage and reformation of double bonds within the olefins. The Goodyear researchers hence termed the reaction “olefin metathesis”\textsuperscript{116} This marked the beginning of olefin metathesis as a potential new chemical synthetic methodology.

Since then, ring closing metathesis reactions have been widely employed in organic synthesis\textsuperscript{117,118}. However, of prime importance is the type of catalyst used. A well defined catalyst should offer flexibility over a wide range of functional groups and at the same time be reactive towards a range of substrates leading to high yields in reactions involving a wide range of functional groups. Two such catalysts have been widely used to date, the Schrock catalyst 112\textsubscript{a} and Grubbs’ catalysts, 112\textsubscript{b} and 112\textsubscript{c}. They have a major advantage over the previously used catalysts for olefin metathesis reactions in that they are highly active, are longer lasting and do not require Lewis acids, co-catalysts or promoters\textsuperscript{114}.

The ruthenium-carbene\textsuperscript{114} catalysts 112\textsubscript{b} and 112\textsubscript{c} are now preferred over the molybdenum complex catalyst 112\textsubscript{a}. The molybdenum complex possesses some shortcomings, which include poor functional group tolerance, high sensitivity to moisture, air and impurities present in solvents, thermal instability, and high production costs. However, the molybdenum complex catalyst has reactivity over a spectrum of substrates, which have steric and electronic variations\textsuperscript{114}.

\begin{center}
\includegraphics[width=\textwidth]{catalysts.png}
\end{center}
The ruthenium-carbene catalysts show reactivity towards a variety of ring closing metathesis processes under mild conditions and exhibit tolerance towards a range of organic functional moieties\textsuperscript{119}. Unlike their molybdenum based counterpart, the sensitivity of the ruthenium catalysts are not significantly reduced when exposed to air, moisture, or minor impurities.

\begin{center}
\textbf{Scheme 3.15-} The catalytic cycle showing the mechanism of ring closing metathesis\textsuperscript{111,112}.
\end{center}

They can be stored over a period of weeks without showing severe signs of decomposition and they are readily available and easy to use. The only negative
aspect is that they often show relatively lower rates of propagation, particularly with sterically bulky substrates\textsuperscript{120}.

The mechanism for ring closing metathesis follows a process involving the formation of a metallacyclobutane which is a key intermediate in these ring closing metathesis reactions. The metallacyclobutane intermediate formed can undergo cycloreversion either to products or back to starting materials. When the olefins of the substrate are terminal, the driving force for ring closing metathesis in this case is the removal of ethene from the reaction mixture\textsuperscript{117,118} (Scheme 3.15).

\textbf{3.2.4. Current project and metathesis reactions}

\textit{Ring closing metathesis}

Ring closing metathesis plays an important role in the synthesis of complex molecules\textsuperscript{111,113,121,122} which incorporate annular double bonds. It offers efficient routes in which the numbers of chemical transformations are minimal. In relation to the current project, it offered a facile route to novel benzo[b]thiophene compounds which had potential for further chemical manipulation at the resultant olefinic functionality. In the syntheses of novel fused benzo[b]thiophene compounds, ring closing metathesis is preferable to alternative processes in terms of ease of availability of substrates, number of steps, synthetic flexibility, the purity of products and yields.

Ring closing metathesis was considered for the synthesis of a number of target compounds incorporating the benzo[b]thiophene moiety in a range of molecular environments. Of the simpler molecules, 78 and 79 were potential candidates for intramolecular ring closing metathesis reactions as the two allyl groups were spaced in a manner that would easily afford a 5-membered ring after metathesis.
For reactions involving these compounds, benzylidene-bis(trichlorohexylphosphine)dichlororuthenium (112c), commonly referred to as Grubbs’ catalyst (or Grubbs’ I catalyst), was used. In the literature, 5-10 mol % of Grubbs’ I catalyst is generally used for the ring closing reaction with dry dichloromethane as the solvent. For reactions involving 78 and 79 (Scheme 3.16), 10 mol % of Grubbs’ I catalyst was employed and the reactions stirred for 20-22 hours at room temperature in dry dichloromethane under an inert atmosphere with removal of ethene. The reactions were monitored by thin layer chromatography and interestingly both reaction mixtures developed a green colouration. Workup of these reactions was smooth requiring only a few purification steps. Filtration of the reaction mixture followed by flash chromatography using dichloromethane afforded the expected dihydropyrrole derivatives 113 and 114 in good yield of 73 % and 80 % for respectively. It was predicted that the new compounds may be both photo- and air sensitive and consequently they were stored at low temperature under nitrogen and in the dark. Full spectroscopic characterization was obtained. In the 1H-NMR of 113 and 114 the olefinic protons were assigned to signals at 4.31 and 4.48 ppm (2x CH2) with the corresponding signals at 5.79 and 5.93 ppm (2x CH). In the 13C-NMR signals at δ 161.7 were assigned to the amide carbonyl groups in 113 and 114 respectively.

Scheme 3.16- Ring closing metathesis of a diallyl benzo[b]thiophene compound to the corresponding dihydropyrroles.
Polymer bound Grubbs’ I catalyst\textsuperscript{122} has also been widely used in ring closing metathesis reactions with a profound effect on the purity and yield of cyclised products\textsuperscript{124}. This catalyst had several advantages over its homogeneous counterpart in that it was reusable up to three times without adversely affecting yield. The ring closing metathesis reaction using 78 as a substrate was attempted with the use of the polymer bound Grubbs’ I catalyst under the same conditions used with the homogeneous catalyst (Scheme 3.16).

Filtration of the reaction mixture after 22 hours gave a colourless solution containing the product. Evaporation of the dichloromethane gave colourless oil that solidified on standing. The product 113, was purified by flash chromatography to remove trace ruthenium contaminants. A comparison between the two forms of the catalyst showed that the polymer bound variety gave a better yield of 85 % \textit{versus} 73 % of 113 and a much cleaner product and, importantly, the catalyst was recovered.

The higher yield of 113 with the use of a polymer supported ruthenium catalyst could be attributed to the greater stability of the polymer supported catalyst, compared to the homogeneous variety that is susceptible to decomposition under normal atmospheric conditions. Comparisons of reaction residues derived from polymer bound catalyst \textit{versus} those from the homogeneous catalyst were informative, with those from the homogeneous catalyst being highly coloured and hence requiring purification by silica chromatography, while the polymer supported product solution was almost colourless after filtration.

\textbf{Ring closing metathesis of molecules containing a tetrahydroisoquinoline motif}

The successful synthesis of the above mentioned new benzo[\textit{h}]thiophene compounds with dihydropyrrole moieties paved the way for the synthesis of
benzo[b]thiophene systems with larger rings. The initial goal was to attempt the synthesis of a 9-membered ring compound (of type E as mentioned in Chapter 1 under project aims) via ring closing metathesis reaction. The cyclisation precursor under investigation needed allyl groups at the C3 position of the benzo[b]thiophene ring and at the C1 position of the 1,2,3,4-tetrahydroisoquinoline ring moiety in the amide. It was therefore necessary to synthesize a benzo[b]thiophene acid with an allyl group at the C3 position that could then be coupled to \( \text{32} \) to give the cyclisation precursor \( 2-(3\text{-allylbenzo}[b]\text{thiophen-2-oyl})\text{-1-allyl-1,2,3,4-tetrahydroisoquinoline (118)} \) (Scheme 3.17). A literature search indicated that no previous work had been conducted on allylation of the C3 position of the benzo[b]thiophene ring system.

The allylation of the C3 position of the benzo[b]thiophene ring was attempted using a Stille coupling method. The conditions used were adapted from a literature method for the reported synthesis of new dibenzothiophene amino acid and cyclophane derivatives\(^\text{125}\), which involved an allyl-aryl intermediate. The starting point for these reactions was the bromination of \( \text{20} \) to afford \( \text{25} \) (Scheme 2.3) which was then converted to its methyl ester to protect the carboxylic acid functionality.

The substitution of the C3 bromine with an allyl group to give methyl 3-allylbenzo[b]thiophene-2-carboxylate \( \text{(116)} \) was attempted using a Stille coupling method in dimethylformamide with 1 mole equivalent of \( \text{115} \) and allyl tributyltin and a half mole equivalent of palladium(II) chloride in the presence of triphenylphosphine (Scheme 3.17).
Scheme 3.17- Synthesis of precursors 118 and 119 for the 9-membered ring compounds through ring closing metathesis.

The reaction was carried out under an inert atmosphere at 110 °C in a sealed tube. These conditions were maintained for one and a half days while the progress of the reaction was monitored using thin layer chromatography (Scheme 3.17). This reaction gave a crude residue which was difficult to workup due to the formation of triphenylphosphine oxide and the presence of tin by-products. Simple filtration was insufficient to eliminate the tin by-products. After a series of chromatographic
purification steps the desired product, methyl 3-allylbenzo[b]thiophene-2-carboxylate (116), was isolated as an oily product in moderate yield (50 %). A trace amount of the starting material was also detected. The structure of 116 was confirmed using spectroscopic techniques. The $^1$H-NMR showed the presence of characteristic signals for the allylic protons at 4.15 (d, $J = 4.2$ Hz, -CH$_2$), 5.04-5.13 (m, 2H, = CH$_2$) and 5.97-6.06 (m, CH), as well as the expected relative integral for signals in the aromatic region. The methyl and methylene protons were confirmed using a DEPT spectrum. The $m/z$ value for the MH$^+$ ion in the high-resolution mass spectrum was consistent with the molecular formula for 116.

The methyl 3-allylbenzo[b]thiophene-2-carboxylate (116) was then hydrolysed to the corresponding acid 117 in high yield (93 %) by heating at reflux in 2 % LiOH solution in tetrahydrofuran, followed by acidification (Scheme 3.17). Evidence for the hydrolysis was apparent in the $^1$H-NMR spectrum of 117 with the loss of the signal for the methyl ester protons. The other peaks remained similar for both the product and the substrate except for a broad singlet resonating downfield at 11.0 ppm consistent with the carboxylic acid proton.

The final step in the synthesis of the cyclisation precursor 118 was the DCC/HOBt mediated coupling of 3-allylbenzo[b]thiophene-2-carboxylic acid (117) with the 1-allyltetrahydroisoquinoline 32 (Scheme 3.17). The tetrahydroisoquinoline 118 was obtained in 50 % yield. The $^1$H-NMR spectrum of 118 was complicated owing to the presence of amide rotamers. Amongst the characteristic proton signals for 118 was the signal associated with the H1 proton at 4.85 ppm and the signals for the H3 and H4 methylene protons on the tetrahydroisoquinoline ring at 3.50ppm and 3.66 ppm respectively. The allyl groups gave overlapping signals but the relative integration was consistent for two such groups. The DEPT NMR spectrum confirmed the
expected number of methylene and methine groups. The $^{13}$C-NMR gave a signal ascribed to the carbonyl carbon at 165 ppm.

A simple molecular modelling study performed on 118 using AM1 as the minimiser suggested that the two allyl groups were disposed geometrically in a manner that would facilitate the ring closing metathesis.

![Figure 3.2](image_url)

**Figure 3.2-** Orientation of the allyl groups on the cyclisation precursor 118 showing their closeness in space, indicating their ease to ring close leading to the 9-membered product 120.

Excellent orbital overlap could be achieved using simple allyl aliphatic bond rotation from a descent appropriate local minimum (Figure 3.2).

For the final step, the precursor 118 was taken up in dry dichloromethane together with 10 mol % of polymer supported Grubbs’ I ruthenium catalyst. The reaction was carried out under argon at room temperature for a period of 15 hours (Scheme 3.18). Workup was relatively easy involving the recovery of the catalyst by
filtration and thin layer chromatographic analysis of the filtrate indicated a single major product (Rf 0.4) was formed. This product was isolated chromatographically and shown to be the novel fused 9-membered ring compound 120. The compound was obtained in 78 % yield.

\[ \text{Scheme 3.18-} \text{ Formation of a 9-membered ring system through ring closing metathesis.} \]

The mass spectrum of 120 showed a peak at \( m/z \) 346 (MH\(^+\)) consistent with the expected cyclised compound 5,8,16,17-tetrahydroazonino[3,4-b]benzo[b]thieno[3,2-a]isoquinolin-14(4bH)-one (120). The \(^1\)H-NMR spectrum of 120 was not as complicated as the corresponding spectrum of its precursor due in part to the absence of lactam rotamers in the ring-closed product. The olefinic protons in the 9-membered ring was 5.99 ppm (H7) and 6.17 ppm (H6), consistent with a \( cis \) double bond; no evidence of the \( trans \) isomer was seen. Preliminary computational studies using
AM1\textsuperscript{96} minimisation suggested that the \textit{cis} arrangement of the olefinic double bond in the ring was about 5 kcal mol\textsuperscript{-1} more stable than its \textit{trans}-counterpart (Figure 3.3).

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure3.3.png}
\caption{3D representations of local AM1\textsuperscript{96} minima found for the \textit{cis} (A, B) and \textit{trans} (C, D) isomers for compound 120.}
\end{figure}

This appears to be in part due to the steric strain induced on the tertiary carbon adjacent to the amide nitrogen. In the case of the \textit{trans} conformer there is a larger deviation from a tetrahedral geometry for this carbon than there is in the same carbon for the \textit{cis} isomer. Furthermore, there is considerable orbital overlap involved in the
C-N amide bond and the *trans* olefinic bond with the centroid-centroid distance between the bonds being only 2.60 Å, as opposed to the analogous distance in the *cis* isomer of 3.7 Å.

The compound 120 was recrystallised from ethanol to give colourless rhombic crystals which were submitted for X-ray crystallographic analysis by Professor A.H. White, University of Western Australia. The X-ray data confirmed the *cis* double bond at C(13) and C(14) as shown in Figure 3.4. The compound 120, which is representative of a new heterocyclic system, was tested for biological activity as an antimalarial agent, a topic that is discussed in more detail in Chapter 4.

**Figure 3.4** - The single crystal X-ray structure of 120 confirming the *cis* nature of the olefinic double bond C(13) and C(14). Note: The X-ray crystallographic numbering is different from the systematic numbering.
In order to assess substituent effects on biological activity, the synthesis of the dimethoxy analogue of 120 was also investigated. The benzo[b]thiophene-based precursor 119 for the ring closing metathesis reaction was prepared from 117 (Scheme 3.17) and the 6,7-dimethoxytetrahydroisoquinoline 34. In the initial synthesis of the amine 34, (Scheme 2.7) the commercially acquired 33 was converted in good yield (75 %) to the corresponding 6,7-dimethoxy-3,4-dihydroisoquinoline 37 using NBS. However, the allylation of the imine 37 using the allyl bromide/ zinc approach was low yielding (42 %). Alternative reagents were thus investigated for the allyl group addition to 37 (Scheme 3.19). Allyltributyltin has been reported to react readily with aldimines to give homoallyl amines in good yield with Lewis acid catalysis\textsuperscript{126,127}. Thus initial studies in the current work focussed on the use of boron trifluoride etherate as the Lewis acid for the addition of allyltributyltin to 37.

\textbf{Scheme 3.19-} The boron trifluoride etherate/ allyltributyltin approach to compound 34.
Boron trifluoride etherate was added to the imine 37 in dry dichloromethane at low temperature (-78 °C), followed by brief warming of the reaction mixture to near room temperature and then re-cooling to -78 °C before the addition of the allyltributyltin was commenced (Scheme 3.19). However, none of the 1-allyl-6,7-dimethoxy-tetrahydroisoquinoline (34) was formed in this reaction. Interestingly, when activated zinc powder was added to the reaction mixture including boron trifluoride etherate, the desired product 34, was obtained in good yield (80 %).

It is possible that, diallylzinc may have been generated \textit{in situ} from the allyltributyltin and it was this reagent that was responsible for the addition of the allyl group to the BF$_3$-complexed imine moiety as shown in Scheme 3.20. Trace amounts of the amine 33 were also observed, and the formation of this compound may have been due to the reduction of the imine 37 by the elemental zinc. The combination of zinc, allyltributyltin and boron trifluoride etherate does not appear to have been reported previously for allyl additions to imines and is worthy of further investigation to ascertain its wider synthetic utility.

\[ 2 \text{Bu}_3\text{Sn} + \text{Zn} \rightarrow \text{Zn} \left( \text{alkene} \right)_2 + \text{Bu}_3\text{Sn-SnBu}_3 \]

\[ \text{Zn} \left( \text{alkene} \right)_2 + \text{MeO} \quad \text{MeO} \quad \text{OMe} \quad \text{OMe} \quad \text{BF}_3 \rightarrow \text{HN} \quad \text{HN} \quad \text{OMe} \quad \text{OMe} \quad \text{34} \]

\textbf{Scheme 3.20-} The proposed formation of 34 where, diallylzinc generated \textit{in situ} from allyltributyltin adds the allyl functionality to the BF$_3$-complexed imine.
The DCC/ HOBt coupling of 3-allylbenzo[b]thiophene-2-carboxylic acid \( \text{117} \) with \( \text{34} \) afforded the diallyl amide 2-(3-allylbenzo[b]thiophen-2-oyl)-1-allyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (\( \text{119} \)) in 57 % yield (Scheme 3.17). The cyclisation of \( \text{119} \) using polymer-supported Grubbs’ I catalyst then gave \( \text{121} \) in good yield (Scheme 3.18), and the presence of the cis double bond in the cyclised product was confirmed by \(^1\text{H}-\text{NMR} \) spectroscopic analysis.

The synthesis of 8-membered ring benzo[b]thiophene-fused analogues of \( \text{120} \) compounds was also considered. One approach to achieve this was to incorporate a vinyl group at the C1 position of the tetrahydroisoquinoline ring portion of the amide precursor for the ring closing metathesis. The synthesis of this isoquinoline precursor for the 8-membered compound was attempted in a one pot reaction. This involved the reaction of 3,4-dihydroisoquinoline with vinylmagnesium bromide in diethyl ether at low temperature, followed by the addition methyl 3-allyl-benzo[b]thiophene carboxylate (\( \text{116} \)) (Scheme 3.21).

\[ \text{Scheme 3.21- The attempted synthesis of the vinyl substituted derivative 122.} \]
The expected product 2-(3-allylbenzo[b]thiophen-2-oyl)-1-vinyl-1,2,3,4-tetrahydroisoquinoline (122) was not isolated after workup of this reaction however only trace amounts of the starting material were observed. Due to time constraints, the reaction using this pathway was only attempted once and was not pursued further.

In summary, the ring closing metathesis reaction was demonstrated to be a feasible method for the synthesis of compounds with 9-membered ring annulation to the benzo[b]thiophene nucleus.

3.2.5. Palladium-Catalysed Cyclisations

Cyclisation reactions mediated by palladium are widely used in synthetic organic chemistry, including the synthesis of heterocyclic systems\textsuperscript{128}.

Apart from the major role played by palladium in hydrogenation and oxidation reactions, it also acts as a catalyst for numerous C-C bond formation reactions. There are three key areas where palladium is used efficiently; the reaction of palladium cation complexes with nucleophiles\textsuperscript{127}, the cross coupling\textsuperscript{129} of organometallic reagents (such as organotin, organoboron and organozinc reagents) with organic halides\textsuperscript{130-133} (e.g. Stille coupling; Scheme 3.22), and the reaction of organic halides (usually aryl/ alkenyl halides) with olefins\textsuperscript{134}.
The catalyst in these reactions is usually a Pd(0) species. However, this is usually generated \textit{in situ} from a Pd(II) salt by reduction with the organometallic reagent, solvent or some other species in the reaction mixture. The oxidative addition of aryl halides and triflates to a Pd(0) species inverts the relative activities of aryl and alkyl halide systems, i.e. in nucleophilic displacement reactions it is expected that the alkyl halide would be more reactive, but in the palladium catalysed reactions the alkyl halides are unreactive.
3.2.6. Palladium-promoted ring closure

Palladium-catalysed cyclisation through C-C bond formation was also investigated in this project because it opened up a way to the preparation of novel fused-derivatives with a benzo[b]thiophene framework.

In recent years, polycyclic compounds bearing an indole ring within their motif have been of immense interest to medicinal chemists. Many of these polycyclic compounds have been successfully synthesised by intramolecular dehydrogenation of aroylindoles with palladium(II) acetate\textsuperscript{136-140}. A similar approach was attempted in this project where highly constrained and planer molecules of benzo[b]thiophene were required (molecules of type (D); as mentioned in Chapter 1 under project aims). The specific target was the new heterocyclic derivative \textbf{126} (Scheme 3.23).

The acid chlorides \textbf{26} and \textbf{27} required for these reactions were synthesised from their precursor benzo[b]thiophene carboxylic acids \textbf{20} and \textbf{25} as explained in chapter two (Scheme 2.4). The acid chlorides were achieved in good yield and were used immediately due to their tendency to hydrolyse.

The coupling of benzo[b]thiophene-2-carbonyl chloride (\textbf{26}) and 3-bromobenzo[b]thiophene-2-carbonyl chloride (\textbf{27}) with indole \textbf{123} was performed after the indolic proton was removed with sodium hydride at low temperature; this afforded the (benzo[b]thiophen-2-oyl)(1H-indol-1-yl)methanone (\textbf{124}) and (3-bromobenzo[b]thiophen-2-yl)(1H-indol-1-yl)methanone (\textbf{125}) cyclisation precursors respectively (Scheme 3.23). Cyclisation reactions with palladium(II) acetate were investigated first with the C3-unsubstituted benzo[b]thiophene derivatives.
Scheme 3.23- The intramolecular ring closure of 124 and 125 by palladium acetate.

The reaction of 26 with indole 123 in dry dimethylformamide and sodium hydride afforded the N-acylated indole 124 in good yield (71 %) after flash column chromatography (Scheme 3.23). The $^1$H-NMR of 124 had characteristic doublet signals at 7.28 ppm and 6.71 ppm for the indolic H2 and H3 protons respectively and a singlet at 7.90 ppm ascribed to the benzo[b]thiophene H3 proton.

The aromatic protons for the benzo[b]thiophene appeared further downfield than those of the indole; however the peak integration for the total number of aromatic protons expected was consistent with the proposed structure 124. The $^{13}$C-NMR signals were consistent with the number of carbons expected and the presence of an amide carbonyl carbon peak at 164.3 ppm was also noted.
Figure 3.5- Two views of a ball and stick representation of an AM196 local minimum conformation of 126.

The oxidative cyclisation of 124 was achieved using 1 mol. equivalent of palladium(II) acetate in glacial acetic acid at reflux for 2 days. The workup was difficult owing to the presence of metallic palladium in the reaction mixture; however, upon purification using column chromatography and preparative TLC, an intense orange product (14 % yield) was isolated whose structure was assigned as 126. The intense colour was consistent with the extensively conjugated π-electron system in 126. An AM196 local minimum-derived molecular model showed that 126 was highly planar (Figure 3.5). An analysis of the mass spectrum showed the presence of a peak at $m/z$ 276 (Cl⁺), consistent with the MH⁺ ion peak expected for 11H-benzo[b]thieno[2,3-c]pyrrolo[2,3-a]indol-11-one (126).

Further structural confirmation of the isolated cyclised product 126 was established by ¹H-NMR in which the H5 proton appeared as a sharp singlet at 6.57
ppm. The $^{13}$C-NMR spectrum of 126 lacked signals which could be assigned to the C2 and C3 methine peaks formerly associated with the indole ring as well as the C3 methine in the benzo[b]thiophene moiety. A new methine carbon signal was apparent at 102.1 and this was ascribed to the C5 carbon. High-resolution mass spectrometry supported the molecular formula for 126. The UV-visible absorption spectrum of 126 showed a moderate absorption band at $\lambda_{\text{max}}$ 264 nm together with stronger bands at $\lambda_{\text{max}}$ 278 nm and 283 nm as might be expected for $\pi \rightarrow \pi^*$ excitations. A weak and broad peak at $\lambda_{\text{max}}$ 369 nm was also observed possibly due to an $n \rightarrow \pi^*$ excitation.

![UV/Visible absorbance spectrum for 126 in absolute ethanol.](image)

**Figure 3.6-** UV/Visible absorbance spectrum for 126 in absolute ethanol.

The fore mentioned reaction sequence (Scheme 3.23) was repeated under the same conditions but using the 3-bromo substituted acid chloride 27 as the starting material. The synthesis of the 3-bromobenzo[b]thiophene-2-carbonyl chloride (27)
was achieved in good yield (67 %) and the acid chloride was used immediately after isolation due to its tendency to hydrolyse. The coupling of 27 with 123 followed a similar route as that mentioned previously (Scheme 3.23), however the cyclisation precursor, 3-bromobenzo[b]thiophen-2-yl)(1H-indol-1-yl)methanone (125) was isolated in only 20 % yield after flash column chromatography. Steric hindrance associated with the bromo- substituent may account for the lower yield in this case.

The structural elucidation of 125 was achieved by comparison of the $^1$H-NMR spectrum with that of 124 where the indolic C2 and C3 proton signals appeared as a doublet at 7.2 ppm and a doublet of doublets at 6.5 ppm respectively. The $^{13}$C-NMR spectrum indicated the relevant number of carbons expected for 125, and a key signal at $\delta$ 164.3 ascribed to the carbonyl group. The peaks observed at 125.5 ppm and 102.7 ppm were assigned to the C2 and C3 indolic carbons respectively. The mass spectrometric data (CI$^+$) revealed a molecular ion peak at $m/z$ 356 (MH$^+$) with characteristic isotopic peaks for the presence of one bromine. A fragmentation peak at $m/z$ 276 associated with the loss of the bromine was also seen. Finally, the molecular formula was determined via high resolution mass spectroscopy and this was consistent with 125 (Scheme 3.23).

The precursor 125 was cyclized in the presence of palladium(II) acetate in boiling glacial acetic acid to give 11H-benzo[b]thieno[2,3-c]pyrrolo[2,3-a]indol-11-one (126) in 23 % yield. From these two approaches to the palladium catalysed benzo[b]thiophene/ indole derivative 126 it was clear that the use of the C3 unsubstituted compound gave better yields of the cyclised product, probably due to a slower attack by the 6-arylpalladium(II) complex intermediate on the hindered benzo[b]thiophene C3 position in the case where $X = \text{Br}$ (Scheme 3.23).
Scheme 3.24- The proposed pathway for the cyclisation to 126, showing possible intermediates.

The low yield observed with the synthesis of the cyclised compound 126 led to further optimisation studies. It had been demonstrated previously by others that these palladium(II)-mediated oxidative cyclisation reactions become catalytic in palladium with the re-oxidation of Pd(0) to Pd(II) with addition of an oxidising agent such as copper(II) acetate\textsuperscript{138,139}.
Scheme 3.25- The attempted synthesis of 126 in the presence of Cu(II) ions with aim to re-oxidise Pd(0) to Pd(II) and hence favor the forward reaction.

The palladium-mediated cyclisation of (benzo[b]thiophen-2-yl)(1H-indol-1-yl)methanone (124) was thus attempted (Scheme 3.25) with a catalytic amount of palladium(II) acetate and 2.5 equivalents of copper(II) acetate in a mixture of glacial acetic acid/ acetic anhydride. The reaction mixture was heated at reflux for 24 hours but no product was isolated. Thin layer chromatographic analysis of the reaction mixture showed only the presence of the starting material, and this was confirmed by mass spectrometric analysis. Even after extended periods of time, there was no indication of product formation (Scheme 3.25).

In summary, the palladium(II) oxidative coupling reactions involving the benzo[b]thiophene skeleton afforded a pathway to a novel heterocyclic system, however, further study is required to improve the yields.
Chapter 4 Biological Testing

The mind-boggling number of compounds with characteristics of drug-like molecules that can be synthesized means that many tools are needed to select those compounds of greatest biological benefit.

*Sophie Petit-Zeman

Introduction

Biological testing of the benzo[b]thiophene amides synthesised as discussed in Chapters 2 and Chapter 3 was undertaken to determine antimalarial activity and to a lesser extent, antibacterial activity. The *in vitro* antimalarial studies were carried out by Dr Sumalee Kamchongwongpaisan at the BIOTEC, NSTDA research laboratories in Thailand, while the antibacterial studies were undertaken through Dr. J. Deadman, Avexa Ltd, Melbourne (formally Amrad).

The antimalarial activities are reported as IC$_{50}$ values in micrograms per millilitre (µg/mL) and as micro-molar (µM) values. The IC$_{50}$ value is a measure of the concentration required to kill 50% of the microbes being tested, in this case the *Plasmodium falciparum* protozoan. The range of benzo[b]thiophene derivatives synthesised during the course of this study exhibited a wide range of biological activity data which in turn provided valuable information on the essential structural requirements for greater activity. This led to the design and synthesis of new molecules which provided further information regarding structure activity relationships.

4.2 Antimalarial testing

4.2.1. Test procedures

Although a study of the synthesis of the compounds presented in this project is in itself of value, the primary purpose for their preparation, in this case, was to investigate their activity as antimicrobial, and in particular, antimalarial agents.
The novel benzo[b]thiophene amide derivatives were tested for *in vitro* activity against two particular strains of the protozoan *Plasmodium falciparum*. These strains were the double mutant K1 CB1, which is a multidrug resistant/antifolate resistant strain, and the wild type TM4/8.2, an antifolate sensitive strain. In this chapter the two parasite strains will be referred to as K1 and TM4 respectively.

### 4.2.2. *In vitro* antimalarial activity of non-fused benzo[b]thiophene amides

The non-fused benzo[b]thiophene amides were synthesized with a framework (Figure 4.1) incorporating a Cl, Br, H or allyl group in the C3 position (X in Figure 4.1) of the benzo[b]thiophene moiety and a ring structure limited largely to a 6-membered ring moiety.

![Figure 4.1- The generic benzo[b]thiophene amide structure.](image)

The lead molecule 30 showed encouraging *in vitro* activity against both strains of *P. falciparum* (Table 4.1; structures are shown in the following fold out sheet and they are arranged in decreasing order of activity) leading to the development of a strategy to discover and identify a second generation of non-fused benzo[b]thiophene amide derivatives which retained the desirable attributes of the lead but with improved potency.
Table 4.1- Antimalarial activities of the non-fused benzo[h]thiophene amide analogues

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Antimalarial activity against the two strains of <em>P. falciparum</em>. (IC₅₀) values reported in µM and µg/mL.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K₁</td>
</tr>
<tr>
<td>30</td>
<td>13.00</td>
</tr>
<tr>
<td>29</td>
<td>16.77</td>
</tr>
<tr>
<td>67</td>
<td>18.20</td>
</tr>
<tr>
<td>81</td>
<td>18.39</td>
</tr>
<tr>
<td>80</td>
<td>20.00</td>
</tr>
<tr>
<td>60</td>
<td>22.70</td>
</tr>
<tr>
<td>54</td>
<td>28.89</td>
</tr>
<tr>
<td>76</td>
<td>37.10</td>
</tr>
<tr>
<td>59</td>
<td>43.06</td>
</tr>
<tr>
<td>70</td>
<td>46.92</td>
</tr>
<tr>
<td>52</td>
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<td>71</td>
<td>58.71</td>
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<tr>
<td>84</td>
<td>65.97</td>
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<tr>
<td>82</td>
<td>71.3</td>
</tr>
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<td>56</td>
<td>74.40</td>
</tr>
<tr>
<td>66</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>72</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>73</td>
<td>&gt; 50</td>
</tr>
<tr>
<td>75</td>
<td>&gt; 20</td>
</tr>
</tbody>
</table>

Note: For clarity purposes, the standard deviation figures for the IC₅₀ values are not indicated here; these values are given in the Appendix.
The synthesis of benzo[b]thiophene amides with methoxy substituents on the tetrahydroisoquinoline moiety at the C6 and C7 positions (amides, 51 and 50) did not seem to improve activity relative to the non-methoxylated parent which showed an activity greater than 20 µM against the K1 strain of the parasite.

The allyl substituted compounds 52 and 53 had weak activity but the bromo-substituted amide 53 exhibited double the activity of the chloro-substituted analogue 52. For comparison purposes, the amide 54 which had no halogen at the C3 position was tested and it showed mild activity against the K1 and the TM4 strains and exhibited similar IC\(_{50}\) values as those for 53 (22.70 µM and 23.50 µM respectively) (Table 4.1).

The presence of a bulky group in the nitrogen containing moiety seemed to increase biological activity as seen in the case of compounds 80 and 81; this group is hydrophobic and hence could increase membrane permeability. Even though the activity for these benzo[b]thiophene amides was not high, a few differences in results were observed, and it is noticeable that while the activities for amides 80 and 81 against the K1 strain have a similar IC\(_{50}\) range, the chloro-substituted derivative 80 showed higher potency (14.3 µM) in activity against the TM4 strain.

The incorporation of an N-benzyl substituent in the piperazine based amide 67 slightly improved the activity (16.77 µM) relative to the parent molecule 63 containing the bulkier group (18.39 µM). Further attempts to improve potency in the piperazine derivatives by attaching substituted aromatic rings (p-chlorophenyl) as in amides 70 and 71 dramatically decreased the activity. The fmoc-protected piperazine 76, which contains a bulky hydrophobic substituent, showed better activity against the TM4 parasite (15.4 µM) than against the K1 strain (23.3 µM).

The much more conformationally constrained bicyclic amide 85 was of interest in this class of piperazines; however, amide 85 had a similar potency (19.1 µM) to that
observed for the boe protected amides. It might thus be deduced that steric constraints were affecting activity in these amides. The activity of the piperidine derivative 82, which lacked a nitrogen at position 4 in the 6-membered ring but had a hydrophilic hydroxyl group at this position, was not promising. The IC$_{50}$ value against the two *Plasmodium* strains for this amide increased to values greater than 50 µM.

The tetrahydrocarbazole-based amides 55 and 56 were also tested and had similar activities. As mentioned in Chapter 2, the indolic proton was substituted with a benzyl moiety giving amides 59 and 60, and preliminary testing indicated a large improvement in activity from IC$_{50}$ values of 65.97 µM to 28.39 µM for the chloro-substituted amide and 74.40 µM to 20.00 µM for the bromo-substituted derivative.

The improvement in activity indicated that an electron rich substituent was vital for activity for these amide derivatives. Researchers at GlaxoSmithKline reported$^{141,142}$ the synthesis of some structurally analogous 2,9-disubstituted 1,2,3,4-tetrahydropyrido[3,4-b]indoles (A and B in Figure 4.2) as inhibitors of bacterial enoyl acyl carrier protein reductase (FabI)$^{141,142}$. However, no antimalarial activity was reported for these compounds.
Figure 4.2- Compounds A and B were reported\textsuperscript{141,142} by Burgess and Miller as inhibitors of FABI, and bear a partial resemblance to compounds C which were synthesised during this project.

Even though the substituents on the benzyl moiety of the compounds vary, their partial resemblance to those synthesised during this project suggested that the benzo[b]thiophene amides also acted on the same biological target. A further substitution of the benzyl functionality in compound 59 with a nitro group at the ortho position to give 61 saw a 10 fold improvement in activity from 28.89 µM to 18.30 µM, but an overall large improvement compared to the parent compound 55 (65.97 µM) to 61 (18.30 µM) in the case of the K1 strain. The change in activity value ranged similarly for the TM4 strain when compared with the K1 strain.
The novel dihydropyrrole amides 113 and 114 (Scheme 3.14), only exhibited weak activity against both strains of *Plasmodium falciparum* (> 50 µM).

From the biological test results (Table 4.1) it is clear that the benzo[b]thiophene derivatives of the bromo-substituted series generally exhibited a slightly better antimalarial activity profile compared to their chloro-substituted counterparts. The increased hydrophobicity of the bromo substituent may enable increased membrane permeability. This is highlighted by the difference in activity observed between amides 66 and 67 where the bromo- substituted derivative (67) is 9 times more potent than the analogous chloro- substituted derivative (66) against the K1 strain parasite.

**Possible mode of action**

The mode of action for the non-fused benzo[b]thiophene amides that exhibited *in vitro* activity against the two strains of *P. falciparum* remains unclear. It is possible however that some of these compounds may be acting as inhibitors of fatty acid biosynthesis by blocking the enoyl acyl carrier protein reductase (FabI)\(^{27,69}\), based on their partial resemblance to some known inhibitors of this bacterial enzyme.

The enoyl acyl carrier protein reductase enzyme is almost exclusively found in bacteria and is crucial in catalysing the ultimate and rate-limiting step of fatty acid synthesis\(^{69,141}\). The *Plasmodium* parasite also possesses this enzyme in the apicoplast and it is possible that some of the benzo[b]thiophene compounds for example those with tetrahydrocarbazole functionality could selectively act against the *Plasmodium* malarial parasite through inhibition of this enzyme. The crystal structure of the enoyl acyl carrier protein reductase complexed to the antimalarial triclosan has been reported\(^{27,69}\) (Figure 1.3). Time constraints prevented further assessment of molecular
modelling studies to the tetrahydro-\(\beta\)-carboline amides and docking in this structure in the context of enzyme inhibition studies.

Figure 4.3- Crystal structure of *Plasmodium falciparum* enoyl-ACP reductase (pfENR) complexed with an antimalarial inhibitor (triclosan- a known FabI inhibitor).
4.2.3. *In vitro* antimalarial activity for the fused benzo[b]thiophene amides

Figure 4.4 - The generic structure for the fused benzo[b]thiophene amide derivatives.

As mentioned previously in Chapter 3, the underlying principle behind the synthesis of the fused benzo[b]thiophene amide derivatives was to assess the effects of increased conformational restriction on biological activity. The ring-fused derivatives were based on the generic structure shown above (Figure 4.4).

Table 4.2- Antimalarial activities of the fused-ring benzo[b]thiophene amides.

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Antimalarial activity against the two strains of <em>P. falciparum</em> (IC$_{50}$) values reported in µM and µg/ mL.</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>K1</td>
<td>KM4</td>
</tr>
<tr>
<td></td>
<td>µM</td>
<td>µg/ mL</td>
</tr>
<tr>
<td>105</td>
<td>3.63</td>
<td>2.01</td>
</tr>
<tr>
<td>92</td>
<td>3.90</td>
<td>1.00</td>
</tr>
<tr>
<td>106</td>
<td>14.20</td>
<td>3.08</td>
</tr>
<tr>
<td>120</td>
<td>&gt; 20.00</td>
<td>-</td>
</tr>
<tr>
<td>96</td>
<td>&gt; 100.00</td>
<td>-</td>
</tr>
</tbody>
</table>

Note: For clarity purposes, the standard deviation figures for the IC$_{50}$ values are not indicated here; these values are given in the Appendix.
The fused benzo[b]thienopyridone 92 showed very promising antimalarial activity against the two strains of *Plasmodium falciparum* (IC$_{50}$ 3.90 µM, 3.70 µM respectively). Compound 92 was only tested as a racemate. It is possible that one of the enantiomers may be more potent than the other. However due to time constraints the enantiomers were not prepared and tested.

The allyl group in compound 92 offered a point for further functionalisation with the potential to substitute with hydrophobic (benzyl and substituted benzyl) moieties as well as hydrophilic groups through either de-allylation, N-substitution or asymmetric dihydroxylation. When 92 was transformed to 105, there was an overall improvement in potency (Table 4.2). The methoxy group in 105 may serve as a H-bond acceptor group at the ether oxygen or as a hydrophobic group. The compound 105 was identified in this project as the most potent antimalarial (K1; 3.63 µM and TM4; 3.32 µM) against both strains of *Plasmodium falciparum*.

The unsubstituted benzyl derivative 104, although synthesised, was not tested due to time constraints. The removal of the hydrophobic allyl group in compound 92 led to a loss of potency (compound 106, K1; 14.20 µM and TM4; > 20 µM). When hydroxylic groups were introduced in 92, to give the corresponding diol 96, a drastic reduction in activity was observed. This decrease in potency indicated that hydrophilic
groups on 96 were unfavourable, through a reduction in membrane permeability or through steric interactions that are unfavourable for appropriate drug-target interaction.

Unfortunately when the benzo[b]thiophene amide moiety was constrained in a 9-membered ring, as in 120, the antimalarial activity was greatly diminished with IC$_{50}$ values greater than 100 µM against both strains of the test protozoan.

4.2.4. Potential benzo[b]thiophene compounds for future drug development

Some of the benzo[b]thiophene amides discussed above exhibited very promising in vitro activity against the two strains of *Plasmodium falciparum*. Table 4.3 (fold out; pg 120) identifies benzo[b]thiophene compounds with IC$_{50}$ values of 20 µM or less.

Compounds 105 and 92 had the greatest potency of all these benzo[b]thiophene derivatives (Table 4.3) and they are of particular interest because further chemistry to include different functional groups which could improve their potency, is still possible. These two compounds provide the basis for new antimalarial lead compounds.
Table 4.3- Antimalarial IC_{50} values with a cut off value at < 20µM for the fused and non-fused benzo[b]thiophene amide derivatives.

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Molecular Weight</th>
<th>Antimalarial activity against two strains of <em>P. falciparum</em> (IC_{50}) values reported in µM and in µg/ mL.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>K1 strain</td>
</tr>
<tr>
<td></td>
<td></td>
<td>µM</td>
</tr>
<tr>
<td>105</td>
<td>554.72</td>
<td>3.63</td>
</tr>
<tr>
<td>92</td>
<td>257.35</td>
<td>3.90</td>
</tr>
<tr>
<td>30</td>
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<tr>
<td>29</td>
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<td>13.70</td>
</tr>
<tr>
<td>106</td>
<td>217.29</td>
<td>14.20</td>
</tr>
<tr>
<td>67</td>
<td>415.34</td>
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</tr>
<tr>
<td>81</td>
<td>469.44</td>
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<td>63</td>
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<td>85</td>
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<td>60</td>
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<td>76</td>
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<tr>
<td>50</td>
<td>387.88</td>
<td>46.92</td>
</tr>
</tbody>
</table>

Note: For clarity purposes, the standard deviation figures for the IC_{50} values are not indicated here; these values are given in the Appendix.
The substitution of 29 on the tetrahydroisoquinoline ring with methoxy groups at the C6 and C7 positions to give amide 50 saw a marked reduction in potency. However, other substituent groups at these positions with different electronic activity should still be investigated. The halogen on the C3 position on the non-fused amides played a vital role depending on the ring type and the substitution on the ring system, but generally the bromo-substituted derivatives exhibited better potency.

A difference in potency for the benzo[b]thiophene amides against the K1 strain and the TM4 strain was apparent with some amides. For example compounds 50 (IC$_{50}$ K1; 46.92 µM, TM4; 16.5 µM, 76 IC$_{50}$ K1; 23.20 µM, TM4 15.4 µM) were both more potent against the TM4 strain, while 106 (K1; 14.20 µM, TM4; >20µM) was more active against the K1 strain. Compounds 50 and 76 belong to the class of non-fused benzo[b]thiophene ring system and probably act as antifolates whereas 106 which is a fused analogue, may bind differently to the drug target.

**4.3 Antibacterial testing**

**4.3.1. Test procedures**

The benzo[b]thiophene antibacterial assay studies were carried out on seven amides against the *Staphylococcus aureus* and four strains of *Enterococcus faecium*; two of these strains were vancomycin resistant (VRE # 449 and VRE # 820) and two were sensitive to vancomycin although still designated as VRE (VRE # 243 and VRE # 987). The amides were made up in dimethyl sulfoxide (DMSO) and the first test control was at 2.5 %. The similar tests were also carried out on vancomycin against the same bacterial cultures, and the results used as a reference. The activities are reported as Minimum Inhibitory Concentration (MIC) values, in micrograms per millilitre (µg/ mL). The MIC value is a standard measure of the lowest concentration of the antibiotic that
results in the inhibition of visible bacteria growth under standard conditions staff at Avexa Ltd (formally AMRAD, Melbourne) carried out the antibacterial assays. The results are summarized in Table 4.4 where some of the compounds showing antibacterial activity are highlighted in colour.

The assay results shown in Table 4.4 indicate that the conformationally restricted amide 92 was weakly active against *Staphylococcus aureus*. This amide was of interest as it had been shown to also have good potency when tested as an antimalarial (Table 4.2). The conformationally restricted 9-membered derivative 120 showed weak activity against VRE # 820 even though the MIC cut-off point was 125 µg/mL. The boc-protected amide 63 also showed weak activity against the same culture (VRE # 820) and perhaps further investigations should be conducted.

<table>
<thead>
<tr>
<th>Compound number:</th>
<th><em>S. aureus</em></th>
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<tbody>
<tr>
<td>VRE # 243</td>
<td>VRE # 449</td>
<td>VRE # 820</td>
</tr>
<tr>
<td>29</td>
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<td>&gt; 125</td>
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<tr>
<td>61</td>
<td>&gt; 125</td>
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<tr>
<td>92</td>
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</tr>
<tr>
<td>Vancomycin</td>
<td>2.5</td>
<td>&lt; 0.98</td>
</tr>
</tbody>
</table>

*Table 4.4- Antibacterial assay results for some selected benzo[b]thiophene amides against cultures of vancomycin resistant *S. aureus* and *E. faecium*.***
Chapter 5 Conclusions and recommendations

Conclusions

A wide range of benzo[b]thiophene amide derivatives were synthesised and characterised during the course of this project. These derivatives included two categories based on their molecular framework, the fused benzo[b]thiophenes and the non-fused benzo[b]thiophene derivatives.

The project was set to design analogues based on the lead molecule 29, and then to develop a structure-activity relationships with a view to optimising antimicrobial activity, particularly antimalarial activity. Benzo[b]thiophene analogues based on 29 were then systematically varied to include hydrophobic, aromatic, hydrogen-bond donor and ionisable groups. Conformationally restricted derivatives were also made. A range of compounds was then tested for antimalarial activity and a more limited number for antibacterial activity.

Two categories of benzo[b]thiophene amide analogues were synthesised: the first was the non-fused category which incorporated different ring systems such as isoquinoline, tetrahydro-β-carboline, piperazine, piperidine and bridged ring systems in the nitrogen component of the benzo[b]thiophene amide framework, and the fused-ring system category which included annulations of the benzo[b]thiophene moiety.

5.2 Non-fused benzo[b]thiophene amides

The non-fused amides were synthesised by coupling reactions involving an acid or an acid chloride with appropriate amines, giving chloro substituted and bromo-substituted analogues. The bromo-substituted analogues required the use of the coupling
reagents DCC/HOBt under specified appropriate conditions to yield the desired amides. An array of benzo[b]thiophene amides were synthesised this way.

The *in vitro* antimalarial (*Plasmodium falciparum*) testing of the non-fused benzo[b]thiophene derivatives revealed some active compounds (Chapter 4, Table 4.1) which were further modified and their potency improved. For example, the amide 55, was transformed to 59 then to 61 with a remarkable improvement in potency. The most potent antimalarial amide found was 2-(4-methoxybenzyl)-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridin-1-one (105).

**5.2.1. Future directions for the non-fused benzo[b]thiophene amides**

The future work for the non-fused benzo[b]thiophene amides (Chapter 2) should ensure the synthesis of analogues with a wider range of substituent groups. Substitution with appropriate groups that would potentially offer better drug/target interactions and hence improved anti-malarial activity should be probed.

Further chemistry on compounds substituted with a benzyl moiety (49, 59, 60, 61, 66 and 67) need to be explored to include other lipophilic groups since the test results suggested this functionality could be a key pharmacophoric element for these amides, especially on compounds exhibiting good antimalarial activity (61 and 67).

The allylation of aldimines (Chapter 2, Scheme 2.7) was a challenge during this project, especially upon substitution of the C6 and C7 positions with methoxy groups, hence other alternative pathways towards synthesising substituted 1-allyl-1,2,3,4-tetrahydroisoquinoline intermediates should be investigated.

The use of Group 4 metal triflates as Lewis acids in the allylation of aldimines has been reported with exceptionally good yields. Even though a related reaction was attempted for the synthesis of 34 during the course of the project, (Chapter 3, Scheme
3.18) there is a need to look into triflates such as those of hafnium, zirconium and scandium with the use of appropriate allyltributylstannanes in order to improve yield for these intermediates.

The synthesis of amides with substitution on the benzo[b]thiophene ring system should also be investigated further since, during this project only a single amide (58) of this nature was synthesised due to time constraints. Other derivatives bearing functionalities such as methoxy, o-, m-, p-nitro- and/or hydroxyl groups should be investigated.

The synthesis of bromo- substituted amide derivatives using DCC/ HOBt coupling methods gave moderate yields but, alternate reagents such as polymer supported HOBt or even the more beneficial 7-aza-1-hydroxybenzotriazole (HOAt) which are commercially available could be employed to improve yield and purity of these amides.

Finally, future work on other chiral benzo[b]thiophene derivatives such as 84 and 85 containing bridged ring systems should also be investigated to allow the assessment of in vitro studies as antimalarials.

### 5.3 Fused benzo[b]thiophene amides.

The ring fused amides involved the synthesis of conformationally restricted heterocyclic molecules (Chapter 3) with the intention of affording better specificity in their biological potency. To attain these fused ring derivatives, free radical reactions involving tributyltin hydride and AIBN, palladium-mediated reactions, and ring closing metathesis reactions using Grubbs’ I catalyst were pursued.
A new approach to the 1,2,3,4-tetrahydrobenzo[b]thieno[2,3-c]pyridine-1-one system via free radical cyclisation was established. The application of this radical cyclisation synthesis to 79 and 53 produced the new compounds 92 and 112 respectively which were crucial to the project (Scheme 3.4 and 3.11).

Benzylated derivatives 104 and 105 were isolated in good yields from 92 and the IC$_{50}$ value for 105 was significantly enhanced making it the most biologically active benzo[b]thiophene derivative. Compound 92 was deallylated to afford 106. This latter compound is of particular importance because it offers a reactive site where further substituents can be attached with ease, compound 106 was also found to be moderately active against the K1 strain of the *Plasmodium falciparum* parasite (14.20 µM).

Attempts to further modify 92 to the corresponding diol 96 using Sharpless chemistry initially failed, however an alternative pathway towards a racemic diol using the osmium tetroxide/ NMO reagent combinations was successful. Compound 96 was, however, found to be inactive IC$_{50} >100$ µM against both strains of the protozoan *Plasmodium falciparum*. However, this data remains useful for analysing the structure activity relationships.

Palladium assisted intramolecular ring closure of benzo[b]thien-2-yl)(1H-indol-1-yl)methanone intermediates 124 and 125 gave the novel derivative 126, a highly strained and planar molecule (Scheme 3.21).

The ring-closing metathesis reaction using Grubbs’ I catalyst was used to cyclise benzo[b]thiophene derivatives with multiple olefinic groups such as 118, 78 and 79. The use of polymer supported Grubbs’ I ruthenium catalyst improved the yield and purity of the derivatives and was hence preferred over the homogeneous catalyst.
The ring closure of compound 118 via metathesis gave the novel 9-membered benzo[b]thiophene ring system derivative 120. The successful synthesis of 120 was seen as a new approach towards synthesising conformationally restricted and larger ring heterocyclic systems. Lactam 120 retains an olefinic double bond which can be further functionalised. The tetrahydroisoquinoline ring remains open for functionalisation and in fact, attempts to model the compound to include methoxy groups at the C6 and C7 positions was attempted, however due to time constraints the substituted 9-membered precursor 119 was not cyclised to its final derivative 121. The compound 120 was found to have antimalarial IC₅₀ values > 20 µM.

5.3.1. Future directions for the fused benzo[b]thiophene amides

Free radical cyclisation approach marked the synthesis of the most biologically active fused benzo[b]thiophene derivatives; this important discovery should be pursued further to investigate possible modifications of the derivatives, in order to enhance potency. Analogues of 105, the most active derivative should be investigated where different functional groups could be substituted on the benzyl ring to potentially enhance activity. The same approach could be applied to 92 where the allyl group remains available for functionalisation. In vitro antimalarial studies for 105 were performed on an unresolved sample, hence the enantiomers of 105 should be isolated and tested to probe enatiomeric potency differences.

Synthetic routes towards the deallylated derivative 106 should also be investigated further to increase yields of this compound.

The chemistry of products related to 126 acquired through palladium assisted reactions should also be extended to include derivatives with substituents on either the
benzo[b]thiophene and/ or the indolic motif. The *in vitro* antimalarial studies on such planar molecules should also be investigated.

Ring closing metathesis towards the fused 9-membered compounds and their derivatives was not fully completed and further research is warranted. The attempt to synthesise the precursor 122 which could then give access to an 8-membered ring derivative failed (Scheme 3.21), and this should be investigated further. The synthetic method depicted under Scheme 3.21 for the synthesis of 122 requires further assessment where alternative reagents and methods could be used.

### 5.4 Future work on *in vitro* studies

The *in vitro* antimalarial studies were carried out on a number of the benzo[b]thiophene derivatives mentioned in this thesis. The results were varied but had a consistent trend and several compounds showed their potential as future leads as antimalarial drugs (Table 4.3).

The biological activity for these compounds was carried out against the K1 CB1 and TM4 8/2 strains of the *Plasmodium falciparum* parasite (Chapter 4). The mode of action for these compounds remains unclear hence a detailed assessment on the mode of action should be undertaken.

From the wide range of benzo[b]thiophene amides synthesised and tested during this project, a training set for molecular modelling studies could be generated and a pharmacophore developed in order to offer a greater understanding for lead modification with the purpose of improving drug/ target interactions.

The crystal structure of one proposed drug target (FabI, Chapter 4, Figure 4.3) for these amides is now well documented, hence docking studies should be carried out to provide an insight on the nature and behaviour of the discussed benzo[b]thiophene
derivatives. Finally, human cell toxicity testing of the active amides should also be assessed.
Chapter 6 Experimental

General

6.1.1. Solvents and Reagents

Reagents were purchased from Sigma-Aldrich Pty Ltd, Lancaster or Merck and were used as supplied except for those compounds purchased as their acid salts which were converted into their free base before use in the procedure described below.

Tetrahydrofuran and diethyl ether were distilled from sodium in the presence of benzophenone. Toluene was distilled from anhydrous calcium hydride. Dichloromethane and hexanes were distilled and stored over molecular sieves (4 Å). When dry dichloromethane was required it was distilled over anhydrous calcium chloride. Anhydrous dimethylformamide was purchased from Sigma-Aldrich Pty Ltd in a Sure-seal® bottle and was stored under an inert atmosphere and used as supplied. Petroleum ether refers to hexanes with a boiling range between 60 °C and 80 °C while ether refers to diethyl ether. All other solvents were used as supplied.

Generation of free bases.

The purchased salt was dissolved in water (20 mL), placed on an ice bath and basified with ammonia solution to pH 11. The free base was then extracted with dichloromethane, dried over anhydrous sodium sulfate and the solvent was evaporated to afford the free base.
6.1.2. Nuclear Magnetic Resonance Spectroscopy

\textit{^1H-NMR spectra.}

Unless otherwise stated, proton NMR spectra were acquired on a Varian Unity 300 NMR spectrometer running at 299.5 MHz at 298 K. Chemical shifts are quoted in δ values in ppm shift relative to TMS in CDCl$_3$, D$_2$O, CD$_3$COCD$_3$ or d$_6$-DMSO. Some spectra (stated in the text) were acquired on a Varian Innova (500.6 MHz). Coupling constants (\(J\)) are reported in Hertz, with signal multiplicity designated as singlet (s), doublet (d), doublet of doublets (dd), triplet (t), quartet (q), quintet (qn), sextet (sx), multiplet (m) and broad (b). The order in which the signal is described in the text is d, multiplicity, coupling constant(s), assignment and number of protons (integral). For example – 7.34 (d, \(J = 3\) Hz, H-a, 2H). The samples were prepared by dissolving approximately 5-6 mg of the products in CDCl$_3$, D$_2$O, CD$_3$COCD$_3$, or d$_6$-DMSO.

\textit{\textsuperscript{13}C-NMR spectra}

Unless otherwise stated, \textsuperscript{13}C-NMR spectra were acquired on a Varian Unity 300 NMR spectrometer running at 74.99 MHz at 298 K. Some spectra (stated in the text) were acquired on a Varian Innova (125.1 MHz). Completely proton decoupled spectra were recorded. Chemical shifts are quoted in δ values in ppm shift relative to TMS in CDCl$_3$, D$_2$O, CD$_3$COCD$_3$ or d$_6$-DMSO.

\textit{Mass spectrometry}

Low-resolution chemical ionization (isobutane, CI\(^+\)) mass spectroscopy (LRMS) were obtained on a Shimadzu CC-17A gas chromatograph equipped with a
QP-5000 mass spectrometer and CI-50 chemical ionization controller. The direct probe insertion method was used to acquire data.

High-resolution mass spectra (HRMS) (methane CI$^+$ and EI$^+$) were determined on a VG Autospec spectrometer using PFK (Perfluorokerosene) as the reference.

**UV spectrometry**

UV spectra (solvent corrected) were recorded on a Shimadzu 2401-PC UV-VIS spectrophotometer.

**Melting points**

The melting point (Mp.) determinations were recorded on a Reichert melting point apparatus and are reported uncorrected.

**6.1.3. Miscellaneous**

The term routine workup refers to the extraction of crude products into organic solvent several times from water, combining the organic fractions, drying over anhydrous sodium sulfate followed by gravity filtration and evaporation under reduced pressure (*in vacuo*) using a Büchi rotary evaporator.

**Crystal growth**

After column chromatography fractions containing the amide derivative were pooled and concentrated *in vacuo*. The product was then dissolved in minimal volume of absolute alcohol (unless otherwise stated) and warmed on hot water bath till completely dissolved, the resulting solution was left to cool slowly overnight. Crystals were collected *via* vacuum filtration on a sintered funnel, washed at least twice with
minimal cold distilled hexane and dried under vacuum. The X-ray crystallography was undertaken by Prof. A.H. White at the University of Western Australia.

6.1.4. Chromatography

Column chromatography was performed using silica gel 60 (230-400 mesh, Merck) or neutral alumina (activated Brockmann 1, standard grade ~150 mesh, 58 Å, Aldrich) Reactions were monitored with thin layer chromatography (0.25 mm aluminium backed silica gel plates) using analytical grade solvents. Chromatographic solvent mixtures are quoted as volume ratios. Column chromatography was performed with the indicated solvents freshly distilled over molecular sieves (4 Å).

Preparative plate

Pre-made silica plates with aluminium backing (0.2 mm thickness) supplied by Merck were used for separating smaller quantities of isolates. Visualization of the separated bands on preparative TLC plates was done using short and long wave UV light.

6.1.5. Biological testing

Antimalarial activity

In-vitro biological tests were undertaken by, Dr. Sumalee Kamchonwongpaisan, National Centre for Genetic Engineering and Biotechnology (BIOTEC) and the National Science and Technology Development Agency (NSTDA), Bangkok. These tests were carried out against the double mutant K1 CB1 strain, which is a multidrug resistant/ antifolate resistant Plasmodium falciparum, and a wild type antifolate sensitive strain TM4/ 8.2 of Plasmodium falciparum.
The activity was recorded as an IC$_{50}$ value in $\mu$M: on the concentration of a compound required to kill 50% of the malarial parasites.

**Antibacterial activity**

The antibacterial testing was carried out against the *Staphylococcus aureus* and four strains of *Enterococcus faecium*. Two on these strains were vancomycin resistant and the other two sensitive too vancomycin although designated as VRE. The staff at Avexa Ltd (formally-Amrad, Melbourne) carried out the antibacterial assays through Dr. J. Deadman. The test samples were made up in dimethyl sulfoxide (DMSO) and the first test control carried out at 2.5% and referenced against vancomycin as the control. The activities are reported in Minimum Inhibitory Concentration (MIC) values, in micrograms per millilitre ($\mu$g/mL). The MIC value is a standard measure of the lowest concentration of the antibiotic that results in the inhibition of visible bacteria growth under standard conditions.
6.2 Synthetic Procedures


*Synthesis of 3-chlorobenzo[b]thiophene-2-carbonyl chloride (23)*\textsuperscript{143,144}.

Thionyl chloride (5.0 mL, 67.56 mmol) was added to a solution of (E)-cinnamic acid (2.0 g, 1.35 mmol), in pyridine (0.3 mL, 1.8 mmol) and toluene (22.2 mL, 20.92 mmol). The stirred reaction mixture was heated at reflux under nitrogen for 60h after which, it was quenched in ice water. The acid chloride was extracted with distilled dichloromethane (2x 25 mL, 2x 10 mL) and the combined organic extracts washed with distilled water (2x 20 mL) and dried. The solvent was evaporated under reduced pressure yielding a crude yellow residue which was purified using flash silica gel chromatography with dichloromethane and recrystallised from dichloromethane: hexane to afford colourless needles of 23 (1.35 g, 43 %). Mp. 115-116 °C, (Lit.\textsuperscript{143} Mp: 114-116 °C). Mass spectrum (Cl\textsuperscript{+}), m/z 231 [MH\textsuperscript{+1}, 35Cl]. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 7.46 (m, 2H), 7.73 (d, J = 6 Hz, 1H), 7.89 (d, J = 6 Hz, 1H). \textsuperscript{13}C-NMR: 134.6 (C2), 129.65 (C3), 125.62 (C4), 126.59 (C5), 126.73 (C6), 122.91 (C7) and 157.88 (C=O).
Synthesis of 3-chloro-5-methoxybenzo[b]thiophene-2-carbonyl chloride (24)\textsuperscript{143}.

[Chemical structure image]

Thionyl chloride (10.0 mL, 136 mmol) was added to a solution of 5-methoxy-(E)-cinnamic acid (4.8 g, 26.96 mmol), in pyridine (0.3 mL, 1.8 mmol) and toluene (50 mL, 47.11 mmol). The stirred reaction mixture was refluxed under nitrogen for 48 h after which it was quenched in ice water and toluene evaporated \textit{in vacuo}. The acid chloride was extracted with distilled dichloromethane (2x 25 mL, 2x 10 mL) and the combined organic extracts washed with distilled water (2x 20 mL) and dried. The solvent was evaporated under reduced pressure yielding a crude yellow residue which was purified using flash silica gel chromatography with dichloromethane to afford 24 (2.62 g, 50 %) as yellow needles. Mp. 141-143 °C, (Lit.\textsuperscript{143} Mp: 143-144 °C). Mass spectrum (Cl\textsuperscript{+}), $m/z$ 261 [MH\textsuperscript{+}, \textsuperscript{35}Cl].

\textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 4.01 (s, -OCH\textsubscript{3}), 7.26 (qn, $J = 2.85$ Hz, 1H), 7.38 (bs, 1H), 7.7 (d, $J = 8.7$ Hz, 1H), \textsuperscript{13}C-NMR: 56.0 (OCH\textsubscript{3}), 104.6 (C4), 122.1 (C6), 123.9 (C7) and 158.9 (C=O).


Bromine (2.48 g, 31.4 mmol) was added drop wise to a solution of commercially acquired benzo[b]thiophene-2-carboxylic acid (20) (1.0 g, 5.61 mmol), and anhydrous sodium acetate (1.0 g, 12.1 mmol) in glacial acetic acid (35 mL). The reaction mixture was stirred at 55 °C under a reflux condenser in a nitrogen atmosphere over 24h. The mixture was poured in ice water and the resulting precipitate filtered under reduced pressure and then washed with distilled water (3x 25 mL) followed by cold ethanol.
(25 mL) to afford a colourless solid. The product was then oven-dried for 10 minutes and recrystallised from hot acetone to afford 25 (0.70 g, 49 %) as colourless needles. Mp. 280-283 °C. Mass spectrum (Cl⁺), m/z 257 [MH⁺¹, ⁷⁹Br]. ¹H-NMR (CD₃COCD₃, 300 MHz): 7.58-7.67 (m, 2H, Ar-H5 and H6), 7.99-8.05 (m, 2H, ArH4 and H7).

¹³C-NMR: 115.2 (C3), 122.8 (C7), 125.5 (C5), 125.8 (C6), 128.3 (C2), 138.7 (C3a), 139.4 (C7) and 161.9 (C=O).

Synthesis of benzo[b]thiophene-2-carbonyl chloride (26).

Thionyl chloride (0.81 mL, 11.1 mmol) was added to a solution of the commercially available benzo[b]thiophene-2-carboxylic acid (20) (0.4 g, 2.24 mmol), in pyridine (0.3 mL, 1.8 mmol) and toluene (15 mL, 14.1 mmol) at reflux. The reaction mixture was quenched in ice water and the toluene evaporated in vacuo. The acid chloride was extracted with distilled dichloromethane (2x 25 mL, 2x 10 mL) and the combined organic extracts washed with distilled water (2x 20 mL) and dried over anhydrous sodium sulfate. The solvent was evaporated under reduced pressure to a brown residue of which was purified using flash chromatography with dichloromethane to yield 26 (0.27 g, 61 %) as a colourless solid. Mass spectrum (Cl⁺), m/z 196 [MH⁺¹, ³⁵Cl]. ¹H-NMR (CDCl₃, 300 MHz): 7.50 (m, 2H), 7.91 (m, 2H), 8.27 (s, 1H). ¹³C-NMR: 123.1 (C3), 125.9 (C7), 126.9 (C4), 128.9 (C5 and C6), 136.1 (C2), 138.3 (C3a), 144.3 (C7) and 161.9 (C=O).

In the same manner as that reported for 26, reaction of 25 (0.60 g, 2.33 mmol) yielded 27 after chromatography (DCM) as a colourless solid (0.43 g, 67%). Mass spectrum (Cl\(^+\)), \(m/z\) 276 [MH\(^+\), \(^{35}\)Cl \(^{79}\)Br]. \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 7.58-7.67 (m, 2H, ArH- H5 and H6), 7.99-8.05 (m, 2H, ArH- H4 and H7). \(^1^3\)C-NMR: 115.2 (C3), 122.8 (C7), 125.5 (C5), 125.8 (C6), 128.3 (C2), 138.7 (C3a), 139.4 (C7), and 161.9 (C=O).

Attempted synthesis of 3-bromobenzo[b]thiophene-2-carbonyl bromide (28).

Thionyl bromide (4.0 mL, 51.00 mmol) was added to a solution of (E)-cinnamic acid (1.0 g, 0.675 mmol), in pyridine (0.3 mL, 1.8 mmol) and toluene (22.2 mL, 20.92 mmol). The stirred reaction mixture was heated at reflux under nitrogen for 60h after and then quenched in ice water. The crude residue was extracted with distilled dichloromethane (2x 25 mL, 2x 10 mL) and the combined organic extracts washed with distilled water (2x 20 mL) and dried. The solvent was evaporated under reduced pressure yielding a crude dark brown residue (R\(_f\) 0.8, 3 % ethyl acetate: hexane) which was purified using flash silica gel chromatography with dichloromethane to afford dark brown solid (0.56 g, 27 %). However, the data collected on the isolated product was not consistent with that of the expected product. Mp. 113-114 °C. Mass spectrum (Cl\(^+\)), \(m/z\) 289, expected: 318 [MH\(^+\)], \(m/z\) 257, 203, 178 and 160. \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 1.25 (t), 1.6 (bs), 3.73 (q, \(J = 7\) Hz), 5.25 (s) and 7.26 (s).
Synthesis of 3,4-dihydroisoquinoline (36)$^{145,146}$.

1,2,3,4-tetrahydroisoquinoline (31) (3.0 g, 22.55 mmol) was dissolved in dichloromethane (50 mL) under nitrogen. To this stirred solution, N-bromosuccinimide (4.41 g, 24.82 mmol) was gradually added over 20 minutes. The mixture was stirred at room temperature for one and half-hours. Sodium hydroxide solution (15 mL, 30 %) was added and stirring continued for one hour. Distilled water (20 mL) was added to the mixture, and stirred for a further 15 minutes. The two phases were separated and the aqueous phase washed with dichloromethane (20 mL). Combined organic phases were washed with water (25 mL) and extracted with 2 M hydrochloric acid (2x 30 mL, 15 mL). Combined aqueous extracts were washed with dichloromethane (25 mL) and basified to pH 11 with concentrated ammonia solution, this afforded a yellow oil which was back extracted with dichloromethane (20 mL, 2x 15 mL), dried and the solvent evaporated under reduced pressure. The yellow oil was distilled under reduced pressure on a kugelrohr distillation apparatus and 36 (2.32 g, 79 %) was collected at 140 °C between 50-70 mbar as a clear oil which turned light yellow on standing. Mass spectrum (CI$^+$), m/z 132 [MH$^+$]. $^{1}$H-NMR (CDCl$_3$, 300 MHz): 2.71 (t, $J = 7.8$ Hz, 2H, H4), 3.74 (t, $J = 6.6$ Hz, 2H, H3), 7.12 (d, $J = 7.5$ Hz, 1H, H8), 7.21-7.34 (m, 3H, H5, H6 and H7) and 8.31 (s, 1H, H1). $^{13}$C-NMR: 25.2 (C4), 47.5 (C3), 127.2 (C7), 127.3 (C5), 128.6 (C8), 131.2 (C6), 136.4 (C8a) and 160.4 (C1).
**Basification of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (33)**\(^{147,148}\).

The hydrochloride salt of 6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline hydrochloride (33) (3.0 g, 13.1 mmol), obtained from Sigma-Aldrich, was dissolved in water (20 mL) and placed on an ice bath and basified with ammonia solution to pH 11. The product was extracted with dichloromethane (2x 30 mL, 15 mL), washed once with water (20 mL) and dried over anhydrous sodium sulfate. Pooled organic solvents were evaporated to dryness on the rotary evaporator to afford a clear oil. The oil was further dried *in vacuo* to afford a white solid of the free-base (2.50 g, 98%). Mp. 82-84 °C. Mass spectrum (CI\(^{+}\)), \(m/z\) 194 \([\text{MH}^{+}]\). \(^{1}\text{H-NMR (CDCl}_3\), 300 MHz\): 2.75 (t, \(J = 7.8\) Hz, 2H, H4), 3.15 (t, \(J = 6.6\) Hz, 2H, H3), 3.83 (s, 6H, 2x CH\(_3\)), 3.97 (s, 1H, H1), 6.51 (s, 1H, ArH- H5), 6.58 (s, 1H, ArH- H8). \(^{13}\text{C-NMR:} \) 25.0 (C4), 47.5 (C3), 56.3 (2x O-CH\(_3\)), 111.2 (C7), 128.6 (C8), 130.3 (C4a), 147.5 (C5) and 151.0 (C6).

**Synthesis of 6,7-dimethoxy-3,4-dihydroisoquinoline (37)**\(^{149}\).

The amine (1.0 g, 1.58 mmol) was dissolved in dichloromethane (50 mL) under nitrogen. To this stirred solution, \(N\)-bromosuccinimide (1.0 g, 5.64 mmol) was gradually added over 20 minutes. The mixture was stirred at room temperature for one and half-hours. Sodium hydroxide solution (15 mL, 30 %) was added and stirring continued for one hour. Distilled water (20 mL) was added to the mixture, and stirred for a further 15 minutes. The two phases were separated and the aqueous phase washed with dichloromethane (20 mL). Combined organic phases were washed with
water (25 mL) and extracted with 2 M HCL (2x 30 mL, 15 mL). Combined aqueous extracts were washed with dichloromethane (25 mL) and basified to pH 11 with concentrated ammonia solution, this afforded a yellow oil which was back extracted with dichloromethane (20 mL, 2x 15 mL), dried over anhydrous sodium sulfate and the solvent evaporated under reduced pressure to afford 37 (0.74 g, 75 %) as an oil.

Mass spectrum (Cl⁺), \(m/z\) 192 [MH⁺]. **1H-NMR** (CDCl₃, 300 MHz): 2.67 (t, \(J = 7.8\) Hz, 2H, H₄), 3.70 (t, \(J = 6.6\) Hz, 2H, H₃), 3.90 (s, 6H, 2x CH₃), 6.67 (s, 1H, ArH-H₅), 6.81 (s, 1H, ArH- H₈), 8.23 (s, 1H, H₁).

**13C-NMR:** 24.9 (C₄), 47.5 (C₃), 56.2 (2x O-CH₃), 110.5 (C₇), 128.3 (C₈), 130.0 (C₄a), 147.9 (C₅), 151.3 (C₆) and 159.8 (C₁).

**Synthesis of 1-allyl-1,2,3,4-tetrahydroisoquinoline (32)**

Allyl bromide (1.83 g, 15.25 mmol) was added to a stirred suspension of zinc powder (1.00 g, 15.38 mmol) in dry tetrahydrofuran (20 mL) under nitrogen. The mixture was cooled to 0 °C where 36 (2.00 g, 15.03 mmol) in dry tetrahydrofuran (30 mL) was added gradually and stirring maintained for two days. Saturated sodium bicarbonate solution (25 mL) was added and the mixture stirred for 15 minutes before being filtered through celite. Organic solvent was removed under reduced pressure and the resulting aqueous mixture extracted with dichloromethane (25 mL, 10 mL). The combined organic mixtures were extracted with 2 M hydrochloric acid, (2x 25 mL). Combined aqueous portions were washed with dichloromethane (25 mL), basified with ammonia solution to pH 11 on an ice bath, and then back extracted with dichloromethane (2x 25 mL, 15 mL).
Combined organic phases were dried, and solvent evaporated under reduced pressure to afford 32 (1.55 g, 59 %) as pale yellow oil. Mass spectrum (Cl⁻), m/z 174 [MH⁺].

\[ ^1H-NMR \text{ (CDCl}_3, \text{ 300 MHz):} \, 2.63; \, 2.49 \text{ (m, 2H, H1' ), 2.69 (qn, J = 1.72 Hz, 2H, H4), 2.93 (qn, J = 4.05 Hz, 2H, H3), 5.15 (m, 2H, H3'), 5.81 (m, 1H, H2'), 4.03 (t, J = 4.5 Hz, 1H, H1), 7.05-7.16 (m, H5, H6, H7 and H8). \]

\[ ^13C-NMR:\] 30.2 (C4), 40.9 (C3), 41.3 (C2'), 55.3 (C1), 118.1 (C3'), 126.0 (C7), 126.2 (C5), 129.5 (C5 and C8), 135.6 (C1'), 135.9 (C8a), and 138.9 (C4a).

**Synthesis of 1-allyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (34)**

Allyl bromide (0.42 g, 3.57 mmol) was added to a stirred suspension of zinc powder (0.24 g, 3.76 mmol) in dry tetrahydrofuran (20 mL) under nitrogen. The mixture was cooled to 0 °C where 37 (0.73 g, 3.78 mmol) in dry tetrahydrofuran (30 mL) was added gradually and stirring maintained for 3 days. Saturated sodium bicarbonate solution (25 mL) was added and the mixture stirred for 15 minutes before being filtered through celite. Organic solvent was removed in vacuo and the resulting aqueous mixture extracted with dichloromethane (30 mL, 10 mL). The combined organic mixtures were extracted with 2 M hydrochloric acid, (2x 25 mL). Combined aqueous portions were washed with dichloromethane (25 mL), basified with ammonia solution to pH 11 on an ice bath, and then back extracted with dichloromethane (2x 25 mL, 2x 15 mL). Combined organic phases were dried and solvent evaporated to dryness to afford 34 (0.38 g, 42 %) as a dark oil. Mass spectrum (EI), m/z 233.

\[ ^1H-NMR \text{ (CDCl}_3, \text{ 300 MHz):} \, 2.41-2.68 (m, 2H, H1'), 2.87 (m, 2H, H4), 3.14 (m, 2H, H3), 3.64 (t, J = 7.95 Hz, 1H, H1), \]
3.76 (bs, 6H, CH₃), 5.08 (t, $J = 12$ Hz, 2H, H₃'), 5.76 (m, 1H, H₂'), 6.49 (s, 1H, ArH-H₅), 6.57 (s, 1H, ArH-H₈), 8.23 (s, 1H, H₁). $^{13}$C-NMR: 26.9 (C₄), 38.8 (C₁'), 38.6 (C₃), 44.8 (C₁), 53.5 (CH₃), 108.0 (C₅), 109.4 (C₈), 115.5 (C₃'), 127.9 (C₈a), 133.1 (C₂'), 144.8 (C₇) and 145.0 (C₆).

*Boron trifluoride etherate/ allyltributyltin approach to 1-allyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (34).*

Boron trifluoride etherate (0.30 mL, 2.12 mmol) was added to a stirred suspension of the amine 37 (0.41 g, 2.13 mmol) and zinc powder (0.13 g, 2.00 mmol) in anhydrous dichloromethane at minus 78 °C (liquid nitrogen: chloroform) under nitrogen. The suspension was then warmed to 10 °C over a period of 20 minutes and then re-cooled to minus 78 °C. Allyltributyltin (0.7 mL, 2.11 mmol) was slowly added to the suspension and the mixture warmed to room temperature with continued stirring overnight. Saturated sodium bicarbonate solution (20 mL) was added and the mixture stirred for 15 minutes before being vacuum filtered through celite. Organic solvent was removed in vacuo and the resulting aqueous mixture extracted with dichloromethane (30 mL, 10 mL). The pooled organic mixtures were extracted with 2 M hydrochloric acid, (2x 25 mL). Combined aqueous portions were washed with dichloromethane (25 mL), basified with ammonia solution to pH 11 on an ice bath, and back extracted with dichloromethane (2x 25 mL, 2x 15 mL). Combined organic phases were dried and solvent evaporated to under reduced pressure to give 34 (0.39 g, 80 %) as an oil. Mass spectrum (El), m/z 233 [MH$^+$]. $^1$H-NMR (CDCl₃, 300 MHz): 2.40-2.65 (m, 2H, H₁'), 2.85 (m, 2H, H₄), 3.14 (m, 2H, H₃), 3.64 (t, $J = 7.9$ Hz, 1H, H₁), 3.76 (bs, 6H, CH₃), 5.08 (t, $J = 12$ Hz, 2H, H₃'), 5.76 (m, 1H, H₂'), 6.49 (s, 1H, ArH- H₅), 6.57 (s, 1H, ArH- H₈), 8.23 (s, 1H, H₁). $^{13}$C-NMR: 26.9 (C₄),
38.8 (C1’), 38.6 (C3), 44.8 (C1), 53.5 (CH3), 108.0 (C5), 109.4 (C8), 115.5 (C3’), 127.7 (C8a), 133.3 (C2’), 145.0 (C7) and 145.0 (C6).

**Synthesis of 1-benzyl-1,2,3,4-tetrahydroisoquinoline (35)**

Benzyl bromide (0.43 g, 2.52 mmol) was added to a stirred suspension of zinc powder (0.14 g, 2.14 mmol) in dry tetrahydrofuran (15 mL) under nitrogen. The temperature was adjusted to 0°C after which freshly prepared 36 (0.30 g, 2.29 mmol) in dry tetrahydrofuran (30 mL) was added gradually. The mixture was warmed to room temperature and stirring continued for two days. Saturated sodium bicarbonate solution (25 mL) was added and the mixture stirred for 15 minutes before being filtered through celite. Organic solvent was removed under reduced pressure and the resulting aqueous mixture extracted with dichloromethane (25 mL, 10 mL). The combined organic mixtures were extracted with 2 M hydrochloric acid, (2x 20 mL). Combined aqueous portions were washed with dichloromethane (20 mL), basified with ammonia solution to pH 11 on an ice bath, and then back extracted with dichloromethane (2x 25 mL, 15 mL). Combined organic phases were dried, and solvent evaporated under reduced pressure to give compound 35 (0.14 g, 29 %) as an oil. Mass spectrum (Cl+), m/z 224 [MH+1].

**1H-NMR (CDCl3, 300 MHz):** 2.69 (qn, J = 1.72 Hz, 2H, H4), 2.93 (qn, J = 4.05 Hz, 2H, H3), 4.03 (t, J = 4.5 Hz, 1H, H1), 5.31 (s, 2H, CH2-benzyl), 7.05-7.16 (m, H5, H6, H7 and H8), 7.28-7.35 (m, 5H, benzyl).

**13C-NMR:** 30.2 (C4), 40.9 (C3), 55.3 (C1), 58.5 (CH2, benzyl), 126.0 (C7), 126.2 (C5), 129.5 (C5 and C8), 130.1 (C4”) 132.2 (C2”, C3”, C5” and C6”), 135.9 (C8a), and 138.9 (C4a).
Synthesis of 2-(3-chlorobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydroisoquinoline (29).

1,2,3,4-tetrahydroisoquinoline (31) (0.5 g, 4.48 mmol), was slowly added to the acid chloride 23 (1.0 g, 4.34 mmol) and anhydrous potassium carbonate (1.0 g, 7.24 mmol), in dry tetrahydrofuran (30 mL). The mixture was heated at reflux under nitrogen for two and half-hours. The mixture was cooled, filtered to remove unreacted potassium carbonate, and solvent evaporated in vacuo to give an oil, which was recrystallised from hot ethanol to yield 29 (0.84 g, 59 %). Mp. 120-121 °C. Mass spectrum (CI +), 328 [MH +1, 35Cl]. HRMS (CI +): Found: 328.0571; required: 328.0577 for C18H14ClNOS. 1H-NMR (CDCl3, 300 MHz): 2.93 (bs, 2H, -CH2) 4.04; 3.70 (bs 2H, N-CH2), 4.96; 4.64 (bs, 2H, N-CH2), 7.20 (bs, 4H, ArH), 7.47 (m, 2H, H5 and H6), 7.83 (m, 2H, H4 and H7). 13C-NMR: 28.3, 30.0, 41.5 (CH2); 119.2, 122.6, 122.8, 125.5, 126.4, 128.2, 132.2 (Ar-CH); 135.8 (C2), 137.8 (C3) and 163.1 (C=O).

Synthesis of 2-(3-bromobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydroisoquinoline (30).

The isoquinoline 31 (0.26 g, 1.95 mmol) was added to a mixture of 25 (0.5 g, 1.95 mmol), 1,3-dicyclohexylcarbodiimide (0.40 g, 1.94 mmol), and 1-hydroxybenzotriazole (0.32 g, 2.37 mmol) in dry DMF (20 mL). The mixture was stirred at room temperature under nitrogen within 24h. The precipitated dicyclohexylurea was removed by vacuum filtration. Solvent was then evaporated under reduced pressure to give an oily residue, which was then taken up in ethyl
acetate (30 mL), and traces of the urea removed by filtration. The filtrate was then washed with distilled water (50 mL), dried and the solvent evaporated to give a yellow oil. Re-crystallisation from absolute alcohol afforded white crystals of 30 (0.59 g, 82 %). Mp.101-103 °C. Mass spectrum (Cl⁺), 372 [MH⁺⁺¹, 81Br]. HRMS (EI) Found: 372.9959 (4.8 ppm), required: 372.9941 for C₁₈H₁₄⁺₂BrNOS. ¹H-NMR (CDCl₃, 300 MHz): 2.98 (bs, 2H, CH₂), 4.09; 3.73 (bs 2H, N-CH₂), 4.96; 4.60 (bs, 2H, N-CH₂), 7.21 (bs, 4H, ArH), 7.85 (m, 2H, H₄ and H₇), 7.49 (m, 2H, H₅ and H₆). ¹³C-NMR: 28.3, 30.0, 41.5 (CH₂); 119.2, 122.6, 122.8, 125.5, 126.4, 128.2, 132.2 (Ar-CH); 135.8 (C₂), 137.8 (C₃) and 164.1 (C=O).

**Synthesis of 1-benzyl-2-(3-bromobenzof[b]thien-2-oyl)-1,2,3,4-tetrahydro isoquinoline (49).** Following the method used to synthesize 30, 49 (0.48g 55 %) was prepared from the acid 30 (0.40g, 1.55 mmol) and 1-benzyl-1,2,3,4-tetrahydroisoquinoline (35) (0.35 g, 1.55 mmol) as a colourless oil after chromatography (30 % ethyl acetate: hexane). Mass spectrum (Cl⁺), 462 [MH⁺⁺⁺⁺¹, 81Br]. HRMS (EI) Found: 461.4014 (1.8 ppm), required: 461.4020 for C₂₃H₂₀⁺²BrNOS. ¹H-NMR (CDCl₃, 300 MHz): 2.69 (qn, J = 1.72 Hz, 2H, H₄), 2.93 (qn, J = 4.05 Hz, 2H, H₃), 4.03 (t, J = 4.5 Hz, 1H, H₁), 5.31 (s, 2H, CH₂ benzyl), 7.05-7.16 (m, H₅’, H₆’, H₇’ and H₈’), 7.28-7.35 (m, 5H, benzyl), 7.85 (m, 2H, H₄ and H₇), 7.49 (m, 2H, H₅ and H₆). ¹³C-NMR: 30.2 (C₄’), 40.9 (C₃’), 55.3 (C₁’), 58.5 (CH₂, benzyl), 126.0 (C₇’), 115.2 (C₃), 122.8 (C₇), 125.5 (C₅), 125.8 (C₆), 126.2 (C₅’), 128.3 (C₂), 129.5 (C₅’ and C₈’), 130.1 (C₄”) 147
32.2 (C2”, C3”, C5” and C6”), 135.9 (C8a’), 138.7 (C3a), 139.4 (C7), and 162.1 (C=O).

**Synthesis of 2-(3-chlorobenzof[b]thien-2-oyl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (50).**

Dry tetrahydrofuran (30 mL) was added to a mixture of 33 (0.89 g, 4.61 mmol), 23 (1.05 g, 4.56 mmol), and anhydrous potassium carbonate (1.0 g, 7.24 mmol) after which the mixture was heated at reflux under nitrogen for two and a half-hours. The mixture was then cooled to room temperature before being filtered to remove unreacted potassium carbonate. The solvent was evaporated under reduced pressure to give a light yellow oil which was chromatographed (30 % ethyl acetate: hexane) and recrystallised from hot ethanol to give white crystals of 50 (1.44 g, 86 %). Mp. 148-150 °C. Mass spectrum (Cl⁺), m/z 388 [MH⁺, 35Cl]; HRMS (EI) Found: 390.0745 (1.7 ppm) required: 390.0738 for C₂₀H₁₉ClNO₃S. ¹H-NMR (CDCl₃, 300 MHz): 2.63 (bs, 2H, CH₂), 3.48 (bs 2H, NCH₂), 3.62 (s, 6H, OMe), 4.64*; 4.37* (bs, 2H, NCH₂), 6.40 (bs, 2H, H₅` and H₈`), 7.26 (m, 2H, H₅ and H₆), 7.62 (m, 2H, H₄ and H₇). ¹³C-NMR: 28.3, 30.0, 41.5 (CH₂), 53.1 (-OMe), 116.2, 118.6, 120.8, 125.5, 126.4, 128.2, 130.2 (Ar); 135.8 (C2), 145.1 (C3), 158, 156.5 (C6`, C7`) and 162.1 (C=O).

Note: The signals marked here as “*” refer to amide rotamers.
Synthesis of 2-(3-bromobenzof[b]thien-2-yl)-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinoline (51).

To a cooled mixture (ice bath) of 33 (0.40 g, 2.07 mmol), 25 (0.51 g, 1.99 mmol), DCC (0.47 g, 2.28 mmol), and HOBt (0.30 g, 2.22 mmol) was added dry DMF (35 mL). The mixture was stirred for 24h at room temperature under nitrogen. The reaction mixture was filtered and solvent evaporated under reduced pressure to give yellow oil. This residue was taken up in ethyl acetate (30 mL), washed with saturated sodium bicarbonate solution (3x 25 mL) and distilled water (25 mL) to remove traces of unreacted acid. The organic portion was dried and the solvent evaporated under reduced pressure to give a yellow oil which was chromatographed (30 % ethyl acetate: hexane) and recrystallised from hot ethanol to afford 51 (0.26 g, 30 %) as a white solid. Mp.165-167 °C; Mass spectrum (Cl⁺), m/z 432 [MH⁺, 79Br], 192 (C₁₁H₁₄NO₂); HRMS (EI) Found: 434.0249 (3.6 ppm), required: 434.0233 for C₂₀H₁₉BrNO₃S.

1H-NMR (CDCl₃, 300 MHz): 2.63 (bs, 2H, CH₂), 3.48 (bs 2H, N-CH₂), 3.62 (s, 6H, -OMe), 4.64*; 4.37* (bs, 2H, NCH₂), 6.40 (bs, 2H, H₅` and H₈`), 7.26 (m, 2H, H₅ and H₆), 7.62 (m, 2H, H₄ and H₇).

13C-NMR: 28.3, 30.0, 41.5 (CH₂), 53.1 (-OMe), 116.2, 118.6, 120.8, 125.5, 126.4, 128.2, 130.2 (Ar); 135.8 (C2), 145.1 (C3), 158, 156.5 (C₆’, C₇’) and 162.1 (C=O).

Synthesis of 2-(3-chlorobenzof[b]thien-2-yl)-1-(2-propenyl)-1,2,3,4-tetrahydroisoquinoline (52).

Following the method used for the synthesis of 50, 52 (0.31 g, 47 %) was prepared from 32 (0.3 g, 1.73 mmol) and 23 (0.5 g, 2.2 mmol) as a
white crystalline solid (hot ethanol) after column chromatography using 30 % ethyl acetate: hexane. Mp.116-117 °C. Mass spectrum (Cl⁺), m/z 368 [MH⁺, 35Cl], m/z 326 [C₃H₅]; HRMS (EI) Found: 368.0876 (3.3 ppm), required: 368.0877 for C₂₁H₁₉₃₅ClNO. ¹H-NMR (CDCl₃, 300 MHz): 2.65 (m, 2H, H1’’), 2.73 (m, Hz, 2H, H4’), 3.37 (m, 2H, H3’), 4.94 (t, J = 4.5 Hz, 1H, H1’), 5.15 (m, 2H, H3’’), 5.81 (m, 1H, H2’’), 7.20 (m, 4H, ArH), 7.47 (m, 2H, H5 and H6), 7.83 (m, 2H, H4 and H7). ¹³C-NMR: 26.0 (C4’); 42.1 (C3’, C1’’), 52.8 (C1’), 119.2 (C3’’), 122.6, 124.2x 2, 125.5, 126.4x 2, 128.2, 132.2 (Ar-CH); 134.8 (C2’’), 135.5 (C7a), 138.1 (C4a) and 162.5 (C=O).

**Indium metal approach to 2-(3-chlorobenzo[b]thien-2-oyl)-1-(2-propenyl)-1,2,3,4-tetrahydroisoquinoline (52).**

To a stirred and cooled mixture (ice bath) of the acid chloride 23 (0.40 g, 1.72 mmol) and the dihydroisoquinoline 36 (0.25 g, 1.90 mmol) was added dry tetrahydrofuran (15 mL) under nitrogen. To this mixture was then added allyl bromide (0.62 g, 5.16 mmol) and stirring continued for a further 10 minutes before indium powder (0.60 g, 3.84 mmol) was added. The reaction was then warmed to room temperature and stirred over 12h. The reaction was stopped with the addition of 1 M HCL (10 mL) and then extracted with diethyl ether (2x 20 mL). The organic portion was washed with distilled water (2x 15 mL) and dried. Solvent was then evaporated to afford a yellow oil (0.4 g). A TLC spotting (30 % ethyl acetate: hexane) of the oil against an authentic sample of 52 indicated a very minute spot corresponding to the desired product, a minor spot which had the same R₇ as 2-(3-chlorobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydroisoquinoline (29) was evident and a major spot (R₇ 0.4) was also present. Mass spectrum (Cl⁺), m/z 368 [MH⁺, 35Cl], m/z 326 and m/z 344. A preparative TLC was used to isolate the components for further identification. A mass spectrum of the
minor component had an MH$^+1$ peak at $m/z$ 326 which was consistent with the amide 2-(3-chlorobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydroisoquinoline (29) probably formed from reduction of the 3,4-dihydroisoquinoline (36) to 1,2,3,4-tetrahydroisoquinoline (31), while the peak at $m/z$ 368 was that of the desired product 52 (less than 15 % overall). The MH$^+1$ peak at $m/z$ 344 was assigned to the major product of the reaction, $N$-(2-(hydroxymethyl)phenyl)-3-chlorobenzo[b]thiophene-2-carboxamide (89) (0.30 g). $^1$H-NMR (CDCl$_3$, 300 MHz): 3.43 (t, $J = 7.2$ Hz, 2H, -CH$_2$-phenyl), 3.81 (q, $J =$ 6.6 Hz, 2H, CH$_2$-NH), 4.13 (s, 2H, CH$_2$-OH), 7.17-7.39 (m, 4H, ArH) and 7.47 (m, 2H, H5 and H6, BTP), 7.83 (m, 2H, H4 and H7, BTP).

**Synthesis of 2-(3-bromobenzo[b]thien-2-oyl)-1-(2-propenyl)-1,2,3,4-tetrahydro isoquinoline (53).**

The isoquinoline 32 (1.0 g, 5.78 mmol) was added to a mixture acid 25 (1.5 g, 5.83 mmol), 1,3-dicyclohexylcarbodiimide (1.23 g, 5.97 mmol), and 1-hydroxybenzotriazole (0.79 g, 5.85 mmol) in dry DMF (70 mL), on an ice bath. The reaction mixture was then stirred at room temperature under nitrogen overnight. After all the substrate had reacted (via TLC), the precipitated dicyclohexylurea was filtered and organic solvent concentrated under reduced pressure to yellow oil. This residue was taken up in ethyl acetate (25 mL) and traces of the precipitate that crushed out were filtered again. The organic layer was washed with distilled water (50 mL) and dried (anhydrous sodium sulfate) and finally evaporated in vacuo to afford a yellow oil which was recrystallised from hot ethanol to give colourless crystals of 53 (1.02 g, 43 %). Mp.125-127 °C. Mass spectrum (Cl$^-$), $m/z$ 412 [MH$^+1$,$^{79}$Br]. HRMS (EI) Found:
414.0350 (-1.2 ppm), required: 414.0355 for C_{21}H_{19}^{81}BrNOS. \textbf{1H-NMR (CDCl}_3, \textbf{300 MHz}): 2.68 (m, 2H, H1’’), 2.70 (m, 2H, H4’’), 3.39 (m, 2H, H3’’), 4.95 (t, J = 4.5 Hz, 1H, H1’’), 5.15 (m, 2H, H3’’), 5.81 (m, 1H, H2’’), 7.22 (m, 4H, ArH), 7.47 (m, 2H, H5 and H6), 7.83 (m, 2H, H4 and H7). \textbf{13C-NMR:} 26.0 (C4’’), 42.1 (C3’, C1’’), 52.8 (C1’), 119.2 (C3’’), 122.6, 124.2x 2, 125.5, 126.4x 2, 128.2, 132.2 (Ar-CH); 134.8 (C2’’), 135.2 (C7a), 138.3 (C4a) and 164.5 (C=O).

\textit{Synthesis of 2-(benzothien-2-oyl)-1-(2-propenyl)-1,2,3,4-tetrahydro-isoquinoline (54).}

Following a similar approach as for the synthesis of 53, amide 54 (0.10 g, 11 %) was prepared from the coupling of amine 32 (0.6, 3.40 mmol) and the acid 20 (0.5 g, 2.81 mmol). After the usual work up procedure, and column chromatography (20 % ethyl acetate: hexane), 54 was obtained as clear oil. Mass spectrum (Cl\(^+\)), \textit{m/z} 334 [MH\(^{+}\)], GC-MS (EI\(^+\)) 292 (C\(_{18}\)H\(_{14}\)NOS\(_{2}\), 35 %), 161 (C\(_{9}\)H\(_{5}\)NOS\(_{2}\), 100 %). \textbf{HRMS (ES)} Found: 334.0755, required: 334.0777 for C\(_{21}\)H\(_{20}\)NOS. \textbf{1H-NMR (CDCl}_3, \textbf{300 MHz}): 2.71 (bs, 2H, H1’’), 2.73 (bs, 2H, H4’’), 3.37 (bs 2H, H3’’), 5.09 (t, J = 4.5 Hz, 1H, H1’’), 5.14 (m, 2H, H3’’), 5.81 (bs, 1H, H2’’), 7.36-7.80 (m, 8H, ArH) and 7.85 (s, 1H, H3). \textbf{13C-NMR:} 26.0 (C4’’); 42.1 (C3’, C1’’), 52.8 (C1’), 119.2 (C3’’), 120.8 (C3), 122.6, 124.2x 2, 125.5, 126.4x 2, 128.2, 132.2 (Ar-CH); 134.8 (C2’’), 135.5 (C7a), 138.1 (C4a) and 162.5 (C=O).
Synthesis of 2-(3-chlorobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydro-9H-pyrido [3,4-b] indole (55).

Dry tetrahydrofuran (55 mL) was syringed onto a flask containing a mixture 23 (1.10 g, 4.78 mmol), 1,2,3,4-tetrahydro[b]carboline (40) (0.77 g, 4.43 mmol), and anhydrous potassium carbonate (1.0 g, 5.61 mmol). The mixture was heated at reflux under nitrogen for two and half-hours. The cooled solution was filtered to remove unreacted potassium carbonate. Solvent was removed in vacuo to give a yellow oil which was crystallised from hot ethanol to give 55 (1.41 g, 81 %) as colourless crystals. Mp.202-205 °C. Mass spectrum (CI+), m/z 367 [MH+1,35Cl]; HRMS (CI) Found: 367.0671, (3.3 ppm), required: 367.0659 C20H1635ClN2OS. 1H-NMR (CD3COCD3, 300 MHz): 2.06 (d, J = 6.6 Hz, 2H, H4’), 2.86 (d, J = 10.2 Hz, 2H, H3’), 3.84; 4.14 (bs, 2H, H1’), 7.05 (m, 2H, ArH5’ and ArH8’), 7.6 (qn, J = 7.58 Hz, 4H, ArH- H5, H6, H6’ and H7’), 7.89 (d, J = 7.5 Hz, 1H, ArH4), 8.09 (d, J = 7.8 Hz, 1H, ArH7). 13C-NMR (δ): 22.4 (C2’), 46.1 (C1’), 47.2 (C3’), 109.6 (C4a’), 118.3-126.0 (Ar-CH), 130.8 (C12a’), 137.4 (C8a’) and 165.4 (C=O).

Synthesis of 2-(3-bromobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydro-9H-pyrido [3,4-b] indole (56).

Dry N,N-dimethylformamide (30 mL) was added to a mixture of 25 (0.40 g, 1.56 mmol), 40 (0.26 g, 1.51 mmol), DCC (0.32 g, 1.55 mmol), and HOBt (0.21 g, 1.55 mmol) on an ice bath. The mixture was stirred under nitrogen overnight at room temperature. The precipitated
dicyclohexylurea was filtered and DMF evaporated under reduced pressure. To the oily residue was added dichloromethane (25 mL), and the organic layer was then washed with sodium bicarbonate solution (3x 25 mL) and then distilled water (25 mL). Pooled organic solvents were dried and the solvent evaporated to afford a yellow oil which was chromatographed (DCM) and recrystallised from hot ethyl alcohol to give 56 (0.22 g, 36 %) as a colourless crystalline solid. Mp. 217-220 °C. Mass spectrum (Cl⁻), m/z 411 [MH⁺,79Br]. HRMS (Cl) Found: 412.0007, (-5.3 ppm) required: 412.0029 for C₂₀H₁₅⁻⁷⁹BrNO₂S. ¹H-NMR (CD₃COCD₃, 300 MHz): 2.09 (d, J = 6.6 Hz, 2H, H₄'), 2.88 (d, J = 10.2 Hz, 2H, H₃'), 3.82; 4.14 (bs, 2H, H₁'), 7.08 (sp, J = 7.31 Hz, 2H, ArH₅' and ArH₈'), 7.6 (qn, J = 7.56 Hz, 4H, ArH- H₅, H₆, H₆' and H₇'), 7.89 (d, J = 7.5 Hz, 1H, ArH₄), 8.09 (d, J = 7.8 Hz, 1H, ArH₇). ¹³C-NMR: 22.4 (C₂'), 46.2 (C¹'), 47.3 (C₃'), 109.6 (C₄a'), 118.4-126.0 (Ar-CH), 130.8 (C₁₂a'), 137.4 (C₈a') and 165.4 (C=O).

Synthesis of 2-(3-chlorobenzof[b]thien-2-oyl)-5-methoxy-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole (57).

Anhydrous tetrahydrofuran (15 mL) was added to a mixture of 24 (0.50 g, 1.92 mmol), 40 (0.33 g, 1.91 mmol), and anhydrous potassium carbonate (0.22 g, 1.59 mmol). The reaction mixture was heated at reflux under nitrogen for two and half-hours. The cooled solution was filtered to remove unreacted potassium carbonate and the solvent evaporated under reduced pressure to give oil. The oil was taken up in DCM (15 mL) and washed with saturated sodium bicarbonate solution (2x 15mL) to remove unreacted 24 (via TLC). Pooled organic layers were washed with distilled water (30
mL) and solvent removed under reduced pressure to give an oil which was
cromatographed (30 % ethyl acetate: hexane) and crystallised from hot ethanol to
give yellow crystals of 57 (0.7 g, 92 %). Mp. 148-150 °C. Mass spectrum (Cl\(^+\)), m/z
397 [MH\(^{+1}\), \(^{35}\)Cl]; \(^1\)H-NMR (CD\(_3\)COCD\(_3\), 300 MHz): 1.70 (d, \(J = 6.6\) Hz, 2H, H4’),
2.88 (d, \(J = 10.2\) Hz, 2H, H3’), 3.85 (s, 3H, CH\(_3\)), 5.10 (s, 2H, H3’), 7.92-7.65 (m,
7H, ArH- H4, H6, H7, H5’, H6’-H8’). \(^13\)C-NMR: 22.5 (C4’), 40.7(C1’), 46.9 (C3’),
55.9 (CH\(_3\)), 104.3 (C4), 109.6 (C4a’), 119.8-126.5 (Ar-C6, C7, C6’-C8’), 130.8
(C12a’), 137.4 (C8a’), 158.2 (C5) and 165.4 (C=O).

**Synthesis of 2-(3-chlorobenzo[b]thien-2-oyl)-5-methoxy-1,2,3,4-tetrahydro-9-
benzyl-pyrido[3,4-b]indole (58).**

Sodium hydride (0.051 g, 2.12 mmol) was dissolved in dry DMF
(15 mL) under nitrogen. The
mixture was then cooled in ice to 5
°C after which 57 (0.40 g, 10.10 mmol), in dry DMF (15 mL) was added and mixture stirred at room temperature for
45 minutes. The reaction mixture was then cooled to minus 60 °C on a dry ice/
acetone bath. Benzyl bromide (0.2 g, 1.17 mmol) dissolved in dry DMF (15 mL) was
added and the mixture stirred overnight at room temperature under nitrogen. Solvent
was evaporated under reduced pressure to give a crude gummy solid. Distilled water
(50 mL) was added to the solid and the mixture stirred vigorously for one hour. The
solid was extracted in dichloromethane (25 mL) and the organic phase washed with
distilled water (30 mL). The organic solvent was concentrated \textit{in vacuo} to afford a
semi solid yellow product which was chromatographed (40 % ethyl acetate: hexane)
to give a clear oil which was then recrystallised from hot ethanol to give 58 (0.12 g, 24 %) as a colourless crystalline solid. Mp. 153-155 °C. Mass spectrum (CI⁺), m/z 487 [MH⁺, 35Cl]; HRMS (CI) Found: 487.1139, required: 487.1130 for C₂₈H₂₄³⁵Cl₂N₂O₂S. ¹H-NMR (CDCl₃, 300 MHz): 2.32 (bs, 2H, H₄′,-CH₂), 2.96 (bs, 2H, H₃′,-CH₂), 3.93 (s, 3H, -CH₃), 4.91(s, 2H, H₁′, CH₂-N), 5.30 (s, 2H, CH₂-benzyl), 7.12-7.68 (m, 12H, ArH). ¹³C-NMR: 22.5 (C₄′), 40.7 (C₁′), 45.9 (C₃′), 47.1 (CH₂-benzyl), 55.9 (-CH₃), 104.3 (C₄), 109.6 (C₄a′), 117.7-123.7 (Ar-C₆, C₇, C₆′-C₈′), 126.5-129.1 (C₂″-C₆″ benzyl),130.8 (C₁₂a′), 137.4 (C₁″), 137.4 (C₈a′), 158.2 (C₅) and 165.4 (C=O).

**Synthesis of 9-benzyl-2-(3-chlorobenzof[b]thien-2-oyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole (59).**

Sodium hydride (0.06 g, 2.50 mmol) was dissolved in dry N,N-dimethylformamide (10 mL) under nitrogen and the mixture cooled to 5 °C on an ice bath. The amide 55, (0.4 g, 1.73 mmol), in dry N,N-dimethylformamide (15 mL) was then added and mixture allowed to warm to room temperature while stirring for 45 minutes. The flask containing the reaction mixture was then cooled to -60 °C using a dry ice/acetone bath before benzyl bromide (0.3 g, 1.76 mmol) dissolved in dry N,N-dimethylformamide (10 mL) was added. The mixture was then stirred at room temperature over 24h after which the substrate had reacted (TLC analysis). DMF was removed under reduced pressure to afford a gummy solid. Distilled water (50 mL) was added to the crude product and then stirred vigorously for 1h. Dichloromethane (25 mL) was poured onto the mixture, separated, and the organic layer washed with distilled water (2x 20 mL) and then dried. Upon evaporation of the DCM, a yellow
semi solid product was obtained which was then chromatographed (40 % ethyl acetate: hexane) to give a clear oil. The oil was crystallised from absolute ethanol to give 59 (0.30 g, 38 %) as a colourless solid. Mp.143-145 °C. Mass spectrum (Cl⁺), m/z 457 [MH⁺, Cl⁺], HRMS (Cl⁺) Found: 457.1141, (0.9 ppm), required: 457.1137 for C_{27}H_{22}ClNOS. \textbf{1H-NMR (CDCl₃, 300 MHz):} 2.94 (bs, 2H, H₄, CH₂), 3.81 (bs, 2H, H₃, CH₂), 4.90 (s, 2H, H₁, CH₂-N), 5.31 (s, 2H, CH₂-benzyl), 7.12-7.68 (m, 12H, ArH). \textbf{13C-NMR:} 22.5 (C₄), 30.5 (C₁), 46.1 (C₃), 47.2 (-CH₂-benzyl), 104.3 (C₄), 109.6 (C₄a or C₇), 118.4-126.8 (Ar-C₆, C₇, C₆'-C₈'), 127.7-129.1 (C₂''-C₆'' benzyl), 130.8 (C₁₂a'), 137.4 (C₁'''), 137.4 (C₈a') and 164.0 (C=O).

\textbf{Synthesis of 9-benzyl-2-(3-bromobenzof[b]thien-2-oyl)-1,2,3,4-tetrahydro-9H-pyrido[3,4-b]indole (60).}

In a nitrogen atmosphere a solution of sodium hydride (0.055 g, 2.2 mmol) in dry DMF (5 mL) was cooled to 5 °C on an ice bath, and 56 (0.4 g, 0.97 mmol) in anhydrous DMF (10 mL) was slowly added. Stirring was continued at room temperature for 1h. The reaction mixture was then cooled to -60 °C in a dry ice/acetone bath. Benzyl bromide (0.16 g, 0.94 mmol) dissolved in dry N,N-dimethylformamide (5 mL) was slowly added \textit{via} syringe into the mixture and stirring continued overnight at room temperature. The solvent was evaporated under reduced pressure to give an oil. Distilled water (50 mL) was added to this oil and a gummy solid was formed immediately. Vigorous stirring of the gummy product was continued for one and half hours before the solid was extracted with dichloromethane (25 mL). Organic solvent was dried and concentrated to give a yellow oily product which was then chromatographed (30 % ethyl acetate: hexane) to
a clear oil. The oil was finally recrystallised from absolute ethanol to give 60 (0.23 g, 48 %) as a colourless crystalline solid.Mp. 138-140 °C. Mass spectrum (Cl−), m/z 501 [MH+1,79Br]; HRMS (EI) Found: 502.0537 (0.1 ppm), required: 502.0537 for C27H21\(\text{Br}_8\)N2OS. \(^{1}\text{H-NMR (CDCl}_3, 300 \text{ MHz)}\): 2.94 (bs, 2H, H4′,-CH2), 3.81 (bs, 2H, H3′,-CH2), 4.90(s, 2H, H1′, CH2-N), 5.31 (s, 2H, CH2-benzyl), 7.12-7.68 (m, 12H, ArH). \(^{13}\text{C-NMR:} \) 22.5 (C4′), 30.5 (C1′), 46.1 (C3′), 47.2 (CH2-benzyl), 104.3 (C4), 109.6 (C4a′ or C7′), 118.4-126.8 (Ar-C6, C7, C6′-C8′), 127.8-129.1 (C2″-C6″ benzyl), 130.8 (C12a′), 137.4 (C1″), 137.4 (C8a′) and 164.0 (C=O).

_Synthesis of 2-(3-chlorobenzothien-2-oyl)-9-o-nitrobenzyl-9H-1,2,3,4-tetrahydro-pyrido[3,4-b]indole (61)._

Following the method used for the synthesis of 60, 61 (C27H21\(\text{ClN}_3\text{O}_3\text{S}\)) (0.34 g, 50 %) was prepared by reacting 55 (0.5 g, 1.36 mmol), with o-nitrobenzyl bromide (0.46 g, 2.1 mmol) as yellow oil after flash chromatography (40 % ethyl acetate: hexane). The oil was recrystallised from hot absolute ethanol to give 61 as a yellow crystalline solid. Mp.191-193 °C. Mass spectrum (Cl−), m/z 502 [MH+1,35Cl]; HRMS (Cl) Found: 502.0992 (1.2 ppm), required: 502.0983 for C27H21\(^{35}\text{ClN}_3\text{O}_3\text{S}\). \(^{1}\text{H-NMR (CDCl}_3, 300 \text{ MHz)}\): 3.00 (bs, 2H, H4′, CH2), 3.86 (bs, 2H, H3′, CH2), 4.87(s, 2H, H1′, CH2-N), 5.83 (s, 2H, CH2-benzyl), 7.14-7.82 (m, 11H, ArH), 8.24(bs, 1H, H3″). \(^{13}\text{C-NMR:} \) 21.4 (C4′), 22.6 (C1′), 40.5 (C3′), 44.9 (CH2-benzyl), 104.3 (C4), 109.5 (C4a′ or C7′), 118.4-126.8 (Ar-C6, C7, C6′-C8′), 127.8-129.1 (C2″-C6″ benzyl), 130.8 (C12a′), 137.4 (C1″), 137.4 (C8a′) 147.1 (C-NO2) and 162.5 (C=O).

Anhydrous tetrahydrofuran (35 mL), was added to a mixture of 23, (0.69 g, 3.0 mmol), amine 44 (0.46 g, 2.20 mmol) and anhydrous potassium carbonate (0.34 g, 2.46 mmol). The mixture was then heated at reflux for 3h under nitrogen. After the reaction was complete (via TLC), the cooled solution was filtered to remove unreacted potassium carbonate and the solvent evaporated to give a yellow oil that solidified rapidly. The product was chromatographed (30 % ethyl acetate: hexane) and then recrystallised from hot ethanol to give 62 (1.09 g, 97 %) as a white crystalline solid. Mp. 124-126 °C. Mass spectrum (Cl⁺) m/z 381 [MH⁺ˌ₃₅Cl]; HRMS (Cl⁺) Found: 380.096, (0.1 ppm), required: 380.0961 for C₁₈H₂₁₃₅ClN₂O₃S. ¹H-NMR (CDCl₃, 300 MHz): 1.40 (s, 3H, CH₃), 3.52 (s, 8H, CH₂), 7.49 (m, 2H, Ar-H5 and H6), 7.84 (m, 2H, Ar-H4 and H7). ¹³C-NMR: 28.7 (3C, CH₃), 42.3 (4C, CH₂), 80.7 (C-CH₃), 122.8 (C7), 122.9 (C4), 125.7, 126.9 (C5 and C6), 130.0 (C3), 135.8 (C3a), 154.1 (C=O, boc), 162.2 (C=O, amide).

Synthesis of 4-tert-butyloxycarbonyl-1-(3-bromobenzo[b]thien-2-oyl)-piperazine (63).

Anhydrous DMF (15 mL) was syringed into a flask containing a mixture of 25 (0.41 g, 1.59 mmol), 44 (0.30 g, 1.6 mmol), HOBt (0.21 g, 1.55 mmol) and DCC (0.32 g, 1.55 mmol) placed on an ice bath. The mixture was stirred at room temperature overnight under nitrogen. The reaction mixture was vacuum filtered to
remove the precipitated by-product dicyclohexylurea and solvent evaporated to give an oily residue which was taken up in ethyl acetate (25 mL) and re-filtered to remove traces of remaining urea. The organic portion was then washed with distilled water (50 mL) and dried. Solvent was evaporated to afford a clear oil which was flash chromatographed (30 % ethyl acetate: hexane) and recrystallised from hot ethanol to give white crystals of 63 (0.57 g, 85 %). Mp. 90-93 °C. Mass spectrum, (Cl⁺) m/z 425 [MH⁺,1.79Br]; HRMS (EI) Found: 426.0435 (4.8 ppm), required: 426.0415 for C₁₈H₂₁₈¹BrN₂O₃S. ¹H-NMR (CDCl₃, 300 MHz): 1.40 (s, 3H, CH₃), 3.52 (s, 8H, CH₂), 7.49 (m, 2H, Ar-H5 and H6), 7.84 (m, 2H, Ar-H4 and H7). ¹³C-NMR: 28.7 (3x CH₃), 42.3 (4C, CH₂), 80.7 (C-CH₃), 122.8 (C7), 122.9 (C4), 125.7, 126.8 (C5; C6), 130.0 (C3), 135.8 (C3a), 154.5 (C=O, boc) and 162.5 (C=O, amide).

**Synthesis of (3-chlorobenzof[b]thien-2-yl)(piperazine-1-yl)methanone (64).**

Trifluoroacetic acid (3.0 mL, 38.95 mmol) was slowly added into a solution 61 (0.71 g, 1.89 mmol) in dry dichloromethane (30 mL) under nitrogen. The mixture was stirred for one and half-hours at room temperature. Solvent was evaporated under reduced pressure to afford a clear oil which was dissolved in dichloromethane (25 mL), washed with saturated sodium bicarbonate solution (2x 25 mL) and distilled water (50 mL). Combined organic portions were dried and the solvent evaporated to afford 64 (0.52 g, 99 %) as a colourless oil. Mass spectrum (Cl⁺) m/z 281 [MH⁺,1.37Cl]. HRMS (EI) Found: 280.2335, (1.8 ppm), required: 280.2375 for C₁₃H₁₃³⁵ClN₂O₅S. ¹H-NMR (CDCl₃, 300 MHz): 1.96 (NH), 2.93 (bs, 2H, H3` and H5`), 3.48, 3.79 (bs, 2H, H2` and H6`), 7.49 (m, 2H, Ar-H5 and H6), 7.84 (m, 2H, Ar-H4 and H7). ¹³C-NMR: 46.6 (C3` and C5`), 49.0 (C2` and C6`).
Synthesis of (3-bromobenzo[b]thien-2-yl)(piperazine-1-yl)methanone (65).

Following the same method used to deprotect amide 64, compound 65 (0.38 g, 83 %) was prepared from 62 (0.61 g, 1.42 mmol) as a clear oil.

Mass spectrum (Cl⁺) \( m/z \) 325 [MH⁺, 35Cl]; HRMS (EI) Found: 324.1255, (1.3 ppm), required: 324.1258 for C₁₃H₁₃BrN₂O₂S. ^1H-NMR (CDCl₃, 300 MHz): 1.96 (NH), 2.93 (bs, 2H, H3` and H5`), 3.48, 3.79 (bs, 2H, H2` and H6`), 7.49 (m, 2H, Ar-H5 and H6), 7.84 (m, 2H, Ar-H4 and H7). ^13C-NMR: 46.6 (C3` and C5`), 49.0 (C2` and C6`), 122.7 (C7), 122.9 (C4), 125.6 (C6), 126.7 (C5), 130.0 (C3), 137.6 (C3a) and 161.7 (C=O).


Benzyl bromide (0.5 mL, 4.11 mmol) was syringed to a mixture 65 (0.42 g, 0.129 mmol) and anhydrous potassium carbonate (0.15 g, 1.08 mmol) in dry tetrahydrofuran (15 mL). The stirring was continued overnight at room temperature under nitrogen. The reaction mixture was filtered to remove unreacted potassium carbonate, and the solvent concentrated under reduced pressure to give a yellow oil which was chromatographed (30 % ethyl acetate: hexane) to give 66 (0.24 g, 44 %) as a clear oil. Mass spectrum (Cl⁺) \( m/z \) 371.
[MH$^{+}$ $^{35}$Cl]; **HRMS (EI)** Found: 370.0235, required: 370.0229 for C$_{20}$H$_{20}$$^{35}$ClN$_{2}$O$_{2}$.

$^1$H-NMR (CDCl$_3$, 300 MHz): 2.51 (bs, 2H, H3’ and H5’), 3.54, (bs, 2H, H2’ and H6’), 3.82 (bs, 2H, CH$_2$ benzyl) 7.22-7.32 (m, 5H, benzyl) 7.44-7.48 (m, 2H, Ar-H5 and H6), 7.84 (m, 2H, Ar-H4 and H7). $^{13}$C-NMR: 46.6 (C3’ and C5’), 49.0 (C2’ and C6’), 60.1 (CH$_2$ benzyl), 122.7 (C7), 122.9 (C4), 125.6 (C6), 126.7 (C5), 127-129.1 (C2’-C6’, benzyl) 131.9 (C3), 137.6 (C3a) and 162.7 (C=O).

**Synthesis of (3-bromobenzof[b]thien-2-yl)(4-benzylpiperazine-1-yl) (67).**

![Chemical structure](attachment:image.png)

To a cooled mixture (ice bath) of **25** (0.40 g, 1.55 mmol), 1-benzylpiperazine (0.30 mL, 1.73 mmol), HOBt (0.19 g, 1.40 mmol) and DCC (0.34 g, 1.65 mmol) was added dry DMF (15 mL). The mixture was then stirred at room temperature overnight under argon after which it was filtered to remove the precipitated urea by-product. DMF was concentrated under reduced pressure and the oily residue taken up in ethyl acetate (25 mL) and re-filtered again to remove traces of the crushed out urea. The organic portion was washed once with distilled water (50 mL) and dried. Solvent was evaporated to afford an oil which was purified via chromatography (30 % ethyl acetate: hexane) to give a colourless oil of **67** (0.47 g, 73 %). Mass spectrum, (Cl$^+$) m/z 415 [MH$^{+}$, $^{81}$Br]; 337 (C$_{14}$H$_{14}$$^{79}$BrN$_{2}$OS$^{-}$); **HRMS (EI)** Found: 414.0415, required: 414.0419 for C$_{20}$H$_{20}$$^{81}$BrN$_{2}$O$_{2}$. $^1$H-NMR (CDCl$_3$, 300 MHz): 2.55 (bs, 2H, H3’ and H5’), 3.55, (bs, 2H, H2’ and H6’), 3.89 (bs, 2H, CH$_2$ benzyl) 7.25-7.35 (m, 5H, benzyl) 7.44-7.49 (m, 2H, Ar-H5 and H6), 7.85 (dd, J = 5.4 Hz, 2H, Ar-H4 and H7). $^{13}$C-NMR: 46.2 (C3’ and C5’), 49.2 (C2’ and C6’), 60.1 (CH$_2$ benzyl), 122.8 (C7), 123.0 (C4), 125.7
(C6), 126.4 (C5), 127-129.1 (C2”-C6”, benzyl) 131.9 (C3), 137.7 (C3a) and 163.0 (C=O).

Synthesis of 1-(3-chlorobenz[b]thien-2-oyl)-4-phenacyl piperazine (68).

Anhydrous THF (15 mL) was added to a mixture of 64 (0.30 g, 1.0 mmol), 2-bromoacetophenone (0.21 g, 1.04 mmol) and anhydrous potassium carbonate (0.15 g, 1.08 mmol). The reaction mixture was heated at reflux for 3h at room temperature under nitrogen. The cooled solution of the reaction mixture was then filtered to remove excess potassium carbonate. The solvent was evaporated to give an orange coloured oil which was column chromatographed to afford 68 (0.17 g, 40 %) as a clear oil. Mass spectrum (Cl⁺) m/z 399, [MH+ 35Cl]; HRMS (EI) Found: 398.0555, (2.8 ppm); required: 398.0545 for C21H20 35ClN2O2S.

1H-NMR (CDCl3, 300 MHz): 2.71 (bs, 4H, CH2-NCH2), 3.60 (s, 2H, CH2-OCH2), 3.90 (bs, 2H, NCH2), 7.43-7.51 (m, 5H, ArH- H5, H6, H3”, H4” and H5””) and 7.78-7.98 (m, 4H, ArH-H4, H7, H2”” and H6”). 13C-NMR: 46.6 (C3’ and C5’), 49.0 (C2’ and C6’), 64.18 (CH2, phenacyl), 119.5 (C3), 122.7-126.7 (C4, C5, C6, C7), 128.2 (C3” and C5””), 128.8 (C2” and C6””), 133.6 (C4””) 135.9 (C1”), 137.6 (C4a), 161.7 (C=O, amide) and 195.8 (C=O, ketone).
Synthesis of 1- (3-bromobenzothien-2-yl)-4-phenacyl piperazine (69).

Following the same method used to synthesise 68, 69 (C\textsubscript{21}H\textsubscript{19}BrN\textsubscript{2}O\textsubscript{2}S) (0.12 g, 38 %) was synthesised by reacting 65 (0.24 g, 0.73 mmol), with 2-bromoacetophenone (0.15 g, 0.72 mmol) and anhydrous potassium carbonate (0.1 g, 0.72 mmol) as a colourless oil after column chromatography. Mass spectrum (CI\textsuperscript{+}) m/z 442 [MH\textsuperscript{+1}, \textsuperscript{81}Br], 365 (C\textsubscript{15}H\textsubscript{14}BrN\textsubscript{2}O\textsubscript{2}S\textsuperscript{+}), 337 (C\textsubscript{14}H\textsubscript{14}BrN\textsubscript{2}O\textsubscript{S} -); HRMS (EI) Found: 441.0435, (4.8 ppm); required: 441.0415 for C\textsubscript{21}H\textsubscript{20}81BrN\textsubscript{2}O\textsubscript{2}S. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 2.71 (bs, 4H, CH\textsubscript{2}-NCH\textsubscript{2}), 3.60 (s, 2H, CH\textsubscript{2}, OCH\textsubscript{2}), 3.90 (bs, 2H, NCH\textsubscript{2}), 7.43-7.51 (m, 5H, ArH- H5, H6, H3”, H4” and H5”) and 7.78-7.98 (m, 4H, ArH-H4, H7, H2” and H6”). \textsuperscript{13}C-NMR: 46.6 (C3` and C5`), 49.0 (C2` and C6`), 64.18 ( CH\textsubscript{2}, phenacyl), 119.5 (C3), 122.7-126.7 (C4, C5, C6 and C7), 128.2 (C3” and C5”), 128.8 (C2” and C6”), 133.6 (C4”), 135.9 (C1”), 137.6 (C4a), 161.7 (C=O, amide) and 195.8 (C=O, ketone).

Synthesis of (3-chlorobenzothien-2-yl)(4-(4-chlorophenyl)piperazine-1-yl) methanone (70).

Following the method used to synthesise 62, 70 (0.35 g, 52 %) was prepared by reacting 23 (0.40 g, 1.73 mmol), and 42 (0.40 g, 1.71 mmol) as a colourless solid after flash chromatography (DCM) and crystallisation from absolute alcohol. Mp. 143-145 °C. Mass spectrum (CI\textsuperscript{+}) m/z 391 [MH\textsuperscript{+1}, \textsuperscript{35}Cl]; HRMS (CI) Found:
391.0438, required: 391.0410 for C\textsubscript{19}H\textsubscript{16}Cl\textsubscript{2}N\textsubscript{2}OS.  \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 3.19 (bs, 4H), 3.91, 3.66 (bs, 4H), 6.88 (d, \textit{J} = 22.8 Hz, 2H, ArH), 7.23 (d, \textit{J} = 6 Hz, 2H, ArH), 7.48 (m, 2H, ArH) and 7.82 (m, 2H, ArH). \textsuperscript{13}C-NMR: 47.4 (C2', C6'), 118.2 (C2'', C6''), 122.8 (C7), 123.9 (C7, C4''), 125.9 (C5, C6), 129.3 (C3, C3'' and C5''), 138.0 (C3a), 149.5 (C1'') and 161.5 (C=O).

\textit{Synthesis of (3-chlorobenzo[b]thien-2-yl)(4-(4-chlorophenyl)piperazine-1-yl) methanone (71).}

\begin{equation*}
\includegraphics[width=0.5\textwidth]{synthesis_diagram}
\end{equation*}

Dry \textit{N,N}-dimethylformamide (15 mL) was added into a mixture of 25 (0.40 g, 1.55 mmol), 42 (0.40 mL, 1.73 mmol), HOBt (0.21 g, 1.55 mmol) and DCC (0.34 g, 1.65 mmol) placed on an ice bath. The mixture was then stirred at room temperature overnight under nitrogen. After the reaction came to completion (\textit{via} TLC) the reaction mixture was filtered to remove precipitated dicyclohexylurea by-product. The DMF was concentrated on the rotary evaporator to give a crude oily residue which was taken up in ethyl acetate (25 mL) and re-filtered to ensure complete removal of the urea. The solvent was washed once with distilled water (50 mL) and dried. Solvent was then evaporated to afford a clear oil which was column chromatographed (DCM) and crystallised from hot ethanol to give 71 (0.65 g, 96 \%) as a colourless crystalline solid. Mp.148-150 \degree C. Mass spectrum (Cl\textsuperscript{+}) \textit{m/z} 435 [MH\textsuperscript{+}, \textsuperscript{35}Cl\textsuperscript{81}Br]; HRMS (CI) Found: 433.9858 (0.1 ppm), required: 433.9854 for C\textsubscript{19}H\textsubscript{16}\textsuperscript{35}Cl\textsuperscript{81}BrN\textsubscript{2}OS. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 3.18 (bs, 4H), 3.61, 3.92 (bs, 4H), 6.83 (d, \textit{J} = 22.8 Hz, 2H, ArH), 7.23 (d, \textit{J} = 6 Hz, 2H, ArH), 7.48 (m, 2H, ArH) and 7.80 (m, 2H, ArH). \textsuperscript{13}C-NMR: 47.4 (C2', C6'), 118.2 (C2'', C6''), 122.8 (C7), 123.9 (C7, C4''), 125.9 (C5, C6), 129.3 (C3, C3'' and C5''), 138.0 (C3a), 149.5 (C1'') and 161.5 (C=O).
Synthesis of (3-chlorobenzof[b]thiophen-2-yl)(4- (pyridin-2-yl) piperazine-1-yl) methanone (72).

Following the same method used to synthesise 62, 72 (0.30 g, 63 %), was prepared by 23 (0.31 g, 1.30 mmol) and the amine 38 (0.21 g, 1.28 mmol) as a colourless crystalline solid after flash chromatography (30 % ethyl acetate: hexane) and re-crystallisation from hot ethanol. Mp.132-134 °C. Mass spectrum (Cl⁺) m/z 358 \([\text{MH}^+ \text{Cl}^{35}]\); HRMS (Cl) Found: 357.0702 (-1.4 ppm) required: 357.0707 for C₁₈H₁₆₃₅ClN₃OS. \(^1\)H-NMR (CDCl₃, 300 MHz): 3.65, 3.92 (bs, 8H, -CH₂, H₂`,H₃`,H₅`, H₆`), 6.67 (q, J = 4.2 Hz, 2H, H₄", H₆"), 7.44-7.52 (m, H₅, H₆, H₅"), 7.84 (dd, J = 2.5 Hz, 2H, H₄ and H₇), 8.20 (s, 1H, H₃"). \(^{13}\)C-NMR: 45.7 (C₂`, C₆`), 47.5 (C₃`, C₅`), 107.6 (C₆"), 114.2 (C₄"), 122.9 (C₇), 125.7 (C₅), 126.8 (C₆), 129.7 (C₃), 137.6 (C₃a), 137.8 (C₅"), 148.1 (C₃"), 159.1 (C₁") and 161.8 (C=O).


In the same manner as that used to prepare 71, 73 (C₁₈H₁₆⁸¹BrN₃OS) (0.30 g, 48 %) was synthesised by reacting 25 (0.40 g, 1.55 mmol), and 38 (0.25 g, 1.53 mmol), to afford a clear oil after chromatography. The oil was then re-crystallised from hot ethanol to give 73 as a colourless highly crystalline solid. Mp.154-155 °C. Mass spectrum, (Cl⁺) m/z 402 \([\text{MH}^+ \text{Br}^{81}]\), 324 (C₁₈H₁₆N₃OS); HRMS (Cl) Found: 401.0197 (8.8 ppm), required: 401.0162 for C₁₈H₁₆⁸¹BrN₃OS. \(^1\)H-NMR (CDCl₃, 300 MHz): 3.65, 3.94 (bs, 8H, -CH₂, H₂`,H₃`,H₅`, H₆`), 6.65 (q, J
= 4.2 Hz, 2H, H4\'', H6\''), 7.46-7.53 (m, H5, H6, H5\''), 7.84 (dd, J = 2.5, 6 Hz, 2H, H4 and H7), 8.18 (s, 1H, H3\''). \textbf{13C-NMR:} 45.7 (C2\'', C6\''), 47.5 (C3\'', C5\''), 107.6 (C6\''), 114.2 (C4\''), 122.9 (C7), 125.7 (C5), 126.8 (C6), 129.7 (C3), 137.6 (C3a), 137.8 (C5\''), 148.1 (C3\''), 159.1 (C1\'') and 161.8 (C=O).

\textit{Synthesis of (benzo[b]thien-2-yl)(4- (pyridin-2-yl) piperazine-1-yl) methanone (74).} In the same manner used to prepare 73, 74 (C_{18}H_{17}N_{3}OS), (0.72 g, 67 %) was synthesised by reacting the acid 20 (0.60 g, 3.3 mmol), and the amine 38 (0.54 g, 3.3 mmol), to afford a yellow solid after column chromatography (30 % ethyl acetate: hexane) which was then re-crystallised from absolute ethanol. Mp.123-125 °C. Mass spectrum, (Cl\(^{+}\)) \textit{m/z} 324 [MH\(^{+}\)]; \textbf{HRMS (Cl)} Found: 323.0207 (6.8 ppm), required: 323.0172 for C_{18}H_{18}N_{3}OS. \textbf{\textit{1H-NMR (CDCl\textsubscript{3}, 300 MHz):}} 3.63 (s, 4H, CH\textsubscript{2}, H2\'', H6\''), 3.89 (s, 4H, -CH\textsubscript{2}, H3\'', H5\''), 6.67 (q, J = 4.2 Hz, 2H, H4\'', H6\''), 7.46-7.52 (m, H5, H6, H5\''), 7.54 (s, 1H, H3), 7.85 (m, 2H, H4 and H7), 8.19 (s, 1H, H3\''). \textbf{13C-NMR:} 45.5 (C2\'', C6\''), 47.5 (C3\'', C5\''), 107.6 (C6\''), 114.2 (C4\''), 122.6 (C3), 124.9 (C7), 125.0 (C5), 126.8 (C6), 138.8 (C5\''), 140 (C3a), 148.2 (C3\''), 159.2 (C1\'') and 164.2 (C=O).
Synthesis of (3-bromobenzo[b]thien-2-yl)(4-(pyrimidin-2-yl)piperazine-1-yl) methanone (75).

In the same manner used to prepare 73, 75 (C_{17}H_{15}N_{4}OS) (0.50 g, 50 %) was synthesised by reacting the acid 25 (0.70 g, 2.72 mmol), and the amine 39 (0.44 g, 2.64 mmol), to afford a colourless solid after column chromatography (30 % ethyl acetate: hexane) which was then re-crystallised from hot ethanol. Mp.188-190 °C. Mass spectrum, (EI⁺) m/z 404 [MH⁺, 81Br], 323 (C_{17}H_{15}N_{4}OS⁺); HRMS (CI) Found: 403.0228 (-1.0 ppm), required: 403.0232 for C_{17}H_{16}^{81}BrN_{4}OS. ¹H-NMR (CDCl₃, 300 MHz): 3.94 (bs, 8H, -CH₂, H₂`, H₆`, H₃`, H₅`), 6.54 (t, J = 4.95 Hz, 1H, H₄`), 7.47 (m, 2H, H₅ and H₆), 7.84 (dd, J = 4.35, 7.35 Hz, 2H, H₄ and H₇), 8.32 (d, J = 4.8 Hz, 2H, H₃” and H₅”). ¹³C-NMR: 45.5 (C₂`, C₆`), 47.5 (C₃`, C₅`), 110.8 (C₄`), 122.8 (C₇), 125.0 (C₅), 126.7 (C₆), 137.4 (C₃a), 138.1 (C₂) 158.0 (C₃” and C₅”), 161.5 (C₁”), and 162.6 (C=O).


Dry THF (15 mL) was added to a mixture of 64 (0.20 g, 0.86 mmol), 46 (0.20 g, 0.77 mmol) and anhydrous potassium carbonate (0.15 g, 1.08 mmol). The mixture was heated at reflux for 3h under nitrogen. After the reaction came to completion (TLC analysis), the cooled reaction mixture was vacuum filtered to remove unreacted potassium carbonate and the organic solvent evaporated under
reduced pressure on a rotary evaporator to give a yellow oil. The oil was purified by column chromatography to afford clear oil, which was then recrystallised from hot ethanol to give colourless crystals of 76 (0.30 g, 69%). Mp. 88-90 °C. Mass spectrum (Cl+) m/z 503 [MH+1, 35Cl]; HRMS (Cl) Found: 503.1196 (1.1 ppm), required: 503.1190 for C28H2435ClN2O3S. 1H-NMR (CDCl3, 300 MHz): 3.50 (bs, 8H, CH2, piperazine), 4.26 (t, J = 11.4 Hz, 1H, CH, H1”), 4.52 (d, J = 6.3 Hz, 2H, OCH2), 7.25-7.42 (m, 6H, ArH- H5, H6, H3”, H4”, H7” and H8”), 7.46-7.56 (m, 2H, ArH- H2” and H8”), 7.74-7.88 (m, 4H, ArH- H4, H7, H5” and H6”). 13C-NMR: 47.7 (C2′ and C6′), 122.8 (C7), 122.9 (C4), 127.3 (C4” and C7”), 128.0 (C2”, C5”, C6” and C8”), 141.5 (C6a” and C6b”), 143.9 (C1a” and C9a”), 156.3 (C=O) and 162.5 (C=O).

**Synthesis of 9H-9-fluorenylmethyl 4-(3-bromobenzol[b]thien-2-oyl)-1-piperazinecarboxylate (77).**

Following the same procedure used to prepare 71, 77 (C28H23BrN2O3S) (0.19 g, 32 %), was prepared by reacting 65 (0.35 g, 1.07 mmol) and 46 (0.31 g, 1.25 mmol) to give 77 as a solid after chromatography and re-crystallisation from hot ethanol. Mp 98-102 °C. Mass spectrum, (Cl+) m/z 547 [MH+1, 81Br]; HRMS (Cl) Found: 546.3121 (1.5 ppm), required: 546.2590 for C28H2481BrN2O3S. 1H-NMR (CDCl3, 300 MHz): 3.53 (bs, 8H, CH2, piperazine), 4.25 (t, J = 11.4 Hz, 1H, CH, H1”), 4.52 (d, J = 6.3 Hz, 2H, OCH2), 7.26-7.42 (m, 6H, ArH- H5, H6, H3”, H4”, H7” and H8”), 7.46-7.57 (m, 2H, ArH- H2” and H8”), 7.73-7.88 (m, 4H, ArH- H4, H7, H5” and H6”). 13C-NMR: 47.7 (C2′
and C6’), 122.8 (C7), 122.9 (C4), 127.3 (C4” and C7”), 128.0 (C2”, C5”, C6” and C8”), 142.0 (C6a” and C6b”), 143.9 (C1a” and C9a”), 158.2 (C=O) and 163.1 (C=O).

**Synthesis of N,N-diallyl-3-chlorobenzo[b]thiophen-2-carboxamide (78).**

Diallylamine (0.266 mL, 2.16 mmol) dissolved in dry tetrahydrofuran (5 mL) was added to a mixture of 23 (0.5 g, 2.17 mmol) and anhydrous potassium carbonate (0.5 g, 3.62 mmol) in dry THF (15 mL). The mixture was heated at reflux for 3h under nitrogen. The reaction mixture was cooled to room temperature and then filtered to remove the unreacted potassium carbonate. Organic solvent was evaporated under reduced pressure to give a yellow oil which was purified via column chromatography (30 % ethyl acetate: hexane). Compound 78 (0.60 g, 95 %) was isolated as a clear oil. Mass spectrum (Cl+), m/z 292 [MH+1, 35Cl]; HRMS (Cl) Found: 291.0176 (1.1 ppm), required: 291.0170 for C15H15^35ClNOS.

\(^1\)H-NMR (CDCl3, 300 MHz): 3.92, 4.19 (bd, 4H, 2x NCH2), 5.24 (sx, \(J = 13.0\) Hz, 4H, CH2), 5.74, 5.81 (bd, \(J = 36.9\) Hz, 2H, 2x CH), 7.45 (qn, \(J = 7.28\) Hz, 2H, ArH, H5 and H6), 7.8 (m, 2H, ArH, H4 and H7). \(^13\)C-NMR: 47.1, 51.1 (C2’), 118.2, 118.8 (C4’), 122.6 (C7), 122.9 (C4), 125.6, 126.6 (C5 and C6), 129.9 (C3), 132.2, 132.9 (C3’), 137.4 (C3a) and 163.1 (C=O).

**Synthesis of N,N-diallyl-3-bromobenzo[b]thiophen-2-carboxamide (79).**

In the same manner used to synthesise 73, 79 (0.35 g, 95 %) was prepared by the reaction of 25 (0.28 g, 1.09 mmol) and 47 (0.14 mL, 1.09 mmol) to give a yellow
oil which was then flash chromatographed to afford 79 as a clear oil. Mass spectrum (CI), \( m/z \) 337 [MH\(^{+1}\), \(^{81}\)Br]; HRMS (CI) Found: 336.3171, required: 336.3170 for C\(_{15}\)H\(_{15}\)\(^{81}\)BrNOS. \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 3.92, 4.19 (bd, 4H, 2x NCH\(_2\)), 5.24 (sx, \( J = 13.08 \) Hz, 4H, CH\(_2\)), 5.74, 5.81 (bd, \( J = 36.9 \) Hz, 2H, 2x CH), 7.45 (qn, \( J = 7.28 \) Hz, 2H, ArH, H5 and H6), 7.8 (m, 2H, ArH, H4 and H7). \(^{13}\)C-NMR: 47.1, 51.1 (C2\(’\)), 118.2, 118.8 (C4\(’\)), 122.6 (C7), 122.9 (C4), 125.6, 126.6 (C5 and C6), 129.9 (C3), 132.2, 132.9 (C3\(’\)), 137.4 (C3a) and 163.1 (C=O).

**Synthesis of N-4-(tert-butyl)-(4S)-2-(3-chlorobenzo[b]thien-2-oyl)-1,2,3,4-tetrahydro-4-isoquinolinecarboxamide(80).**

Following the same method as that used to synthesise 76, 80 (0.75 g, 90 %) was prepared by reacting 23 (0.45 g, 1.95 mmol) and 45 (0.45 g, 1.93 mmol). Compound 80 was obtained as colourless crystals after purification by chromatography and re-crystallisation from ethanol. Mp 162-164 °C. Mass spectrum (CI) \( m/z \) 427 [MH\(^{+1}\), \(^{35}\)Cl], 391 (C\(_{23}\)H\(_{23}\)N\(_2\)O\(_2\)S\(’\)). HRMS (EI) Mass Found: 426.1168 (7.5 ppm), required: 426.1136 for C\(_{23}\)H\(_{23}\)\(^{35}\)ClN\(_2\)O\(_2\)S. \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 1.31 (s, 9H, 3x CH\(_3\)), 3.14; 3.48 (dd, 2H, H3\(’\)), 4.62 (s, 2H, H1\(’\)), 5.28 (bs, 1H, H4\(’\)), 6.93-7.48 (m, 4H, ArH- H5\(’\), H6\(’\), H7\(’\) and H8\(’\)),7.53 (m, 2H, ArH, H5 and H6), 7.88 (m, 2H, ArH- H4 and H7). \(^{13}\)C-NMR: 29.0 (3x CH\(_3\)), 47.7 (C4\(’\)), 51.8 (C1\(’\) and C-CH\(_3\)), 54.6 (C3\(’\)), 122.9 (C7), 124.0 (C5), 125.7 (C6), 126.7; 127.0 (C6 or C7\(’\)), 128.0; 128.5 (C3, C4), 134.1 (C4a\(’\)), 137.2 (C3a), 164.3 (C=O) and 168.5 (C=O-NH).
Synthesis of N-4-(tert-butyl)-(4S)-2-(3-bromobenzothien-2-yl)-1,2,3,4-tetrahydro-4-isoquinolinecarboxamide (81).

Dry N,N-dimethylformamide (20 mL) was added to an ice-cooled mixture of 25 (0.42 g, 1.63 mmol), 45 (0.35 g, 1.56 mmol), HOBt (0.21 g, 1.55 mmol) and DCC (0.35 g, 1.69 mmol). The mixture was then warmed to room temperature and stirring continued overnight under nitrogen. The mixture was filtered to remove the precipitated urea. The filtrate was then evaporated to give an oil which was dissolved in ethyl acetate (25 mL) and re-filtered. The organic portion was washed once with distilled water (50 mL) and dried. Solvent was evaporated to afford a clear oil which solidified rapidly. The solid was then chromatographed (30 % ethyl acetate: hexane) and re-crystallised from dichloromethane: hexane to give 81 (0.45 g, 60 %) as a colourless crystalline solid. Mp 148-150 °C. Mass spectrum (Cl+) m/z 471 [MH+\(^{\text{81}}\)Br]; \textbf{HRMS (EI) Found:} 472.0643 (1.2 ppm), required: 472.0637 for C\(_{23}\)H\(_{23}\)\(^{\text{81}}\)BrN\(_2\)O\(_2\)S. \(^{1}\)H-NMR (CDCl\(_3\), 300 MHz): 1.31 (s, 9H, 3x CH\(_3\)), 3.14; 3.46 (dd, 2H, H\(_{3}\)'), 4.62 (s, 2H, H1'), 5.28 (bs, 1H, H4'), 6.93-7.48 (m, 4H, ArH- H5', H6', H7' and H8'), 7.53 (m, 2H, ArH- H5 and H6), 7.88 (m, 2H, ArH, H4 and H7). \(^{13}\)C-NMR: 29.0 (3x CH\(_3\)), 47.7 (C4'), 51.8 (C1' and C-CH\(_3\)), 54.6 (C3'), 122.9 (C7), 124.0 (C5), 125.7 (C6), 126.7; 127.0 (C6' or C7'), 128.0; 128.5 (C3, C4), 134.1 (C4a'), 137.2 (C3a), 164.3 (C=O) and 168.5 (C=O-NH).
Synthesis of 1-(3-chlorobenzo[b]thien-2-oyl)-4-(4-chlorophenyl)-4-hydroxy-piperidine (82).

Following the same method as that used to synthesise 76, 82 (0.40 g, 45 %) was synthesised by reacting 23 (0.5 g, 2.17 mmol) and 48 (0.45 g, 2.13 mmol) to give a clear oil after chromatography. Mass spectrum (Cl⁺) m/z 406 [MH⁺, 35Cl]; HRMS (EI) Found: 406.3254 (1.2 ppm), required: 406.0357 for C₂₀H₁₇Cl₂NO₂S. ¹H-NMR (CDCl₃, 300 MHz): 1.73-1.87 (bs, 4H, 2x CH₂), 2.06 (s, 1H, OH), 3.36;3.63 (bs, 4H, 2x CH₂), 7.3 (m, 4H, ArH, phenyl), 7.46 (m, 2H, ArH, H5 and H6), 7.82 (m, 2H, ArH, H4 and H7). ¹³C-NMR: 35.2 (2x N-CH₂), 38.0 (2x CH₂), 71.3 (C4’), 119.3 (C3), 122.7, 122.9 (C4, C7), 125.6 (C5, C6), 128.6 (C1”, C2” and C5”), 129.9 (C4”), 137.5 (C3a, C1”), 146.3 (C7a) and 161.6 (C=O).

Synthesis of 1-(3-bromobenzo[b]thien-2-oyl)-4-(4-chlorophenyl)-4-hydroxy-piperidine (83).

Following the same method as that used to synthesise 81, 83 (0.71 g, 93 %) was prepared from 25 (0.37 g, 1.74 mmol) and 48 (0.37 g, 1.74 mmol) to afford a clear oil after chromatography (30 % ethyl acetate: hexane). Mass spectrum (Cl⁺) m/z 449 [MH⁺, ⁸¹Br⁺]; HRMS (EI) Found: 448.7764 (3.2 ppm), required: 448.9851 for C₂₀H₁₇⁸¹BrCl₂NO₂S. ¹H-NMR (CDCl₃, 300 MHz): 1.73-1.87 (bs, 4H, 2x CH₂), 2.06 (s, 1H, OH), 3.36;3.63 (bs, 4H, 2x CH₂), 7.3 (m, 4H, ArH, phenyl), 7.46 (m, 2H, ArH, H5 and H6), 7.82 (m, ArH, H4 and H7). ¹³C-NMR: 35.2 (2x N-CH₂), 38.0
Synthesis of 2-(3-chlorobenzothien-2-yl)(5-(4-chlorophenyl)-2,5-diaza bicyclo[2.2.1]heptan-2-yl)methanone (84).

The amine 43, (0.07 g, 0.33 mmol) in its free base state was reacted with 23 (0.07 g, 0.30 mmol), and anhydrous potassium carbonate (0.06 g, 0.43 mmol) in anhydrous THF (15 mL). The mixture was heated at reflux for 3 h under nitrogen. The mixture was then cooled to room temperature and vacuum filtered to remove unreacted base. The filtrate was evaporated under reduced pressure to give light yellow oil, which was then purified via chromatography (30% ethyl acetate: hexane) to give a colourless solid of 84 (0.04 g, 33%). Mp. 114-120 °C. Mass spectrum (Cl⁺) m/z 403 [MH⁺, 35Cl]; HRMS (Cl) Found: 402.0356 (2.2 ppm), required: 402.0360 for C₂₀H₁₇₃₅Cl₂N₂OS.

1H-NMR (CDCl₃, 300 MHz): 1.90; 2.08 (d, J = 9.5 Hz, 2H, H7'), 2.64 (t, J = 58.1 Hz, 1H, H3'), 3.33 (dd, J = 9.0, 5.02, Hz 2H, H5'), 4.52 (d, J = 9.3 Hz, 2H, H2'), 4.39 (q, J = 24.6 Hz, 1H, H6'), 6.42-6.90 (m, 2H, ArH-phenyl, H2"; H6", 2H,), 7.13-7.20 (m, 2H, ArH-phenyl, H3"; H5", 2H,), 7.46 (m, 2H, ArH- H5 and H6), 7.85 (m, 2H, ArH- H4 and H7). 13C-NMR: 37.0 (C7'), 47.4 (C2', C5'), 55.0 (C6'), 57.0 (C3'), 118.2 (C2", C6"), 122.8 (C7), 123.9 (C7, C4"), 125.9 (C5, C6), 129.3 (C3, C3", C5"), 138.0 (C3a), 150.0 (C1") and 164.0 (C=O).
Synthesis of 2- (3-bromobenzo[b]thien-2-yl)(5-(4-chlorophenyl)-2,5-diaza bicyclo [2.2.1]heptan-2-yl)methanone (85).

Following the same method as that used to synthesise 81, 85 (0.20 g, 28 %) was prepared by reacting the bromo- substituted acid 25 (0.40 g, 1.55 mmol) and the amine 44 (0.30 g, 1.40 mmol) in its free base form. Compound 85 was obtained as a colourless solid after chromatography and re-crystallisation from ethanol. Mp.147-150 °C. Mass spectrum, (Cl⁺) m/z 449 [MH⁺, 79Br]; 369 (C₂₀H₁₆ClN₂O₅S'). ¹H-NMR (CDCl₃, 300 MHz): 2.08 (d, J = 9.5 Hz, 2H, H7'), 2.66 (t, J = 58.1 Hz, 1H, H3'), 3.33 (dd, J = 9.0, 4.05 Hz, 2H, H5'), 4.49 (d, J = 9.3 Hz, 2H, H2'), 4.40 (q, J = 24.6 Hz, 1H, H6'), 6.40-6.91 (m, 2H, ArH-phenyl, H2”; H6”, 2H), 7.13-7.22 (m, 2H, ArH-phenyl, H3”; H5”, 2H), 7.47 (m, 2H, ArH- H5 and H6), 7.83 (m, 2H, ArH- H4 and H7). ¹³C-NMR: 37.4 (C7'), 47.3 (C2’, C5’), 55.1 (C6’), 57.1 (C3’), 118.3 (C2”, C6”), 122.7 (C7), 123.9 (C7, C4”), 125.9 (C5, C6), 129.3 (C3, C3”, C5”), 137.8 (C3a), 152.0 (C1”) and 164.0 (C=O).

Ethyl 3-chlorobenzo[b]thiophene-2-carboxylate (86).

To a solution of 23 (0.30 g, 1.30 mmol) in absolute ethyl alcohol (5 mL) was added 2 drops of concentrated sulfuric acid and the mixture heated at reflux overnight. After all the substrate had reacted (via TLC), the mixture was cooled to room temperature and solvent evaporated in vacuo to give a yellow oil which solidified rapidly. The solid was purified by re-crystallisation from DCM: hexane to give 86 (0.25 g, 81 %). Mass spectrum, (Cl⁺) m/z 241 [MH⁺, 35Cl]. ¹H-NMR
(CDCl₃, 300 MHz): 1.43 (t, J = 7.2 Hz, 3H, CH₃), 4.43 (q, J = 7.1 Hz, 2H, CH₂), 7.51 (m, 2H, H5 and H6), 7.81 (d, J = 5.7 Hz, 1H, H4), 7.98 (d, J = 6.9 Hz, 1H, H7). ¹³C-NMR: 15.1 (CH₃), 61.1 (CH₂), 122.6 (C7), 122.9 (C4), 125.6, 126.6 (C5 and C6), 129.9 (C3), 137.4 (C3a) and 163.1 (C=O).
6.2.2. Experimental for Chapter 3: The synthesis of fused benzo[b]thiophene amides.

Synthesis of 2-allyl-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridin-1-one (92).

To a solution of 79 (0.54 g, 1.61 mmol) heated at reflux in dry toluene (20 mL) under argon was added dropwise using an addition funnel a mixture of tributyltin hydride (1.0 mL, 3.4 mmol) and azobis(isobutyronitrile) (0.28 g, 1.70 mmol) in dry toluene (35 mL) over a period of one hour forty-five minutes. The mixture was heated at reflux for a further three and half hours. The reaction mixture was then cooled to room temperature and the solvent evaporated under reduced pressure which gave a yellow oil. The oil was then dissolved in diethyl ether (25 mL) and a saturated solution of potassium fluoride (25 mL) added with continued stirring for a further 1h. The organic layer was then separated, washed with distilled water (2x 25 mL) and dried. Organic solvent was evaporated in vacuo to give a crude yellow oil which was purified via chromatography (20 % ethyl acetate: hexane) to give 92 (0.20 g, 49 %) as colourless oil. Mass spectrum (Cl\(^+\)) m/z 258 [MH\(^+\)], 242 (C\(_{14}\)H\(_{12}\)NOS\(^-\)); HRMS (EI) Found: 257.0874 (4.5 ppm), required: 257.0862 for C\(_{15}\)H\(_{15}\)NOS.

\(^1\)H-NMR (CDCl\(_3\), 300 MHz): 1.33 (d, J = 2.4 Hz, 3H, CH\(_3\)), 3.33;3.87 (d and q, J\(_a\) = 6 Hz, J\(_b\) = 6 Hz, 2H, H3), 3.98 (d, J = 6.6 Hz, 2H, H1\(^-\)), 4.36-4.38 (m, 1H, H4), 5.19-5.31 (m, 2H, H3\(^-\)), 5.81-5.90 (m, 1H, H2\(^-\)), 7.40 (m, 2H, ArH- H7 and H8), 7.85 (m, 2H, ArH- H6 and H9). \(^13\)C-NMR: 18.6 (C5, CH\(_3\)), 28.8 (C4), 49.2 (C1\(^-\)), 53.0 (C3), 118.6 (C3\(^-\)), 122.6 (C9), 122.9 (C6), 125.6, 126.6 (C7 and C8), 137.4 (C5a), 138.8 (C2\(^-\)), 142.5 (C5b), 143.7 (C3a) and 161.4 (C=O).
Synthesis of N,N-diallyl-2-bromobenzamide (94).

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\text{o-Bromobenzoyl chloride (91) (4.0 g, 1.83 mmol) in THF (15 mL) was heated at reflux in 2 % LiOH (15 mL) for 1h after which it was treated with 2 M HCl to precipitate 2-bromobenzoic acid (93) (3.7 g, 81 %) as a white solid. The precipitate was washed with distilled water (20 mL) and dried in vacuo. Anhydrous DMF (15 mL) was then added to a mixture of the acid 93 (2g, 10.0 mmol), HOBt (1.35g, 10.0 mmol), DCC (2.06 g, 10.0 mmol) and diallylamine (47). The reaction mixture was stirred overnight at room temperature under nitrogen after which it was filtered to remove the precipitated dicyclohexyl urea. The filtrate was concentrated under reduced pressure to give a dark oil which was dissolved in ethyl acetate and filtered. The ethyl acetate was evaporated to give an oil which was purified via column chromatography to afford 94 (1.9 g, 68 %) as a clear oil. Mass spectrum (Cl⁻) m/z 280 [MH⁺], 200 (C₁₃H₁₄NO).}^{1}H-NMR (CDCl₃, 300 MHz): 3.77 (bs, 4H, 2x H₁'), 5.03-5.30 (m, 4H, 2x H₃'), 5.58; 5.85 9m, 2H, 2x H₂'), 7.22 (t, J = 7.1 Hz, 2H, ArH- H₄ and H₅), 7.49 (bs, 1H, H₃), 7.71 (bs, 1H, H₆).^{13}C-NMR: 49.1 (2x C₁'), 118.4 (2x C₃'), 119.1 (2x C₃'), 125.4 (C₅), 126.3 (C₆), 127.6 (2x C₂'), 130.4 (C₃'), 132.3 (C₄), 137.9 (C₁) and 169.3 (C=O).

Synthesis of 2-allyl-4-methyl-1,2,3,4-tetrahydro-1-isoquinolinone (95).

To a stirred solution of 94 (1.0 g, 3.58 mmol) heated at reflux in dry toluene (20 mL) under argon was added dropwise using an addition funnel, a mixture of tributyltin hydride (1.0 mL, 3.46 mmol) and AIBN (0.30 g, 1.73 mmol) in dry toluene (20 mL) over a period of one hour forty-five minutes. The mixture was further heated at
reflux overnight. The reaction mixture was then cooled to room temperature and the toluene evaporated under reduced pressure to give a yellow oil. The oil was then dissolved in diethyl ether (20 mL) and to it a saturated solution of potassium fluoride (25 mL) was added with stirring for 1h. The mixture was filtered and the organic layer was separated, washed with distilled water (2x 25 mL) and dried. Organic solvent was evaporated \textit{in vacuo} to give an oil which was purified \textit{via} chromatography (20 % ethyl acetate: hexane) to give 95 (0.36 g, 50 %) as a clear oil. Mass spectrum (Cl\(^+\)) \(m/z\) 202 [MH\(^+\)]; \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 1.35 (d, \(J = 2.4\) Hz, 3H, CH\(_3\)), 3.35;3.87 (m, 2H, H3), 3.94 (d, \(J = 6.6\) Hz, 2H, H1’), 4.34-4.38 (m, 1H, H4), 5.20-5.33 (m, 2H, H3’), 5.81-5.90 (m, 1H, H2’), 7.44 (m, 2H, ArH- H7 and H8), 7.82 (m, 2H, ArH- H6 and H9). \(^13\)C-NMR: 18.7 (C5, CH\(_3\)), 32.3 (C4), 49.8 (C1’), 51.8 (C3), 118.3 (C3’), 122.7 (C6), 127.1 (C8), 127.8 (C9) 128.6 (C7), 133.0 (C2’), 133.2 (C9a), 143.2 (C5a) and 161.4 (C=O).

\textit{Attempted synthesis of the diol 96 using the Sharpless reagent (AD-mix \(\alpha\)).}

\begin{center}
\begin{tikzpicture}
\end{tikzpicture}
\end{center}

To a mixture of tert-butyl alcohol (3 mL) and distilled water (3 mL) was added AD-mix \(\alpha\) followed by the addition of methyl sulfonamide (0.04 g, 0.42 mmol) and the reaction stirred at room temperature until fully mixed (yellow solution). The solution was then cooled to 0 °C (ice bath) and the olefin 95 (0.08 g, 0.39 mmol) dissolved in minimal tert-butyl alcohol added. Stirring was continued with periodic TLC monitoring. After 7h, no reaction of the starting material 95 was apparent. The reaction mixture was then stirred at 0 °C (ice bath) for a further 5h; however, starting material was still evident. The temperature was then raised to 10 °C and stirring
continued for 12h. TLC analysis (30 % ethyl acetate: hexane, visualisation: iodine vapour) showed no reaction progress hence the reaction mixture was warmed to room temperature and stirred overnight. The reaction was then quenched at low temperature (0 °C) with the addition of sodium sulfite (0.1 g, 0.79 mmol) and stirring at room temperature for 1h (clear solution). The solution was taken up in ethyl acetate (15 mL) and washed once with 2 M potassium hydroxide solution (10 mL) to remove any methyl sulfonamide present and then once with distilled water (15 mL). The organic portion was then dried and evaporated on the rotary evaporator to give a yellow oil which on TLC and spectroscopic analysis was shown to be the starting material 95 (0.05 g).

Attempted synthesis of the model diol 98 from allyl bromide using the Sharpless reagent AD-mix α.

Following a similar method as that used to attempt the synthesis of 96, allyl bromide (97) (0.1 g, 0.83 mmol) was reacted with AD-mix α. Workup of the reaction mixture after 10h indicated that the expected diol was not formed, however the starting material 96 (0.07 g) was recovered. Mass spectrum (CI+) m/z 121 [MH+1, 81Br]. ¹H-NMR (CDCl₃, 300 MHz): 3.15 (d, J = 4.8 Hz, 2H, H1), 5.25 (t, J = 7.0 Hz, 2H, H3), 5.84-5.90 (m, 1H, H2). ¹³C-NMR: 53.6 (C1), 117.3 (C3) and 136.1 (C2).
Synthesis of 2-(2,3-dihydroxypropyl)-4-methyl-1,2,3,4-tetrahydro-1-isquinolinone (96).

To a stirred solution of the olefin 95 (0.34 g, 1.69 mmol) in a mixture of AR grade acetone (5 mL) and distilled water (2 mL) was added potassium osmate (0.034 g, 10%) and N-methylmorpholine-N-oxide (0.5g, 4.21mmol). The reaction mixture was stirred at room temperature for 24h. The reaction was then quenched by adding sodium sulfite (0.5 g, 3.52 mmol) and stirring continued for 1hr. The mixture was extracted with ethyl acetate (2x 15 mL). The aqueous portion was then washed with ethyl acetate (2x 10 mL) and the pooled organic solvents concentrated under reduced pressure to give a dark oil (0.29 g, 74%). The oil was further purified by column chromatography (10 % methanol: DCM) to give 96 (0.12 g, 31%) as a colourless oil. Mass spectrum (Cl⁺) m/z 236 [MH⁺].

\[ ^1H-NMR \ (CDCl_3, \ 300 \text{ MHz}): \ 1.35 \ (d, \ J = 2.4 \text{ Hz, } 3\text{H, CH}_3), \ 2.45 \ (bs, \ 2\text{H, 2x OH}), \ 3.31-3.92 \ (m, \ 8\text{H, H1', H3', H3, H4, H2'}), \ 7.48 \ (m, \ 2\text{H, ArH- H7 and H8}), \ 7.82 \ (d, \ J = 6.0, \text{ Hz, 1H, H6}) \text{ and } 8.03 \ (t, \ J = 3.5, \text{ Hz, 1H, H9}). \]

\[ ^13C-NMR: \ 18.7 \ (C5, \text{ CH}_3), \ 32.3 \ (C4), \ 51.0 \ (C1'), \ 55.4 \ (C3), \ 64.0 \ (C3'), \ 66.8 \ (C2'), \ 122.7 \ (C6), \ 127.1 \ (C8), \ 127.8 \ (C9) \ 128.6 \ (C7), \ 133.2 \ (C9a), \ 143.2 \ (C5a) \text{ and } 162.4 \ (C=O). \]

Synthesis of 2-(2,3-dihydroxypropyl)-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridin-1-one (99).

To a stirred solution of the olefin 92 (0.35 g, 1.36 mmol) in a mixture of acetone (5 mL, AR grade) and distilled water (2 mL) was added potassium osmate (0.04 g, 10%) and N-methylmorpholine-N-oxide (0.5g, 4.21mmol).
N-oxide (0.39 g, 3.36 mmol). The reaction mixture was stirred at room temperature overnight. The reaction was then quenched by adding sodium sulfate (0.1g, 0.70 mmol) and stirring continued for 1hr. The mixture was extracted with ethyl acetate (2x 15 mL). The aqueous portion was then washed with ethyl acetate (2x 10 mL) and the pooled organic solvents concentrated under reduced pressure to give a dark oil which was purified by column chromatography (10 % methanol: DCM) to give 99 (0.32 g, 82 %) as colourless oil. Mass spectrum (Cl⁺) m/z 292 [MH⁺]. $^{1}H$-NMR (CDCl₃, 300 MHz): 1.33 (d, $J = 2.4$ Hz, 3H, CH₃), 2.44 (bs, 2H, 2x OH), 3.33-3.92 (m, 8H, H1’, H3’, H3, H4, H2’), 7.48 (m, 2H, ArH- H7 and H8), 7.85 (dd, $J = 3.45$, 5.2, Hz, 2H, ArH- H6 and H9). $^{13}C$-NMR: 18.6 (C5, -CH₃), 28.9 (C4), 50.2 (C1’), 56.1 (C3), 70.9 (C3’), 71.2 (C2’), 122.6 (C9), 122.9 (C6), 125.6, 126.6 (C7 and C8), 137.4 (C5a), 142.5 (C5b), 143.7 (C3a) and 161.6 (C=O).

**Synthesis of N-benzyl-3-bromobenzo[b]thiophene-2-carboxamide (100).**

To a cooled mixture (ice bath) of the 3-bromo substituted acid 25 (0.60 g, 2.33 mmol), benzyl amine (0.24 g, 2.24 mmol), DCC (0.48 g, 2.33 mmol) and HOBT (0.31 g, 2.29) was added anhydrous DMF (15 mL). The reaction mixture was then warmed to room temperature and stirred overnight under nitrogen after which time all the substrate had reacted (via TLC). The reaction mixture was vacuum filtered to remove precipitated urea by-product. The organic solvent was then evaporated under reduced pressure to give a dark oil which was dissolved in ethyl acetate (20 mL) and then re-filtered. The ethyl acetate was evaporated to afford an oil which was chromatographed (30 % ethyl acetate: hexane). The product had a
tendency to crystallise in the column and hence a short column was run under
pressure to give 100 (0.78 g, 98 %) as a colourless, highly crystalline solid. Mp. 115-
117 °C. Mass spectrum (Cl\(^+\)) \(m/z\) 346 [MH\(^+\), \(^{81}\)Br]; 266 (C\(_{16}\)H\(_{12}\)NOS'). **HRMS (Cl)**

Found: 344.9822 (-1.0 ppm), required: 344.9826 for C\(_{16}\)H\(_{12}\)\(^{81}\)BrNOS. ¹H-NMR

(CDC\(_3\), 300 MHz): 4.72 (d, \(J = 5.7\) Hz, 2H, CH\(_2\)), 7.31-7.45 (m, 5H, ArH benzyl),
7.48 (m, 2H, ArH- H5 and H6), 7.83 (dd, \(J = 3.45\), 5.3 Hz, 2H, ArH- H4 and H7).

¹³C-NMR: 44.5 (CH\(_2\)), 106.6 (C3), 122.9 (C7), 124.7 (C4), 125.8 (C5 and C6), 127.9
(C4'), 128.0 (C2'and C6'), 129.1 (C3' and C5'), 137.7 (C3a), 138.6 (C1'), 138.7
(C2), and 161.1 (C=O).

**Synthesis of N-allyl-N-benzyl-3-bromobenzo[b]thiophene-2-carboxamide (101).**

![Chemical structure of 101](image)

To a cooled solution (5 °C, ice bath) of sodium hydride (0.055 g, 2.29 mmol) in anhydrous DMF
(5 mL) was slowly added 100 (0.50 g, 1.44 mmol) in dry DMF (15 mL). Stirring was
continued at room temperature for 1h. The reaction mixture was then cooled to -60 °C
( dry ice/ acetone bath) and allyl bromide (0.17 g, 1.41 mmol) dissolved in dry DMF
(10 mL) added slowly. The reaction was slowly warmed to room temperature and
stirring continued overnight. The solvent was evaporated under reduced pressure to
give an oil. Distilled water (50 mL) was added to the oil and a gummy solid was
rapidly formed. Vigorous stirring of the gummy product in water was continued for
one and half hours after which it was extracted with dichloromethane (25 mL). The
organic extract was dried and concentrated to give an oily residue which was then
chromatographed (30 % ethyl acetate: hexane). The amide 101 (0.50 g, 91 %) was
obtained. Mass spectrum (EI\(^+\)), \(m/z\) 385 [MH\(^{+1, 81}\)Br], 306 \((C_{10}H_{16}NOS')\), 238 \((C_9H_4BrOS')\). **HRMS (EI)** Found: 387.0115 (-1.9 ppm), required: 387.0122 for \(C_{19}H_{16}^{81}\)BrNOS. **\(^1\)H-NMR (CDCl\(_3\), 300 MHz):** 3.78 (t, \(J = 11.2\) Hz, 2H, H2’), 4.12 (s, 2H, CH\(_2\)-benzyl), 5.16-5.30 (m, 2H, H3’), 5.60-5.83 (bs, 1H, H2’), 7.31-7.36 (m, 5H, ArH benzyl), 7.44 (m, 2H, ArH- H5 and H6), 7.83 (dd, \(J = 3.45, 5.30\) Hz, 2H, ArH- H4 and H7). **\(^{13}\)C-NMR:** 46.8 (C1’), 50.5 (C-CH\(_2\), benzyl), 114.3 (C3’), 118.6 (C3), 122.8 (C7), 123.8 (C4), 125.8 (C5), 126.6 (C6), 130.1 (C4”), 132.0-132.2 (C2”, C3”, C5” and C6”), 137 (C1”), 137.9 (C3a), 159.3 (C7a) and 164.1 (C=O).

**Synthesis of N-(4-methoxybenzyl)-3-bromobenzo[b]thiophene-2-carboxamide (102).**

Following the same procedure as that used to synthesise 100, 102 \((C_{17}H_{14}BrNO_2S)\), (1.14 g, 91 %) was synthesised from 25 (0.85 g, 3.30 mmol) and 4-methoxybenzyl amine (0.43 mL, 3.12 mmol) and obtained as a highly crystalline colourless solid after chromatography (30 % ethyl acetate: hexane). Mp. 123-124 °C. Mass spectrum (EI\(^+\)) \(m/z\) 376 [MH\(^{+1, 81}\)Br]; **HRMS (EI)** Found: 374.9928 (-0.3 ppm), required: 374.9929 for \(C_{17}H_{14}^{81}\)BrNO_2S. **\(^1\)H-NMR (CDCl\(_3\), 300 MHz):** 3.80 (s, 3H, OMe), 4.65 (d, \(J = 5.7\) Hz, 2H, CH\(_2\)), 6.91 (m, 2H, H3’ and H5’), 7.34 (m, 2H, H2’and H6’), 7.46 (m, 2H, ArH- H5 and H6), 7.83 (dd, \(J = 3.45, 5.28\) Hz, 2H, ArH- H4 and H7). **\(^{13}\)C-NMR:** 44.0 (CH\(_2\)), 55.5 (CH\(_3\)), 106.6 (C3), 114.4 (C3’ and C5’) 122.9 (C6), 124.7 (C5), 125.7 (C4), 129.4 (C2’ and C6’), 135.1 (C1’), 138.7 (C3a), 159.4 (C-O) and 161.0 (C=O).
**Synthesis of N-allyl-N-(4-methoxybenzyl)- 3-bromobenzo[b]thiophene-2-carboxamide (103).**

Following the same method as that used to prepare 101, 103 (C\textsubscript{20}H\textsubscript{18}BrNO\textsubscript{2}S), (0.34 g, 72 %) was synthesised from 102 (0.43 g, 1.14 mmol) and allylbromide (0.13 g, 1.08 mmol) and obtained as a colourless oil after chromatography. Mass spectrum (CI\textsuperscript{+}) m/z 416 [MH\textsuperscript{+1}, \textsuperscript{81}Br]; **HRMS (EI)** Found: 418.0299 (-0.2 ppm), required: 418.0300 for C\textsubscript{20}H\textsubscript{19} \textsuperscript{81}BrNO\textsubscript{2}S. **\textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz):** 3.73 (bs, 3H, OMe), 4.07(bs, 2H, H1\texttextsuperscript{`}), 4.48 (s, 2H, CH\textsubscript{2}-benzyl), 5.08-5.27 (m, 2H, H3\texttextsuperscript{`}), 5.60-5.83 (bs, 1H, H2\texttextsuperscript{`}), 6.87 (d, J = 1.95 Hz, 2H, H3 and H5\texttextsuperscript{``}), 7.10 (d, J = 2.25, Hz, 2H, H2\texttextsuperscript{``}and H6\texttextsuperscript{``}), 7.45 (m, 2H, ArH- H5 and H6), 7.78 (dd, J = 3.45, 5.30 Hz, 2H, ArH-H4 and H7). **\textsuperscript{13}C-NMR:** 21.3 (CH\textsubscript{3}), 46.8 (C1\texttextsuperscript{`}), 50.5 (C-CH\textsubscript{2}, benzyl), 114.3 (C3\textsuperscript{`}), 118.6 (C3), 122.8 (C7), 123.8 (C4), 125.8 (C5), 126.6 (C6), 130.1 (C4\textsuperscript{``}), 132.0-132.2 (C2\textsuperscript{``}, C3\textsuperscript{``}, C5\textsuperscript{``} and C6\textsuperscript{``}), 137 (C1\textsuperscript{``}), 137.9 (C3a), 159.4 (C7a) and 164.2 (C=O).

**Synthesis of 2-benzyl-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c] pyridin-1-one (104).**

Following the same method as that used to prepare 92, 104 (C\textsubscript{19}H\textsubscript{17}NOS), (0.90 g, 80 %) was synthesised from 101 (0.90 g, 1.14 mmol) and obtained as a straw yellow oil after chromatography. Mass spectrum (CI\textsuperscript{+}) m/z 308 [MH\textsuperscript{+1}]; **HRMS (EI)** Found: 307.1030 (1.0 ppm), required: 307.1027 for C\textsubscript{19}H\textsubscript{17} NOS. **\textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz):** 1.33 (d, J = 2.4 Hz, 3H, CH\textsubscript{3}), 3.33;3.87 (d J\textsubscript{a} = 6 Hz, J\textsubscript{b} = 6 Hz, 2H, H3),
4.36-4.38 (m, 1H, H4), 4.57 (s, 2H, CH2-benzyl), 7.31-7.36 (m, 5H, ArH benzyl),
7.40 (m, 2H, ArH- H7 and H8), 7.85 (dd, \( J = 3.45, 5.3 \) Hz, 2H, ArH- H6 and H9).

**\(^{13}\)C-NMR:** 18.6 (C5, CH3), 28.8 (C4), 50.5 (C-CH2, benzyl), 53.0 (C3) 122.6 (C9),
122.9 (C6), 125.6, 126.6 (C7 and C8), 130.1 (C4’) 132.2 (C2’, C3’, C5’ and C6’),
137.1 (C1’), 137.4 (C5a), 142.5 (C5b), 143.7 (C3a) and 161.8 (C=O).

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**Synthesis of 2-(4-methoxybenzyl)-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]
thieno[2,3-c] pyridine-1-one (105).**

Following the same method as that used to prepare 92, 105 (C\(_{20}H_{19}NO_2S\)), (0.90 g, 80 %) was synthesised from 103 (0.90 g, 1.14 mmol) as an oil after chromatography. Mass spectrum (Cl\(^+\)) \( m/z \) 338 [MH\(^{+1}\)];

**HRMS (Cl)** Found: 338.1214 (1.8 ppm), required: 338.1208 for C\(_{20}H_{20} NO_2S\). **\(^1\)H-NMR (CDCl\(_3\), 300 MHz):** 1.36 (d, \( J = 2.4 \) Hz, 3H, CH3), 3.34;3.86 (d \( J_a = 6 \) Hz, \( J_b = 6 \) Hz, 2H, H3), 4.33-4.35 (m, 1H, H4), 4.55 (s, 2H, CH2-benzyl), 6.87 (d, \( J = 1.95 \) Hz, 2H, H3’ and H5’), 7.10 (d, \( J = 2.25 \) Hz, 2H, H2’and H6’), 7.40 (m, 2H, ArH- H7 and H8), 7.85 (dd, \( J = 3.45, 5.27 \) Hz, 2H, ArH- H6 and H9). **\(^{13}\)C-NMR:** 18.6 (C5, CH3),
28.8 (C4), 50.5 (C-CH2, benzyl), 53.0 (C3) 122.6 (C9), 122.9 (C6), 125.6, 126.6 (C7 and C8), 130.3 (C4’), 132.2 (C2’, C3’, C5’ and C6’), 137.2 (C1’), 137.4 (C5a), 142.5 (C5b), 143.7 (C3a) and 161.4 (C=O).
Synthesis of 4-methyl-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c]pyridin-1-one (106).

To a mixture of 92, (0.24 g, 0.93 mmol), triphenylphosphine (0.24 g, 0.91 mmol) and palladium(II) acetate (0.23 g, 1.02 mmol) was added formic acid (10 mL) under nitrogen and the mixture heated at reflux for 1h. The mixture was then cooled to room temperature and then vacuum filtered. Solvent was evaporated under reduced pressure to give a dark crude residue which was then chromatographed (40 % ethyl acetate: hexane) to give 106 (0.17 g, 85 %) as a yellow oil; due to traces of impurities, the oil was further purified by successive preparative plate chromatography (0.05 g, 25 %). Mass spectrum (EI⁺) m/z 217, 202 (C₁₁H₈NOS). HRMS (CI) Found: 218.0639 (2.6 ppm), required: 218.0633 for C₁₂H₁₂NO₂S. ¹H-NMR (CDCl₃, 300 MHz): 1.38 (d, J = 2.4 Hz, 3H, CH₃), 3.38-3.70 (m, 2H, H₃), 3.92-3.98 (m, 1H, H₄), 7.46 (m, 2H, ArH- H7 and H8), 7.91 (dd, J = 3.45, 5.3 Hz, 2H, ArH- H6 and H9). ¹³C-NMR: 18.2 (CH₃), 28.6 (C4), 47.8 (C3), 122.9 (C9), 123.7 (C5), 125.1 (C7; C8) 142.3 (C5b), 145.6 (C5a), and 164.7 (C=O).

Attempted allyl deprotection of (92) using rhodium chloride.

To a mixture of 2-allyl-4-methyl-1,2,3,4-tetrahydrobenzo[4,5]thieno[2,3-c] pyridin-1-one (92) (0.2 g, 0.77 mmol) and rhodium(III) chloride (0.15 g, 0.72 mmol) under nitrogen was added ethanol (15 mL). The mixture was then heated at reflux for 1hr, after which time no 92 could be detected by TLC analysis. The reaction mixture was then cooled to room temperature and vacuum filtered. The filtrate was evaporated under reduced pressure to give a black residue which was further purified via
chromatography to give a yellow oil (0.01g, 6 %). Mass spectrum (Cl+) \( m/z \) 177 [MH\(^{+1}\)], \( m/z \) 161, \( m/z \) 133. This product was not identified. The expected product 106 was not successfully synthesised through this method.

**Synthesis of N-benzylprop-2-en-1-amine (107).**

Anhydrous tetrahydrofuran (20 mL) was added to a mixture of benzylamine (1.0 mL), allyl bromide (0.6 mL) and an excess of potassium carbonate (1.5 g, 10.86 mmol) under nitrogen and the reaction mixture heated at reflux for 1h. The reaction mixture was then cooled to room temperature and vacuum filtered to remove unreacted potassium carbonate. Solvent was then evaporated under reduced pressure to give 107 (1.05 g, 76 %) as a yellow oil. Mass spectrum (Cl+) \( m/z \) 148 [MH\(^{+1}\)] (C\(_{10}\)H\(_{13}\)N, base peak), \( m/z \) 188 [MH\(^{+1}\)] (C\(_{13}\)H\(_{17}\)N). The mass spectrum showed an [MH\(^{+1}\)] peak at \( m/z \) 188 consistent with the undesired tertiary amine N-allyl-N-benzylprop-2-en-1-amine (108). \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 3.15 (d, \( J = 4.8 \) Hz, 2H, H1’), 3.59 (s, 2H, CH\(_2\)- benzyl), 5.25 (t, \( J = 10.0 \) Hz, 2H, H3’), 5.84-5.90 (m, 1H, H2’), 7.20-7.34 (m, 6H, ArH). \(^{13}\)C-NMR: 53.6 (C1’), 55.8 (CH\(_2\)- benzyl), 117.3 (C3’), 127.0 (C4), 126.1 (C3 and C5), 129.0 (C2 and C6), 136.1 (C2’) and 138.2 (C1).

Tertiary amine (108) (C\(_{13}\)H\(_{17}\)N): 3.08 (d, \( J = 4.8 \) Hz, 4H, H1’), 3.57 (s, 2H, CH\(_2\)- benzyl), 5.18 (t, \( J = 10.0 \) Hz, 4H, H3’), 5.83-5.91 (m, 2H, H2’), 7.20-7.34 (m, 6H, ArH). \(^{13}\)C-NMR: 56.6 (2x C1’), 57.8 (CH\(_2\)- benzyl), 117.6 (2x C3’), 127.0 (C4), 126.4 (C3 and C5), 129.1 (C2 and C6), 136.1 (C2’) and 139.6 (C1).
Synthesis of 2-(2-bromobenzoyl)-1-(prop-2-enyl)-1,2,3,4-tetrahydro isoquinoline (109).

To a stirred solution of 32 (1.5 g, 8.87 mmol) in dry dichloromethane was added consecutively triethylamine (5.0 mL, 36.25 mmol), DMAP (0.11 g, 0.90 mmol) and \( o \)-bromobenzoyl chloride (2.46 g, 11.23 mmol) in dry DCM under nitrogen. Stirring was maintained for 5h after which time all the substrate had reacted (TLC analysis). To the reaction mixture was then added distilled water (25 mL) and the organic layer separated and washed further with water (2x 15 mL) and then with 1 M HCL (20 mL), and finally with sodium bicarbonate solution (20 mL). The organic portion was then dried and solvent evaporated under reduced pressure to give an oily residue which was chromatographed (20 % ethyl acetate: hexane) and then re-crystallised from ethanol to give 109 (0.88 g, 48 %) as a crystalline solid. Mp. 94-95 °C. Mass spectrum (Cl\(^+\)) \( m/z \) 356 [MH\(^+\), \({\text{\( ^81 \)}}\)Br]; HRMS (Cl) Found: 356.0639 (2.6 ppm), required: 356.0633 for C\(_{19}\)H\(_{19}\)\({\text{\( ^81 \)}}\)BrNO. \(^1\)H-NMR (CDCl\(_3\), 300 MHz): 2.39; 2.66 (m, 2H, H\(_1\)), 2.787 (bs, 2H, H4), 3.28; 3.36 (m, 2H, H3), 5.05- 5.14 (m, 3H, H1 and H3'), 5.83-6.12 (m, 1H, H2'), 7.07-7.23 (m, 4H, H5, H6, H7, H8), 7.35 (m, 2H, ArH-H4” and H5”), 7.57 (dd, \( J = 3.45, 5.27 \) Hz, 2H, ArH- H3” and H6”).

\(^{13}\)C-NMR: 29.3 (C4), 41.4 (C1’), 42.0 (C3), 51.2 (C1), 117.8 (C3’), 119.7 (C2’), 126.8 (C7), 127.1 (C6), 127.6 (C5 and C5’), 127.9 (C8), 130.6 (C3”), 134.0 (C2’), 138.0 (C1”) and 168.5 (C=O).
Synthesis of N-benzoyl-7-methyl-6,7,8,9-tetrahydro-5H-benzocycloheptane-5,8-imine (110).

Following the same general method (free radical cyclisation reaction) as that used to synthesise 92, 110 (0.53 g, 76 %) was synthesised from 109 (0.90 g, 2.52 mmol) and obtained as a clear oil after chromatography.

Mass spectrum (CI+) m/z 277 [MH+1]; HRMS (CI)

Found: 278.1445 (1.0 ppm), required: 278.1440 for C19H20NO.

1H-NMR (CDCl3, 300 MHz): 1.02 (s, 3H, CH3), 1.74; 1.87 (bm, 1H, H7), 2.65-2.93 (m, 2H, H9), 3.72-3.80 (m, 1H, H8), 4.66-4.82 (m, 1H, H5), 7.12 (bs, 4H, H1-H4), 7.34 (bs, 3H, ArH-3',4', 5') and 7.47 (bs, 2H, ArH- H2' and H6').

13C-NMR: 16.6 (CH3), 27.2 (C9), 30.2 (C7), 33.4 (C6), 46.0 (C5), 60.6 (C8), 125.0-127.0 (9Ar-C), 135.2 (C1') and 167.9 (C=O).

Synthesis of N-benzof[b]thien-2-oyl-7-methyl-6,7,8,9-tetrahydro-5H-benzo cycloheptene-5,8-imine (112).

Following the same method (free radical cyclisation reaction) as that used to synthesise 92, 112 (0.50 g, 89 %) was synthesised from 53 (0.70 g, 1.70 mmol) was obtained as a clear oil after chromatography (30 % ethyl acetate: hexane). A GC-MS analysis indicated 3 peaks of similar mass (m/z 333) corresponding to isomeric products. Further purification was attempted via multi-development preparative plate chromatography, but mixtures were still obtained. Mass spectrum (Cl+) m/z 334 [MH+1]. HRMS (EI)

Found: 333.1187 (4.9 ppm), required: 333.1171 for C21H19NOS.
preparative plate was analysed as follows by GC-HRMS (EI). Peak 1 band 2 Found: 333.1187 (1.7 ppm), required: 333.1181 for C\textsubscript{21}H\textsubscript{19}NOS; Peak 2 band 2 Found: 333.1187 (0.8 ppm), required: 333.1184 for C\textsubscript{21}H\textsubscript{19}NOS; Peak 3 band 2 Found: 333.1187 (3.8 ppm), required: 333.1174 for C\textsubscript{21}H\textsubscript{19}NOS. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 1.02 (s, 3H, CH\textsubscript{3}), 1.74; 1.87 (bm, 1H, H7), 2.65-2.93 (m, 2H, H9), 3.72-3.80 (m, 1H, H8), 4.66-4.82 (m, 1H, H5), 7.45 (m, 2H, ArH- H5 and H6), 7.78 (dd, J = 3.45, 5.3 Hz, 2H, ArH- H4 and H7), 7.81 (s, 1H, H3\textsuperscript{′}). \textsuperscript{13}C-NMR: 16.6 (CH\textsubscript{3}), 27.2 (C9), 30.2 (C7), 33.4 (C6), 46.0 (C5), 60.6 (C8), 119.6 (C3), 122.8 (C7), 123.8 (C4), 125.8 (C5), 126.6 (C6), 167.9 (C=O).

**Synthesis of 1-(3-chlorobenzof[b]thien-2-oyl)-2,5-dihydropyrrole(113).**

Anhydrous dichloromethane (30 mL) was added to a mixture of 78 (0.70 g, 2.41 mmol) and benzylidenebis(tricyclohexylphosphine)dichlororuthenium. (Grubbs’ I catalyst) (0.0335 g, 0.042 mmol, 10 %). The mixture was stirred under nitrogen at room temperature overnight. The reaction mixture was then vacuum filtered and solvent evaporated to give a dark green residue which was then flash chromatographed (DCM) to give 113 (0.46 g, 73 %) as a colourless oil which solidified rapidly. Mp. 104-106 °C. Mass spectrum (Cl\textsuperscript{−}), m/z 265 [MH\textsuperscript{−}, \textsuperscript{35}Cl]; HRMS (CI) Found: 264.0249 (+ 0.0 ppm), required: 264.0249 for C\textsubscript{10}H\textsubscript{11}\textsuperscript{35}ClNOS. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 4.31; 4.48 (d, J = 3.3 Hz, 2H, 2x CH\textsubscript{2}), 5.79; 5.93 (q, J = 3 Hz, 1H, 2x CH), 7.53 (m, 2H, ArH- H5 and H6), 7.88 (dd, J = 3.45, 5.28 Hz, 2H, ArH- H4 and H7). \textsuperscript{13}C-NMR: 64.1 (C2\textsuperscript{′}; C5\textsuperscript{′}), 122.7 (C7).
122.9 (C4), 125.6 (C5), 126.7 (C6), 128.5 (C3’; C4’), 129.7 (C3), 135.8 (C3a), 137.6 (C2a) and 161.7 (C=O).

**Synthesis of 113 using polymer supported Grubbs’ I catalyst.**

Dry dichloromethane (25 mL) was added to a mixture of 23 (0.46 g, 1.56 mmol) and polymer supported benzylidene-bis(tricyclohexylphosphine)dichlororuthenium (polymer supported Grubbs’ I catalyst) (0.0330 g, 0.042 mmol). The mixture was then stirred overnight at room temperature under argon. The reaction mixture was vacuum filtered and the recovered catalyst washed once with dichloromethane, dried in vacuo and stored under nitrogen. The combined filtrate and washings were evaporated to afford a clear oil, which was flash chromatographed (DCM). This gave the product as a clear oil initially which then solidified rapidly to give colourless crystals of 113 (0.35 g, 85 %). Mp.105-106 °C. Mass spectrum (CI+), m/z 265 [MH+1, 35Cl]. The 1H-NMR and 13C-NMR data was consistent with that of the desired product 113 as shown above.

**Synthesis of 1-(3-bromobenzo[b]thien-2-oyl)-2,5-dihydropyrrole(114).**

Following the same method as that used to prepare 113, 114 (0.24 g, 80 %) was prepared from 25 (0.34 g, 1.01 mmol) and homogeneous Grubbs’ I catalyst (0.030 g, 0.037 mmol) as a crystalline solid after flash chromatography (DCM). Mp.118-120 °C. Mass spectrum (Cl+) m/z 309 [MH+1, 81Br]; HRMS (Cl) Found: 307.9744 (2.0 ppm), required: 307.9738 for C10H1181BrNOS. 1H-NMR (CDCl3, 300 MHz): 4.31; 4.48 (d, J = 3.3 Hz, 2H, 2x CH2), 5.79; 5.93 (q, J = 3 Hz, 1H, 2x CH), 7.53 (m, 2H, ArH- H5 and H6), 7.88 (dd, J = 3.45, 5.3 Hz, 2H, ArH- H4 and H7). 13C-NMR: 64.1
(C2’, C5’), 122.7 (C7), 122.9 (C4), 125.6 (C5), 126.7 (C6), 128.5 (C3’, C4’), 129.7 (C3), 135.8 (C3a), 137.6 (C2a) and 161.7 (C=O).

Synthesis of methyl 3-allylbenzo[b]thiophene-2-carboxylate (116).

The acid 25 (1.0 g, 3.89 mmol) was converted to its methyl ester derivative 115 by heating it at reflux in methanol (15 mL) and a catalytic amount of concentrated sulfuric acid overnight. Compound 115 was obtained as a white solid (0.9 g, 90%). A glass high-pressure tube was flushed with nitrogen and a mixture of palladium(II) chloride (0.1 g, 0.85 mmol) and triphenylphosphine (0.23 g, 0.87 mmol) added, and to this mixture was then added the methyl ester 115 (0.5 g, 1.85 mmol) in anhydrous DMF (15 mL) and stirring continued. Allyltributyltin (0.6 mL) was then injected into the reaction mixture and the tube sealed. The mixture was heated at 90 °C with stirring for 2h, and then at 100 °C for 36h. The reaction mixture was then cooled to room temperature and the solvent evaporated under reduced pressure to give a yellow oil. The oil was then purified via chromatography (10 % ethyl acetate: 40-60 pet. spirit) to give 116 (0.21 g, 50%) as a yellow oil. Mass spectrum (Cl+) m/z 233 [MH+]; HRMS (Cl) Found: 232.0558 (1.0 ppm), required: 232.0555 for C13H13O2S. 1H-NMR (CDCl3, 300 MHz): 3.91 (s, 3H, CH3), 4.15 (d, J = 4.2 Hz, 2H, H1’), 5.04-5.13 (m, 2H, H3’), 5.97-6.06 (m, 1H, H2’), 7.46 (m, 2H, ArH- H5 and H6), 7.85 (m, 2H, ArH- H4 and H7). 13C-NMR: 31.6 (C1’), 52.4 (CH3), 116.2 (C3’), 122.9 (C7), 124.1 (C4), 124.7 (C5), 124.8 (C6), 127.4 (C2), 135.4 (C2’), 140.9 (C3a), 142.7 (C3b) and 163.6 (C=O).

A mixture of 116 (0.17g, 0.73 mmol) and 2 % aqueous LiOH (10 mL) in tetrahydrofuran (15 mL) was heated at reflux for 3h. The reaction mixture was then cooled to room temperature and the solvent was concentrated under reduced pressure and then the residue acidified with 1 M HCl at 5-10 °C (ice bath). The precipitate was vacuum filtered, washed with distilled water (20 mL) and then dried under vacuum to give 117 (0.14g, 93 %) as a white solid. Mass spectrum (Cl+) m/z 218 [MH+]. $^1$H-NMR (CD$_3$COCD$_3$, 300 MHz): 4.13 (d, $J = 4.2$ Hz, 2H, H1), 4.97; 5.14 (m, 2H, H3), 5.96; 6.05 (m, 1H, H2), 7.52 (m, 2H, ArH- H5 and H6), 7.97 (dd, $J = 3.45$, 5.26 Hz, 2H, ArH- H4 and H7). $^{13}$C-NMR: 31.6 (C1), 116.2 (C3), 122.9 (C7), 124.1 (C4), 124.7 (C5), 124.8 (C6), 127.4 (C2), 135.4 (C2'), 140.9 (C3b), 142.7 (C3a) and 163.6 (C=O).

**Synthesis of (1-allyl-1,2,3,4-tetrahydroisoquinolin-2-yl)(3-allylbenzo[b]thien-2-yl)methanone (118).**

The isoquinoline 32 (0.11 g, 0.063 mmol) was added to a cooled mixture (ice bath) of 117 (0.14 g, 0.61 mmol), 1,3-dicyclohexylcarbodiimide (0.13 g, 0.63 mmol), and 1-hydroxybenzotriazole (0.10 g, 0.74 mmol) in dry DMF (10 mL). The mixture was stirred at room temperature under nitrogen for 24h. The precipitated dicyclohexylurea was removed by vacuum filtration. Solvent was then evaporated under reduced pressure to give an oily residue which was then taken up in ethyl acetate (20 mL) and the mixture urea re-filtered. The filtrate was then washed with distilled water (40 mL), dried and the solvent evaporated to give a yellow oil. The oil was further purified via column chromatography (20 % ethyl
acetate: 40-60 pet. spirit) to give 118 (0.10 g, 55 %) as clear oil. Mass spectrum (CI’),
372 [MH+]'; 332 (C_{21}H_{18}NOS'). HRMS (EI) Found: 332.1500 (4.8 ppm), required:
332.1503 for C_{21}H_{18}NOS. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 2.70;2.95 (m, 2H, H1” allyl
THIQ), 3.50;3.66 (m, 2H, H4’), 3.66-3.88 (m, H3’ and H1” allyl BTP), 4.85-5.18 (m,
5H, 2x H3” (CH\textsubscript{2}) and H1”), 5.91-6.02 (m, 2H, 2x H2’”), 7.11-7.36 (m, 4H, ArH
THIQ), 7.42 (m, 2H, ArH- H5 and H6), 7.79 (m, 2H, ArH- H4 and H7). \textsuperscript{13}C-NMR:
29.8 (C4’), 31.7 (C1”, allyl BTP), 41.5 (C1”, allyl THIQ), 41.8 (C3’), 52.2 (C1’),
116.6 (C3”, THIQ), 118.1 (C3”, BTP), 122.7 (C7), 123.3 (C4), 124.7 (C6), 125.5
(C5), 127.0 (C5’), 135.0 (C2’, THIQ), 135.4 (C2), 136.6 (C2’,BTP) and 164.7 (C=O).

Note: BTP- benzo[b]thiophene and THIQ- tetrahydroisoquinoline

\textit{Synthesis of (1-allyl-6,7-dimethoxy-1,2,3,4-tetrahydroisoquinolin-2-yl)(3-
allylbenzo[b]thien-2-yl)methanone (119).}

Following a similar synthetic method as
that used to prepare 118, 119
(C_{26}H_{27}NO_{3}S) (0.19 g, 57 %) was
prepared from 34 (0.20 g, 0.85 mmol) and
117 (0.17 g, 0.77 mmol) and obtained as
a colourless oil after chromatography (30 % ethyl acetate: 40-60 pet. spirit). Mass
spectrum (CI’), 434 [MH’+]; 392 (C_{23}H_{22}NOS’). HRMS (CI) Found: 332.1721 (3.2
ppm), required: 332.1718 for C_{26}H_{28}NO_{3}S. \textsuperscript{1}H-NMR (CDCl\textsubscript{3}, 300 MHz): 2.70;2.95
(m, 2H, H1” allyl THIQ), 3.50;3.66 (m, 2H, H4’), 3.66-3.81 (m, H3’ and H1” allyl
BTP), 3.86 (s, 3H, 2x CH\textsubscript{3}), 4.85-5.18 (m, 5H, 2x H3” (CH\textsubscript{2}) and H1”), 5.91-6.02 (m,
2H, 2x H2’”), 6.57 (s, 2H, ArH THIQ), 7.42 (m, 2H, ArH- H5 and H6), 7.79 (m, 2H,
ArH- H4 and H7). \textsuperscript{13}C-NMR: 29.8 (C4’), 31.7 (C1”, allyl BTP), 41.5 (C1’”, allyl

Dry dichloromethane (10 mL) was added to a mixture of 118, (0.1 g, 0.26 mmol) and polymer supported Grubbs’ I catalyst (0.15 g) under nitrogen and the mixture heated at reflux over 12h. The reaction mixture was then cooled to room temperature and then filtered to recover the catalyst. The filtrate was evaporated under reduced pressure to afford a clear oil which solidified rapidly. The crude product was further purified via column chromatography (30 % ethyl acetate: pet. spirit) to give a colourless solid which was re-crystallised from ethanol to 120 (0.07 g, 78 %) as a give highly crystalline solid. Note: A similar reaction was also attempted using the homogeneous Grubbs’ I catalyst to give 120 (0.18 g, 75 %) as a crystalline solid after chromatography and re-crystallisation. Mp. 233-245 °C. Mass spectrum (Cl+), 346 [MH+1]; HRMS (Cl) Found: 345.1187 (1.3 ppm), required: 345.1182 for C22H19NOS.

1H-NMR (CDCl3, 300 MHz): 2.73-2.86 (m, 4H, H5 and H16), 3.71 (dd, J = 16.65, Hz, 2H, H17), 3.71; 4.10 (q, J = 13.8 Hz, 2H, H8), 5.02 (t, J = 6.6 Hz, 1H, H15a), 5.99 (q, J = 3.3 Hz, 1H, H7), 6.17 (q, J = 10.3 Hz, 1H, H6), 6.92-7.16 (m, 4H, ArH-H1-H4), 7.46 (m, 2H, ArH- H5 and H6), 7.82 (dd, J = 3.45, 5.3 Hz, 2H, ArH- H4 and H7). 13C-NMR: 27.9 (C8), 28.2 (C17), 36.2 (C5) 36.7 (C16), 59.0 (4b), 122.8 (C9
and C12), 125.8-127.1 (C1-C3, C10 and C11), 129.4 (C7), 130.2 (C6), 133.2 (C13a), 138.0 (C4a), 138.9 (C17a), 140.0 (C8a) and 164.3 (C=O).

**Attempted synthesis of (3-allylbenzo[b]thien-2-yl)(1-vinyl-1,2,3,4-tetrahydroisoquinolin-2-yl)methanone (122).**

To a cooled solution (ice bath, sodium chloride) of 3,4-dihydroisoquinoline (36) (0.80 g, 6.06 mmol) in dry ether (15 mL) was added a 1 M solution in THF of vinylmagnesium bromide (2.4 mL) under nitrogen. The reaction mixture was stirred for 5 minutes after which 116 (0.87 g, 3.75 mmol) in dry diethyl ether (10 mL) was added. The temperature was raised to room temperature and stirring continued with periodic TLC monitoring for 2h. The reaction was worked up by evaporating the solvent under reduced pressure and the dark oil obtained was taken up in diethyl ether (25 mL) and washed once with distilled water (15 mL). Organic solvent was dried and evaporated to give an oil. A TLC analysis of the oil revealed no products and only starting material was present.

**Synthesis of (benzo[b]thien-2-yl)(1H-indol-1-yl)methanone (124).**

Anhydrous DMF (10 mL) was syringed in to a flask containing sodium hydride (0.082 g, 3.51 mmol) under nitrogen and the solution cooled to 5 °C (ice bath) with stirring. Indole (123) (0.16 g, 1.36) dissolved in dry DMF (10 mL) was then added and stirring maintained for 45 minutes between 0-5 °C. The reaction mixture was then warmed to room temperature and stirring continued for a further 45 minute after which time the temperature was
dropped to -60 °C (liquid nitrogen: chloroform). The acid chloride 26 (0.27 g, 1.37) dissolved in dry DMF (15 mL) was then added slowly and the mixture warmed to room temperature and stirring maintained over 12h. After all the substrate had reacted, (TLC analysis) the solvent was concentrated in vacuo to give a brown solid residue. Distilled water (20 mL) was added to the flask containing the residue and the mixture stirred for 1h, after which time it was extracted with dichloromethane (2x 25 mL). The aqueous layer was washed once with dichloromethane (15 mL). Pooled organic solvents were then dried and evaporated under reduced pressure to give a brown oil which solidified rapidly. The solid was purified by column chromatography (40 % ethyl acetate: hexane) to give 124 (0.27 g, 71 %) as a brown crystalline solid.

Mp. 44-47 °C. Mass spectrum (Cl⁺), 278 [MH⁺]; 161 (C₉H₅OS⁻). **HRMS (Cl) Found:** 278.1223 (3.2 ppm), required: 278.1220 for C₁₇H₁₂NOS.

**¹H-NMR (CDCl₃, 300 MHz):** 6.71 (d, J = 3.6 Hz, 1H, H3), 7.28 (d, J = 15.6 Hz, 1H, H2), 7.33-7.46 (m, 4H, H5; H6, H5’; H6’), 7.74 (d, J = 3.9 Hz, 1H, H4), 7.90 (s, 1H, H3’), 7.93 (dd, J = 3.45, Hz, 5.3 2H, ArH- H4 and H7). 8.44 (dd, J = 1.2, 2.6 Hz, 1H, H7). **¹³C-NMR:** 102.8 (C3), 111.2 (C7), 120.0 (C4), 120.9 (C6), 121.2 (C3’), 122.2-125.5 (C5, C6, C4’- C7’), 125.8 (C2), 130.4 (C3a) and 138.7 (C7a)
Synthesis of (3-bromobenzof[b]thien-2-yl)(1H-indol-1-yl)methanone (125).

In the same manner as that reported for 124, reaction of the bromo substituted acid chloride 27 (0.60 g, 2.33 mmol) and indole 123 (0.34 g, 2.90 mmol) yielded 125 after chromatography (40 % ethyl acetate: hexane) as a brown solid (0.20 g, 45 %). Mass spectrum (Cl\(^+\)), \(m/z\) 356 [MH\(^{+1}\), \(^{81}\)Br], 276 (C\(_{17}\)H\(_{10}\)NOS\(^-\)), 239 (C\(_9\)H\(_4\)BrOS\(^-\)) and 211 (C\(_8\)H\(_4\)BrS\(^-\)). **HRMS (Cl)** Found: 356.1211 (3.2 ppm), required: 356.1213 for C\(_{17}\)H\(_{11}\)\(^{81}\)BrNOS. **\(^1\)H-NMR** (CDCl\(_3\), 300 MHz): 6.71 (d, \(J = 3.6\) Hz, 1H, H3), 7.26 (d, \(J = 15.6\) Hz, 1H, H2), 7.33-7.44 (m, 4H, H5; H6, H5\(^-\); H6\(^-\)), 7.74 (d, \(J = 3.9\) Hz, 1H, H4), 7.93 (dd, \(J = 3.45, 5.26\) Hz, 2H, ArH- H4 and H7). 8.44 (dd, \(J = 1.2, 2.5\) Hz, 1H, H7). **\(^{13}\)C-NMR**: 102.6 (C3), 111.3 (C7), 120.0 (C4), 120.9 (C6), 122.2-125.5 (C5, C6, C4\(^-'\)-C7\(^-'\)), 125.8 (C2), 130.2 (C3a), 138.7 (C7a) and 164.3 (C=O).


To a mixture of 124 (0.22 g, 0.79 mmol) and palladium(II) acetate (0.17 g, 0.75 mmol) was added glacial acetic acid (70 mL) and the mixture heated to 100 °C with continuous stirring under nitrogen for 7h. After the reaction came to completion (TLC monitoring; 30 % ethyl acetate: hexane, bright yellow spot), the reaction mixture was cooled to room temperature and solvent evaporated under reduced pressure to give a black residue. The crude residue was dissolved in dichloromethane (15 mL) and vacuum filtered through celite to remove suspended palladium(0) particles. After solvent evaporation, the impure solid was purified by
chromatography (40 % ethyl acetate: hexane) to give 126 (0.03 g, 14 %) as a deep orange coloured solid. The product was further purified by multi-development preparative plate chromatography (20 % ethyl acetate: hexane) and 3 bands isolated of which the (most polar) 3rd band (6.6 mg, 3 %) was the compound 126. Mass spectrum (Cl), m/z 276 [MH+]; HRMS (Cl) Found: 276.0483 (0.3 ppm), required: 276.0483 for C17H10NOS. 1H-NMR (CDCl3, 300 MHz): 6.57 (s, 1H, H5), 7.00-7.17 (m, 2H, H2 and H3), 7.25-7.55 (m, 3H, H1, H7 and H8), 7.72 (d, J = 3.9 Hz, 1H, H4), 8.06 (m, 2H, H6 and H9). 13C-NMR: 102.1 (C5), 111.4 (C1), 119.6 (C2), 121.7 (C3 and C9), 124.7-124.9 (C5a, C7 and C8), 128.0 (C4a) 135.2 (C12a) and 162.9 (C=O).

**Synthesis of 126 from 125.**

In the same manner as that described for 124 above, 125 (0.20 g, 0.75 mmol) was reacted to form 126 (0.05 g, 23 %), which was isolated after chromatography as a deep orange coloured solid. Mass spectrum (Cl), m/z 276 [MH+]; HRMS (Cl) Found: 276.0479 (1.2 ppm), required: 276.0479 for C17H10NOS. 1H-NMR (CDCl3, 300 MHz): 6.58 (s, 1H, H5), 7.01-7.16 (m, 2H, H2 and H3), 7.28-7.57 (m, 3H, H1, H7 and H8), 7.70 (d, J = 3.9 Hz, 1H, H4), 8.06 (m, 2H, H6 and H9). 13C-NMR: 102.1 (C5), 111.2 (C1), 119.7 (C2), 121.5 (C3 and C9), 124.7-124.9 (C5a, C7 and C8), 128.3 (C4a) 135.2 (C12a) and 162.9 (C=O).

** Attempted synthesis of 126 in the presence of copper(II) acetate.**

To a mixture of 124 (0.40 g, 1.43 mmol) and palladium(II) acetate (0.2 g, 0.88 mmol) and glacial acetic acid (70 mL) was added copper(II) acetate (0.72 g, 3.61 mmol) in acetic anhydride (15 mL) and the mixture heated at reflux with continuous stirring under nitrogen for 12h. After 12h, a TLC analysis of the reaction mixture indicated
primarily the presence of the starting material. The reaction conditions were maintained for a further 48h with periodic monitoring by TLC and mass spectroscopy, however the formation of 126 was not evident. The reaction mixture was then cooled to room temperature, and solvent evaporated under reduced pressure to give a crude residue which was chromatographed (40 % ethyl acetate: hexane) to give what was identified as the starting material 124 (0.38g) as a brown solid. Mass spectrum (Cl⁺), 278 [MH⁺]. ^1H-NMR (CDCl₃, 300 MHz): 6.71 (d, J = 3.6 Hz, 1H, H3), 7.26 (d, J = 15.6 Hz, 1H, H2), 7.33-7.46 (m, 4H, H5, H6, H5', H6'), 7.74 (d, J = 3.9 Hz, 1H, H4), 7.90 (s, 1H, H3'), 7.95 (dd, J = 3.45, 5.24 Hz, 2H, ArH- H4 and H7). 8.44 (m, 1H, H7).
6.3 Experimental for Chapter 4 (biological testing)

6.3.1. Antibacterial assay

Sample preparation.

Pure samples of the benzo[b]thiophene derivatives were carefully weighed out (approx. 2 mg) on an analytical balance and packed in small glass sample tubes. These samples were then sent for antibacterial assays to the staff at Avexa Ltd (formally-Amrad, Melbourne) who carried out the antibacterial assays through Dr. J. Deadman. All assays used the \textit{Staphylococcus aureus} strain ATCC 6538P, or vancomycin resistant enterococci (VRE) strains 243, 449, 820 and 987 (\textit{Enterococcus faecium}). It should be noted however that two strains, VRE 243 and 987, although designated as VRE, were in fact sensitive to vancomycin.

Antibacterial testing methodology.

The Mueller-Hinton Broth (MHB) medium culture media was prepared with final concentrations of 1 $\mu$g/ mL MgCl$_2$ and 2 $\mu$g/ mL CaCl$_2$ and was pre-warmed for 2-3h at 37 °C before use. Mueller-Hinton Agar (MHA) medium culture was then prepared at concentrations of 1.5 % agar (Merck Agar 1.01614). \textit{Staphylococcus aureus} was streaked onto MHA and the plate was incubated overnight at 37 °C. From this plate, 10 cryovial were prepared by looping several colonies into 0.5 mL of 20 % glycerol solution and immediately stored at minus 140 °C. A cryovial was removed from minus 140 °C storage and thawed to room temperature. The MHA plate was streaked with bacterial suspension and incubated at 37 °C overnight to create a parent plate (P1). The parent plate was then stored at 4 °C. A daughter plate (D1) was incubated at 37 °C overnight and its loop of colony used to inoculate a 125 mL flask.
containing 20 mL of MHB containing 25 µg/ mL CaCl₂ 2H₂O) and 12.5 µg/ mL MgCl₂ 6H₂O. The flask was swirled at 260 rpm at 37 °C for 18h on an orbital incubator shaker. The parent plates P1 and P2 were each used twice to generate two daughter plates (D1 and D2) before being discarded.

The standardised inocula for assays was prepared as 1/10 dilution of seed cultures by adding 250 µL of the cultures to 2,250 µL of MHB in a disposable cuvette and the required dilution factor was calculated by dividing the observed OD₆₅₀.

Sufficient volumes of the final inoculum cultures were prepared in pre-warmed MHB (37 °C) by diluting the standardised cultures to the required final concentration (10⁸ dilution).

**Assay Procedure for 96-well Microtitre Plates.**

To each well of the 96-well microtitre plate was added 50 µL of liquid medium and 50 µL of peptoid test solution initially prepared by dissolving in 2.5 % DMSO and added in triplicate to the top of the microtitre plate. A vancomycin control set (triplicate) and a compound negative control set (triplicate) were also set up on each plate. The inoculated culture medium was incubated at 37 °C for 30 min with continuous shaking at 130 rpm. Using the multi-channel pipette and multi-stepper pipette the adding, transferring and mixing of the inoculum were performed on the wells of the plates. The plates were then incubated at 37 °C on a 90 % relative humidity environment for 18h with continued shaking (100 rpm). The activities were reported in minimum inhibitory concentration (MIC) value which is a standard measure of the lowest concentration of the antibiotic that results in the inhibition of visible bacteria growth under standard conditions. The MIC was also determined for DMSO (2.5 %) as a control measure. The test results are summarised in Table 4.4.
6.3.2. Antimalarial assay

Sample preparation.

Purified samples of the benzo[h]thiophene derivatives to be tested were carefully weighed out (10-12 mg) on an analytical balance and packed in small glass sample tubes. These samples were then sent for antimalarial testing at the National Centre for Genetic Engineering and Biotechnology (BIOTEC), National Science and Technology Development Agency (NSTDA), Bangkok. The in vitro tests were undertaken by Dr. Sumalee Kamchonwongpaisan.

Antimalarial assay method.

Samples were made up in DMSO solution and using the Microdilution Radioisotope Technique, the in vitro antimalarial activity testing was carried out. Two strains of *Plasmodium falciparum* were used for these tests, the double mutant K1 CB1 strain, which is a multidrug resistant/ antifolate resistant *Plasmodium falciparum*, and a wild type antifolate sensitive strain TM4/ 8.2 of *Plasmodium falciparum*. The test sample (25 µl, in the culture medium) was placed in triplicate in a 96-well plate where parasitised erythrocytes (200 µl) with a cell suspension (1.5 %) of parasitemia (0.5-1 %) were then added to the wells. The range of the final concentrations of the samples was varied from $1 \times 10^{-5}$ to $1 \times 10^{-8}$ g/ mL with 0.1% of the organic solvent. The plates were then cultured under standard conditions for 24h after which $^3$H-hypoxanthine (25 µL, 0.5 mCi) was added. The culture was incubated for 18-20h after which the DNA from the parasite was harvested from the culture onto glass fibre filters and a radiation counter used to determine the amount of $^3$H-hypoxanthine present. The inhibitory concentration of the sample was determined from its dose-response curves or by calculation.
The Trager and Jensen method\textsuperscript{153} was used to culture \textit{Plasmodium falciparum} K1 CB1 strain. The parasites were maintained in human red blood cells in a culture medium. RPMI 1640 was supplemented with 25 mM HEPES, 0.2 \% sodium bicarbonate, and 8 \% human serum, at 37 °C in a carbon dioxide gas incubator\textsuperscript{154}. The test results are given in Table 4.1, Table 4.2 and in Appendix 1.
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Appendices

Appendix 1: Antimalarial activities for both fused and non-fused benzo[b]thiophene derivatives showing the standard deviation figures for the IC$_{50}$ values

<table>
<thead>
<tr>
<th>Compound Number</th>
<th>Antimalarial activity against K1 CB1 and TM4/8.2 strains of <em>P. falciparum</em>. The (IC$_{50}$) values are reported in µM and µg/mL.</th>
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<tr>
<td></td>
<td>K1</td>
</tr>
<tr>
<td></td>
<td>µM</td>
</tr>
<tr>
<td>92</td>
<td>3.9 +/- 2.5</td>
</tr>
<tr>
<td>105</td>
<td>3.63 +/- 0.34</td>
</tr>
<tr>
<td>30</td>
<td>13.0 +/- 4.4</td>
</tr>
<tr>
<td>29</td>
<td>13.7 (18.2, 9.2)</td>
</tr>
<tr>
<td>106</td>
<td>14.2 +/- 0.89</td>
</tr>
<tr>
<td>81</td>
<td>17.9 +/- 6.1</td>
</tr>
<tr>
<td>80</td>
<td>18.2 +/- 5.2</td>
</tr>
<tr>
<td>61</td>
<td>18.3 +/- 4.3</td>
</tr>
<tr>
<td>63</td>
<td>18.4 +/- 3.2</td>
</tr>
<tr>
<td>85</td>
<td>19.1 +/- 6.5</td>
</tr>
<tr>
<td>60</td>
<td>20.0 +/- 3.5</td>
</tr>
<tr>
<td>54</td>
<td>22.7 +/- 7.5</td>
</tr>
<tr>
<td>76</td>
<td>23.2 +/- 9.5</td>
</tr>
<tr>
<td>53</td>
<td>23.5 +/- 3.5</td>
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<tr>
<td>59</td>
<td>28.89 +/- 2.82</td>
</tr>
<tr>
<td>70</td>
<td>37.1</td>
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<tr>
<td>62</td>
<td>43.0 +/- 2.36</td>
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<tr>
<td>50</td>
<td>46.92</td>
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<tr>
<td>52</td>
<td>51.1</td>
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<tr>
<td>51</td>
<td>54.13</td>
</tr>
<tr>
<td>71</td>
<td>&gt; 50.0</td>
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<tr>
<td>84</td>
<td>58.71 +/- 4.0</td>
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<tr>
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<td>113</td>
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<tr>
<td>120</td>
<td>&gt; 20</td>
</tr>
<tr>
<td>96</td>
<td>&gt; 100</td>
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</table>
Appendix 2: $^1$H-NMR of benzo[b]thiophene derivative 92 synthesised via free radical cyclisation.
Appendix 3: $^1$H-NMR of the de-allylated benzo[b]thiophene derivative 106.
Appendix 4: $^1$H-NMR of the 9-membered ring derivative 120 synthesised via ring closing metathesis reaction using Grubbs’ I catalyst.
Appendix 5: $^1$H-NMR of benzo[b]thiophene derivative 126 acquired through palladium-assisted cyclisation reaction.
Appendix 6: $^1$H-NMR of 2,5-dihydropyrrole derivative 114.