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# **Novel Antimalarial and Antitubercular Agents Based on Natural Products**



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**University of Cape Town  
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# Novel Antimalarial and Antitubercular Agents Based on Natural Products

A thesis submitted to the University of Cape Town in partial  
fulfilment of the requirements for the degree of  
Doctor of Philosophy

By

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**A dedication to my loving parents Isabella and Willem Hans and my siblings: Adrian,  
Doris, Irene and Manfred for their inspiring strength, encouragement, support,  
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## Abstract

Malaria and tuberculosis are listed among the major infectious diseases. They are responsible for severe morbidity and mortality especially in resource-poor settings where control interventions are inaccessible, unaffordable and plagued by widespread resistance. According to current estimates, malaria afflicts over 40% of the world's population and claims the lives of 1-3 million annually. The epidemiology of tuberculosis is just as grim. About one third of the world population is reported to be infected with *Mycobacterium tuberculosis* and it is responsible for 2-3 million deaths annually. Of particular interest to this project, is the fact that natural products have always been on the frontline in the battle against these diseases, that is, most of the clinically used drugs in antimalarial and antitubercular chemotherapy are of natural product-origin. In this project we therefore focussed on the design, synthesis, characterization and biological evaluation of novel antimalarial and antitubercular agents obtained by synthetically hybridizing and decorating scaffolds based on natural products or derivatives - with a history in the aforementioned disease models. Scaffolds selected include the thiolactone ring system, a key intermediate of the natural product thiolactomycin, the non-peptidic natural product isatin and the chalcone scaffold. In this way a series of hybrids were constructed which can be subdivided into three main groups: (i) thiolactone-isatin hybrids, (ii)  $\beta$ -amino alcohol thiolactone-chalcone and isatin-chalcone hybrids, and (iii) dihydroartemisinin-isatin, dihydroartemisinin-chalcones and other miscellaneous hybrids. These were evaluated for antiplasmodial activity against the chloroquine resistant (W2) and chloroquine sensitive (D10) strains of *Plasmodium falciparum* as well as for inhibitory activity against cysteine proteases. Evaluation of antimycobacterial activity of the synthesized compounds against the drug sensitive H<sub>37</sub>Rv strain of *M. tuberculosis* was also undertaken.

(i) For the first group of hybrids we used the C-4 hydroxyl group of the thiolactone ring as a handle for functionalization by attaching it *via* a variable, non-hydrolyzable alkyl linker to the isatin scaffold. Most striking, is the operational simplicity of the synthesis methodology employed and how it led to the discovery of a novel tetracyclic ring system. Identified from the latter is the compound **3.8p** which is the most active antimalarial from this series with an IC<sub>50</sub> of 6.92  $\mu$ M in the W2 strain. Some of the hybrids (**3.7** and **3.8**) were more active than the monomers and the parent drug thiolactomycin, thus demonstrating the potential of hybridization as a drug discovery tool. Antimalarial structure activity relationships for the novel tetracycles **3.8** revealed the importance of substitution at C-5 of the isatin scaffold and

the need for increased lipophilicity. Although the antitubercular activity of the hybrids was inferior compared to the control drugs, a number of advanced intermediates were identified which displayed promising activity against both fast growing and slow-growing, persistent forms of *M. tuberculosis*.

(ii) The second group of hybrids consisted of a 36-member library obtained by the covalent linkage of methoxylated chalcones with the thiolactone ring and the isatin scaffold. Incorporated in their design is the  $\beta$ -amino alcohol moiety, a known bioactiphore. For the synthesis of these hybrids we employed the copper-catalyzed Huisgen 1,3-dipolar cycloaddition reaction (also known as “click” chemistry) which in addition to expediting structure activity relationship studies yielded the 1,2,3-triazole ring system. The antiplasmodial results showed that the thiolactone-chalcones, with  $IC_{50}$ s ranging from 0.68 to 6.08  $\mu$ M, were more active against the W2 strain than the isatin-chalcones ( $IC_{50}$  = 2.09 - 14.90  $\mu$ M). More so, structure activity relationships delineated for the former indicated the preference for triOMe substitution on ring A of the chalcone scaffold. The most active compound for this series **4.14f** [ $IC_{50}$  = 0.68  $\mu$ M (W2)] is 10-fold less active than chloroquine but has a greater efficacy than the parent natural product thiolactomycin. Results obtained for cysteine protease activity showed that the isatin-chalcone hybrids inhibited falcipain-2 activity, whereas the thiolactone-chalcone hybrids were devoid of enzyme inhibitory activity. With regard to antitubercular activity, the advanced intermediates were more active than the hybrid constructs. The most promising antitubercular agent identified is the acetylenic chalcone **4.10f** (MIC = 13.1  $\mu$ M) which is 2-fold more active than one of the controls, moxifloxacin (MIC = 31.1  $\mu$ M) against the slow-growing persistent forms of *M. tuberculosis*.

(iii) The final group of compounds is a limited series of semi-synthetic artemisinin analogues obtained by hybridizing the first generation analogue, dihydroartemisinin with previously mentioned scaffolds (isatin, chalcones, thiolactone) and other biologically relevant scaffolds such as the 4-aminoquinoline unit and azidovudine (AZT). As with the previous series we utilized the ‘click’ reaction to effect the synthesis of these hybrids. The most active compound identified is the intermediate **5.4** [ $IC_{50}$  = 6.13 nM (W2)] which is more active than the parent natural product artemisinin [ $IC_{50}$  = 10.84 nM (W2)], 16 times more active than chloroquine and 2-times less active than dihydroartemisinin. The lack of antitubercular activity of compounds in this series moreover confirmed the antimalarial specificity of artemisinin analogues.

## List of Abbreviations

ACC	Acetyl-CoA Carboxylase
ACP	Acyl Carrier Protein
ACT	Artemisinin Combination Therapy
ADME	Absorption, Distribution, Metabolism, and Excretion
AIDS	Acquired Immune Deficiency Syndrome
ART	Artemisinin
ATCC	American Type Culture Collection
AZT	Zidovudine
br	Broad (in $^1\text{H}$ NMR)
$\text{Br}_2$	Bromine
$\text{CaH}_2$	Calcium hydride
$\text{CD}_3\text{OD}$	Deuteromethanol
$\text{CDCl}_3$	Deuteriochloroform
CFU	Colony forming unit
$\text{CHCl}_3$	Chloroform
$\text{CH}_3\text{COSH}$	Thiolacetic acid
$\text{CH}_3\text{I}$	Methyl iodide
$^{13}\text{C}$ NMR	Carbon Nuclear Magnetic Resonance
CoA	Coenzyme A
CQ	Chloroquine
CQR	Chloroquine-resistant
CQS	Chloroquine-sensitive
d	Doublet (in $^1\text{H}$ NMR)
dd	Double doublet or doublet <i>of</i> doublets (in $^1\text{H}$ NMR)
dq	Doublet of quartets (in $^1\text{H}$ NMR)
DBU	Diaza(1,3)bicyclo[5.4.0]undecane
DCM	Dichloromethane
DEET	Diethyl toluamide
DEPT	Distortionless Enhancement by Polarization Transfer
DHA	Dihydroartemisinin
DHFR	Dihydrofolate reductase
DHPS	Dihydropteroate synthase



DMF	Dimethylformamide
DMSO	Dimethylsulfoxide
DMSO-d <sub>6</sub>	Deuterodimethylsulfoxide
D <sub>2</sub> O	Deuterium oxide
DOC	Dissolved Oxygen Concentration
DOTS	Direct Observation of Treatment Short-course
DST	Drugs Susceptibility Testing
E-64	<i>L-trans</i> -epoxy-succinylleucylamido(4-guanidino)butane.
EC <sub>50</sub>	50% Effective Concentration
ECPI	Endoperoxide Cysteine Protease Inhibitor
EI	Electron Impact
Eq	Equivalent(s)
ESI	Electrospray Ionization
Et <sub>3</sub> N	Triethylamine
EtOAc	Ethylacetate
EtOH	Ethanol
FAS	Fatty acid synthase
Fas	Fatty acid synthesis
FDA	Food and Drug Administration
FP	Falcipains
FTIR	Fourier transform infrared
FV	Food vacuole
GI	Growth Index
h	Hour(s)
HAP	Histo-aspartic protease
HCl	Hydrochloric acid
Hex	Hexane
<sup>1</sup> H - <sup>1</sup> H COSY	Proton-proton COrrrelation SpectroscopY
HIV	Human Immunodeficiency Virus
<sup>1</sup> H NMR	Proton Nuclear Magnetic Resonance
HMBC	Heteronuclear Multiple Bond Correlation
HRMS	High Resolution Mass Spectrometry
HSQC	Heteronuclear Single Quantum Coherence
Hz	Hertz

IC <sub>50</sub>	50% Inhibitory Concentration
INH	Isoniazid
IR	Infrared
ITN	Insecticide-treated bednets
IUPAC	International Union of Pure and Applied Chemistry
KAS	Ketoacyl-Acyl Carrier Protein Synthase
KBr	Potassium bromide
K <sub>2</sub> CO <sub>3</sub>	Potassium carbonate
KF/Al <sub>2</sub> O <sub>3</sub>	Potassium fluoride on alumina
KOH	Potassium hydroxide
LiClO <sub>4</sub>	Lithium perchlorate
LiHMDS	Lithium bis(trimethylsilyl)amide
LORA	Low Oxygen Recovery Assay
LRMS	Low Resolution Mass Spectrometry
LTBI	Latent tuberculosis infection
MabA	Mycolic acid biosynthesis A
MABA	Microplate Alamar Blue assay (MABA)
MDR	Multi-drug resistant
MetHb	Methaemoglobin
MIC	Minimum Inhibitory Concentration
μM	MicroMolar
m	multiplet (in <sup>1</sup> H NMR)
mL	MilliLiter
m. p.	melting point
MeOH	Methanol
MOX	Moxifloxacin
nM	NanoMolar
NADH	Reduced form of Nicotinamide adenine dinucleotide
NADPH	Reduced form of Nicotinamide adenine dinucleotide phosphate
NaH	Sodium hydride
NaHMDS	Sodium bis(trimethylsilyl)amide
Na <sub>2</sub> SO <sub>4</sub>	Sodium sulphate
NaN <sub>3</sub>	Sodium azide
NCE	New Chemical Entities

NH <sub>4</sub> Cl	Ammonium chloride
NRP	Non-replicating persistent
PfATP6	<i>Plasmodium falciparum</i> Calcium ATPase
PBS	Phosphate buffered saline
PfCRT	<i>Plasmodium falciparum</i> Chloroquine Resistance Transporter
Pgh	P-glycoprotein homologue
Pgp	P-glycoproteins
PM	Plasmepsins
ppm	Parts per million
q	quartet (in <sup>1</sup> H NMR)
quint	quintet (in <sup>1</sup> H NMR)
Rec-FP-2	Recombinant Falcipain-2;
R <sub>f</sub>	Retention factor
RI	Resistance Index
RLU	Relative Light Unit
RMP	Rifampin or rifampicin
s	singlet (in <sup>1</sup> H NMR)
SAR	Structure activity relationship(s)
SERCA	Sarco/endoplasmic reticulum Ca <sup>2+</sup> -ATPase
SM	Streptomycin
t	triplet (in <sup>1</sup> H NMR)
TB	Tuberculosis
TBAH	Tetrabutylammonium hydroxide
TCTP	Translationally Controlled Tumor Protein
THF	Tetrahydrofuran
TLC	Thin layer chromatography
TLM	Thiolactomycin
TMS	Tetramethylsilane
TMSCl	Chlorotrimethylsilane
TMSN <sub>3</sub>	Trimethylsilyl azide
uv	Ultraviolet
UPLC	Ultra performance liquid chromatography
WHO	World Health Organization
XDR	Extensively drug resistant

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## Chapter 1

### INTRODUCTION I

#### 1.0. Malaria

##### 1.1. History of Malaria

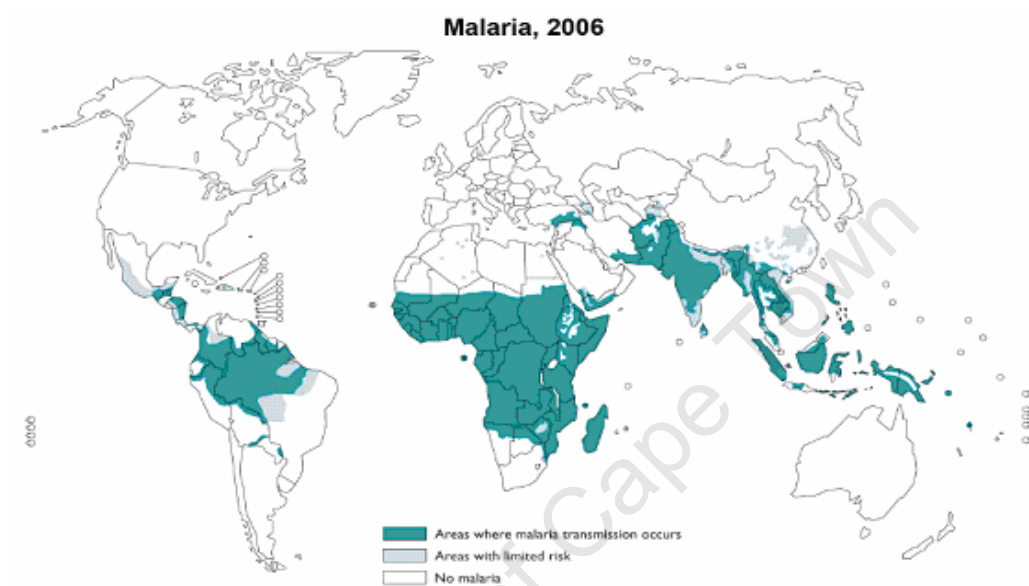
The origin of malaria has been traced to ancient times and the devastating effect it had and is still having on mankind is well documented.<sup>1,2</sup> Ancient Italians coined the term “mal’aria” meaning “bad air” in reference to a substance that arose from swamps and marshes and which, at the time, was believed to be the cause of malaria. A landmark event in the history of malaria is French pathologist Charles Alphonse Laveran’s discovery in 1880 of the plasmodial parasite as the causative agent.<sup>1</sup> This was followed by the groundbreaking work of malariologists Ronald Ross and Giovanni Batissta Grassi who identified the *Anopheles* mosquito as the vector of malaria.<sup>3</sup> These discoveries, although marked by feuds and scepticism at their revelation, have made a significant contribution to our current understanding of the disease and the development of methods to combat it.

A recent milestone which has become pivotal in the fight against malaria is the completion of the *Plasmodium falciparum* genome sequence by Gardner and co-workers.<sup>4</sup> The genomic data allowed a better understanding of the biochemistry, cellular and molecular biology of the parasite, revealed new targets for drug development<sup>4</sup> and shed light on the molecular mechanism of drug resistance.<sup>5</sup>

##### 1.2. Global Distribution and Burden of Malaria

Malaria is endemic to over 90 countries and affects about 40% of the world’s population. It is distributed throughout tropical areas such as sub-Saharan Africa, South East Asia, the Pacific islands and Central and South America (fig. 1.1).<sup>6</sup> Moreover, the disease is reported to have spread to regions previously declared malaria-free such as Central Asia and Eastern Europe<sup>7a</sup> with clinical cases in the U.S.A, reported to average 1300 per year.<sup>7b</sup> Over 500 million malaria related clinical cases are reported each year, of which 90% are confined to sub-Saharan Africa.<sup>8</sup> The severity of the disease is further highlighted by the fact that 2-3 million malaria related deaths are reported annually, with most of the casualties being children. According to the World Health Organization (WHO) estimates, one child under the age of 5 years dies every 30 seconds which translates to 3000 deaths per day.<sup>9a</sup>

The strong and treacherous link between malaria and poverty has been well documented.<sup>9</sup> Indeed, one of the challenges faced by people in resource-poor settings is the difficulty of gaining access to health care facilities. This causes them to miss out on an early diagnosis and effective treatment which constitutes “the cornerstone of the global malaria control strategy”.<sup>10</sup>



**Figure 1.1:** Global distribution of malaria, 2006<sup>9c</sup>

### 1.3. Life Cycle of the Malaria Parasite

The causative agent of malaria is a unicellular, protozoan parasite of the genus *Plasmodium*. It is transmitted from one human host to another through the bite of an infected female *Anopheles* mosquito. There exist 156 species of *Plasmodium* but only four are known to infect humans, that is, *P. vivax*, *P. ovale*, *P. malariae* and *P. falciparum* of which the latter is the most virulent.



manifestation of the disease which is characterized by high fever, convulsions, chills and anemia. In the case of severe malaria delirium, metabolic acidosis and multi-organ systems failure may occur which, in the absence of timely medical intervention, may result in coma and death.

When the erythrocyte ruptures (**6**, fig. 1.2), it releases merozoites which can infect other erythrocytes to generate a new asexual cycle. The parasite proliferates continuously in this manner. Some parasites develop into gametocytes (**7**, fig. 1.2) which can be taken up by another mosquito (**8**, fig. 1.2). In the stomach of the mosquito, the gametocytes develop into male and female gametes (**C**, fig. 1.2) which upon fertilization, produces an oocyst (**11**, fig. 1.2). The mature oocyst ruptures to release infectious sporozoites, which migrates to the mosquito's salivary glands (**12**, fig. 1.2) ready for the next bloodmeal.

#### 1.4. Combating Malaria

The complexity of malaria as an infectious parasitic disease requires the use of multiple approaches for its control. Preventative and curative measures currently employed are summarized below:

- (i) Vector control is primarily aimed at reducing the mosquito population density and therefore the risk of exposure to infectious mosquitoes. Traditional approaches to make residences 'mosquito-proof' entail the use of screens and indoor residual spraying with pyrethroids, permethrin or deltamethrin. Other personal protective measures include the application of insect repellent-containing diethyl toluamide (DEET) on exposed skin<sup>6,12a</sup> and the use of insecticide-treated bed nets (ITNs). The efficacy of vector control interventions is hampered by the inconsistent application of the above measures and the ability of the vector to develop resistance to insecticides.<sup>12b</sup>
- (ii) Chemoprophylaxis is a preventative method and has the primary goal of prevention and/or suppression of clinical malaria. Antimalarials use in prophylactic treatment include proguanil (Paludrine<sup>®</sup>), mefloquine (Lariam<sup>®</sup>), atovaquone-proguanil (Malarone<sup>®</sup>) and doxycycline.<sup>13</sup> These chemoprophylactic regimens are reported to provide between 75% and 95% protection and are thus not totally effective.<sup>12a</sup> As with vector control, chemoprophylaxis is threatened by resistance.
- (iii) The severity of *falciparum* malaria requires early diagnosis and the initiation of prompt treatment. At the onset, malaria commonly presents with fever.

Unfortunately because of this general manifestation the misdiagnosis of malaria is common among travellers returning from malaria endemic regions and those residing in these regions.

- (iv) Chemotherapy refers to the drug treatment of clinical cases and will be elaborated on in section 1.4.1.

#### **1.4.1. Antimalarial Chemotherapy**

The erythrocytic cycle is the major life cycle target for antimalarials.<sup>14</sup> Also, there is a limited arsenal of antimalarial drugs each with their own set of liabilities. These include availability, toxicity, cost and resistance, with the latter being the key motivator for “continued innovation”.<sup>15</sup>

Several factors need to be considered when choosing an effective and safe antimalarial regimen. The species of the malaria parasite and the site of contraction of infection, to ascertain the likely pattern of resistance, are important initial requirements.<sup>16</sup> Effective antimalarial treatment also requires an understanding of the clinical manifestations of the disease, the different categories of patients and the immune status of the host.<sup>17</sup> For example, treatment of uncomplicated malaria which is commonly found in semi-immune adults differs from that for severe malaria which is characterized by anemia and cerebral malaria. Indeed, in tropical Africa, people who have acquired partial immunity require less supportive antimalarial therapy even with severe parasitaemia.<sup>18</sup> Immunocompromised patients such as pregnant women and young children aged under 5 years, who are at risk of contracting severe malaria, require special treatment.<sup>19</sup> It seems then that there is a need for personalized medication in antimalarial chemotherapy which the current arsenal cannot sufficiently provide for. This clearly supports the need for new and better antimalarials.

#### 1.4.1.1. Classification of Antimalarial Drugs

Antimalarials can be classified according to their structure, life-cycle's stage specificity and their mechanism of action. The commonly used antimalarials (Fig.1.3) can be structurally subdivided into five classes of compounds.<sup>6</sup>

- (a) quinolines and aryl aminoalcohols: quinine **1.1**, primaquine **1.2**, chloroquine **1.3**, mefloquine **1.4**, lumenfantrine **1.5**, halofantrine **1.6**, tafenoquine **1.7**,
- (b) antifolates: pyrimethamine **1.8**, sulfadoxine **1.9**, dapsone **1.10**, proguanil **1.11**, chlorproguanil **1.12**,
- (c) hydroxynaphthaquinones: atovaquone **1.13**,
- (d) artemisinin derivatives: artemisinin **1.14**, dihydroartemisinin **1.15**, artemether **1.16**, arteether, **1.17**, artesunate **1.18**,
- (e) antibiotics: tetracycline **1.19**, doxycycline **1.20**, clindamycin **1.21**.

Another method of classifying antimalarials, as mentioned above, is based on their life-cycle's stage specificity (fig. 1.2). A blood schizontocide for example, targets the erythrocytic stage. Tissue schizontocides kill the liver stage and therefore prevent relapse of malaria. It is now widely accepted that *P. falciparum* is not characterized by recrudescence, thus treatment with a blood schizontocidal suffices. Clearance of parasitaemia caused by *P. vivax* and *P. ovale* requires both blood schizontocides and tissue schizontocides; a treatment method known as a 'radical cure' of malaria.<sup>20</sup> Gametocidal drugs kill off the non-pathogenic gametes. The latter are essential for transmission between hosts and also contributes to the development and spread of drug resistance.<sup>18</sup>

#### 1.4.1.2. Clinically Established Antimalarial Drugs

In this section, some clinically established antimalarial regimens are briefly introduced. The emphasis is on their stage or life cycle specificity and their use in curative and/or prophylactic therapy. Also covered is their side-effect profiles and other limitations; all of which is to demonstrate the constant need for safer, more effective and affordable drugs. A discussion of the mechanism of action of some of these drugs is reserved for section 1.4.2.

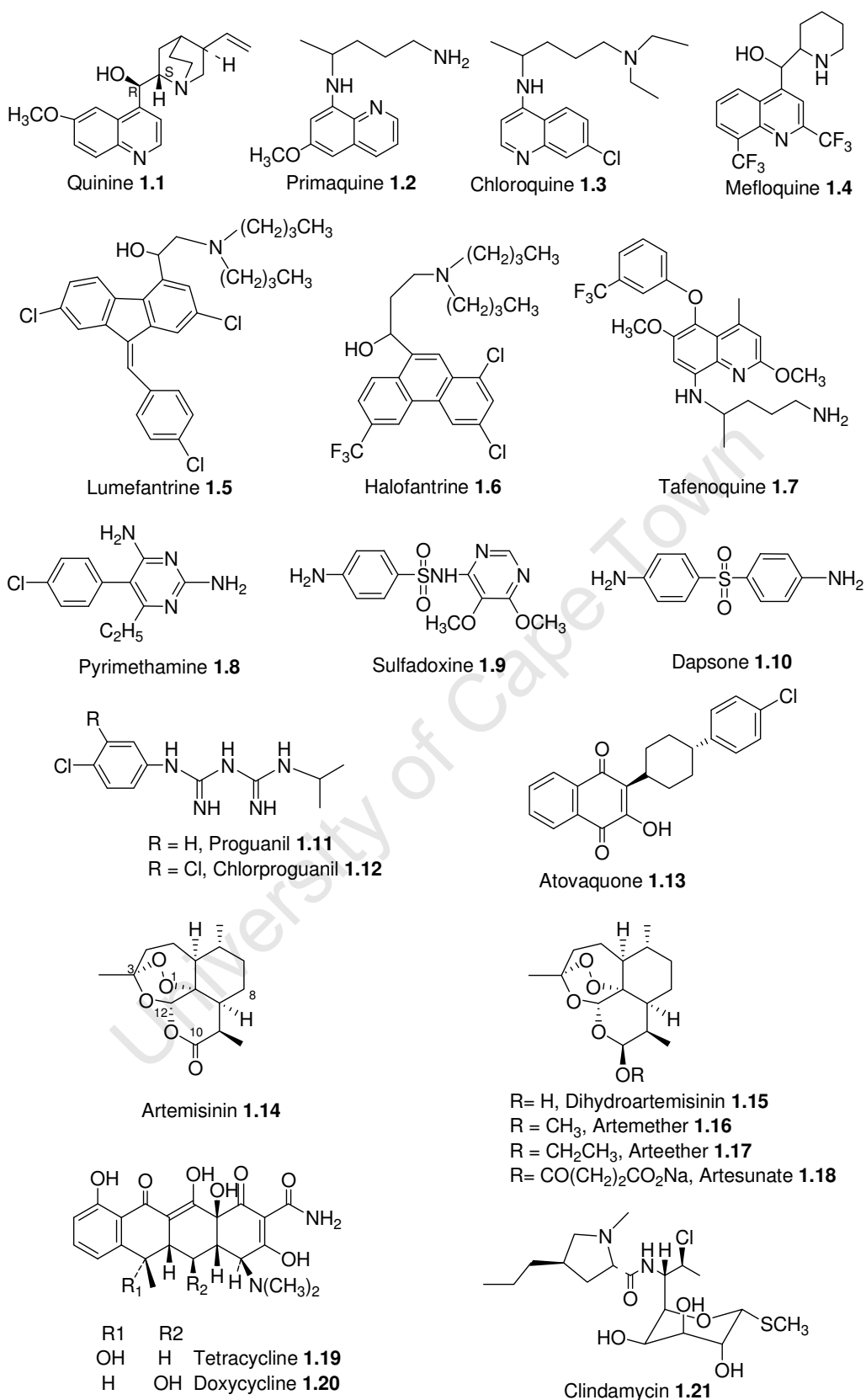


Figure 1.3: Chemical structures of some common antimalarials

Quinine **1.1**<sup>\*</sup> is a blood schizontocide with a side-effect profile that includes dysphoria, tinnitus and high-tone deafness.<sup>16</sup> In spite of this it remains the drug of choice and is administered intravenously in the treatment of severe malaria. For areas marked by quinine resistance the drug is partnered with slow-acting antibiotics such as clindamycin **1.21**, tetracycline **1.19** or the structurally related doxycycline **1.20**. The latter two antibiotics, however, are contraindicated in children and pregnant women.<sup>21</sup> Primaquine **1.2** and tafenoquine **1.7** belong to the class of 8-aminoquinolines. The aforementioned drug is an effective tissue schizontocide. It kills off *P. vivax* and *P. ovale* hypnozoites<sup>16</sup> but is ineffective as a blood schizontocide and thus not ideal for treating *P. falciparum* infections. Side-effects associated with the use of **1.2** include methaemoglobin (MetHb) toxicity and haemolysis in glucose-6-phosphate dehydrogenase deficient individuals.<sup>22</sup> The congener **1.7** is reported to be more active and less toxic.<sup>22</sup>

The 4-aminoquinoline chloroquine **1.3** is a blood schizontocide which acts on the trophozoite stage of the parasite. Although hampered by resistance, most countries in Africa still use it as a first line drug.<sup>23</sup> Mefloquine **1.4**, another blood schizontocide is used in the oral treatment of uncomplicated malaria and is active against various resistant strains of *Plasmodium*. Its use as a prophylactic stems from its long half-life of 14-21 days.<sup>21</sup> However, being structurally related to quinine it has been implicated in cross-resistance and is also associated with various neuropsychiatric side-effects.<sup>24</sup> Halofantrine **1.6**, like mefloquine is an expensive drug which is effective in treating chloroquine resistant *falciparum* malaria, but there are doubts about its safety. Common side effects such as abdominal pain, diarrhoea, purists and skin rash have been reported.<sup>16</sup> The combination drug Fansidar<sup>®</sup> represents a cheap first-line treatment in most African countries. It consists of two blood schizontocides pyrimethamine **1.8** and sulfadoxine **1.9** and is used for the treatment of uncomplicated malaria. Fansidar is reportedly the drug of choice for pregnant women. Malarone, a combination of the prodrug proguanil **1.11** and atovaquone **1.13** is used in multi-drug resistant malaria and also as a prophylactic in chloroquine resistant areas. A major setback is its high cost making it virtually unavailable in malaria-endemic, poverty stricken countries in Africa.<sup>25</sup>

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\* All bold numbers in this section refer to figure 1.3.

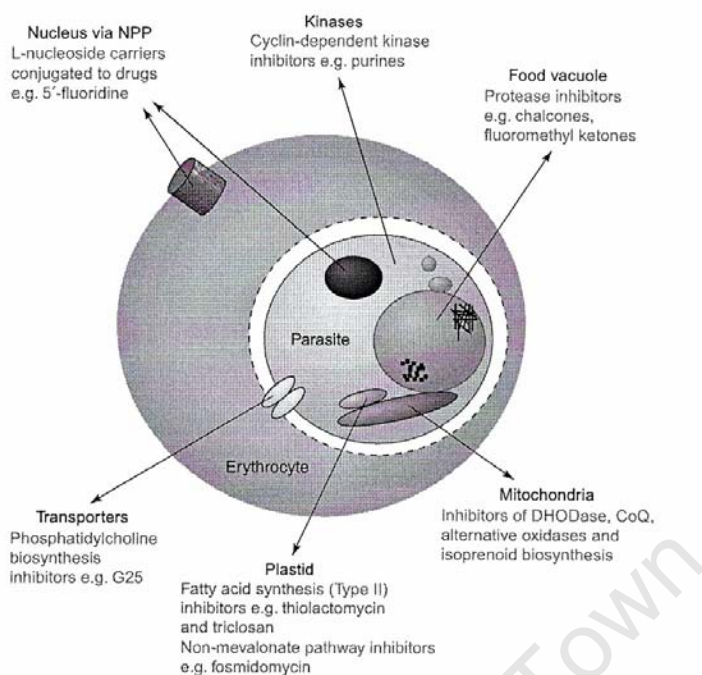


An affordable alternative to Fansidar is the chlorproguanil **1.12** and dapsone **1.10** combination (Lapdap<sup>®</sup>), which has proven to be effective in the treatment of uncomplicated malaria.<sup>18</sup>

The most effective and fast-acting class of antimalarials are represented by the sesquiterpene lactone peroxides, artemisinin **1.14** and derivatives (dihydroartemisinin **1.15**, artemether **1.16**, arteether **1.17** and artesunate **1.18**). They are tissue -, blood schizontocidal and gametocidal and therefore eliminate all stages of the malarial parasite. To prevent the development of resistance, treatment with artemisinin and derivatives is limited to severe multidrug resistant *falciparum* malaria. The therapeutic value of this class is limited by their low solubility, neurotoxicity<sup>26a</sup> and short half-life which account for the reported recrudescence when artemisinin and its derivatives are used in monotherapy. Because of the latter and the threat of drug resistance the WHO introduced Artemisinin Combination Therapies (ACTs) whereby the artemisinins are partnered with slow-acting antimalarials.<sup>26b</sup> One example is the fixed-ratio combination of artemether **1.16** with lumefantrine **1.5**, a structural analogue to halofantrine **1.6**.

#### **1.4.2. Selected Targets for Antimalarial Chemotherapy**

The *P. falciparum* genome project has been instrumental in the identification of new parasite-specific targets which are critical to combat emerging resistance. Validated drug targets include cytosolic parasite membrane targets, food vacuole targets, mitochondrial targets and apicoplast targets.<sup>27,28</sup> Some of these targets and their location in the malaria parasite are shown in fig 1.4. For purposes of this report the discussion of antimalarial drug targets has been restricted to fatty acid synthesis which occurs in the apicoplast and the food vacuole targets; haemozoin formation and cysteine proteases. Oxidative stress which is mediated by the artemisinins in malaria-infected erythrocytes is also of particular relevance to this project.



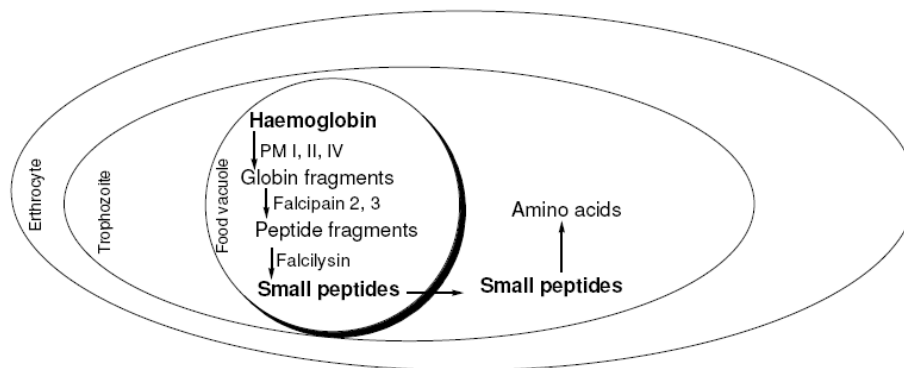
**Figure 1.4:** Drug targets for antimalarial chemotherapy<sup>27</sup>

#### 1.4.2.1. The Acidic Food Vacuole

The intraerythrocytic parasite is highly dependent on the host for nutrients. During the intraerythrocytic cycle, the metabolically active trophozoite and early schizont forms<sup>29</sup> of the malaria parasite takes up the cytoplasm of the erythrocyte which is rich in haemoglobin *via* a process known as endocytosis. This entails the invagination of the parasitophorous vacuolar membrane or cytosome formation followed by the fusion of (transport) vesicles pinched off from the cytosome with the food vacuole.<sup>30</sup> The digestive, lysosomal food vacuole in *P. falciparum* is a single-membrane organelle with a pH between 5.2-5.6,<sup>31</sup> which plays host to a number of biochemical processes essential for the growth and survival of the parasite. These include acidification, peptide transport, haemoglobin degradation and haem polymerization. Of these the latter two have been studied more extensively.

##### 1.4.2.1.1. Haemoglobin Degradation Pathway

The malaria parasite hydrolyses up to 80% of the host haemoglobin to source amino acids for protein synthesis,<sup>32a</sup> to maintain osmotic stability<sup>32b</sup> and to create space for the growing parasite.<sup>33a</sup> Haemoglobin degradation is catalyzed by proteases such as the aspartic proteases [plasmepsins (PM) I, II, IV and histo-aspartic protease (HAP)],<sup>34</sup> cysteine proteases (falcipains-2 and -3)<sup>33</sup> and the metallopeptidase, falcilysin.<sup>35</sup>



**Figure 1.5:** Haemoglobin degradation pathway<sup>36</sup>

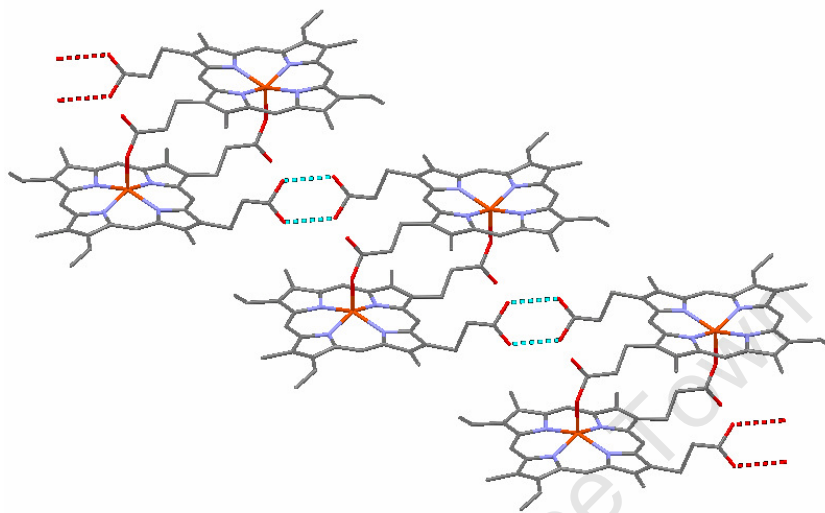
The highly ordered process of haemoglobin degradation starts with the initial cleavage of haemoglobin by aspartic proteases (PM I, II, IV) to generate globin fragments (fig. 1.5).<sup>34b,37</sup> Gluzman and coworkers<sup>34b</sup> demonstrated the substrate specificity of vacuolar proteases through *in vitro* studies. They claimed that the plasmepsins are the only proteases able to cleave native haemoglobin. A different view was shared by Gamboa de Dominguez and Rosenthal. The isolation of intact native haemoglobin from cultured parasites incubated with cysteine proteases, led the authors to conclude that cysteine proteases participate in the cleavage of native haemoglobin.<sup>38</sup>

The further breakdown of globin fragments to peptides consisting of 10-15 amino acids, is mediated by falcipains-2 and -3 (fig 1.5). These peptides are then cleaved to smaller peptides or oligomers of 6-8 amino acids by the metalloprotease, falcilysin. Interestingly, haemoglobin is not degraded to free amino acids in the food vacuole. Instead smaller peptides generated by falcilysin are transported from the food vacuole to the cytoplasm of the parasite where they are degraded to amino acids by the action of serine proteases.

#### **1.4.2.1.2. Haemozoin Formation as a Target in Antimalarial Chemotherapy**

Large quantities of haem are released during haemoglobin degradation; these are oxidized to haematin [aquaferriprotoporphyrin IX or  $\text{H}_2\text{O}-\text{Fe(III)PPIX}$ ], through an unknown process.<sup>39</sup> Accumulated haematin is toxic to the parasite<sup>40</sup> and contributes to the formation of reactive oxygen intermediates which in turn cause damage to the cellular components of the parasite such as lipids and proteins.<sup>30c,41</sup> The parasite circumvents this problem by crystallizing the oxidized ferriprotoporphyrin (FP) into an insoluble, inert crystal known as haemozoin (malaria pigment). Studies have shown that approximately 95% of haematin is

incorporated into haemozoin.<sup>39b</sup> It is now accepted that haemozoin formation occurs via a biocrystallization process with the end product being a hydrogen bonded network of FP dimers. As shown in figure 1.6 these dimers are formed by reciprocal iron to side chain carboxylate bonds.<sup>40,41c</sup>



**Figure. 1.6:** Structure of haemozoin with the bonds between iron and the carboxylate group indicated as red lines and the hydrogen bonds between FP-dimers as light blue dotted lines<sup>42</sup>

#### 1.4.2.1.2. 1. Inhibitors of Haemozoin Formation

Most of the studies on inhibitors of haemozoin formation were modelled on synthetic haematin or  $\beta$ -haematin, a cyclic dimer of ferriprotoporphyrin IX which shows structural and chemical similarity to haemozoin.<sup>40,43</sup> Both the quinolines (quinine **1.1**, chloroquine **1.3**, mefloquine **1.4**) and aryl alcohol antimalarials (lumefantrine **1.5**, halofantrine **1.6**) are concentrated in the food vacuole and are thought to exert their activity by forming complexes with the haem through  $\pi$ - $\pi$  stacking of their planar aromatic structures.<sup>44</sup> Intriguingly, in a recent publication by Egan and co-workers<sup>45</sup> the crystal structure of the aryl alcohol, halofantrine **1.6** complexed with ferriprotoporphyrin IX [Fe(III)PPIX] was presented for the first time. The model proposed from the latter revealed, in addition to  $\pi$ - $\pi$  stacking between the phenanthrene and porphyrin ring systems, the coordination of **1.6** through its benzylic alcohol to the Fe(III) center and hydrogen bonding between **1.6** and Fe(III)PPIX. These interactions provided insight into the mechanism of action of this class of drugs and are expected to guide future research into non-quinoline antimalarials which target haemozoin formation.<sup>45</sup>

All in all, the interaction of the quinolines and aryl alcohol antimalarials with haem prevents it from being incorporated into haemozoin. Inhibition of crystal growth subsequently leads to the accumulation of toxic haem and thus to the demise of the parasite.

#### 1.4.2.1.3. Oxidative Stress Mediated by the Artemisinins

The mechanism of action of artemisinin **1.14** and its semisynthetic and synthetic analogues (hereon referred to as artemisinins) has been the topic of ongoing and intense debate as evidenced by the number of reviews published to date. It is generally accepted that the artemisinins undergo a reductive activation which leads to the homolytic cleavage of the peroxide bond and the subsequent formation of various cytotoxic radical species. These radicals reportedly interact with various biomolecules in the parasite which ultimately results in parasite death.<sup>46</sup> It is also reported that the artemisinins display nanomolar activity against the malaria parasite but concentrations in the micromolar range are required for growth inhibitory activity against mammalian cells. This selective toxicity has been ascribed to an enhanced uptake of artemisinins by the infected erythrocytes compared to normal or uninfected erythrocytes as revealed by isotopic labelling studies with **1.14** (fig 1.3) and **1.15**.<sup>46b</sup>

Despite the plethora of proposed hypotheses, which unfortunately cannot be extensively covered in this report, there is a general consensus that the peroxide bridge is essential for the antimalarial activity of this class of compounds. Evidence presented in support of this, is the observed lack of activity for structural analogues devoid of this moiety (section 5.2). With regard to the activation of the latter, one proposal put forth is that activation and the subsequent generation of toxic radicals is mediated by ferrous ( $\text{Fe}^{2+}$ ) haem.<sup>47a</sup> Moreover, the alkoxy radicals so generated are believed to undergo rearrangement to form C-centered radicals which act as alkylating agents of haem,<sup>47</sup> essential parasitic proteins<sup>48</sup> and the *P. falciparum* translationally controlled tumor protein (TCTP).<sup>49</sup> Evidence presented in support of the haem-mediated activation theory, is the isolation of haem-artemisinins adducts from parasite cultures treated with pharmacologically relevant doses of the artemisinin.<sup>47c,50a</sup> The fact that these adducts lack *in vitro* antimalarial activity, contests this theory.<sup>48c,50b</sup> Studies also showed that fluorescent derivatives of artemisinin are distributed throughout the parasite and not confined or localized to the acidic food vacuole where the degradation of haemozoin takes place (section 1.4.2.1.1).<sup>51</sup> Other inconsistencies with the haem-hypothesis include the reported activity of artemisinins against the youngest life cycle stage (ring forms) of the

parasite which are known to have low concentration of haem<sup>52a,b</sup> and the discovery of potent artemisinin derivatives which cannot chemically react with haem.<sup>52c,d</sup> Further evidence against haem as a source of iron for activation of the endoperoxide bridge<sup>46a</sup> and the role of haem alkylation in the mechanism of action of artemisinins<sup>53a</sup> has been presented.

Presented as an alternative is the proposal that “exogenous” or free iron(II) may be responsible for the reductive cleavage of the peroxide bridge in the artemisinins.<sup>53b-d</sup> The observation that iron chelators, known for their inability to isolate or sequester haem-iron, inhibits the parasitocidal effect of artemisinins, supports this proposal.<sup>54a,b</sup> Further support for the iron-mediated activation theory was presented in the study conducted by Eckstein-Ludwig and coworkers.<sup>51</sup> More importantly, however, is the convincing evidence they presented on the inhibition of PfATP6 - the sarco/endoplasmic reticulum Ca<sup>2+</sup>-transporting ATPase (SERCA) of *P. falciparum* - by the artemisinins. In this study, PfATP6 expressed in *Xenopus laevis* oocytes was used to demonstrate the antimalarial equipotency between artemisinin **1.14** and thapsigargin, a natural occurring specific inhibitor of mammalian SERCAs. The further showed that the artemisinins selectively inhibits PfATP6 and not mammalian SERCAs.<sup>51</sup>

Another possible mechanism proposed by Haynes and coworkers is that the artemisinins undergo activation after binding to an active site.<sup>52c,53c</sup> This was based on *in vitro* studies which showed that replacement of the methyl group C-3 of **1.14** (fig 1.3) with sterically hindering groups, for example the phenylethyl group, resulted in a reduction of antimalarial activity.<sup>54c</sup> Inhibition of *P. falciparum* endocytosis<sup>55a</sup> and interference with mitochondrial function<sup>55b,c</sup> has also been attributed to the action of artemisinins. Furthermore, the inhibition of malarial cysteine proteases prevent the parasite from acquiring the essential amino acid needed for growth and development, as will be discussed in section 1.4.2.1.4. Of interest therefore is the specific inhibition of malarial cysteine proteases by **1.14** as demonstrated by Chauhan and coworkers in *P. yoelli*.<sup>47b</sup>

As rightfully pointed out by O’Neill and Posner,<sup>46a</sup> some of the studies conducted to prove or disprove the theories proposed to date on the mechanism of action of the artemisinins, need to graduate from biomimetic investigations to studies in animal models (malaria parasites) whereas some need to be extended from studies in animal forms of the parasite to human forms. This will hopefully aid the formulation of a more ‘consolidated’ mechanism of action,

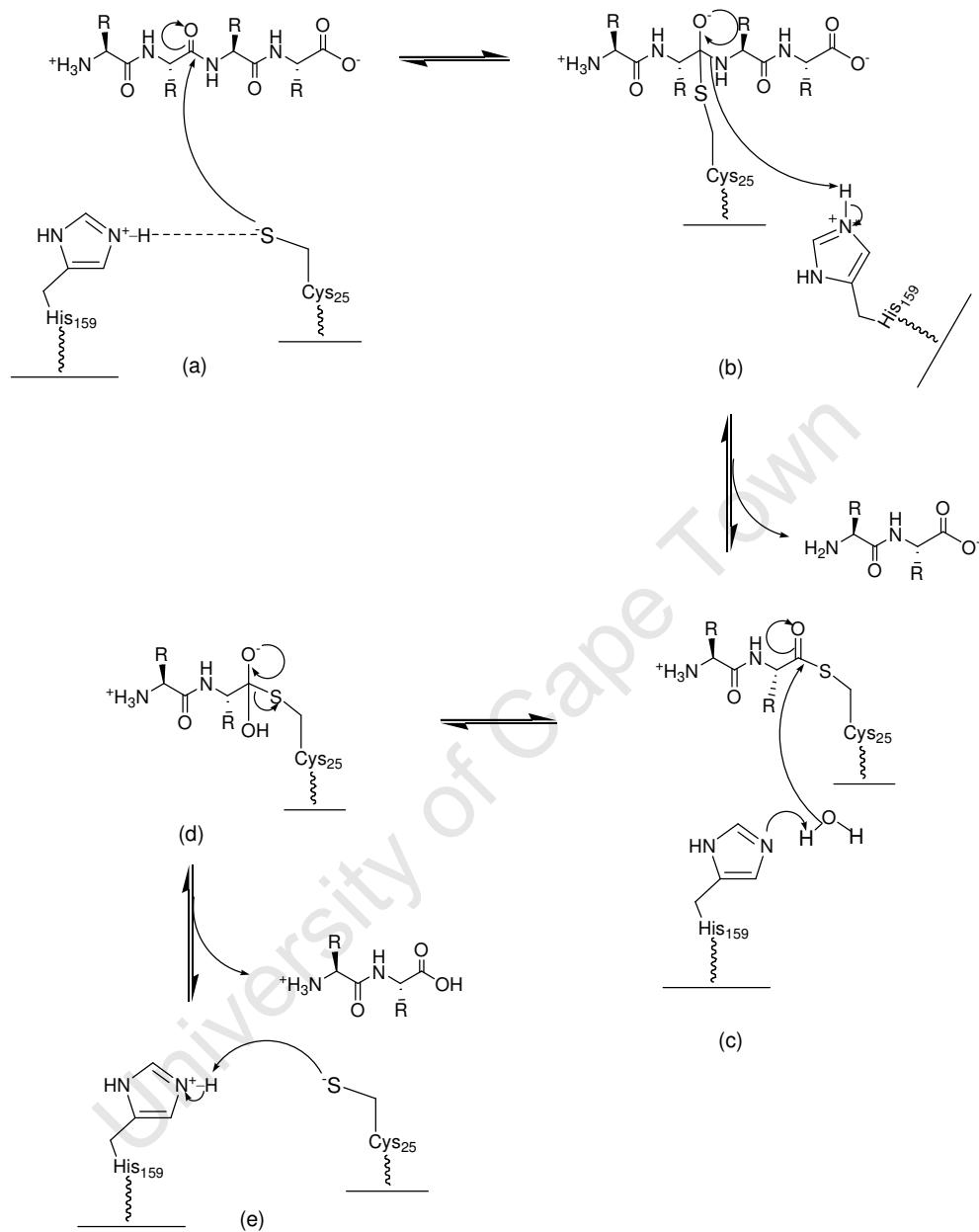
which is now urgently needed to guide future research and to stem the tide of resistance against this valuable class of compounds. Recent developments pertaining to the latter will be elaborated on in section 1.5.

#### **1.4.2.1.4. Cysteine Proteases as Targets in Antimalarial Chemotherapy**

As mentioned earlier, cysteine proteases perform critical roles in the erythrocytic life cycle of the parasite. These include merozoite release from red blood cells, invasion of red blood cells and most importantly the hydrolysis of haemoglobin.<sup>33a</sup> Although the *P. falciparum* genome codes for other cysteine proteases, the papain-family are the only isolated and extensively characterized cysteine proteases. Three papains (or falcipains), are expressed during the erythrocytic stages of *P. falciparum*. It includes the principal cysteine proteases falcipain-2 and falcipain-3 which show a 68% identity in sequence,<sup>56a</sup> and are thus homologous. The observation that the development of a falcipain-1 knockout malaria parasite is uncompromised or comparable to a wild-type parasite led researchers to conclude that the structurally distinct falcipain-1 is not important for the growth and survival of the intraerythrocytic parasite. Reports about a possible role of falcipain-1 in erythrocyte rupture and invasion remain unconfirmed.<sup>56b</sup> Falcipain-2 and falcipain-3 are critical for the survival and growth of the parasite and have been the focus of antimalarial drug discovery efforts.

##### **1.4.2.1.4.1. Mechanism of Proteolysis**

Cysteine proteases are proteolytic enzymes, which utilize the cysteine thiol located at the active site as a catalytic nucleophile for peptide bond cleavage.<sup>33a</sup> The proposed mechanism by which they mediate peptide hydrolysis is shown in figure 1.7. It is the imidazole group of histidine (His159) - in close proximity to the active site - which polarizes the enzyme cysteine thiol group (Cys25) thus allowing for the deprotonation to occur at close to neutral pH. This leads to the formation of a highly nucleophilic thiolate/imidazolium ion pair [fig. 1.7(a)]. Attack of the thiolate anion on the carbonyl carbon of the scissile amide bond, results in the formation of a tetrahedral intermediate [fig. 1.7(b)]. This intermediate is reportedly stabilized by hydrogen bonding to the NH backbone of Cys25 and to the NH<sub>2</sub> group of the Gln19 side chain. Acylation of enzyme makes the imidazolium ion acidic allowing for it to protonate the nitrogen of the leaving group; release of the first product follows immediately [fig. 1.7(b)]. A second tetrahedral intermediate is formed by the base-catalyzed hydrolysis of the acyl enzyme [fig. 1.7(c)]. The collapse of the second intermediate results in product release and enzyme regeneration [fig. 1.7(d)].



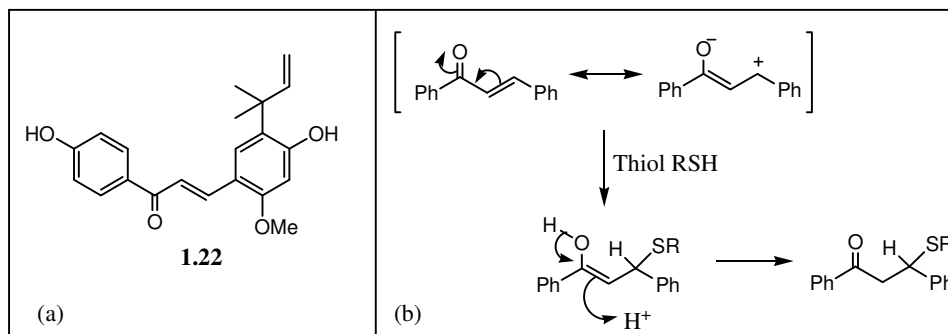
**Figure 1.7:** Proposed catalytic mechanism of peptide hydrolysis catalyzed by cysteine proteases



#### 1.4.2.1.4.2. Inhibitors of Cysteine Proteases

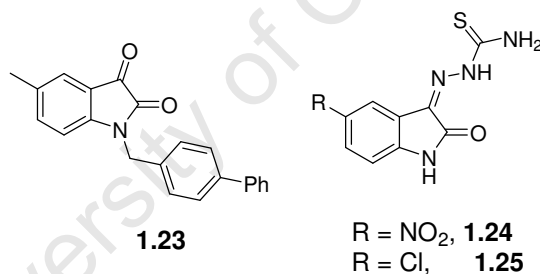
In two independent studies Rosenthal and Bailly convincingly demonstrated the role of cysteine proteases in haemoglobin degradation by incubating the parasite with a specific cysteine proteases inhibitor, E-64 (*L-trans*-epoxy-succinylleucylamido(4-guanidino)butane). The appearance of a swollen, dark-stained food vacuole coupled with the isolation of large quantities of undegraded haemoglobin was evidence of the blockage of haemoglobin degradation.<sup>33b,57</sup> These studies also showed that although inhibition of both aspartic and cysteine proteases lead to the retardation of haemoglobin hydrolysis, inhibitors of cysteine proteases produced a more “specific morphological abnormality”.<sup>33b</sup> Peptide-based cysteine protease inhibitors include the fluoromethyl ketones,<sup>33b,58</sup> vinyl sulfones,<sup>58b,c</sup> peptidyl aldehydes and  $\alpha$ -ketoamides.<sup>59</sup> The therapeutic utility of some of these peptidyl inhibitors is however limited by poor bioavailability, instability due to the cleavable nature of the amide bond and poor selectivity which accounts for host toxicity.<sup>60</sup>

Molecular modelling studies confirmed that the antimalarial activity of the nonpeptidyl acyl hydrazines and chalcones can be attributed to their ability to inhibit cysteine proteases.<sup>61</sup> Interest in chalcones as antimalarials was sparked by the discovery of the natural product licochalcone A **1.22** [fig. 1.8(a)], an oxygenated chalcone which displayed potent *in vitro* and *in vivo* antimalarial activity.<sup>62a</sup> A key structural feature of chalcones is the enone linker which is stable at vacuolar pH where cysteine proteases operate.<sup>62b</sup> More importantly, as a Michael acceptor it can undergo conjugate addition with the sulfhydryl group of the cysteine side chain [fig.1.8(b)]. Evidence to support the significance of this structural motif for antimalarial activity was found in a study conducted by Li and coworkers.<sup>63</sup> When they reduced the double bond of the enone linker they observed a 10 fold decrease in antimalarial activity. From this same study it was concluded that antimalarial activity of chalcones cannot always be attributed to cysteine protease inhibition.<sup>63</sup>



**Figure 1.8:** (a) Chemical structure of licochalcone A, **1.22**, (b) Reaction of a representative chalcone (Michael acceptor) and a nucleophilic thiol (RSH)<sup>64</sup>

Non-peptidyl isatin derivatives have found application as inhibitors of cysteine and serine proteases.<sup>65</sup> Studies undertaken in our laboratory showed the inhibitory activity against recombinant falcipain-2 of the *N*-substituted derivative **1.23** and the thiosemicarbazone derivatives **1.24-1.25** (fig. 1.9), with IC<sub>50</sub> values less than 10  $\mu$ M. These were identified from an exploratory compound library modelled on the isatin scaffold.<sup>65c</sup>

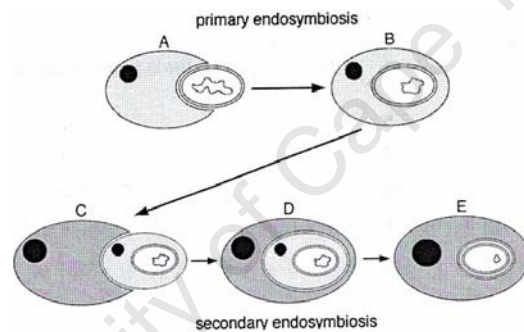


**Figure 1.9:** Chemical structure of isatin-derived cysteine protease inhibitors

#### 1.4.2.2. The Apicoplast

The malaria parasite belongs to the phylum Apicomplexan.<sup>66</sup> *Plasmodium* and other apicomplexan parasites possess a plastid, an organelle found predominantly in plants and algae.<sup>67</sup> It is thought to have been acquired from an ancestral cyanobacterium (blue-green algae) through a process of secondary endosymbiosis. As shown in figure 1.10, primary endosymbiosis describes the endosymbiotic relationship between a prokaryote (cyanobacteria) and eukaryote (red or green algae). In secondary endosymbiosis the primary plastid-containing eukaryote is engulfed by a second heterotrophic eukaryote, for example *Plasmodium*. The presence of extra membranes surrounding the apicoplast lends support to the hypothesis of it being a secondary plastid.<sup>68</sup>

The plastid possesses a 35kb circular genome but most of its proteins are encoded for by genes in the nuclear genome of the eukaryotic host (*Plasmodium*). This is the result of evolutionary processes which facilitated the transfer of genes to the host and which stripped the engulfed eukaryote from non-essential functions such as photosynthesis.<sup>69</sup> The plastid is, however, indispensable in *Plasmodium*, having been identified as the site of biosynthetic pathways which are essential for parasite growth and survival.<sup>70</sup> These metabolic pathways are specific therapeutic targets by virtue of the prokaryotic origin of the plastid. They include the non-mevalonate isoprenoid synthesis, apicoplast DNA replication, transcription, translation, fatty acid synthesis and several steps of haeme synthesis.<sup>71</sup> Of all the metabolic pathways occurring in the apicoplast, fatty acid synthesis is reportedly the most well-studied function. It is also of particular interest to this project and will be discussed in more detail in the next section.



**Figure 1.10:** Origin of the plastid<sup>72</sup>

#### 1.4.2.2.1. Fatty Acid Synthesis

Fatty acids constitute an important component of cellular and intracellular membranes and also function as an energy source. The synthesis thereof is important for living cells. It was initially claimed that *P. falciparum* acquire fatty acids by scavenging them from human host cells.<sup>73</sup> The completion of the *P. falciparum* genome disproved this long held belief. The *de novo* synthesis of fatty acids in *P. falciparum* occurs in the apicoplast. Genetic evidence in support of this includes the discovery of nucleus-encoded fatty acid synthesis genes such as *acpP*, *fabH* and *fabZ* whose translation products - the acyl carrier protein, FabH and FabA/FabZ, respectively - are targeted to the apicoplast.<sup>66a,74</sup> Biochemical studies such as the incorporation of <sup>14</sup>C-labelled acetate CoA and malonyl CoA in fatty acids (C<sub>10</sub> and C<sub>14</sub>) *in vitro* and *in vivo* in *P. falciparum* provided further support.<sup>66b</sup>

The organization of the fatty acid synthesis system is essentially the same in all organisms and can be divided into two distinct forms called type I and type II. In *P. falciparum* fatty acid synthesis is mediated by the type II fatty acid synthase (FAS<sup>†</sup>) or dissociated pathway.<sup>74</sup> The type II pathway which operates in most bacteria, plants and yeast, is distinct from the type I or associated pathway.<sup>75a</sup> The latter is characterized by a single multidomain polypeptide located in the cytosol and is found in mammals, fungi and some mycobacteria.<sup>75b</sup> In contrast, type II FAS is a multienzyme system. These structural differences allows for the development of parasite-selective drugs to target individual enzymes of type II fatty acid synthesis (fas).<sup>69,71b</sup>

A recent landmark study conducted by the research groups of Fidock and Kappe showed, that type II fas is only necessary for the development of the late liver stage of the malaria parasite.<sup>76</sup> They moreover demonstrated, in the rodent malaria parasite *P. yoelli*, that type-II fas-deficient parasites could not form the infectious exo-erythrocytic merozoites necessary for the initiation of the erythrocytic stage. The difference in the number of hepatic merozoites ( $\pm 40\ 000$ <sup>77a</sup>) produced compared to the 8-36 erythrocytic merozoites produced per invasive cycle,<sup>77b</sup> reportedly accounts for the increased demand for fatty acids by the liver stage forms of the parasite.<sup>76</sup> This discovery raises the question as to how the parasite obtains fatty acids to sustain the other life cycle stages; more importantly, however, is the new direction it offers for future research into antimalarial type II fas inhibitors.<sup>76a</sup>

#### 1.4.2.2.2. Mechanism of Type II Fatty Acid Synthesis

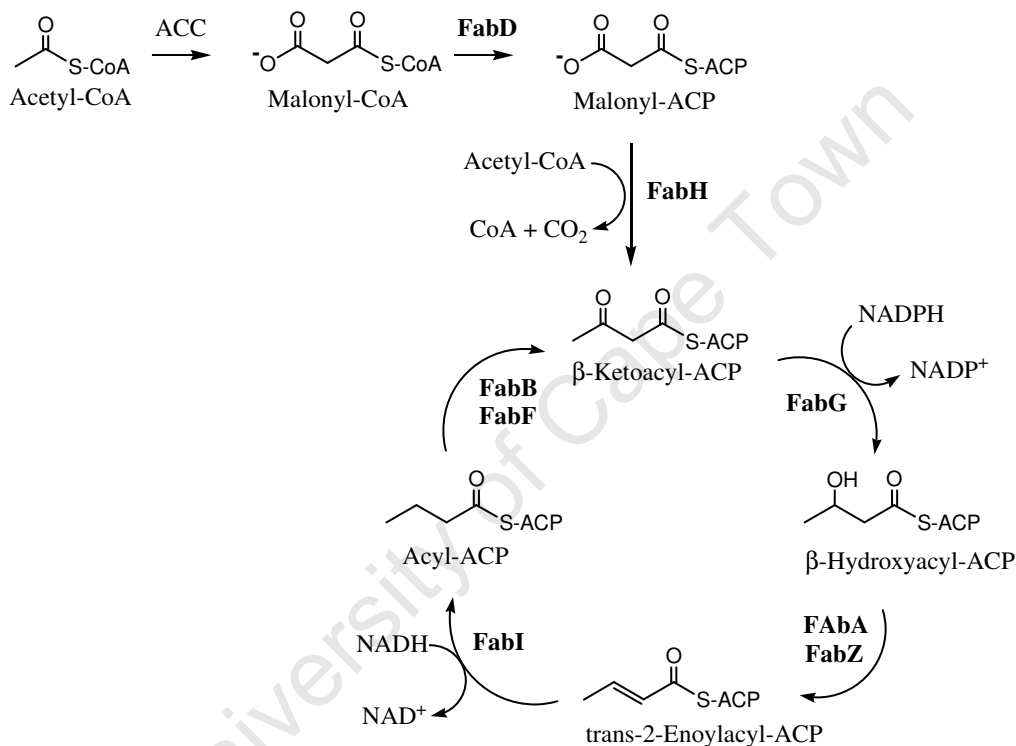
Fatty acid biosynthesis in *E. coli* has been extensively studied. As depicted in figure 1.11 it comprises two stages - initiation and elongation. In the initiation step acetyl-CoA is carboxylated to yield malonyl-CoA. This step is reportedly catalyzed by the biotin containing enzyme acetyl-CoA carboxylase (ACC) with bicarbonate acting as the source of the carboxy group. Malonyl-CoA is then transferred to the acyl carrier protein (ACP) by the enzyme malonyl-CoA:ACP transacylase (FabD).

The elongation step is initiated by the condensation of malonyl-ACP and acetyl-CoA catalyzed by the  $\beta$ -ketoacyl-ACP synthase III (FabH). The product so formed,  $\beta$ -ketoacyl-ACP undergoes a NADPH-dependent reduction catalyzed by the enzyme  $\beta$ -ketoacyl-ACP

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<sup>†</sup> In the literature the abbreviation FAS is used for fatty acid synthases and fas for fatty acid synthesis.

reductase (FabG) to yield  $\beta$ -hydroxyacyl-ACP. The reduction step is then followed by the dehydration of  $\beta$ -hydroxyacyl-ACP by the enzyme  $\beta$ -hydroxyacyl-ACP dehydratase (FabA or FabZ). The double bond in the dehydrated product, *trans*-2-enoylacyl-ACP is reduced by enoyl-ACP reductase (FabI) in the presence of NADH to form butyryl-ACP. This product then re-enters the elongation cycle catalyzed by either  $\beta$ -ketoacyl-ACP synthase I (FabB) or  $\beta$ -ketoacyl-ACP synthase II (FabF). Each cycle of the elongation extends the acyl-ACP product by two carbons.<sup>72,78</sup>



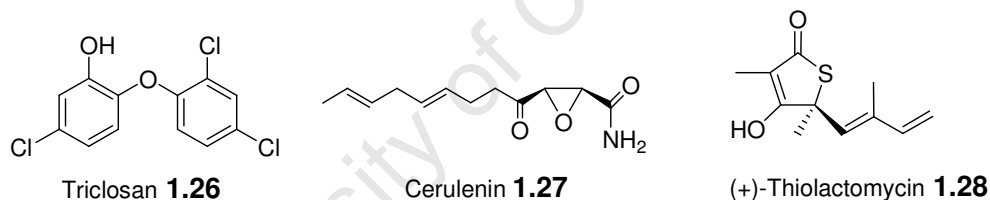
**Figure 1.11:** Type II fatty acid biosynthesis in *E. coli*<sup>78</sup>

The type II fas in *Plasmodium* occurs in an analogous manner to the bacterial type II fas discussed above; despite organizational and structural differences in the enzymes. It is reported that in addition to other enzymes of type II FAS, plants and bacteria possess three KAS enzymes, KAS I, KAS II, and KAS III, with the *E. coli* equivalents being FabB, FabF and FabH, respectively as shown in figure 1.11.<sup>79</sup> Furthermore, in *E. coli* the homologous FabB and FabF share a 40% identity, but show a poor homology with FabH in spite of the similar architecture that they display at the active sites.<sup>79</sup> In contrast, the *P. falciparum* genome data revealed only two different KAS enzymes. One is a single enzyme with a 37%

and 31% identity to the FabB and FabF of *E. coli*, respectively. This enzyme is denoted KASI/II or more specifically, *pf*KASI/II.<sup>80</sup> The other enzyme, KAS III (or *pf*KASIII) is homologous to the FabH of *E. coli*. It is reported that the catalytic domain of the former share a 35% identity with the latter.<sup>75a,80</sup>

#### 1.4.2.2.3. Inhibitors of Type II Fatty Acid Synthesis

Studies conducted by Surolia and coworkers revealed that the inhibition of type II fas rapidly compromises the growth of the parasite. This is in contrast to the ‘delayed death’ elicited by inhibitors of other apicoplast functions such as DNA replication, transcription and translation.<sup>81</sup> To illustrate, comparative studies on the parasitocidal effect of triclosan **1.26** (inhibitor of type II fas, fig. 1.12) and the antibiotic clindamycin **1.21** (inhibitor of apicoplast transcription, fig. 1.3), showed that the latter required a prolonged incubation time (about 72-84 hours, compared to the 48 hours required for the completion of a life cycle in *P. falciparum*) to exert maximum effect in a *P. falciparum* growth inhibition assay. This is in contrast to the fast-killing effect of triclosan.<sup>60c,66</sup>



**Figure 1.12:** Chemical structures of antimalarial type II fas inhibitors

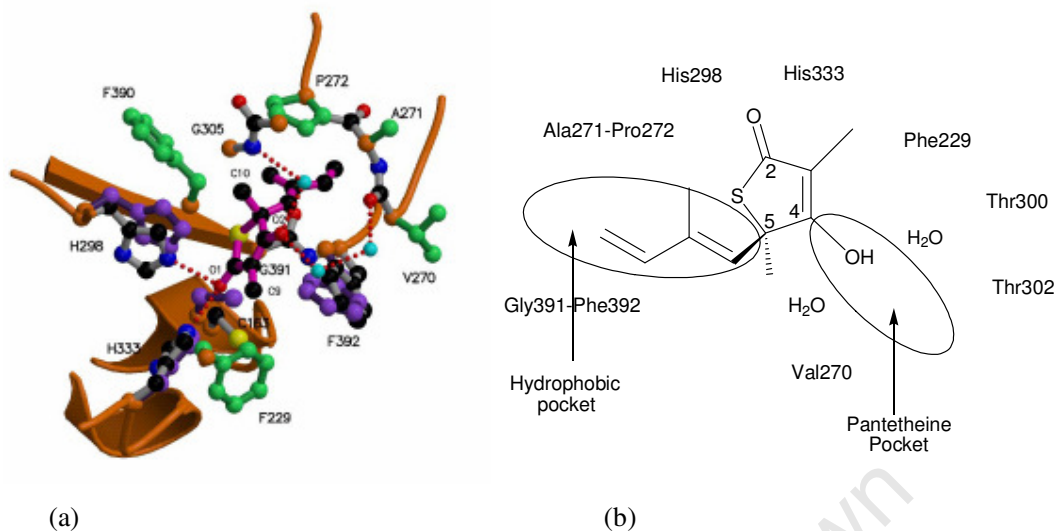
The bisphenol, **1.26** is a broad-spectrum antibiotic which displays *in vitro* and *in vivo* growth-inhibitory activity in *P. falciparum* and specifically targets *P. falciparum* enoyl reductase (FabI in *E. coli*, fig. 1.11). Although **1.26** inhibits growth of the malaria parasite with an  $IC_{50}$  value of  $\sim 1 \mu M$ ,<sup>66b</sup> it displays poor bioavailability and is also implicated in glucuronidation.<sup>82</sup> Contrary to the findings of Surolia and coworker, a recent study demonstrated through gene knockout studies, that FabI is not principal target of **1.26**.<sup>76b</sup> This was supported by the findings of Vaughan *et al* who showed that the FabI enzyme is not important for the blood stage development of the *P. falciparum*.<sup>76a</sup> Another FAS inhibitor, the fungal metabolite cerulenin **1.27** (fig. 1.12) is reported to covalently bind to the active site of its primary targets FabB/F (or *pf*KAS I/II). It furthermore inhibits growth of *P. falciparum* with  $IC_{50}$  values ranging between 10-20  $\mu M$ .<sup>66b,75a</sup> Listed among the shortcomings of

cerulenin is its ability to irreversibly inhibit both type I fas and type II fas and its instability in mammalian systems.<sup>79</sup> Derivatives of **1.27** have been considered for the development of *pf*KAS I/II inhibitors.<sup>83</sup>

A selective reversible inhibitor of type II fas is the natural product (+)-thiolactomycin **1.28** (fig. 1.12). It is believed to inhibit both FabH and FabB/F by mimicking malonyl-ACP (fig. 1.11) in its binding site.<sup>84</sup> Waller and coworkers showed that racemic **1.28** inhibits the growth of the multi-drug resistant W2mef strain of *P. falciparum* with an IC<sub>50</sub> value of 50 μM.<sup>75a</sup> In a separate study, Prigge and co-workers revealed **1.28** to be a poor inhibitor of *pf*KAS III (IC<sub>50</sub> > 330 μM) and attributed the observed inhibitory activity to the uncharacterized *pf*KASI/II enzyme.<sup>80</sup> The synthesis of derivatives of **1.28** to improve potency has been the focus of many investigations. In this endeavour, Price and coworkers solved the crystal structure of **1.28** bound to the FabB enzyme in *E. coli* (fig. 1.13).<sup>84c</sup> Among the most important interactions revealed by the FabB-thiolactomycin model is that:

- thiolactomycin imitates the transition state adopted by the natural substrate, the thiomalonate intermediate;
- the carbonyl group at C-2 (fig. 1.13) is shown to be hydrogen bonded to two histidines (His298 and His333);
- the isoprenoid moiety at C-5 extends into an incompletely-filled hydrophobic crevice in the active site of the enzyme
- the hydroxyl group at C-4 (fig. 1.13) is housed in an incompletely filled pantetheine binding pocket and is stabilized through water-assisted hydrogen bonding interaction with the amino acid residues Val270 and Gly305.<sup>84b,c</sup>

In addition to elucidating the architecture of the KAS active site, the crystal structure provided valuable information on enzyme-ligand binding interactions and has greatly contributed to the design of thiolactomycin analogues (section 2.2.3.1).<sup>84b,c</sup>



**Figure 1.13:** (a) Structure of the FabB-thiolactomycin (TLM) complex showing **1.28** with magenta bonds. The hydrophobic residues are shown in green, water molecules in light blue and hydrogen bonds as red dotted lines;<sup>84c</sup> (b) Schematic diagram of the FabB-TLM complex showing the key interactions<sup>84b</sup>

### 1.5. Drug Resistance

The increase in mortality,<sup>85a</sup> morbidity<sup>85b</sup> and the negative effects on public health<sup>10</sup> caused by the increase in drug resistance has been documented. Of the four human plasmodia, resistance has been reported only for *P. falciparum* and *P. vivax* with particular attention given to the former because of its virulence. First reports of the development of resistance to quinine **1.1** (fig. 1.3.) surfaced in 1910.<sup>86</sup> The appearance of the chloroquine resistant (CQR) strain of *P. falciparum* was first reported in the 1950's in Southeast Asia and South America and detected in Africa in the late 1970's. CQR strains can now be found in most of the malaria endemic regions (fig 1.1).<sup>87</sup> It has consequently been replaced as the first line drug with a cheap, alternative, sulfadoxine-pyrimethamine (marketed as Fansidar<sup>®</sup>) in most of Africa.<sup>88</sup> However, resistance to the latter was reported in the late 1980's in Africa and is spreading at an alarming rate.

Increased *in vitro* IC<sub>50</sub>s, prolonged clearance of parasitaemia and treatment failure are listed as indicators of clinical resistance to the artemisinins.<sup>89a</sup> A recently conducted clinical study involved the treatment of 40 patients [western Cambodia (20) and northwestern Thailand (20)] suffering from uncomplicated falciparum malaria, with artesunate and the artesunate-



mefloquine combination.<sup>89b</sup> The results obtained (table 1.1) demonstrate the *in vivo* susceptibility to artesunate in the western Cambodia study group, as evidenced by the prolonged clearance of parasitaemia. Of interest, however, is the *in vitro* susceptibility data (table 1.1) which appears to be relatively normal for both study groups, when compared to the activity against the reference 3D7 strain of *P. falciparum*. Notwithstanding, results from this study serve as an early indication of the emergence of clinical resistance to the artemisinin along the Thai-Cambodian border.<sup>89b</sup>

**Table 1.1:** Parasitologic responses and IC<sub>50</sub> of the western Cambodia and northwestern Thailand study groups<sup>89b</sup>

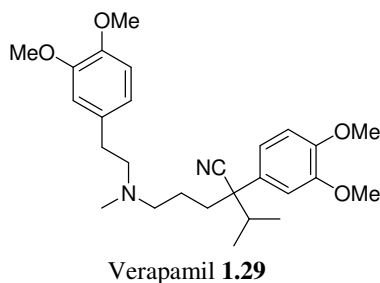
	Western Cambodia	Northwestern Thailand
<b><u>Median Parasite Clearance time (hours)</u></b>		
Artesunate	84	54
Artesunate-Mefloquine	72	48
<b><u>Recrudescence (%)<sup>a</sup>:</u></b>		
Artesunate	30	10
Artesunate-Mefloquine	5	5
<b><u><sup>b</sup>In vitro testing: Median IC<sub>50</sub> (nM)</u></b>		
<sup>c</sup> Artesunate	1.9	3.3
<sup>d</sup> Dihydroartemisinin	2.3	1.5

<sup>a</sup> Calculated as a percentage relative to the total (N = 20); <sup>b</sup>Conducted on parasites from patients in study groups; the 3D7 strain was used as reference; <sup>c</sup>IC<sub>50</sub> against 3D7 strain strain: 2.7 nM; <sup>d</sup>IC<sub>50</sub> against 3D7 strain strain: 2.3 nM

### 1.5.1. Mechanism of Drug Resistance

The discussion of the mechanism of drug resistance has been limited to the genetic proposals put forward to explain the development of resistance against CQ. It was established as early as the 1960s that the chloroquine-resistant (CQR) parasite has a significantly lower concentration of the drug at the target site, i.e. the food vacuole, compared to chloroquine-sensitive (CQS) parasites.<sup>90a</sup> The principal mechanism proposed for verapamil-sensitive CQ resistance in *P. falciparum* involves mutations in the gene, *pfcr1*. This gene encodes for the *Plasmodium falciparum* chloroquine resistance transporter (PfCRT) an integral membrane protein which is located in the lysosome-like food vacuole where CQ exerts its mechanism of action.<sup>90</sup> It is believed that PfCRT brings about changes in the pH of the food vacuole which in turn may affect the pH-dependant interaction of CQ and other quinoline antimalarials with

haeme.<sup>90b,91</sup> Numerous mutations in PfCRT have been reported. The CQ resistant phenotype, however, requires a specific mutation which is the replacement of the amino acid threonine (T) with lysine (K) at position 76 in PfCRT, also known as the K76T mutation.<sup>90d,92</sup> The complexity of the CQR resistance mechanism makes provision for the consideration of other polymorphisms in *pfcr*t and/or the participation of several genes in the development of CQ resistance.<sup>92</sup>



**Figure 1.14:** Chemical structure of verapamil **1.29**

The discovery of similarities between multidrug resistance (MDR) in mammalian cancer and CQR in *P. falciparum* suggested a similar mechanism of resistance; both can be reversed by the chemosensitizer verapamil **1.29**<sup>93a</sup> (fig. 1.14) and both are reportedly linked to the overexpression and/or presence of P-glycoproteins (Pgp).<sup>93b,c</sup> The Pgp is an energy-dependent, membrane-bounded protein encoded by *mdr* genes. In *P. falciparum* the protein product of the gene *pfmdr1*, a *mdr* homologue, is a P-glycoprotein homologue 1 (Pgh-1) which like PfCRT is localized to the membrane of the food vacuole;<sup>93c</sup> where it actively transports chloroquine out of the food vacuole. It is notable that mutations such as increased expression of Pgh-1, only account for a low-level drug resistance to chloroquine in *P. falciparum*.<sup>94</sup> This was supported by the findings of Cowman and coworkers who discovered equal amounts of Pgh-1 in both CQS and CQR strains.<sup>93c</sup> Polymorphism in *pfmdr1* has, in addition to modulating chloroquine resistance as mentioned above, been implicated in resistance to halofantrine **1.6** and quinoline drugs (quinine **1.1**, mefloquine **1.4**).<sup>95</sup>

### 1.5.2. Measures to Manage the Spread of Drug Resistance

Development of resistance to previously cheap, effective drugs requires the deployment of new treatment strategies. One such strategy is the abandoning of monotherapies replacing them with effective combination therapies. This strategy is currently being used in the treatment of HIV/AIDS, tuberculosis and leprosy.<sup>96</sup> The combination of two antimalarial drugs, each exerting a different mechanism of action or targeting two different metabolic pathways of the parasite, retards the development of resistance. The rationale is that for resistance to develop against both drugs the occurrence of two independent and simultaneous mutations in the same parasite are required which is highly improbable.<sup>88</sup> Against this background, artemisinin-based combination therapies (ACTs) were recommended by the WHO in 2001.<sup>26b</sup> ACTs has been approved in over 40 countries as first or second line malaria treatment in spite of the high cost.<sup>97</sup>

Another method of slowing down the development of resistance entails the use of resistance reversing agents or chemosensitisers such as verapamil **1.29** (fig. 1.14). Reversal of resistance is characterized by the reduction of the IC<sub>50</sub> value of CQ against the resistant strain and the increase of CQ accumulation in the acidic food vacuole of CQR.<sup>98</sup> It is noteworthy that reversal agents identified so far, neither restore CQ levels in the food vacuole nor do they restore the IC<sub>50</sub> values. These reversal agents only mediate a partial reversal of resistance in the CQR strains of *P. falciparum*.<sup>94</sup>

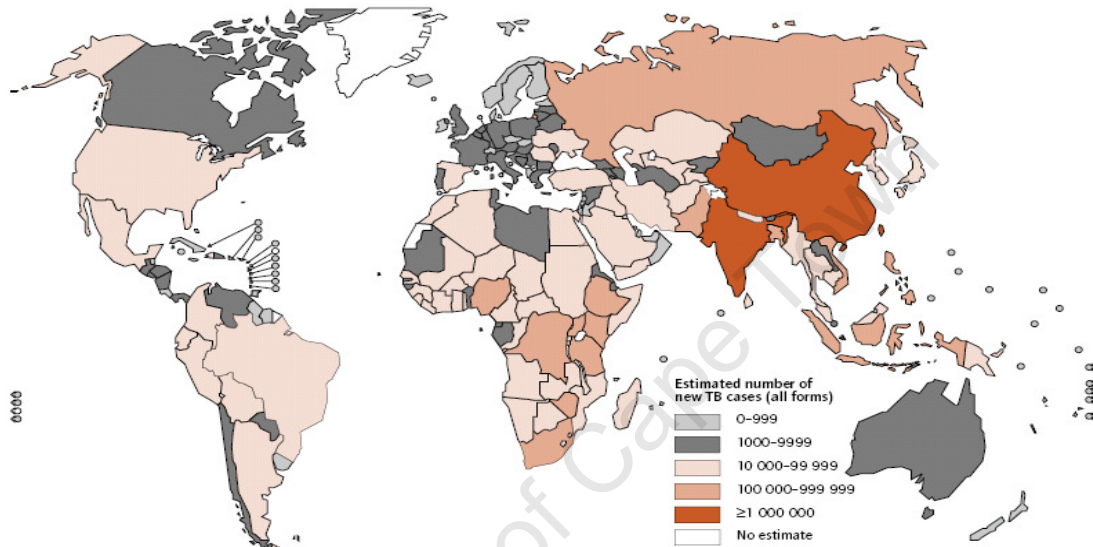
A third measure entails the redesigning of drugs in order to restore their efficacy. A number of comprehensive reviews cover the structural changes in the quinoline class of antimalarials. The discussion of this aspect is beyond the scope of this project.

### 1.6.0. Tuberculosis

#### 1.6.1. Epidemiology

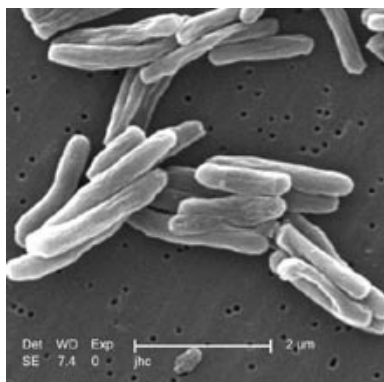
According to WHO estimates 8.8 million new cases of TB were reported in 2003 of which 1.7 million resulted in deaths. The grim reality is that 27% of these cases and 31% of the deaths took place in poverty stricken Africa, home to approximately 11% of the world's population.<sup>99a</sup> The most recent assessment of the TB epidemic puts the estimated new cases for 2007 at 9.27 million.<sup>99b</sup> The geographical distribution of these cases are shown in figure 1.15.

In an attempt to stem a global TB epidemic the WHO launched the “Framework for effective TB control” in 1994.<sup>99c</sup> This entails a five-point strategy which includes the *direct observation of treatment short-course* (DOTS); this protocol involves the provision of drugs directly to the patient and requires observing the patient taking the drugs. In spite of the adoption of this programme, increases in the incidences of TB has been reported for the former Soviet Union and sub-Saharan Africa.<sup>99d</sup>



**Figure 1.15:** Estimated new tuberculosis cases, 2007<sup>99b</sup>

In sub-Saharan Africa, the continuing increase in the incidence of TB is due to its close association with the HIV/AIDS epidemic.<sup>100</sup> Approximately 12% of the total deaths due to TB which occurred in 2000 were attributed to HIV co-infection.<sup>101</sup> The burden of TB is not only compounded by the synergy between TB and HIV/AIDS but also by the emergence of multi-drug resistant (MDR) and extensively drug resistant (XDR) forms of the disease. On realizing the threat resistance poses and the ease with which it can develop into the virtually untreatable XDR-TB, the WHO introduced the DOTS Plus strategy for the control of MDR-TB. XDR-TB cases have been reported in 45 countries according to a WHO global survey. This survey covered the period 2002 to 2006 and included about 90 000 TB patients in 81 countries.<sup>102</sup> With limited representative data from Africa, considering that only six countries participated in the survey, and the knowledge that most African countries lack the proper laboratory infrastructure to perform second line drugs susceptibility testing (DST) a detailed assessment of the MDR- and XDR-TB burden in Africa is yet to be made.



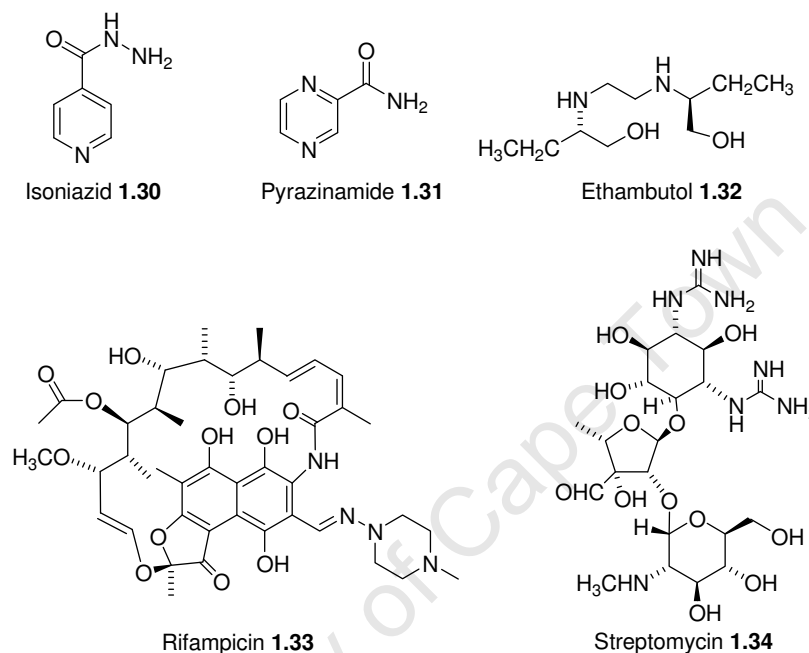
**Figure 1.16:** Electron microscope view of the bacilli of *M. tuberculosis*<sup>103</sup>

The causative agent of tuberculosis (TB) in humans are small, rod-shaped mycobacteria (fig. 1.16). Of the 85 species in the genus *Mycobacterium* those commonly affecting humans are *M. tuberculosis*, *M. bovis*, *M. africanum*, *M. microti*, and *M. canetti* and are collectively known as the *Mycobacterium tuberculosis* complex.<sup>104</sup> Tuberculosis is a contagious disease generally transmitted from a person with active pulmonary TB in the form of droplets of saliva or mucus containing the TB bacilli. This occurs during sneezing or coughing and when sharing eating and drinking utensil. Pulmonary tuberculosis represents the contagious form of the disease and is characterised by a persistent cough, the coughing of blood, chest pains, fatigue, weight loss, fever and night sweats. However, not all TB infections develop into the active pulmonary TB. Approximately 90% of all *M. tuberculosis* infection can be ascribed to latent TB infection (LTBI). This asymptomatic, non-contagious form of the disease occurs when the bacilli remain dormant in the host after inhalation. The main risk associated with latent TB is that 10% of patients diagnosed with it can develop active TB at a later stage, that is, the bacilli can become reactivated when the immune system of the host becomes compromised. Miliary or disseminated TB is a non-contagious but extreme form of the disease and is common in infants and immunocompromised persons.<sup>105</sup> It occurs when the disease spreads from the lungs *via* the bloodstream to internal organs such as the central nervous system (meningitis), the renal and genital tract (genitourinary tuberculosis) and bones and joints including the spine (Pott's disease).<sup>106</sup>

### 1.6.2. Tuberculosis Chemotherapy

Tuberculosis is a preventable and treatable infectious disease. The WHO-recommended multidrug therapy consists of a cocktail of frontline drugs (fig 1.17) initiated by the four-drug combination: isoniazid **1.30**, pyrazinamide **1.31**, ethambutol **1.32** and rifampicin (or

rifampin) **1.33** or streptomycin **1.34** taken over a period of 2 months. These drugs all exhibit bactericidal activity and kill off the fast-growing (replicating) and semi-dormant bacilli.<sup>107</sup> The bactericidal phase of the treatment is followed by a course of isoniazid **1.30** and rifampin **1.33** for a further 4-7 months. This constitutes the sterilizing phase of the treatment and is aimed at killing the slow-growing or non replicating bacilli.<sup>108</sup>



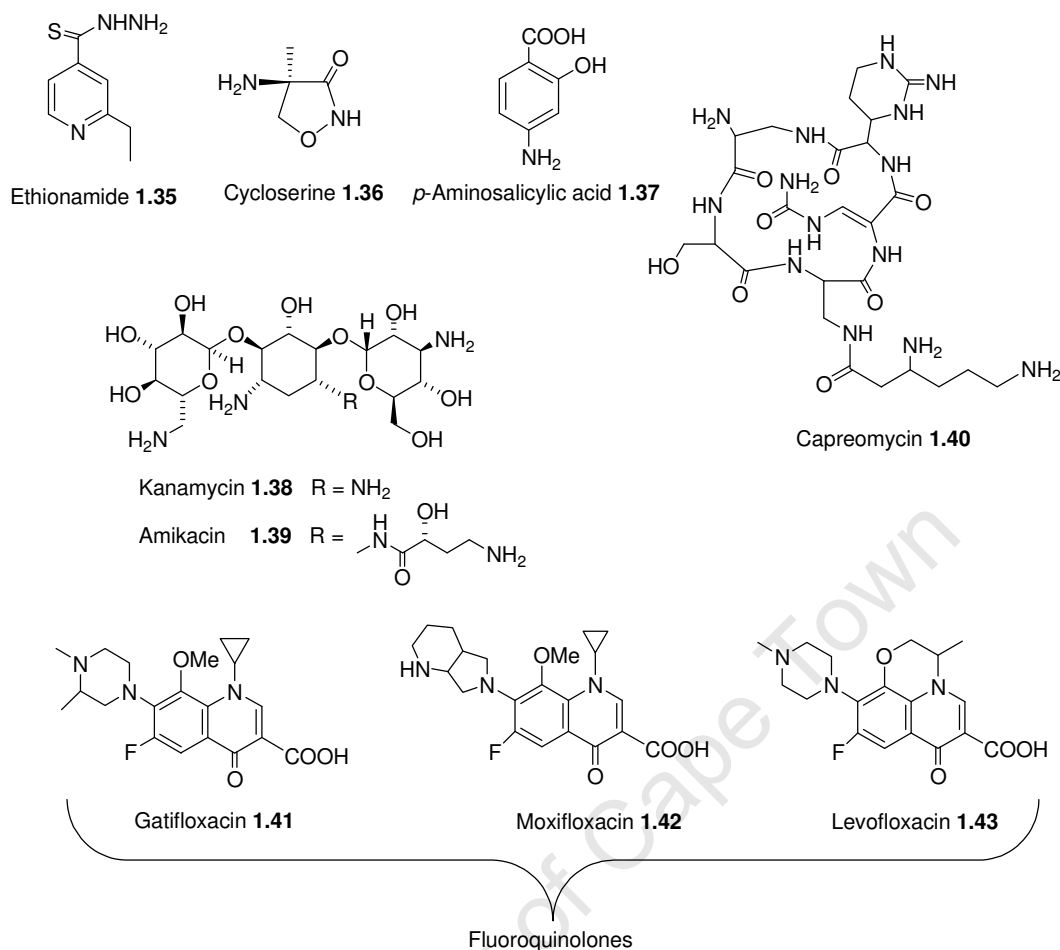
**Figure 1.17:** Chemical structures of the first-line antitubercular drugs

Tuberculosis patients are reported to become non-infectious after the first two weeks of treatment; the remainder of 6-9 months of treatment is needed to eradicate the persistent, slow-metabolizing and non-growing bacilli and thus prevent relapse. The presence of this heterogeneous population of bacilli (actively growing, slow growing and nongrowing) is directly responsible for the lengthy treatment and presents additional challenges. For example, a consequence of the lengthy course of treatment is patient non-compliance which has been implicated in the emergence of resistance.

Failure of treatment with the first line drugs, because of drug resistance or intolerance requires treatment with second line drugs (fig. 1.18). This forms part of the DOTS Plus programme introduced in 1999 for the treatment of MDR-TB.<sup>109</sup> These drugs consist of ethionamide **1.35** and cycloserine **1.36**, *para*-aminosalicylic acid **1.37**, kanamycin **1.38**, amikacin **1.39**, capreomycin **1.40**, and the fluoroquinolones (gatifloxacin **1.41**, moxifloxacin

**1.42**, levofloxacin **1.43**). As would be expected, this treatment regime requires a much longer duration (18-24 months) and has a lower success rate than the first line drugs. In addition, this cocktail is reported to be more toxic and more expensive.<sup>108</sup> The treatment outlook is poor for both HIV negative and HIV positive TB patients. However, in the case of HIV-related TB treatment is further challenged by the drug-drug interaction between antiretrovirals and antitubercular drugs such as rifampin **1.33**.<sup>110</sup>

The shortcomings of TB chemotherapy have been identified and incorporated in the objectives of the Global Alliance for TB drug development.<sup>111</sup> These include the shortening of treatment regimes from 6-9 months to  $\leq 2$  months, the development of drugs for treatment of MDR-TB, XDR-TB and latent TB. Also included in the objectives is the development of drugs which are compatible with antiretrovirals, exert new mechanisms of actions and which are low cost by virtue of the association of the disease with poverty. These objectives need to be incorporated in any antitubercular drug development endeavour.



**Figure 1.18:** Chemical structures of the second-line antitubercular drugs

### 1.6.3. Drug Resistance

The emergence of drug resistance in tuberculosis was first noted in the early 1990's. WHO estimates put new infection with the multi-drug resistant strain of tuberculosis at 1.1%.<sup>99b</sup> According to the WHO, multidrug-resistance is defined as resistance to rifampicin and isoniazid. MDR requires treatment with the less effective second-line drugs (fig. 1.18) which incidentally are more expensive, toxic and less accessible especially in Africa.<sup>100</sup> The treatment success rate for MDR-TB stands at 75%. However, in the case of inadequate treatment this strain can develop into the highly resistant form of XDR-TB. Extensively drug-resistant (XDR) TB is defined as being resistant to at least rifampicin and isoniazid, in addition to any fluoroquinolone and at least one injectable second-line agent (kanamycin **1.38**, amikacin **1.39** and capreomycin **1.40**, fig. 1.18).<sup>102,112</sup> The mortality rate for XDR is much higher (89%) than MDR and treatment options are severely limited.<sup>109</sup>



#### 1.6.4. Type II Fatty Acid Synthesis and Mycolic Acid Biosynthesis as Targets for Tuberculosis Chemotherapy

As was the case with malaria, the completion of the genomic sequence of the H<sub>37</sub>Rv strain of *M. tuberculosis* in 1998 and the follow-up re-annotation thereof has been instrumental in the identification and validation of novel drug targets.<sup>113</sup> A bulk of the work on the identification of genes, and their protein products, essential for *M. tuberculosis* growth (or “survivasome”) was carried out by Sasetti and coworkers.<sup>114</sup> Among the enzymes identified are those responsible for mycolic acid biosynthesis.<sup>114,115</sup> The latter constitutes a vital component of the lipid-rich cell wall and is important in the intracellular survival of the mycobacteria.

Mycolic acids can be subdivided into three main classes ( $\alpha$ -mycolates, methoxymycolates and ketomycolates) and are structurally characterized as high-molecular weight  $\alpha$ -alkyl,  $\beta$ -hydroxy fatty acids consisting of 60-90 carbon atoms. In mycobacteria, mycolic acid synthesis is complex and involves both type I and type II FAS systems.<sup>116</sup> The eukaryotic type I FAS catalyzes the *de novo* fatty acid synthesis to yield medium chain length (C<sub>16,18</sub> or C<sub>24,26</sub>) fatty acids, whereas prokaryotic type II FAS is responsible for the elongation of the type I FAS products into long chain mycolic acid precursors.<sup>116c,117</sup> With most of the enzyme activity of the type I FAS restricted to a single polypeptide which shares structural similarities with the type I FAS system of the human host, the potential targets for antimycobacterial drugs are the enzymes of the type II fas; this has been confirmed by a number of researchers.<sup>118</sup> The type II FAS system in *M. tuberculosis* include the  $\beta$ -ketoacyl reductase (MabA or KAR, FabG in *E.coli*), trans-2-enoyl reductase (InhA, FabI in *E. coli*)<sup>119</sup> and the condensing enzymes  $\beta$ -ketoacyl-ACP synthases (KAS). The latter category includes *mtKas A* which is responsible for extending the type I fas product (palmitoyl CoA) to C<sub>40</sub> products, *mtKas B* which extends the *mtKas A* product to a product of average size C<sub>54</sub><sup>118b</sup> and *mtFabH*.

#### 1.6.4.1. Inhibitors of Type II Fatty Acid Synthesis and Mycolic Acid Biosynthesis

The frontline antitubercular prodrug isoniazid **1.30** (fig. 1.17) has a remarkable specificity for mycobacteria. This was confirmed by the observed minimum inhibitory concentration (MIC) of isoniazid which was found to be greater than 500  $\mu\text{g/mL}$  for *E.coli* whereas as little as 0.05  $\mu\text{g/mL}$  is required for the inhibition of *M. tuberculosis*.<sup>120</sup> The primary target for the active form of isoniazid is InhA.<sup>121</sup> There is existing evidence which implicates the condensing enzyme *mtKas A* as an additional target.<sup>122</sup> In order to exert its antimycobacterial effect, InhA requires activation which is mediated by an endogenous catalase-peroxidase, *katG*.<sup>123</sup> Another prodrug ethionamide used in the treatment of MDR-TB also targets InhA. This was concluded from the discovery that a single or point mutation in the *inhA* gene which encodes for InhA conferred resistance to both isoniazid and ethionamide.<sup>121</sup> Triclosan **1.26** (fig. 1.12) has been identified as an inhibitor of InhA.<sup>124a</sup> It displayed *in vitro* antimycobacterial activity against both the isoniazid-resistant and sensitive strains of *M. tuberculosis* with MIC in the range 20-60  $\mu\text{M}$ .<sup>124b</sup> Parrish and coworkers<sup>125</sup> recorded minimum inhibitory concentrations ranging between 1.5 and 12.5 mg/L for cerulenin **1.27** (fig. 1.12) against various pathogenic (*M. bovis*, H<sub>37</sub>Rv and multidrug-resistant strains of *M. tuberculosis*) and non-pathogenic mycobacteria species.

A well characterized inhibitor of type II fas, which is of particular interest to this project, is the thiolactone containing antibiotic thiolactomycin **1.28** (fig. 1.12). It exerts its antimycobacterial activity by inhibiting fatty acid synthesis and mycolic acid biosynthesis.<sup>118a</sup> It is furthermore reported to selectively inhibit the  $\beta$ -ketoacyl synthase condensing enzyme *mtKas A* and *mtKas B* but appears to be a poor inhibitor of the condensing enzyme *mtFabH* which serves as the link between the type II and type I pathway in mycolic acid synthesis. No cross-resistance to other antitubercular agents has been reported.<sup>118a,126</sup> Thiolactomycin analogues with enhanced antimycobacterial activity, include the biphenyl based,<sup>127a</sup> aromatic acetylenic derivatives<sup>127b</sup> and analogues devoid of the C-5 isoprene unit.<sup>127c</sup> *In vivo* activity of these analogues against *M. tuberculosis* is, however, still pending.

### 1.7. References

1. Poser, C.M.; Bruyn, G.W. *An Illustrated History of Malaria*, Parthenon publishing, UK, **1999**
2. World Health Organization, *World Malaria Report*, Geneva, **2008**
3. Phillips, R.S. Malaria in *Studies in Biology*, Arnold, E. (ed), Chamelot Press, Great Britain, **1983**
4. Gardner, M.J.; Hall, N.; Fung, E.; White, O.; Berriman, M.; Hyman, R.W.; Carlton, J. M.; Pain, A.; Nelson, K.E.; Bowman, S.; Paulsen, I.T.; James, K.; Eisen, J.A.; Rutherford, K.; Salzberg, S.L.; Craig, A.; Kyes, S.; Chan, M.S.; Nene, V.; Shallom, S.J., Suh, B.; Peterson, J.; Angiuolli, S.; Perlea, M.; Allen, J.; Selengut, J.; Haft, D.; Mather, M.W.; Vaidya, A.B.; Martin, D.M.; Fairlamb, A.H.; Fraunholz, M.J.; Roos, D.S.; Ralph, S.A.; McFadden, G.I.; Cummings, L.M.; Subramanian, G.M.; Mungall, C.; Venter, J.C.; Carucci, D.J.; Hofmann, S.L.; Newbold, C.; Davis, R.W.; Fraser, C.M.; Barrell, B. *Nature*, **2002**, 419, 498
5. Macreadie, I.; Ginsburg, H.; Sirawaraporn, W.; Tilley, L. *Parasitol. Today*, **2000**, 16, 438
6. Ashley, E.; McGready, R.; Proux, S.; Nosten, F. *Travel Medicine and Infectious Disease*, **2006**, 4, 159
7. (a) Mundy, S.B.; White, A.C. Jr.; Hines, J.S.; Marino, B.J.; Young E.J. *Southern Medical Journal*, **1996**, 89, 616; (b) Filler, S.; Causer, L.M.; Newman, R.D.; Barber, A.M.; Roberts, J.M.; MacArthur, J.; Parise, M.E.; Steketee, R.W. *MMWR Surveillance Summaries*, **2003**, 52, 1
8. (a) Greenwood, B.; Mutabingwa, T. *Nature*, **2002**, 415, 570; (b) Snow, R.W.; Guerra, C.A.; Noor, A.M.; Myint, H.Y.; Hay, S.I. *Nature*, **2005**, 343, 214; (c) World Health Organization, *The World Health Report: Making a Difference*, Geneva, **1999**
9. (a) World Health Organization, Severe *falciparum* malaria, *Trans. Roy. Soc. Trop. Med. Hyg.*, **2000**, 94 (Suppl. 1), S1-S90; (b) Teklehaimanot, A.; Mejia, P. *Ann. N. Y. Acad. Sci.*, 2008, 1132, 32; (c) <http://www.who.int/malaria/malariaendemiccountries.html>
10. Björkman, A.; Bhattarai, A. *Acta Tropica*, **2005**, 94, 163
11. [www.dpd.cdc.gov/dpdx](http://www.dpd.cdc.gov/dpdx)
12. (a) Chen, L.H.; Wilson, M. E. *J. Am. Med. Assoc.*, **2006**, 296, 2234; (b) Lengeler, C. *Cochrane Database Syst. Rev.*, **2004**, 2, CD000363
13. (a) Toovey, S. *Continuing Medical Education*, **2005**, 23, 113; (b) Checkley A.M.; Hill, D.R. *Trends Parasitol*, **2007**, 23; 462
14. Demidov, V.V. *Drug Discovery Today*, **2003**, 8, 913
15. Ridley, R.G. *Nature*, **2002**, 415, 686

16. (a) Newton, P.; White, N. *Annu. Rev. Med.*, **1999**, *50*, 179; (b) Francis, S.; Gluzman, I.; Oksman, A.; Banerjee, D.; Goldberg, D.E. *Mol. Biochem. Parasitol.*, **1996**, *83*, 189
17. Brown, G.V.; Beck, H.P.; Molyneux, M.; Marsh, K. *Parasitol. Today*, **2000**, *16*, 448
18. Winstanley, P.A. *Parasitol Today*, **2000**, *16*, 146
19. Briand, V.; Cottrell, G.; Massougbodji, A.; Cot, M. *Malarial J.*, **2007**, *6*, 160
20. Kumar, A.; Katiyar, S.B.; Agarwal, A.; Chauhan, P.M.S. *Curr. Med. Chem.*, **2003**, *10*, 1137
21. Kremsner, P.G.; Radloff, P.; Metzger, W.; Wildling, E.; Mordmuller, B.; Philipps, J.; Jenne, L.; Nkeyi, M.; Prada, J.; Bienzle, U.; Kremsner, P.G.; Radloff, P.; Metzger, W. *Antimicrob. Agents Chemother.*, **1995**, *39*, 1603
22. (a) Jain, M.; Vangapandu, S.; Sachdeva, S.; Singh, S.; Singh, P.P.; Jena, G.B.; Tikoo, K.; Ramarao, P.; Kaul, C.L.; Jain, R. *J. Med. Chem.*, **2004**, *47*, 285; (b) Peters, W.; Robinson, B.L.; Milhous, W.K. *Ann. Trop. Med. Parasitol.*, **1993**, *87*, 547
23. (a) Foote, S.J.; Cowman, A.F. *Acta Tropica*, **1994**, *56*, 157; (b) Foley, M.; Tilley, L. *Int. J. Parasitol.*, **1997**, *27*, 213
24. (a) Tripathi, R.P.; Mishra, R.C.; Dwivedi, N.; Tewari, N.; Verma, S.S. *Curr. Med. Chem.*, **2005**, *12*, 2643; (b) Van Riemsdijk, M.M.; Sturkenboom, M.C.J.M.; Pepplinkhuizen, L.; Stricker, B. H. *J. Clin. Psychiatry*, **2005**, *66*, 199
25. Looareesuwan, S.; Chulay J. D.; Canfield, C. J.; Hutchinson, D. B. *Am. J. Trop. Med. Hyg.*, **1999**, *60*, 533
26. (a) Gordi, T.; Lepist, E.I. *Toxicol. Lett.*, **2004**, *147*, 99; (b) World Health Organization, *Antimalarial Drug Combination Therapy: Report of WHO Technical Consultation*, **2001**, Geneva, WHO/CDS/RBM/2001.35;
27. Rosenthal P.J. *J. Exp. Biol.*, **2003**, *206*, 3735
28. Biagini, G.A.; O'Neill, P.M.; Nzila, A.; Ward, S.A.; Bray, P.G. *Trends Parasitol.*, **2003**, *19*, 479
29. Yayon, A.; Vande Waa, J. A.; Yayon, M.; Geary, T. G.; Jensen, J. B. *J. Protozoology*, **1983**, *30*, 642
30. (a) Olliaro, P.L.; Goldberg, D.E. *Parasitol Today*, **1995**, *11*, 294; (b) Rosenthal, P.J.; Meshnick, S.R. *Mol. Biochem. Parasitol.*, **1996**, *83*, 131; (c) Francis, S.E.; Sullivan, Jr. D.J.; Goldberg, D.E. *Annu. Rev. Microbiol.*, **1997**, *51*, 97
31. (a) Krogstad, D.J.; Schlesinger, P.H.; Gluzman, I.Y. *J. Cell Biol.*, **1985**, *101*, 2302; (b) Yayon, A.; Cabantchik, Z.I.; Ginsburg, H. *EMBO J.*, **1984**, *3*, 2695
32. (a) Maser, P.; Sutterlin, C.; Kralli, A.; Kaminsky, R. *Science*, **1999**, *285*, 242; (b) Lew,

- V.L.; Tiffert, T.; Ginsburg, H. *Blood*, **2003**, *101*, 4189
33. (a) Rosenthal, P. J. *Int. J. Parasitol.*, **2004**, *34*, 1489; (b) Rosenthal, P.J.; McKerrow, J.H.; Aikawa, M.; Nagasawa, H.; Leech, J.H. *J. Clin. Invest.*, **1988**, *82*, 1560
34. (a) Carter, N. *J. Biol. Chem.*, **1995**, *270*, 28153; (b) Gluzman, I.Y.; Francis, S.E.; Oksman, A.; Smith, C.E.; Duffin, K.L.; Goldberg, D.E. *J. Clin. Invest.*, **1994**, *93*, 1602
35. Eggleston, K.K.; Duffin, K.L.; Goldberg, D.E. *J. Biol. Chem.*, **1999**, *274*, 32411
36. Banerjee, R.; Goldberg, D.E. In *Antimalarial Chemotherapy: Mechanism of Action, Resistance and New Directions in Drug Discovery*, Rosenthal, P.J. (ed.), Humana Press Inc., Totowa, **2001**, 43
37. Goldberg, D.E. *Proc Natl. Acad. Sci. U.S.A.*, **1990**, *87*, 2931
38. Gamboa de Dominguez, N.D.; Rosenthal, P.J. *Blood*, **1996**, *87*, 4448
39. (a) Egan T.J. *Mol. Biochem. Parasitol.*, **2008**, *157*, 127; (b) Egan, T.J.; Combrinck, J.M.; Egan, J.; Hearne, G.R.; Marques, H.M.; Ntenti, S.; Sewell, B.T.; Smith, P.J.; Taylor, D.; Van Schalkwyk, D.; Walden, J.C. *Biochem. J.*, **2002**, *365*, 343
40. Pagola, S.; Stephens, P.W.; Bohle, D.S.; Kosar, A.D.; Madsen, S.K. *Nature*, **2000**, *404*, 307
41. (a) Schmitt, T.H.; Frezzatti, W.A. Jr.; Schreier, S. *Arch. Biochem. Biophys.*, **1993**, *307*, 96; (b) Ryter, S.W.; Tyrrell, R.M. *Free Radical Biology and Medicine*, **2000**, *28*, 289; (c) Hempelmann, E.; Egan, T.J. *Trends Parasitol.*, **2002**, *18*, 11
42. [http://en.wikipedia.org/wiki/File:Hemozoin\\_structure.png](http://en.wikipedia.org/wiki/File:Hemozoin_structure.png)
43. Bohle, D.S. *J Biol. Chem.*, **1997**, *272*, 713
44. Mungthin, M.; Bray, P.; Ridley, R.; Ward, S. *Antimicrob. Agents Chemother.*, **1998**, *42*, 2973
45. De Villiers, K.A.; Marques, H.M.; Egan, T.J. *J. Inorg. Biochem.*, **2008**, *102*, 1660
46. (a) O'Neill, P.M.; Posner, G.H. *J. Med. Chem.*, **2004**, *47*, 2945; (b) Meschnick, S.R.; Taylor, T.E.; Kamchonwongpaisan, S. *Microbiol. Rev.*, **1996**, *60*, 301; (c) Olliaro, P.L.; Haynes, R.K.; Meunier, B.; Yuthavong, Y. *Trends Parasitol.*, **2001**, *17*, 122
47. (a) Meshnick, S.R.; Thomas, A.; Ranz, A.; Xu, C.M.; Pan, H.Z. *Mol. Biochem. Parasitol.*, **1991**, *49*, 181; (b) Pandey, A. V., Tekwani, B. L., Singh, R. L., Chauhan, V. *S. J. Biol. Chem.*, **1999**, *274*, 19383; (c) Zhang, F.; Gosser, D.K.; Meschnick, S.R. *Biochem. Pharmacol.*, **1992**, *43*, 1805
48. (a) Yang, Y.Z.; Little, B.; Meshnick, S.R. *Biochem. Pharmacol.*, **1994**, *48*, 569; (b) Yang, Y.Z.; Little, B.; Asawamasakda, W.; Meshnick, S.R. *Biochem.*

- Pharmacol.*, **1993**, *46*, 336; (c) Asawamahasakda, W.; Ittarat, I.; Chang, C.C.; McElroy, P.; Meshnick, S.R. *Antimicrob. Agents Chemother.*, **1994**, *38*, 1854
49. (a) Bhisutthibhan, J.; Philbert, M.A.; Fujioka, H.; Aikawa, M.; Meshnick, S.R. *Eur. J. Cell. Biol.*, **1999**, *78*, 665; (b) Bhisutthibhan, J.; Meshnick, S.R. *Antimicrob. Agents Chemother.*, **2001**, *45*, 2397
50. (a) Hong, Y.-L.; Yang, Y.-Z.; Meshnick, S.R. *Antimicrob. Agents Chemother.*, **1994**, *38*, 1854; (b) Cazelles, J.; Camuzat-Dedenis, B.; Provot, O.; Robert, A.; Mayrargue, J.; Meunier, B.; *J. Chem. Soc., Perkin Trans. 1*, **2000**, 1265
51. Eckstein-Ludwig, U.; Webb, R.J.; Van Goethem, I.D.; East, J.M.; Lee, A.G.; Kimura, M.; O'Neill, P.M.; Bray, P.G.; Ward, S.A.; Krishna, S. *Nature*, **2003**, *424*, 957
52. (a) ter Kuile, F.; White, N.J.; Holloway, P.; Pasvol, G.; Krishna, S. *Exp. Parasitol.*, **1993**, *76*, 85; (b) Angus, B.J.; Chotivanich, K.; Udomsangpetch, R.; White, N.J. *Blood*, **1997**, *90*, 2037; (c) Haynes, R.K.; Ho, W.-Y.; Chan, H.-W.; Fugmann, B.; Stetter, J.; Croft, S.L.; Vivas, L.; Peters, W.; Robinson, B.L. *Angew. Chem. Int. Ed.*, **2004**, *43*, 1381; (d) O'Neill, P.M.; Bishop, L.P.; Searle, N.L.; Maggs, J.L.; Ward, S.A.; Bray, P.G.; Storr, R.C.; Park, K.B., *Tetrahedron Lett.*; **1997**, *38*, 4263
53. (a) Haynes, R.K.; Monti, D.; Taramelli, D.; Basilico, N.; Parapini, S.; Olliaro, P. *Antimicrob. Agents Chemother.*, **2003**, *47*, 1175; (b) Wu, W.-M.; Wu, Y.; Wu, Y.-L.; Yao, Z.-J.; Zhou, C.-M.; Li, Y.; Shan, F.; *J. Am. Chem. Soc.*, **1998**, *120*, 3316; (c) Haynes, R.K.; Vonwiller, S.C.; *Tetrahedron Lett.*, **1996**, *37*, 257; (d) O'Neill, P.M.; Bishop, L.P.; Searle, N.L.; Maggs, J.L.; Storr, R.C.; Ward, S.A.; Park, K.B.; Mabbs, F. *J. Org. Chem.*, **2000**, *65*, 1578
54. (a) Posner, G.H.; Meshnick, S.R. *Trends Parasitol.*, **2001**, *17*, 266; (b) Meshnick, S.R.; Yang, Y.-Z.; Lima, V.; Kuypers, F.; Kamchonwongpaisan, S.; Yuthavong, Y. *Antimicrob. Agents Chemother.*, **1993**, *37*, 1108; (c) Avery, M.A.; Mehrotra, S.; Johnson, T.L.; Bonk, J.D.; Vroman, J.A.; Miller, R. *J. Med. Chem.*, **1996**, *39*, 4149
55. (a) Hoppe, H.C.; Van Schalkwyk, D.A.; Wiehart, U.I.M.; Meredith, S.A.; Egan, J.; Weber, B.W. *Antimicrob. Agents Chemother.*, **2004**, *48*, 2370; (b) Li, W., Mo, W., Shen, D., Sun, L., Wang, J., Lu, S.; Gitschier, J.M.; Zhou, B. *PLoS Genet.*, **2005**, *1*, e36; (c) Krungkrai, J., Burat, D.; Kudan, S.; Krungkrai, S.; Prapunwattana, P. *Southeast Asian J. Trop. Med. Public Health*, **1999**, *30*, 636
56. (a) Rosenthal, P. J.; Puran, S.S.; Singh, A.; Shenai, B.R. *Curr. Pharm. Des.*, **2002**, *8*, 1659; (b) Sijwali, P.S.; Kato, K.; Seydel, K.B.; Gut, J.; Lehman, J.; Klemba, M.; Goldberg, D.E.; Miller, L.H.; Rosenthal, P.J. *Proc. Nat. Acad. Sci. U.S.A.*, **2004**, *101*,

8721

57. Bailly, E.; Jambou, R.; Savel, J.; Jaureguiberry, G. *J. Protozool.*, **1992**, *39*, 593
58. (a) Rosenthal, P.J.; Wollish, W.S.; Palmer, J.T.; Rasnick, D. *J. Clin. Invest.*, **1991**, *888*, 1467; 51; (b) Olson, J. E.; Lee, G.K.; Seemenov, A.; Rosenthal, P.J. *Bioorg. Med. Chem.*, **1999**, *7*, 633; (c) Roush, W.R.; Gwaltney II, S.L.; Cheng, J.; Scheit, K.A.; McKerrow, J.H.; Hansell, E. *J. Am. Chem. Soc.*, **1998**, *120*, 10994
59. Lee, B.J.; Singh, A.; Chiang, P.; Kemp, S.J.; Goldman, E.A.; Weinhouse, M.I.; Vlasuk, G.P.; Rosenthal, P.J. *Antimicrob. Agents Chemother.*, **2003**, *47*, 3810
60. (a) Leung-Tong, R.; Li, W.; Tam, T.F.; Karimian, K. *Curr. Med. Chem.*, **2002**, *9*, 979; (b) Zhu, S.; Hudson, T.H.; Kyle, D.E.; Lin, A.J. *J. Med. Chem.*, **2002**, *45*, 3491; (c) Leung, D.; Abbenante, G.; Fairlie, D.P. *J. Med. Chem.*, **2000**, *43*, 305
61. Ring, C.S.; Sun, E.; McKerrow, J.H.; Lee, G.K.; Rosenthal, P.J.; Kuntz, I.D.; Cohen, F.E. *Proc. Nat. Acad. Sci. U.S.A.*, **1993**, *90*, 3583
62. (a) Chen, M.; Theander, T.G.; Christensen, S.B.; Hviid, L.; Zhai, L.; Kharazmi, A. *Antimicrob. Agents Chemother.*, **1994**, *38*, 1470; (b) Mishra, N.; Arora, P.; Kumar, B.; Mishra, L.C.; Bhattacharya, A.; Awasthi, S.K.; Bhasin, V.K. *Eur. J. Med. Chem.*, **2007**, *1*
63. Li, R.; Kenyon, G.L.; Cohen, F.E.; Chen, X.; Gong, B.; Dominguez, J.N.; Davidson, E.; Kurzban, G.; Miller, R.E.; Nuzum, E.O.; Rosenthal, P.J.; McKerrow, J.H. *J. Med. Chem.*, **1995**, *38*, 5031
64. Go, M.L.; Wu, X.; Liu, X.L. *Curr. Med. Chem.*, **2005**, *12*, 483
65. (a) Lyer, R.A.; Hanna, P.E. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 89; (b) Webber, S.E.; Tikhe, J.; Worland, S.T.; Fuhrman, S.A.; Hendrickson, T.F.; Matthews, D.A.; Love, R.A.; Patick, A.K.; Meador, J.W.; Ferre, R.A.; Brown, E. L., DeLisle, D.M.; Ford, C.E.; Binford, S.L. *J. Med. Chem.*, **1996**, *39*, 5072; (c) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P.J.; McKerrow, J.H.; Chibale, K. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3527
66. (a) Surolia, N.; RamachandraRao, S. P.; Surolia, A. *BioEssays*, **2002**, *24*, 192; (b) Surolia, N.; Surolia, A. *Nature Medicine*, **2001**, *7*, 167
67. (a) McFadden, G. I.; Reith, M.; Munholland, J.; Lang-Unnasch, N. *Nature*, **1996**, *381*, 482; (b) Wilson, R.J.M.; Denny, P.W.; Preiser, P.R.; Rangachari, K.; Roberts, K.; Roy, A.; Whyte, A.; Strath, M.; Moore, D.J.; Moore, P.W.; Williamson, D.H. *J. Mol. Biol.*, **1996**, *261*, 155
68. McFadden G.I.; Roos, D.S. *Trends Microbiol.*, **1999**, *7*, 328
69. Waller, R.F.; McFadden, G.I. *Curr. Iss. Mol. Biol.*, **2005**, *7*, 57
70. McConkey, G.A.; Rogers, M.J.; McCutchan, T.F. *J. Biol. Chem.*, **1997**, *272*, 2046

71. (a) Ralph, S.A.; Van Dooren, G.G.; Waller, R.F.; Crawford, M.J.; Fraunholz, M.J.; Foth, B.J.; Tonkin, C.J.; Roos, D.S.; MacFadden, G. I. *Nat. Rev. Microbiol.*, **2004**, 2, 203; (b) Ralph, S.A.; D’Ombrian, M.C.; McFadden, G.I. *Drug Resist. Updates*, **2001**, 4, 145;
72. Tazdemir, D. *Phytochem. Rev.*, **2006**, 5, 99
73. (a) Haldar, K.; Ferguson, M.A.J.; Cross, G.A.M. *J. Biol. Chem.*, **1985**, 260, 4969; (b) Ramya, T.N.C.; Surolia, N.; Surolia, A. *Life*, **2005**, 57, 371
74. Waller, R.F.; Keeling, P.J.; Donald, R.G.K.; Striepen, B.; Handman, E.; Lang-Unnasch, N.; Cowman, A.F.; Besra, G.S.; Roos, D.S.; McFadden, G.I. *Proc. Natl. Acad. Sci. USA*, **1998**, 95, 12352
75. (a) Waller R.F.; Ralph S.A.; Reed, M.B.; Su, V.; Douglas, J.D.; Minnikin, D.E.; Cowman, A.F.; Besra, D. S.; McFadden, G.I. *Antimicrob. Agents Chemother.*, **2003**, 47, 297; (b) Smith, S. *FASEB J.*, **1994**, 8, 1248
76. (a) Vaughan, A.M.; O’Neill, M.T.; Tarun, A.S.; Camargo, N.; Phuong, T.M.; Aly, A.S.I.; Cowman, A.F.; Kappe, S.H.I. *Cell. Microbiol.*, **2009**, 11, 506; (b) Min Yu, M.; Kumar, S.T.R.; Nkrumah, L.J.; Coppi, C.; Retzlaff, S.; Li, C.D.; Kelly, B.J.; Moura, P.A.; Lakshmanan, V.; Freundlich, J.S.; Valderramos, J.-C.; Vilcheze, C.; Siedner, M.; Tsai, J.-H.C.; Falkard, B.; Sidhu, A.B.; Purcell, L.A.; Gratraud, P.; Kremer, L.; Waters, A.P.; Schiehser, G.; Jacobus, D.P.; Janse, C.J.; Ager, A.; Jacobs W.R.Jr.; Sacchettini, J.C.; Heussler, V.; Sinnis, P.; Fidock, D.A. *Cell Host & Microbe*, **2008**, 4, 567
77. (a) Shortt, H.E.; Fairley, N.H.; Covell, G.; Shute, P.G.; Garnham, P.C.C. *Tr. Roy. Soc. Trop. Med. Hyg.*, **1951**, 44, 405; (b) Cowman, A.F.; Crabb, B.S. *Cell*, **2006**, 124, 755
78. Carballeira, N.M. *Prog. Lipid Res.*, **2008**, 47, 50
79. Heath, R.J.; White, S.W.; Rock, C.O. *Prog. Lipid Res.*, **2001**, 40, 467
80. Prigge, S.T.; He, X.; Gerena, L.; Waters, N.C.; Reynolds, K.A. *Biochemistry*, **2003**, 42, 1160.
81. (a) Surolia, A.; Ramya, T.N.C.; Ramya, V.; Surolia, N. *Biochem. J.*, **2004**, 383, 401; (b) Fichera, M.E.; Roos, D.S. *Nature*, **1997**, 390, 407
82. McLeod, R.; Muench, S.P.; Rafferty, J.B.; Kyle, D.E.; Mui, E.J.; Kirisits, M.J.; Mack, D.G.; Roberts, C.W.; Samuel, B.U.; Lyons, R.E.; Dorris, M.; Milhous, W.K.; Rice, D.W. *Int. J. Parasitol.*, **2001**, 31, 109
83. Lu, J.Z.; Lee, P.J.; Waters, N.C.; Prigge, S.T. *Comb. Chem. High Throughput Screen*, **2005**, 8, 15
84. (a) Kodali, S.; Galgoci, A.; Young, K.; Painter, R.; Silver, L.L.; Herath, K.B.; Singh, S.B.; Cully, D.; Barrett, J.F.; Schmatz, D.; Wang, J. *J. Biol. Chem.*, **2005**, 280, 1669; (b)



- McFadden, J.M.; Medghalchi, S.M.; Thupari, J.N.; Pinn, M.L.; Vadlamudi, A.; Miller, K.I.; Kuhajda, F.P.; Townsend, C.A. *J. Med. Chem.*, **2005**, *48*, 946; (c) Price, A.C.; Choi, K.H.; Heath, R.J.; Li, Z.M.; White, S.W.; Rock, C.O. *J. Biol. Chem.*, **2001**, *276*, 6551
85. (a) Trape, J.F.; Pison, G.; Preziosi, M.P.; Enel, C.; Desgrees du Loû, A.; Delaunay, V.; Samb, B.; Lagarde, E.; Molez, J.F.; Simondon, F. *Compte Rendu de l'Academie des Sciences III*, **1998**, *321*, 689; (b) Bloland, P.B.; Lackritz, E.M.; Kazembe, P.N.; Were, J.B.O.; Steketee, R.; Campbell, C.C. *J. Infect. Dis.*, **1993**, *167*, 932
86. Wongsrichanalai, C.; Pickard, A.L.; Wernsdorfer, W.H.; Meshnick, S.R. *Lancet*, **2002**, *2*, 209
87. Zhang, H.; Paguio, M.; Roepe, P.D. *Biochem.*, **2004**, *43*, 8290
88. Hastings, I.M.; D'Alessandro, U. *Parasitol. Today*, **2000**, *16*, 340
89. (a) Noedl, H. *Trends Parasitol.*, **2005**, *21*, 404; (b) Dondorp, A.M.; Nosten, F.; Yi, P.; Das, D.; Phyto, A.P.; Tarning, J.; Lwin, K.M.; Ariey, F.; hanpithakpong, W.; Lee, S.J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; An, S.S.; Yeung, S.; Singhasivanon, P.; Day, N.P.J.; Lindegardh, N.; Socheat, D. White, N.J. *New Engl. J. Med.*; **2009**, *361*, 455
90. (a) Fitch, C.D. *Proc. Natl. Acad. Sci. U.S.A.*, **1969**, *64*, 1181; (b) Su, X-Z.; Kirkman, L.S.; Wellems, T.E. *Cell*, **1997**, *91*, 593; (c) Fidock, D.A.; Nomura, T.; Talley, A.K.; Cooper, R.A.; Dzekunov, S.M.; Ferdig, M.T.; Ursos, L.M.B.; Sidhu, A.B.S.; Naude, B.; Deitsch, K.W.; Su, X.-Z.; Wootton, J.C.; Roepe, P.D.; Wellems, T.E. *Mol. Cell*, **2000**, *6*, 861; (d) Bray, P.G.; Park, K.B. *J. Med. Chem.*, **2003**, *46*, 4933
91. Waller, K.L.; Muhle, R.A.; Ursos, L.M.B.; Horrocks, P.; Verdier-Pinard, D.; Sidhu, A.B.S.; Fujioka, H.; Roepe, P.D.; Fidock, D.A. *J. Biol.Chem.*, **2003**, *278*, 33593
92. (a) Cooper, R.A.; Hartwig, C.L.; Ferdig, M.T. *Acta Tropica*, **2005**, *94*, 1700; (b) Cooper, R.A.; Ferdig, M.T.; Su, X.-Z.; Ursos, L.M.; Mu, J.; Nomura, T.; Fujioka, H.; Fidock, D.A.; Roepe, P.D.; Wellems, T.E. *Mol. Pharmacol.*, **2002**, *61*, 35
93. (a) Martin, S.K.; Oduola, A.M. *Science*, **1987**, *235*, 899; (b) Gottesman, M.M.; Pastan, I.; Ambudkar, S.V. *Curr. Opin. Genet. Dev.*, **1996**, *6*, 610; (c) Cowman, A.F.; Karcz, S.; Galatis, D.; Culvenor, J.G. *J. Cell Biol.*, **1991**, *113*, 1033
94. Van Schalkwyk, D.A.; Egan, T.J. *Drug Resistance Update*, **2006**, *9*, 211
95. Reed, M.B.; Saliba, K.J.; Caruana, S.R.; Kirk, K.; Cowman, A.F. *Nature*, **2000**, *403*, 906
96. RBM Partnership Consensus Statement: Assuring Access to Effective Malaria

- Case Management, Roll Back Malaria Partnership Secretariat, **2004**,  
[http://rbm.who.int/partnership/wg/wg\\_management/docs/RBMConsensusStatement\\_ACT.pdf](http://rbm.who.int/partnership/wg/wg_management/docs/RBMConsensusStatement_ACT.pdf)
97. Traoré, F.N. *Trans. Royal Soc. Trop. Med. Hyg.*, **2005**, 99, 403
98. Krogstad, D.J.; Gluzman, I.Y.; Kyle, D.E.; Oduola, A.M.J.; Martin, S.K.; Milhous, W.K.; Schlesinger, P.H. *Science*, **1987**, 238, 1283
99. (a) World Health Organization, Antituberculosis Drug Resistance in the World, Geneva, **2005**; (b) World Health Organization, *Global Tuberculosis Control – Epidemiology, Strategy, Financing*, Geneva, **2009**, WHO/HTM/TB/2009.411; (c) World Health Organization, *Global Tuberculosis Programme. Framework for Effective Tuberculosis Control*, Geneva, **1994**, WHO/TB/94.179; (d) World Health Organization, *Global Tuberculosis Control: Surveillance, Planning, Financing*, Geneva, **2006**, WHO/HTM/TB/2006.362
100. Corbett, E.L., Marston, B., Churchyard, G.J., De Cock, K. M. *Lancet*, **2006**, 367, 926
101. World Health Organization, *TB/HIV: A Clinical Manual*, Geneva, **2004**, WHO/HTM/TB/2004.329
102. World Health Organization, *Antituberculosis Drug Resistance in the World*, 4<sup>th</sup> Global Report, Geneva, **2008**, WHO/HTM/TB/2008.394
103. <http://www.cbc.ca/health/story/2006/03/17/tb-who060317.html?ref=rss>
104. (a) Zignol, M.; Hosseini, M.S.; Wright, A.; Weezenbeek, C.L.; Nunn, P.; Watt, C.J.; Williams, B.G.; Dye, C. *J. Infect. Dis.*, **2006**, 194, 479; (b) Lui, J.; Ren, H.P. *Anti-infective Agents in Medicinal Chemistry*, **2006**, 5, 331
105. Kim, J.-Y.; Park, Y.B.; Kim, Y.S.; Kang, S.B.; Shin, J.W.; Park, I.W.; Choi, B.W. *Int. J. Tuberc. Lung Dis.*, **2003**, 7, 359
106. Frieden, T.R.; Sterling, T.R.; Munsiff, S.S.; Watt, C.J.; Dye, C. *Lancet*, **2003**, 362, 887
107. Basso, L.A.; Santos, D.S. *Med. Chem. Rev. online*, **2005**, 2, 393
108. Blumberg, H.M.; Burman, W.J.; Chaisson, R.E.; Daley, C.L.; Etkind, S.C.; Friedman, L.N.; Fujiwara, P.; Grzemska, M.; Hopewell, P.C.; Iseman, M.D.; Jasmer, R.M.; Koppaka, V.; Menzies, R.I.; O'Brien, R.J.; Reves, R.R.; Reichman, L.B.; Simone, P.M.; Starke, J.R.; Vernon, A.A. *Am. J. Respir. Crit. Care Med.*, **2003**, 167, 603
109. Aziz, M.A. *Lancet*, **2006**, 368, 2142
110. Boulle, A.; Van Cutsem, G.; Cohen, K.; Hilderbrand, K.; Mathee, S.; Abrahams, M.; Goemaere, E.; Coetzee, D.; Maartens, G. *J. Am. Med. Assoc.*, **2008**, 300, 530
111. Global Alliance for TB Drug Development, [www.tballiance.org](http://www.tballiance.org)

112. Maartens, G.; Wilkinson, R.J. *Lancet*, **2007**, *370*, 2030
113. (a) Cole, S.T.; Brosch, R.; Parkhill, J.; Garnier, T.; Churcher, C.; Harris, D.; Gordon, S.V.; Eiglmeier, K.; Gas, S.; Barry III, C.E.; Tekaia, F.; Badcock, K.; Basham, D.; Brown, D.; Chillingworth, T.; Connor, R.; Davies, R.; Devlin, K.; Feltwell, T.; Gentles, S.; Hamlin, N.; Holroyd, S.; Hornsby, T.; Jagels, K.; Krogh, A.; McLean, J.; Moule, S.; Murphy, L.; Oliver, K.; Osborne, J.; Quail, M.A.; Rajandream, M.-A.; Rogers, J.; Rutter, S.; Seeger, K.; Skelton, J.; Squares, R.; Squares, S.; Sulston, J.E.; Talyor, K.; Whitehead, S.; Barrell, B.G. *Nature*, **1998**, *393*, 537; (b) Camus, J.C.; Pryor, M. J.; Medigue, C.; Cole, S.T. *Microbiology*, **2002**, *148*, 2967
114. (a) Sasseti, C.M.; Boyd, D.H.; Rubin, E.J. *Mol. Microbiol.*, **2003**, *48*, 77; (b) Sasseti, C.M.; Rubin, E.J. *Nature Medicine*, **2007**, *13*, 279
115. Bhatt, A.; Kremer, L.; Dai, A. Z.; Sacchettini, J.C.; Jacobs, W.R., Jr. *J. Bacteriol.*, **2005**, *187*, 7596
116. (a) Brindley, D.N.; Matsumura, S.; Bloch, K. *Nature*, **1969**, *224*, 666; (b) Bloch K. *Methods Enzymol.*, **1975**, *35*, 84; (c) Bloch, K. *Advances in Enzymology*, **1977**, *45*, 1
117. Schaeffer, M.L.; Agnihotri, G.; Volker, C.; Kallender, H.; Brennan, P.J.; Lonsdale, J.T. *J. Biol. Chem.*, **2001**, *276*, 47029
118. (a) Slayden, R.A., Lee, R.E., Armour, J.W., Cooper, A.M., Orme, I.M., Brennan, P.J., Besra, G.S. *Antimicrob. Agents Chemother.*, **1996**, *40*, 2813; (b) Kremer, L., Dover, L.G., Carrere, S., Nampoothiri, K.M., Lesjean, S., Brown, A.K., Brennan, P.J., Minnikin, D.E., Lochi, C., Besra, G.S. *Biochem. J.*, **2002**, *364*, 4232
119. (a) Banerjee, A.; Sugantino, M.; Sacchettini, J.C.; Jacobs, W.R. Jr. *Microbiol.*, **1998**, *144*, 2697
120. (a) Rosner, J.L. *Antimicrob. Agents Chemother.*, **1993**, *37*, 2251; (b) Winder, F.G., In *The Biology of Mycobacteria*, Ratledge, C.; Stanford, J.L. (eds.), Academic Press, San Diego, **1982**, 353
121. Banerjee, A.; Dubnau, E, Quémard, A.; Balasubramanian, V.; Um, K.S.; Wilson, T.; Collins, D.; De Lisle, G.; Jacobs, W. R. Jr. *Science*, **1994**, *263*, 227
122. Mdluli, K.; Slayden, R. A.; Zhu, Y.; Ramaswamy, S.; Pan, X.; Mead, D.; Crane, D. D.; Musser, J. M.; Barry, C.E. III *Science*, **1998**, *280*, 1607
123. Johnson, K.; Schultz, P.G. *J. Am. Chem. Soc.*, **1994**, *116*, 7425
124. (a) Parikh, S.L.; Xiao, G.; Tonge, P.J. *Biochem.*, **2000**, *39*, 7645; (b) Kuo, M.R.; Morbidoni, H.R.; Alland, D.; Sneddon, S.F.; Gourlie, B.B.; Staveski, M.M.; Leonard, M.; Gregory, J.S.; Janjigian, A.D.; Yee, C.; Musser, J.M.; Kreiswirth, B.; Iwamoto, H.;

- Perozzo, R.; Jacobs, W.R. Jr.; Sacchettini, J.C.; Fidock, D.A. *J. Biol. Chem.*, **2003**, 278, 20851
125. Parrish, N.M.; Kuhajda, F.P.; Heine, H.S.; Bishai, W.R.; Dick, J.D. *J. Antimicrob. Chemother.*, **1999**, 43, 219
126. Choi, K.H.; Kremer, L.; Besra, G.S.; Rock, C.O. *J. Biol. Chem.*, **2000**, 275, 28201
127. (a) Senior, S.J.; Illarionov, P.A.; Gurcha, S.S.; Campbell, I.B.; Schaeffer, M.L.; Minnikin, D.E.; Besra, G.S. *Bioorg. Med. Chem. Lett.*, **2003**, 13, 3685; (b) Senior, S.J.; Illarionov, P.A.; Gurcha, S.S.; Campbell, I.B.; Schaeffer, M.L.; Minnikin, D.E.; Besra, G.S. *Bioorg. Med. Chem. Lett.*, **2004**, 14, 373; (c) Kamal, A.; Shaik, A.A.; Sinha, R.; Yadav, J.S.; Arora, S.K. *Bioorg. Med. Chem. Lett.*, **2005**, 15, 1927

## Chapter 2

### INTRODUCTION II

#### 2.1. Natural Products (Secondary Metabolites)

Rollinger and coworkers broadly defined natural products or secondary metabolites as “complex extracts and their chemical entities, which are biosynthesized in nature”.<sup>1</sup> There is a general consensus that the boundary between primary and secondary metabolites is blurred. Nonetheless, secondary metabolites are believed to be of limited distribution in nature and do not directly participate in the growth and development of an organism compared to primary metabolites such as carbohydrates, proteins, lipids and nucleic acids. Moreover, these metabolites can be sourced from both terrestrial and marine organisms. By virtue of the role they play in the survival of organisms (defence, secretion, attraction and signalling) they have over the years undergone an evolutionary refinement or natural selection to emerge as potent, structurally diverse<sup>2</sup> and biologically relevant molecules. Secondary metabolites of interest to man are dyes, pigments, polymers, drugs, oils, perfumes and flavouring agents to name but a few. Relevant to this project and to be discussed from hereon is the utilization of natural products in the drug discovery process.

#### 2.2. Natural Products in Drug Discovery

Throughout the ages, humans exploited nature in sourcing medicine to treat various ailments.<sup>3</sup> The utilization of traditional medicine is still commonplace. In the 19<sup>th</sup> century, technological advances such as spectroscopy contributed significantly to the isolation and characterization of bioactive principles from natural resources and thus facilitated the transition from plant extracts to single, purified products. Classical natural product research is, however, regarded as labour-intensive, time consuming and expensive. Additional deterrents to pursuing this route include the difficulties of obtaining an adequate supply of the drug from the natural source and securing intellectual property rights.<sup>4</sup> It was for these reasons that the early 1990s witnessed a decline in natural product research with most pharmaceutical companies opting for genomics and/or high-throughput-screening of small drug-like molecules from combinatorial libraries.<sup>5a</sup> The counter-productivity of deemphasizing natural product research by the major pharmaceutical companies was made evident by the observed decline in the number of new chemical entities (NCE).<sup>5c</sup> This is a disappointing but perhaps anticipated outcome considering that the discovery and

development of most of the drugs in clinical use not only preceded the genomic era but also originated from, or are, natural products.

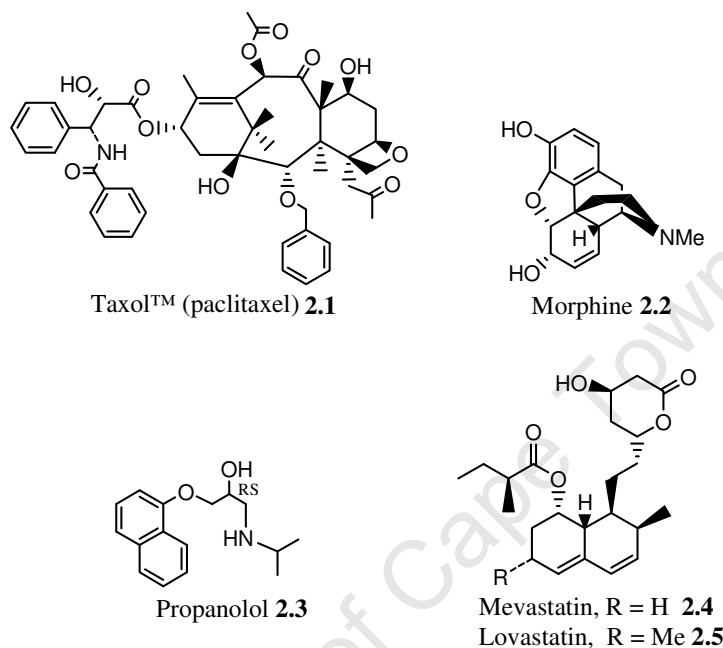
Natural products represent a consistent and abundant source of drugs and drug leads. Indeed only a small fraction, 5-15% of the about 250 000 species of higher plants have been tested for bioactive principles. Additionally, less than 1% of bacterial species and less than 5% of fungal species have been explored whereas other sources such as marine life remain virtually unexplored.<sup>5</sup> About 39% of the approximately 520 new drugs approved by the Food and Drug Administration (FDA) between 1983 and 1994 are reported to be natural products or derivatives thereof.<sup>5c</sup> The same survey revealed that 60% of the cancer drugs and 75% of drugs used in the treatment of infectious diseases were derived from natural products.<sup>5</sup> It is also noteworthy that about 74% of the 119 chemical substances currently considered as important drugs arose from the phytochemical studies of traditional medicinal plants.<sup>6</sup>

### 2.2.1. Natural Products as Drugs and Drug Templates in Non-infectious Diseases

An example of a natural product turned drug is the anticancer agent Taxol™ **2.1** (paclitaxel, fig. 2.1), which was isolated from the bark of the Pacific yew tree, *Taxus brevifolia*. Its utility in cancer chemotherapy includes the treatment of ovarian and breast cancer as well as cancers of the head and neck. Analogue synthesis guided by mechanistic and structure activity relationship (SAR) studies yielded the analogue taxotere which displayed improved selectivity and bioavailability.<sup>7</sup> Another confirmatory example is the analgesic drug morphine **2.2** (fig. 2.1), isolated from the latex of the opium poppy, *Papaver somniferum* and which has revolutionized the treatment of severe pain. It initiated the production of a vast range of synthetic analogues which retained the analgesic activity but are devoid of the addictive qualities.<sup>7</sup>

Natural products also made significant contributions towards the treatment of non-communicable diseases, which recently surpassed infectious diseases as the leading cause of death globally.<sup>8</sup> Propranolol **2.3** (fig. 2.1) served as template for the development of a wide variety of cardioactive  $\beta$ -adrenoceptor blocking agents (beta-blockers); a class of drugs used in the management of hypertension and treatment of other cardiovascular conditions.<sup>7</sup> The discovery of cholesterol lowering agents mevastatin **2.4** and lovastatin **2.5** (fig. 2.1) took the pharmaceutical industry by storm.<sup>5a</sup> Derived from these molecules were a series of analogues with better activity and pharmacokinetic profiles such as fluvastatin, simvastatin, pravastatin,

rosuvastatin and atorvastatin all of which have surpassed the parent molecule in terms of efficacy.<sup>7,9a</sup> A recent article reported on the superior inhibitory activity of atorvastatin compared to other statins against chloroquine-resistant and chloroquine-sensitive strains of *P. falciparum*.<sup>9b</sup> This serves as a further demonstration of the far reaching effects of natural products when used as starting points in drug discovery.



**Figure 2.1:** Chemical structures of some natural product drugs used in the treatment of non-infectious diseases

### 2.2.2. Natural products as Drugs and Drug Templates in Infectious Diseases

The greatest concern with infectious diseases is the increasing pace at which resistance against established drugs develops. This renders the current arsenal almost ineffective, increases the demand for new agents and with the reduced number of NCEs presents a precarious situation especially for resource-poor developing countries with high endemicity for these diseases. Undoubtedly, for most of these countries traditional medicines still play a center-stage role on grounds of accessibility, affordability and efficacy. Among the common infectious diseases are malaria and tuberculosis which are the main focus of this project.

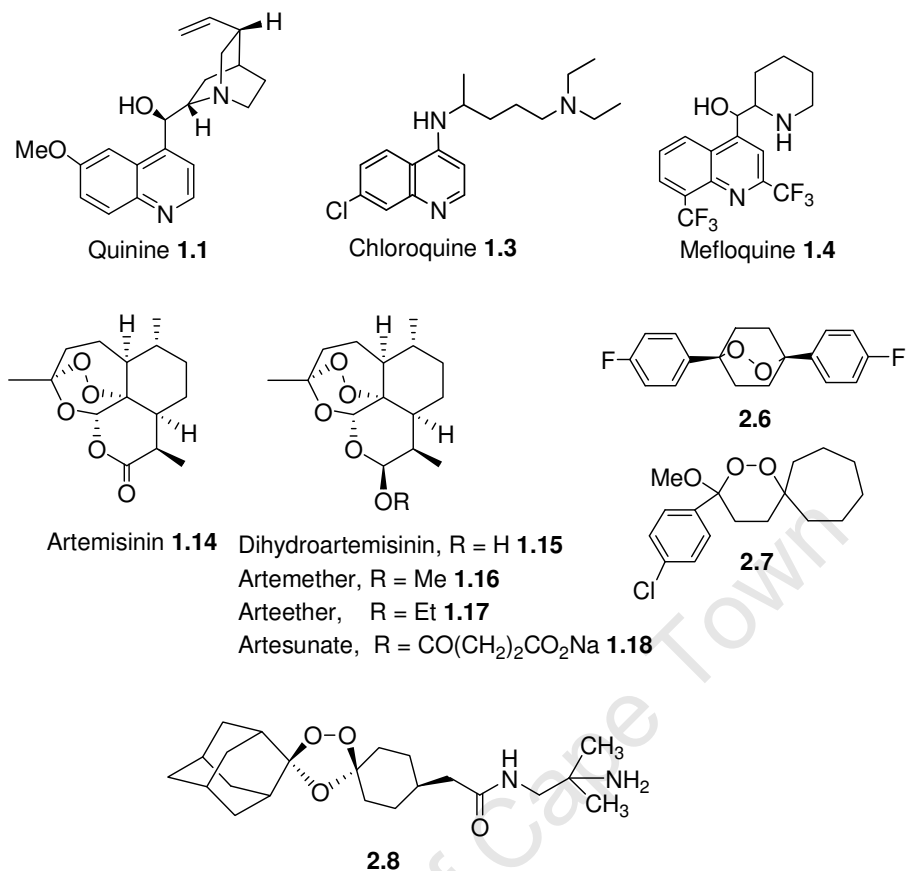
#### 2.2.2.1. Natural Products in Antimalarial Chemotherapy

In the fight against malaria the most typical lead is quinine **1.1** (fig. 2.2) a natural product isolated from the bark of the Peruvian *Cinchona* tree. It represents one of the oldest malaria remedies and joined the battle as early as the 1600s.<sup>10</sup> The quinoline core of quinine has since

served as a template for the synthesis of quinoline-containing antimalarials currently in clinical use such as chloroquine **1.3** and mefloquine **1.4**.<sup>11</sup> Ethnopharmacological research on Chinese herbal remedies led to the discovery of a rare sesquiterpene 1,2,4-trioxane lactone with an unusual endoperoxide moiety, artemisinin **1.14** (fig. 2.2). The latter was isolated from an extract from the wormwood, *Artemisia annua*. Using artemisinin as a template, semisynthetic derivatives such as dihydroartemisinin **1.15** the oil-soluble artemether **1.16** and arteether **1.17** and the water-soluble artesunate **1.18** have been prepared. All these derivatives in addition to having enhanced bioavailability are active against the sexual and asexual blood stage forms of the parasite.<sup>7,12</sup>

Much of the benefit of natural products lies in the fact that by virtue of their origin and thus interaction with biological systems, structural information about the target(s) is not a requirement in analogue design. Artemisinin **1.14**, like various other natural products discovered in the pre-genomic era, serves as an example to this effect. As mentioned earlier (section 1.4.2.1.3), previous SAR studies identified the endoperoxide bridge as a key structural feature which is essential for antimalarial activity.<sup>13</sup> With this recognition simpler and easy to synthesize compounds **2.6** and **2.7** were introduced which in addition to bearing the peroxy bridge, still retain nanomolar antimalarial activity.<sup>14</sup> An exceptional example is the 1,2,4-trioxolane derivative OZ277 **2.8** (fig. 2.2) designed and synthesized by Vennerstrom and coworkers.<sup>15</sup> Like artemisinin **1.14** it contains a shielded endoperoxide bridge but shows increased water solubility, a good toxicity profile and potent *in vitro* and *in vivo* antimalarial activity which surpasses that of first generation artemisinin analogues artemether **1.16** and artesunate **1.18**. It is currently undergoing Phase II clinical trials.<sup>15</sup>



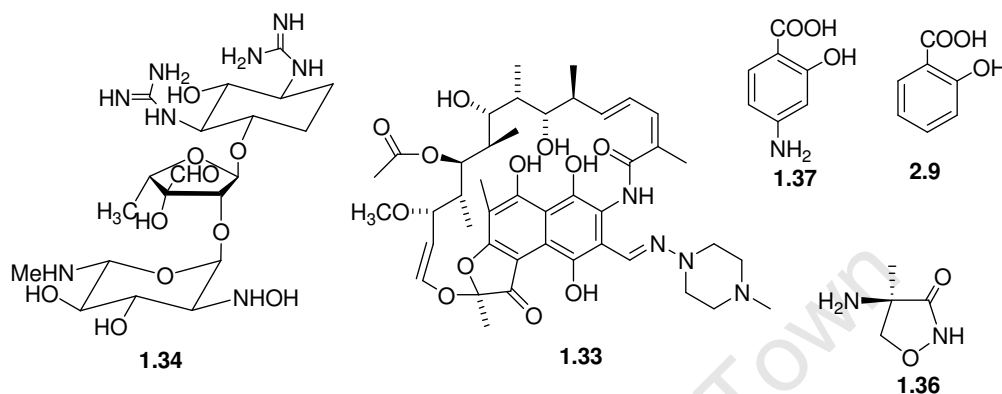


**Figure 2.2:** Chemical structures of natural product and natural product-derived antimalarials

### 2.2.2.2. Natural Products in Tuberculosis Chemotherapy

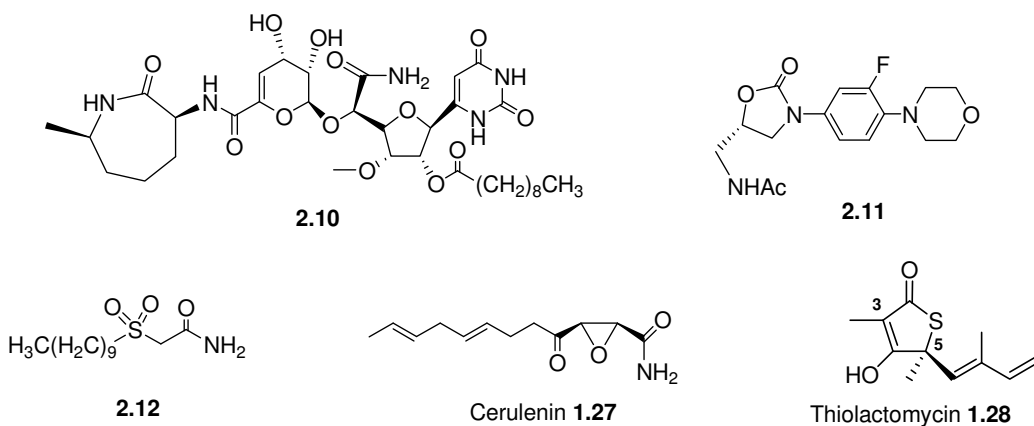
The global burden of TB, the exacerbation thereof by the HIV pandemic and the ever increasing pace at which drug resistance is developing are evidence of the urgent need for new classes of anti-TB drugs. Historically, natural product chemistry has contributed significantly towards the armamentarium of antitubercular drugs. The search for antibiotics from micro-organisms was stimulated by the discovery of the antibiotic penicillin from the fungus, *Penicillium notatum*.<sup>16</sup> Antimycobacterial drug research was soon after rewarded with the discovery of the natural product streptomycin **1.34**<sup>17</sup> (fig. 2.3) which was cultivated from cultures of a strain of *Streptomyces griseus*.<sup>7</sup> Currently this aminoglycoside antibiotic forms part of the frontline antitubercular drugs. Rifamycin, a polyketide produced by cultures of *Ammycolatopsis mediterranei*<sup>7,18</sup> is an important anti-TB drug lead, which aided the discovery of the semisynthetic rifampin **1.33** (fig. 2.3), a first-line anti-TB drug. Other semisynthetic derivatives of rifamycin, which display enhanced anti-TB activity and pharmacological properties include rifapentin, rifametane and rifabutin.<sup>19</sup> The latter deserves

special mention, for its compatibility with antiretrovirals has made it invaluable in the treatment of HIV-related TB.<sup>20</sup> The discovery of *p*-aminosalicylic acid **1.37** (fig. 2.3) as an antitubercular agent in 1949 was an incidental result from the discovery of the rapid metabolism of the natural product salicylic acid **2.9** by *Mycobacterium tuberculosis*.<sup>21</sup>



**Figure 2.3:** Chemical structures of natural product and natural product-derived antitubercular agents

Another streptomycete-derived natural product is the D-alanine mimic, cycloserine **1.36** (fig. 2.3) which exerts its mechanism of action by inhibiting cell wall biosynthesis in *M. tuberculosis*. It is produced by cultures of *Streptomyces orchidaceus* and is used clinically as a second-line antitubercular agent.<sup>7</sup> Advances in screening technologies for detection of anti-TB activity in natural product extracts provided further examples of new classes of natural product-derived anti-TB compounds which are undergoing preclinical development. These include the capuramycin derivative **2.10**<sup>22a</sup> (fig. 2.4) the oxazolidinone derived linezolid **2.11**<sup>22b</sup> which is to be used for the treatment of MDR-TB<sup>22c</sup> as well as the  $\beta$ -sulfonylcarboxamide derivative **2.12**.<sup>22d</sup> The latter mimics the transition state of the condensing enzyme of type II Fas,  $\beta$ -ketoacyl synthase in much the same way as the antitubercular natural products cerulenin (**1.27**, fig. 2.4) and thiolactomycin **1.28**<sup>22d,23</sup> (section 1.4.2.2.3).



**Figure 2.4:** Chemical structures of natural product antitubercular agents

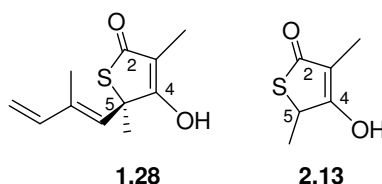
### 2.2.3. Natural Product Scaffolds as Templates in the Discovery of Anti-infective Agents

In rationalizing the use of natural products scaffolds as “blueprints”, Henkel and coworkers<sup>24</sup> showed through detailed statistical analysis that natural products, compared to synthetic compounds, generally have higher molecular weights and therefore do not always comply with Lipinski’s rule of five for oral drugs.<sup>25</sup> It was also found that these products exhibit a different distribution of heteroatoms and are sterically more complex by containing more rings and chiral centers.<sup>24</sup> This chemical and structural diversity coupled with the inherent biological activity of natural products is believed to confer quality to compound libraries modelled on them.<sup>26</sup>

Furthermore, the benefits of incorporating natural product scaffolds or derivatives thereof in the synthesis of compound libraries is epitomised by the concept of “privileged structures”.<sup>5a</sup> This term was first introduced in the 1980s by Evans and coworkers, after witnessing the indiscriminate binding of the 1,4-benzodiazepine-2-one scaffold to various receptors.<sup>27a</sup> It is nowadays used to describe ligands that bind various, unrelated enzymes and receptors with high affinity.<sup>27b,c</sup> By utilizing privileged structures, of which the majority are natural products or derivatives thereof, compound libraries can be designed or modelled upon one core scaffold and screened against various receptors; several active compounds have been obtained in this manner.<sup>27c</sup> A further expectation is that the incorporation of natural products or privileged structures into compound libraries will result in the transfer of favourable, drug-like properties for example bioactivities and bioavailabilities. This approach is also reputed to offer libraries of active compounds with high hit rates at smaller library size compared to larger libraries obtained through pure synthetic or combinatorial efforts.<sup>28</sup>

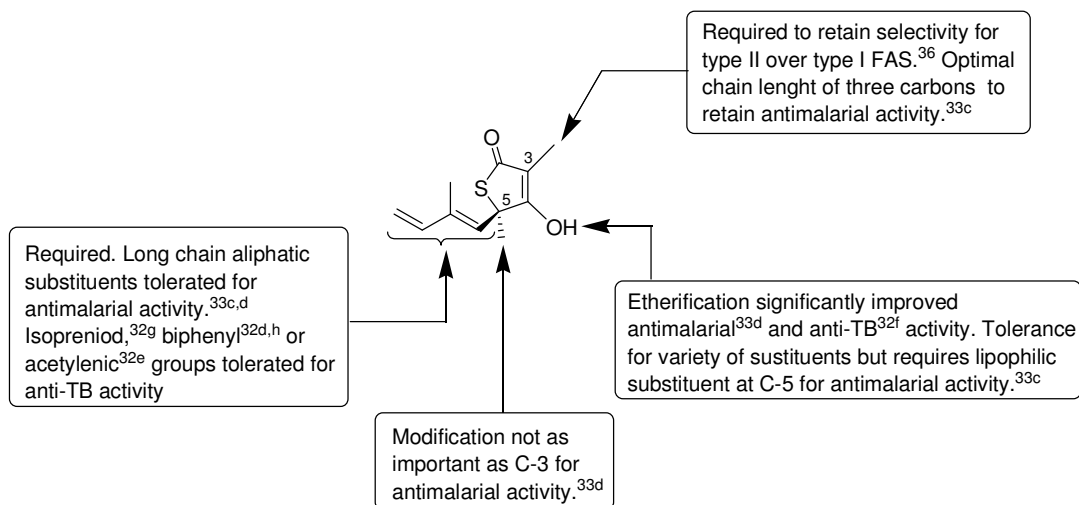
In our study we were particularly interested in the natural products thiolactomycin, isatin and chalcone. A brief account on the discovery, biological profiles, analogue synthesis and SAR studies of each of these scaffolds has therefore been undertaken and is presented below.

### 2.2.3.1. Thiolactomycin as Template for Antimalarial and Antitubercular Agents



**Figure 2.5:** Chemical structures of thiolactomycin **1.28** and key intermediate thiolactone **2.13**

Thiolactomycin ((4S)(2E,5E)-2,4,6-trimethyl-3-hydroxy-2,5,7-octatriene-4-thiolide, **1.28**, TLM) is a broad-range antibiotic isolated from the fermentation broth of a soil bacteria from the genus *Nocardia*. The structure and antibiotic properties of **1.28** was first reported in 1982 by Oishi and coworkers.<sup>29</sup> It displays a number of characteristics which makes it an attractive lead in the search for anti-infective agents. These include its low toxicity in mice,<sup>29a</sup> the fact that it is well-absorbed orally and selectively inhibits type II fatty acid synthases (FAS)<sup>30</sup> which operates in a number of pathogenic organisms. The diverse biological activity of **1.28** and analogues against *Gram* negative bacteria,<sup>29b,29</sup> *M. tuberculosis*,<sup>30,32</sup> *P. falciparum*,<sup>33</sup> *Trypanosoma brucei*<sup>33c,e</sup> and *Toxoplasma gondii*<sup>33e</sup> - the causative agent of AIDS-related opportunistic infections and congenital birth defects - has been reported. Despite favourable properties such as low molecular weight, high water solubility and appropriate lipophilicity<sup>32g</sup> the therapeutic utility of **1.28** is limited by synthetic accessibility and stability issues.<sup>34</sup> These liabilities coupled with the selectivity of **1.28** has attracted the attention of synthetic chemists. Synthesis methodologies have been developed for racemic,<sup>35a</sup> non-natural<sup>35b-d</sup> and naturally occurring **1.28**.<sup>35d-g</sup> Also reported are the synthesis and biological evaluation of analogues obtained either through modification of **1.28** or the key intermediate thiolactone **2.13** (fig. 2.5). Some key structure-activity relationships delineated from these studies are outlined in figure 2.6.



**Figure 2.6:** Summary of structure-activity relationships for thiolactomycin **1.28**

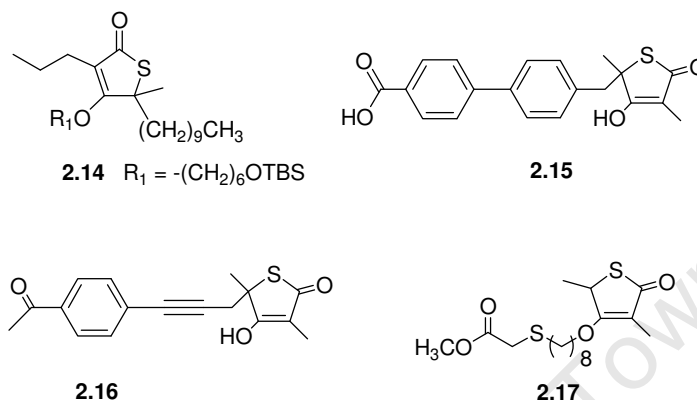
The elucidation of the crystal structure of **1.28** bound to *E. coli*'s FabB (section 1.4.2.2.3) revealed important interactions between TLM and the enzyme's active site. One important interaction, is the accommodation of the isoprene group at C-5 of **1.28** in an incompletely filled hydrophobic crevice.<sup>23,36</sup> Most of the synthesis of thiolactomycin analogues focussed on the replacement and/or modification of the isoprenoid side-chain at C-5, with a bulk of the work carried out by Jones and coworkers.<sup>33c</sup> They initially introduced hydrophobic alkyl groups at position C-3 and C-5 and generated a series of racemic analogues which were evaluated for *in vitro* growth inhibitory activity against *P. falciparum*, *T. brucei* and *T. cruzi*.<sup>33c</sup> In order to effect detailed SAR studies (fig 2.6) the authors - in a follow-up study - explored, in addition to further derivatization of the C-3 and C-5 position, the replacement of the sulfur atom with oxygen and nitrogen as well as alkylation of the hydroxyl group at C-4.<sup>33d</sup> The most significant enhancement of antimalarial activity - a 100-fold increase compared to **1.28** - was observed for the 4-*O*-alkylated analogues and in particular **2.14** (fig. 2.7). All of the analogues synthesized were assessed for inhibitory activity against recombinant *pfKASIII*. However, in failing to correlate antiplasmodial activity with *pfKASIII* inhibition the authors concluded that the latter "is not the primary target for TLM analogues".<sup>33d</sup>

Within the context of antitubercular activity, thiolactomycin **1.28** is believed to exert its mechanism of action by targeting the  $\beta$ -ketoacyl-acyl-carrier protein synthases; *mtKas A*, *mtKas B*,<sup>30</sup> and to a lesser extent *mtFabH*<sup>32b</sup> - all members of the type II Fas system of *M.*

*tuberculosis* (section 1.6.4). Douglas and coworkers<sup>32c</sup> synthesized racemic thiolactomycin analogues with aliphatic substituents at the C-5 position. These were evaluated for inhibitory activity against mycolic acid biosynthesis and type II FAS in extracts of *M. smegmatis* and for *in vivo* activity against the H<sub>37</sub>Rv strain of *M. tuberculosis*. The authors found that although **1.28** inhibited both type II Fas and mycolate synthesis, analogues with longer side chains showed improved mycolate synthase inhibition but appeared to stimulate type II Fas activity.<sup>32c</sup> In a separate study, Senior and coworkers synthesized analogues with biphenyl<sup>32d,h</sup> and acetylene<sup>32e</sup> substituents at the C-5 position which were screened for inhibitory activity against recombinant *mtFabH*. Tolerance for these functionalities was demonstrated by compound **2.15** (fig. 2.7) from the biphenyl-based series which showed a 4-fold increase in activity compared to **1.28** whereas the most active acetylenic analogue **2.16** (fig. 2.7) showed a 18-fold increase in activity.

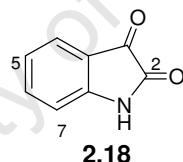
Of particular interest to this project is the thiolactomycin analogues synthesized by Kamal and coworkers.<sup>32f</sup> By etherification of the C-4 hydroxyl group of **2.13** (fig. 2.5) they obtained a series of compounds of which the most active **2.17** (fig. 2.7) showed anti-TB activity with MICs ranging between 1.0-4.0 µg/mL against drug sensitive and resistant strains of *M. tuberculosis*. Speculatively, the latter study challenges the requirement of an isoprene unit or substitution at C-5 in the design of antitubercular thiolactomycin analogues. However, a detailed structure-activity relationship study conducted by Kim and coworkers<sup>32g</sup> confirmed the importance of this unit for anti-TB activity. By subjecting the isoprenoid side chain of fermentation-derived **1.28** to systematic modification (i.e. selective reduction of the double bonds, demethylation and methylation) they obtained a series of analogues which were tested for inhibitory activity against *mtKas A*, *mtKas B* and *mtFabH*. Consistent with earlier findings they failed to correlate whole-cell activity with enzyme inhibitory activity for some of the analogues. Also of importance is the research undertaken by McFadden and coworkers.<sup>36</sup> It involved the synthesis of thiolactomycin analogues modified at C-3, C-4, C-5 and was aimed at sourcing inhibitors for type I Fas or more particularly, cancer and obesity treatment. The reason for targeting these two disease models reportedly stem from the higher level of type I FAS expressed by cancer and tumour cells compared to normal cells and the weight loss resulting from the inhibition of type I FAS activity.<sup>37</sup> Overall, this study showed that a methyl group at C-3 is required for thiolactomycin analogues, to maintain selectivity for type II over type I Fas.<sup>36</sup>

The comparison of biological activities obtained from different studies as done above may be questionable, because of different experimental or assay conditions used. However, the intention in this project was to demonstrate the utility and validity of the thiolactomycin scaffold as a template for the discovery of new anti-infective agents.



**Figure 2.7:** Chemical structures of antimalarial and antitubercular thiolactomycin analogues

### 2.2.3.2. Isatin as Template for Antimalarial and Antitubercular Agents

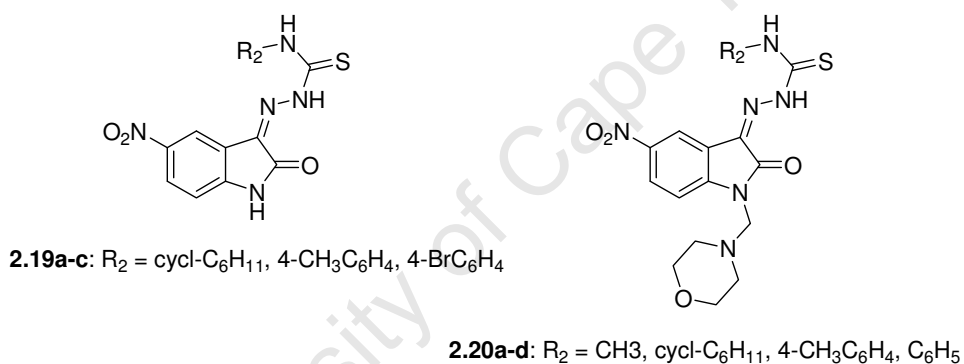


**Figure 2.8:** Chemical structure of isatin **2.18**

Isatin (1H-indole-2,3-dione or 2,3-dioxindoles, **2.18**, fig. 2.8) is widely distributed in nature. Sources include the plant genus *Isatis*,<sup>38a</sup> the microbial *Alteromonas* specie, the parotid glands of *Bufo* frogs<sup>38b</sup> and it has also been identified as a metabolite of adrenaline in humans.<sup>38c</sup> This natural product consists of a pyrrole ring fused to a benzene ring, with oxygen substituents at positions 2 and 3 (fig. 2.8). It furthermore represents a versatile natural product scaffold with diversity points represented by the ketonic carbonyl at C-3, the nitrogen in the five membered ring and the C-5, C-7 positions on the aromatic ring.

Consistent with the privileged status of the isatin scaffold, its derivatives exhibit a wide range of biological activity. For example, isatin sulphonamides are selective inhibitors of the caspase 3 and 7 cysteine proteases which are involved in programmed cell death for cancer

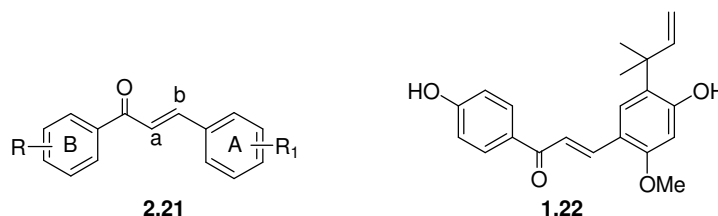
therapy<sup>39</sup> whereas *N*-alkylated-5-isatin carboxamide derivatives inhibits the human rhinovirus - causative agent of the common cold.<sup>40</sup> Also, Schiff and *N*-Mannich base derivatives of isatin show a wide spectrum of activities which include, anticonvulsant,<sup>41a</sup> antibacterial,<sup>41b</sup> antiviral,<sup>41c</sup> anti-inflammatory,<sup>41d</sup> antifungal,<sup>41e</sup> antiprotozoal,<sup>41f</sup> antihelminthic,<sup>41g</sup> anti-HIV,<sup>41h</sup> anti-TB<sup>41i-k</sup> and anticancer<sup>41l</sup> activity. The antimalarial activity and in particular cysteine protease inhibitory activity of thiosemicarbazone derivatives of isatin has been discussed in section 1.4.2.1.4.2. With regards to antitubercular activity, Karali and coworkers<sup>41k,42</sup> designed and synthesized 1*H*-indole-2,3-dione derivatives of which 5-nitro-1*H*-indole-2,3-dione -3-thiosemicarbazones **2.19a-c** (fig. 2.9) and 1-morpholinomethyl derivatives **2.20a-d** showed promising *in vitro* activity against *M. tuberculosis* with 90-96% inhibition at a MIC of > 6.25 µg/mL. A major shortcoming of these compounds, however, is their toxicity.<sup>42</sup>



**Figure 2.9:** Chemical structures of compounds **2.19** and **2.20**

The chemistry and synthesis of isatin and its analogues has been extensively studied and reported in a number of comprehensive reviews and monographs<sup>43</sup> and because of its voluminous nature has not been included in this report.

### 2.2.3.3. Chalcone as Template for Antimalarial and Antitubercular Agents

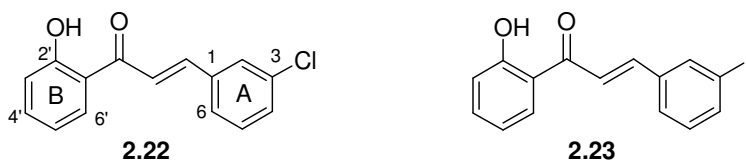


**Figure 2.10:** General structure of chalcones **2.21** and chemical structure of lichochalcone A **1.22**



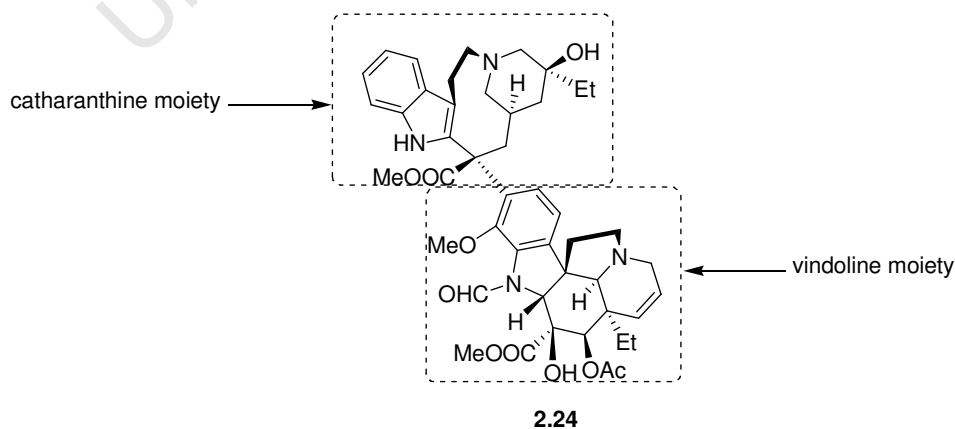
Chalcones (1,3-diarylprop-2-en-1-one, **2.21**, fig. 2.10) represent a major class of natural products which act as precursors in the synthesis of flavonoid derivatives.<sup>7</sup> Structurally, they consist of two aromatic rings joined by a conjugated  $\alpha,\beta$ -unsaturated ketone linker. By convention, the aldehyde-derived ring is labelled “A” and the acetophenone-derived ring “B”.<sup>44</sup> Furthermore, they comprise of a class of natural product privileged structures which, depending on the substituents on the aromatic rings, displays a wide range of biological activities. These include anti-inflammatory,<sup>45</sup> antileishmanial,<sup>46</sup> antibacterial,<sup>47</sup> antifungal,<sup>48</sup> antitumour,<sup>49</sup> antimalarial<sup>44,50</sup> and antiTB activity.<sup>51</sup> With regard to antimalarial activity, cysteine protease inhibition is the most likely mode of action by which these derivatives exert their antimalarial activity (section 1.4.2.1.4.2). It has also been demonstrated that some alkoxyated and hydroxylated chalcones inhibit new permeability pathways (NNP) induced by the parasite in the erythrocyte membrane.<sup>50g</sup> These pathways reportedly allows the intracellular parasite access to nutrients, organic and inorganic ions, to which an otherwise uninfected erythrocyte is impermeant to.<sup>52</sup> This study investigated the effect of alkoxyated and hydroxylated chalcones on the haemolysis of infected erythrocytes caused by sorbitol which entered through parasite-induced channels. The results showed a good correlation between antiplasmodial activity and inhibition of parasite-induced channels, especially for some mono- and dimethoxychalcones. More interesting is the observation that some of the potent chalcones did not inhibit parasite-induced channels which lends support to earlier observations that antiplasmodial chalcone derivatives, depending on the substituents and substitution pattern, may exert their activity *via* “different routes or target additional pathways”<sup>50g</sup>

Chalcones and derivatives are easily prepared by base-catalyzed Claisen-Schmidt condensation of an appropriately substituted acetophenone with an appropriately substituted benzaldehyde. Invariable yields obtained for some chalcone derivatives led to the modification of the Claisen-Schmidt method and included among others the exploration of catalysts other than the conventional NaOH and KOH. The use of basic catalysts such as Ba(OH)<sub>2</sub>,<sup>53a</sup> hydrotalcites,<sup>53b</sup> calcined NaNO<sub>3</sub>/natural phosphates,<sup>53c</sup> LiHMDS<sup>53d</sup> and acidic catalysts such AlCl<sub>3</sub>,<sup>53e</sup> dry HCl,<sup>53f</sup> TiCl<sub>4</sub><sup>53g</sup> and BF<sub>3</sub>-OEt<sub>2</sub><sup>53h</sup> have been reported in the literature. Also reported is the utilization of ultrasound and microwave<sup>54</sup> in facilitating rapid SAR studies and the application and limitations of alternative synthesis methodologies such as Suzuki coupling<sup>55</sup> and Mukaiyama-type aldol condensation.<sup>56</sup>



**Figure 2.11:** Chemical structures of compounds **2.22** and **2.23**

The pioneering chalcone antimalarial, licochalcone A (**1.22**, fig. 2.10) was isolated from the roots and rhizomes of *Glycyrrhiza inflata* also known as the Chinese liquorice.<sup>50a</sup> Confirmation of the cysteine protease inhibitory activity of chalcones was obtained *via* computer modelling studies.<sup>50c,f</sup> The key structural requirement identified for antimalarial activity is the conjugated  $\alpha,\beta$ -unsaturated ketone linker which imparts rigidity thus forcing the chalcone to adopt an almost planar structure that can fit into the long cleft of the active site of the enzyme.<sup>44b,50c</sup> Antimalarial structure-activity relationship studies showed a preference for alkoxyated as opposed to hydroxylated chalcones.<sup>44a</sup> In a separate study, Larsen and coworkers evaluated a series of *Z*- and *E*-isomers of chalcones for antiplasmodial activity and revealed the thermodynamically stable *E*-isomer as the bioactive conformer.<sup>50e</sup> Incidentally, **1.22** also served as a model for the design of analogues with antileishmanial and anti-TB activity. Friis-Möller and coworkers reported a minimum inhibitory activity (MIC) of  $\leq 20\text{mg/L}$  for **1.22** against *M. tuberculosis*, *M. bovis*, *M. kansasii*, *M. xenophii* and *M. marinum*.<sup>51b</sup> Antitubercular structure-activity relationship studies also showed a preference for halogen substituents at the C-3 position (ring A) of 2'-hydroxychalcone as typified by compounds **2.22** and **2.23** (fig. 2.11) which showed a 90-92% inhibition at a drug concentration of  $12\ \mu\text{g/mL}$ .<sup>51a</sup>

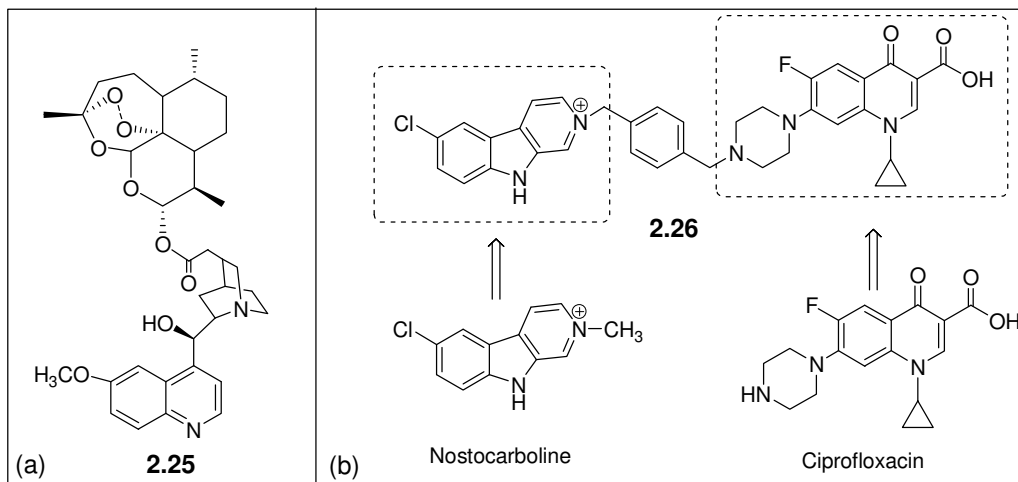


**Figure 2.12:** Chemical structure of the natural product hybrid vincristine **2.24**

#### 2.2.4. Natural Product and Natural product-like Hybrids as a Source of Leads in Drug Discovery

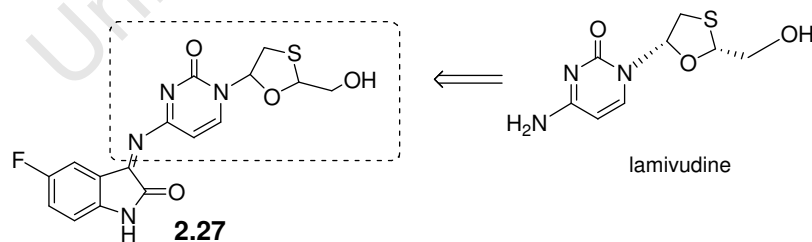
Hybridization is a strategy long used by nature; it involves the covalent linkage of two or more molecules derived from different biosynthetic pathways.<sup>57a</sup> Apart from being structurally diverse, biologically active natural products so derived have been found to exhibit enhanced or even new properties compared to the monomers. One example is the natural hybrid construct, vincristine **2.24** (fig. 2.12) which has revolutionized the treatment of childhood leukaemia. It has been found that the monomeric alkaloids individually do not display useful biological activity.<sup>57b</sup> The strategy of hybrid (chimera) construction has since filtered into drug discovery programs. It offers advantages such as (i) a reduced propensity for the development of resistance when two scaffolds with different modes of action are linked, (ii) improved toxicity and bioavailability profile, (iii) enhanced and/or new properties, as will be demonstrated by the examples presented below.

The first example, is the artemisinin-quinine hybrid **2.25** [fig. 2.13(a)] synthesized by Walsh and coworkers.<sup>58</sup> Synthesis of this hybrid entailed the linking of dihydroartemisin **1.15** (fig. 2.2) to quinine **1.1** via its modified vinylic side chain; whilst retaining functionalities necessary for antimalarial activity as guided by previous SAR studies.<sup>59</sup> Intriguingly, the hybrid showed superior antimalarial activity compared to (i) artemisinin **1.14**, (ii) **1.1** and (iii) a fixed ratio (1:1) combination of **1.1** and **1.14**. The authors ascribed the dramatic increase in potency to enhanced cellular uptake and anticipated an improved side effect profile, that is, compared to the individual drugs.<sup>58</sup> This approach was further validated by the linking of the natural product nostocarbolone, a phytotoxic agent with the natural product derived antibacterial agent, ciprofloxacin *via* an aryl linker to yield **2.26** [fig. 2.13(b)]. The hybrid **2.26** retained both activities with an MIC of 0.7 $\mu$ M against *E. coli* and 1  $\mu$ M against the cyanobacterium *M. aeruginosa*.<sup>60</sup>



**Figure 2.13:** Chemical structures of natural product hybrids (a) artemisinin-quinine **2.25**, and (b) nostocarboline-ciprofloxacin **2.26**

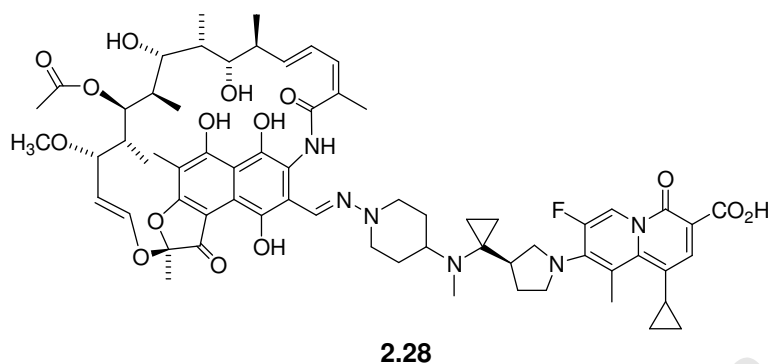
Examples of natural product-like hybrids include the Schiff base **2.27** (fig. 2.14), which was designed with the aim of sourcing compounds that can suppress HIV-replication and inhibit *M. tuberculosis* activity. The hybrid consisted of lamivudine (3TC) a clinically used nucleoside HIV reverse transcriptase inhibitor and 5-fluoro substituted isatin.<sup>61</sup> *In vitro* antiretroviral studies showed that the hybrid **2.27** displayed anti-HIV activity with a median effective concentration ( $EC_{50}$ ) of 0.0742  $\mu\text{M}$  comparable to that of lamivudine ( $EC_{50} = 0.1 \mu\text{M}$ ) as well as antimycobacterial activity with a 82% inhibition at a drug concentration of 6.25  $\mu\text{g/mL}$ . This is a promising find in light of the unfavourable drug-drug interaction currently experienced in the treatment of HIV-related TB.



**Figure 2.14:** Chemical structure of natural product-like hybrid **2.27**

The retardation of resistance development was cited as the main motivation behind the dual-action concept applied in the synthesis of rifamycin-quinolone hybrids. Identified from a pool of 300 hybrids is the antibacterial CBR-2092 **2.28** (fig 2.15) which displayed broad-

spectrum activity *in vitro* and a reduced propensity for development of resistance. It is currently undergoing phase I clinical trials.<sup>62</sup>



**Figure 2.15:** Chemical structure of natural product-like hybrid **2.28**

### 2.3. Aims and Objectives of Study

The hypothesis formulated for this study is that novel antimalarial and antitubercular agents with enhanced activity *in vitro* can be identified by synthetically hybridizing and appropriately decorating scaffolds based on natural products or derivatives thereof. We therefore sought to achieve the following aims:

- To synthesize and investigate structure-activity relationships *in vitro* of natural product hybrids and derivatives based on thiolactomycin, chalcone and isatin.
- To demonstrate the potential of 'click chemistry' for the generation of compounds based on the thiolactomycin, chalcone and isatin scaffolds with hitherto unexplored structures.
- To pharmacologically evaluate synthesized compounds *in vitro* for antimalarial and antitubercular activity.
- To gain insight into the mechanism of action of the appropriate (most) potent novel compounds with respect to inhibition of cysteine proteases in the malaria parasite *Plasmodium falciparum*.

**2.4. References**

1. Rollinger, J. M.; Langer, T.; Stuppner, H. *Curr. Med. Chem.*, **2006**, *13*, 1491
2. Bajorath, J. *Nat. Rev. Drug Discov.*, **2002**, *1*, 882
3. Cordell, G.A. *Phytochem.*, **2000**, *55*, 463
4. Kartal, M. *Phytother. Res.*, **2007**, *2*, 113
5. (a) Cragg, G.M.; Newman, D.J. *Pure Appl. Chem.*, **2005**, *77*, 7; (b) Cragg, G.M.; Newman, D.J.; Snader, K.M. *J. Nat. Prod.*, **1997**, *60*, 52; (c) Newman, D.J.; Cragg, G.M.; Snader, K.M. *J. Nat. Prod.*, **2003**, *66*, 1022; (d) Vuorela, P.; Leinonen, M.; Saikku, P.; Tammela, P.; Rauha, J.-P.; Wennberg, T.; Vuorela, H. *Curr. Med. Chem.*, **2004**, *11*, 1375
6. (a) Farnsworth, N. R.; Akerele, O.; Bingel, A.S.; Soejarto, D.D.; Guo, Z. *Bull. WHO*, **1985**, *63*, 965; (b) Arvigo, R.; Balick, M. *Rainforest Remedies*, Lotus Press, Twin Lakes, **1993**
7. Dewick, P.M. *Medicinal Natural Products: A Biosynthetic Approach*, John Wiley and Sons Ltd, England, **2001**, p. 107-481
8. World Health Organization, *World Health Statistics*, Geneva, **2008**
9. (a) Gilson, M. *Origin of Drugs in Current Use: The Story of Statins*, **2000**, [http://www.world-of-fungi.org/Mostly\\_Medical/Mark\\_Gilson/Mark\\_Gilson.htm](http://www.world-of-fungi.org/Mostly_Medical/Mark_Gilson/Mark_Gilson.htm)  
(b) Pradines, B.; Torrentino-Madamet, M.; Fontaine, A.; Henry, M.; Baret, E.; Mosnier, J.; Briolant, S.; Fusai, T.; Rogier, C. *Antimicrob. Agents Chemother.*, **2007**, *51*, 2654
10. Poser, C.M.; Bruyn, G.W. *An Illustrated History of Malaria*, Parthenon Publishing, UK, **1999**, 75
11. Newman, D.J.; Cragg, G. M.; Snader, K.M. *Nat. Prod. Rep.*, **2000**, *17*, 215
12. Mital, A. *Curr. Med. Chem.*, **2007**, *14*, 759
13. Posner, G.H.; Parker, M.H.; Northrop, J.; Elias, J.S.; Ploypradith, P.; Xie, S.; Shapiro, T.A. *J. Med. Chem.*, **1999**, *42*, 300
14. (a) Posner, G.H.; Tao, X.; Cummings, J.N.; Klinedinst, D.; Shapiro, T.A. *Tetrahedron Lett.*, **1996**, *37*, 7225; (b) Posner, G.H.; O'Dowd, H.; Ploypradith, P.; Cummings, J.N.; Xie, S.; Shapiro, T.A. *J. Med. Chem.*, **1998**, *41*, 2164
15. Vennerstrom, J.L.; Arbe-Barnes, S.; Brun, R.; Charman, S.A.; Chiu, F.C.K.; Chollet, J.; Dong, Y.; Dorn, A.; Hunziker, D.; Matile, H.; McIntosh, K.; Padmanilayman, M.; Santo, T.J.; Scheurer, C.; Scoreaux, B.; Tang, Y.; Urwyler, H.; Wittlin, S.; Charman, W.N. *Nature*, **2004**, *430*, 900
16. (a) Fleming A. *Proc. R. Soc. London, Ser. B*, **1922**, *93*, 306; (b) Demain, A.L. *Nat.*

- Biotechnol.*, **1998**, *16*, 3
17. Waksman, S.A. *Adv. Appl. Microbiol.*, **1969**, *11*, 1
  18. Floss, H.G.; Yu, T.-W. *Chem. Rev.*, **2005**, *105*, 621
  19. (a) Rothstein, D.M.; Shalish, C.; Murphy, C. K.; Sternlicht, A.; Campbell, L. A. *Expert Opin. Invest. Drugs*, **2006**, *15*, 603; (b) Portero, J.-L.; Rubio M. *Expert Opin. Ther. Pat.*, **2007**, *17*, 617
  20. Narita, M.; Stambaugh, J.J.; Hollender, E.S.; Jones, D.; Pitchenik, A.E.; Ashkin, D. *Clin. Infect. Dis.*, **2000**, *30*, 210
  21. (a) Gutierrez-Lugo, M.-T.; Bewley, C.A. *J. Med. Chem.*, **2008**, *51*, 2606; (b) Lehmann, J. *Dis. Chest*, **1949**, *16*, 684
  22. (a) Protopopova, M.; Hanrahan, C.; Nikonenko, B.; Samala, R.; Chen, P.; Gearhart, J.; Einckj, L.; Nacy, C.A.; *J. Antimicrob. Chemother.*, **2005**, *56*, 968; (b) Sood, R.; Bhadauriya, T.; Rao, M.; Gautam, R.; Malhotra, S.; Barman, T. K. *Infect. Disord. Drug Targets*, **2006**, *6*, 343; (c) Erturan, Z.; Uzun, M. *Int. J. Antimicrob. Agents*, **2005**, *26*, 78; (d) Parrish, N. M.; Houston, T.; Jones, P. B.; Townsend, C.; Dick J. D. *Antimicrob. Agents Chemother.*, **2001**, *45*, 1143
  23. Price A.C.; Choi, K.H.; Heath, R.J.; Li, Z.; White, S.W.; Rock, C.O. *J. Biol. Chem.*, **2001**, *276*, 6551
  24. Henkel, T.; Brunne, R.M.; Muller, H.; Reichel, F. *Angew. Chem. Int. Ed. Engl.*, **1999**, *38*, 643
  25. Lipinsky, C.A.; Lombardo, F. *Adv. Drug. Del. Rev.*, **1997**, *23*, 3
  26. Bindseil, K.U.; Jakupovic, J.; Wolf, D.; Lavayre, J.; Leboul, J.; Van der Pyl, D. *Drug Discovery Today*, **2001**, *6*, 840
  27. (a) Evans, B.; Rittle, K.E.; Bock, M.G.; Dipardo, R.M.; Freidinger, R.M.; Whitter, W.L.; Lundell, G.F.; Veber, D.F.; Anderson, P.S.; Chang, R.S.; Lotti, V.J.; Cerino, D.J.; Chen, T.B.; Kling, P.J.; Kunkel, K.A.; Springer, J.P.; Hirshfield, J.J. *J. Med.Chem.*, **1988**, *31*, 2235; (b) Nicolaou, K.C.; Pfefferkorn, A.; Mitchell, H.J.; Roecker, A.J.; Barluenga, S.; Cao, G. -Q.; Affleck, R.L.; Lillig, J.E. *J. Am. Chem. Soc.*, **2000**, *122*, 9939; (c) Horton, D.A.; Bourne, G.T.; Smythe, M.L. *Chem. Rev.*, **2003**, *103*, 893
  28. Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P.J.; McKerrow, J.H.; Chibale, K. *Bioorg. Med. Chem. Lett*, **2003**, *13*, 3527
  29. (a) Oishi, H.; Noto, T.; Sasaki, H.; Suzuki, K.; Hayashi, T.; Okazaki, H.; Ando, K.; Sawada, M. *J. Antibiot. (Tokyo)*, **1982**, *35*, 391; (b) Noto, T.; Miyakawa, S.; Oishi, H.; Endo, H.; Okazaki, H. *J. Antibiot. (Tokyo)*, **1982**, *35*, 401



30. Kremer, L.; Douglas, J.D.; Baulard, A.R.; Morehouse, C.; Guy, M.R.; Alland, D.; Dover, L.G.; Lakey, J.H.; Jacobs, W.R. Jr.; Brennan, P.J.; Minnikin, D.E.; Besra, G.S. *J. Biol. Chem.*, **2000**, *275*, 16857
31. Hamada, S.; Fujiwara, T.; Shimauchi, H.; Ogawa, T.; Nishihara, T.; Koga, T.; Nehashi, T.; Matsuno, T. *Oral Microbiol. Immunol.*, **1990**, *5*, 340
32. (a) Slayden, R.A.; Lee, R.E.; Armour, J.W.; Cooper, A.M.; Orme, I.M.; Brennan, P.J.; Besra, G.S. *Antimicrob. Agents Chemother.*, **1996**, *40*, 2813; (b) Choi, K.H.; Kremer, L.; Besra, G.S.; Rock, C.O. *J. Biol. Chem.*, **2000**, *275*, 28201; (c) Douglas, J.D.; Senior, S.J.; Morehouse, C.; Phetsukuri, B.; Campbell, I.B.; Besra, G.S.; Minnikin, D.E. *Microbiology*, **2002**, *148*, 3101; (d) Senior, S.J.; Illarionov, P.A.; Gurcha, S. S.; Campbell, I.B.; Schaeffer, M.L.; Minnikin, D.E.; Besra, G.S. *Bioorg. Med. Chem. Lett.*, **2003**, *13*, 3685; (e) Senior, S.J.; Illarionov, P.A.; Gurcha, S.S.; Campbell, I.B.; Schaeffer, M.L.; Minnikin, D.E.; Besra, G.S. *Bioorg Med Chem. Lett.*, **2004**, *14*, 373; (f) Kamal, A.; Shaik, A.A.; Sinha, R.; Yadav, J.S.; Arora, S.K. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 1927; (g) Kim, P.; Zhang, Y-M.; Shenoy, G.; Nguyen, Q-A.; Boshoff, H.I.; Manjunatha, U.H.; Goodwin, M.B.; Lonsdale, J.; Price, A.C.; Miller, D.J.; Duncan, K.; White, S.W.; Rock, C.O.; Barry, C.E. III; Dowd, C.S. *J. Med. Chem.*, **2006**, *49*, 159; (h) Bhowruth, V.; Brown, A.K.; Senior, S.J.; Snaith, J.S.; Besra, G.S. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 5643
33. (a) Waller, R.F.; Keeling, P.J.; Donald, R.G.K.; Striepen, B.; Handman, E.; Lang-Unnasch, N.; Cowman, A.F.; Besra, G.S.; Roos, D.S.; McFadden, G.I. *Proc. Natl. Acad. Sci. U.S.A.*, **1998**, *95*, 12352; (b) Waters N.C.; Kopydlowski, K.M.; Guszczynski, T.; Wei, L.; Sellers, P.; Ferlan, J.T.; Lee, P.J.; Li, Z.; Woodard, C.L.; Shallom, S.; Gardner, M.J.; Prigge, S.T. *Mol. Biochem. Parasitol.*, **2002**, *123*, 85; (c) Jones, S.M.; Urch, J.E.; Brun, R.; Harwood, J.L.; Berry, C.; Gilbert, I.H. *Bioorg. Med. Chem.*, **2004**; *12*; 683; (d) Jones, S.M.; Urch, J.E.; Kaiser, M.; Brun, R.; Harwood, J.L.; Berry, C.; Gilbert, I.H. *J. Med. Chem.*, **2005**, *48*, 5932; (e) Morita, Y.S.; Paul, K.S.; Englund, P.T. *Science*, **2000**, *288*, 140
34. Heath R. J.; White, S. W.; Rock, C. O. *Prog. Lipid Res.*, **2001**, *40*, 467
35. (a) Wang, C-L. J.; Salvino, J.M. *Tetrahedron Lett.*, **1984**, *25*, 5246; (b) Chambers, M.S.; Thomas, E.J. *J. Chem. Soc. Chem. Commun.*, **1989**, 23; (c) Chambers, M.S.; Thomas, E.J. *J. Chem. Soc. Perkin Trans. 1*, **1997**, 417; (d) Ohata, K.; Terashima, S. *Tetrahedron Lett.*, **2006**, *47*, 2787; (e) McFadden, J.M.; Frehywot, G.L.; Townsend, C.A. *Org. Lett.*, **2002**, *4*, 3859; (f) Toyama, K.; Tauchi, T.; Mase, N.; Yoda, H.; Takabe, K. *Tetrahedron*

- Letts.*, **2006**, *47*, 7163; (g) Dormann, K.L.; Brückner, R. *Angew. Chem. Int. Ed.*, **2007**, *46*, 1160
36. McFadden, J.M.; Medghalchi, S.M.; Thupari, J.N.; Pinn, M.L.; Vadlamudi, A.; Miller, K.I.; Kuhajda, F.P.; Townsend, C.A. *J. Med. Chem.*, **2005**, *48*, 946
37. (a) Zhou, W.; Simpson, J.P.; McFadden, J.M.; Townsend, C.A.; Medghalchi, S.M.; Vadlamudi, A.; Pinn, M.L.; Ronnett, G.V.; Kuhajda, F.P. *Cancer Res.*, **2003**, *63*, 7330; (b) Rashid, A.; Pizer, E.S.; Moga, M.; Milgraum, L.Z.; Zahurak, M.; Pasternack, G.R.; Kuhadja, F.P.; Hamilton, S.R. *Am. J. Pathol.*, **1997**, *150*, 201; (c) Loftus, T.M.; Jaworsky, D.E.; Frehywot, G.L.; Townsend, C.A.; Ronnett, G.V.; Lane, D.V.; Kuhadja, F.P. *Science*, **2000**, *288*, 2379
38. (a) Guo, Y.; Chen, F. *Zhongcaoyao*, **1986**, *17*, 8; (b) Wei, L.; Wang, Q.; Liu, X. *Yaowu Fenxi Zazhi*, **1982**, *2*, 288; (c) Ischia, M.; Palumbo, A.; Prota, G. *Tetrahedron*, **1988**, *44*, 6441
39. Lee, D.; Long, S.A.; Murray, J.H.; Adams, J.L.; Nuttall, M.E.; Nadeau, D.P.; Kikly, K.; Winkler, J.D.; Sung, C.M.; Ryan, M.D.; Keller, P.M.; Levy, M.A.; DeWolf, W.E. Jr. *J. Med. Chem.*, **2001**, *44*, 2015
40. Webber, S.E.; Tikhe, J.; Worland, S.T.; Fuhrman, S.A.; Hendrickson, T.F.; Matthews, D.A.; Love, R.A.; Patrick, A.K.; Meador, J.W.; Ferre, R.A.; Brown, E.L.; DeLisle, D.M.; Ford, C.E.; Binford, S.L. *J. Med. Chem.*, **1996**, *39*, 5072
41. (a) Krishnan, S.S.; Pandeya, N.S.; Stables, J.P.; Ramesh, A. *Eur. J. Pharm. Sci.*, **2002**, *16*, 129; (b) Daisley, R.W.; Shah, V.K. *J. Pharm. Sci.*, **1984**, *73*, 407; (c) Varma, R.S.; Nobles, W.L. *J. Med. Chem.*, **1967**, *10*, 972; (d) Sridhar, S.K.; Ramesh, A. *Biol. Pharm. Bull.*, **2001**, *24*, 1149; (e) Piscopo, E.; Diurno, M.V.; Gogliardi, R.; Cucciniello, M.; Veneruso, G. *Boll. Soc. Ital. Biol. Sper.*, **1981**, *63*, 827; (f) Varma, R.S.; Khan C.A. *Pol. J. Pharmacol. Pharm.*, **1977**, *29*, 549; (g) Sarciron, S.E.; Audin, P.; Delebre, I.; Gabrion, C.; Petavy, A.F.; Paris, J. *J. Pharm. Sci.*, **1993**, *82*, 605; (h) Pandeya, S.N.; Sriram, D.; De Clercq, E.; Panne-Couque, C.; Witurouw, M. *Indian J. Pharm. Sci.*, **1998**, *60*, 207; (i) Karali, N.; Terzioğlu, N.; Gürsoy, A. *Arzneim.-Forsch.*, **1998**, *48*, 758; (j) Sriram, D.; Yogeewari, P.; Gopal, G. *Eur. J. Med. Chem.*, **2005**, *40*, 1373; (k) Karali, N.; Gürsoy, A.; Kandemirli, F.; Shvets, N.; Kaynak, F.B.; Özbey, S.; Kovalishyn, V.; Dimoglo, A. *Bioorg. Med. Chem.*, **2007**, *15*, 5888; (l) Karali, N. *Eur. J. Med. Chem.*, **2002**, *37*, 909
42. Terzioğlu, N.; Karali, N.; Gürsoy, A.; Pannecouque, C.; Leysen, P.; Paeshuyse, J.; Neyts, J.; De Clercq, E. *ARKIVOC*, **2006**, *i*, 109
43. (a) Sumpter, W.C. *Chem. Rev.*, **1954**, *34*, 407; (b) Popp, F.D.; in *Advance in Heterocyclic*

- Chemistry*, Katritzky, A.R.; Bolton, A.J. (eds), vol. 18, **1975**, p. 1-58; (c) Da Silva, J.F.M.; Garden, S.J.; Pinto, A.C. *J. Braz. Chem.*, **2001**, *12*, 273; (d) Shvekhgeimer, M.-G.A. *Chemistry of Heterocyclic Compounds*, **1996**, *32*, 249
44. (a) Liu, M.; Wilairat, P.; Go, M.-L. *J. Med. Chem.*, **2001**, *44*, 4443; (b) Mishra, N.; Arora, P.; Kumar, B.; Mishra, L.C.; Bhattacharya, A.; Awasthi, S.K., Bhasin, V.K. *Eur. J. Med. Chem.*, **2008**, *43*, 1530
45. Herencia, F.; Ferrandiz, M.L.; Ubeda, A.; Domiguez, J.N.; Charris, J.E.; Lobo, G.M.; Alcaraz, M.J. *Bioorg. Med. Chem.*, **1998**, *8*, 1169
46. Nielsen S.F.; Christensen, S.B.; Cruciani, G.; Kharazmi, A.; Lijefors, T. *J. Med. Chem.*, **1998**, *41*, 4819
47. (a) Nielsen, S.F.; Bosen, T.; Larsen, M.; Schonning, K.; Kromann, H. *Bioorg. Med. Chem.*, **2004**, *12*, 3047; (b) Nielsen, S.F.; Larsen, M.; Bosen, T.; Schonning, K.; Kromann, H. *J. Med. Chem.*, **2005**, *48*, 2667; (c) Liu, X.L.; Xu, Y.J.; Go, M.L. *Eur. J. Med. Chem.*, **2008**, *43*, 1681; (d) Ansari, F.L.; Nazir, S.; Noureen, H.; Mirza, B. *Chemistry & Biodiversity*, **2005**, *2*, 1656
48. (a) Bhakuni, D.S.; Chaturvedi, J. *J. Nat. Prod.*, **1984**, *47*, 585; (b) Tsuchiya, H.; Sato, M.; Akagiri, M.; Takagi, N.; Tanaka, T.; Iinuma, M. *Pharmazie*, **1994**, *49*, 756
49. (a) Go, M.L.; Wu, X.; Liu, X.L. *Curr. Med. Chem.*, **2005**, *12*, 483; (b) Edwards, M.L.; Stemerick, D.M.; Sunkara, P.S. *J. Med. Chem.*, **1990**, *33*, 1948; (c) Ducki, S.; Forrest, R.; Hadfield, J.A.; Kendall, A.; Lawrence, N.J.; MCGown, A.T.; Rennison, D. *Bioorg. Med. Chem. Lett.*, **1998**, *8*, 1051
50. (a) Chen, M.; Theander, T.G.; Christensen, B.S.; Hviid, L.; Zhai, L.; Kharazmi, A. *Antimicrob. Agents Chemother.*, **1994**, *38*, 1470; (b) Domínguez, J.N.; León, C.; Rodrigues, J.; Gamboa de Domínguez, N.; Gut, J.; Rosenthal, P.J. *J. Med. Chem.*, **2005**, *48*, 3654; (c) Li, R.; Kenyon, G.L.; Cohen, F.E.; Chen, X.; Gong, B., Domínguez, J.N.; Davidson, E.; Kurzban, R.E.; Miller, R.E.; Nuzum, E.O.; Rosenthal, P.J.; McKerrow, J.H. *J. Med. Chem.*, **1995**, *38*, 5031; (d) Domínguez, J.N.; Charris, J.E.; Lobo, G.; Gamboa de Domínguez, N.; Moreno, M.M.; Riggione, F.; Sanchez, E.; Olson, J.; Rosenthal, P.J. *Eur. J. Med. Chem.*, **2001**, *36*, 555; (e) Larsen, M.; Kromann, H.; Kharazmi, A.; Nielsen, S.F. *Bioorg. Med. Chem. Lett.*, **2005**, *15*, 4858; (f) Li, R.; Chen, X.; Gong, B.; Selzer, P.M.; Li, Z.; Davidson, E.; Kurzban, G.; Miller, R.E.; Nuzum, E.O.; McKerrow, J.H.; Fletterick, R.J.; Gilmor, S.A.; Craik, C.S.; Kuntz, I.D.; Cohen, F.E.; Kenyon, G.L. *Bioorg. Med. Chem.*, **1996**, *4*, 1421; (g) Go, M.L.; Liu, M.; Wilairat, P.; Rosenthal, P.J.; Saliba, K.J. *Antimicrob. Agents Chemother.*, **2004**, *48*, 3241

51. (a) Lin, Y.-M.; Flavin, M.T.; Zhou, L.-M.; Nie, W.; Chen, F.-C. *Bioorg. Med. Chem.*, **2002**, *10*, 2795; (b) Friis-Moller, A.; Chen, M.; Fursted, K.; Christensen, S.B.; Kharazmi, A. *Planta Med.*, **2002**, *68*, 416
52. (a) Kirk, K. *Physiol. Rev.*, **2001**, *81*, 495; (b) Ginsburg, H.; Kutner, S.; Krugliak, M.; Cabantchik, Z.I. *Mol. Biochem. Parasitol.*, **1985**, *14*, 313
53. (a) Garcia-Raso, A. *Synthesis*, **1984**, 502; (b) Climent, M.J.; Corma, A.; Iborra, S.; Primo, J. *J. Catal.*, **1995**, *151*, 60; (c) Sebt, S.; Solhy, A.; Tahir, R.; Boulaajaj, S.; Mayoral, J.A.; Fraile, J.M.; Kossir, A.; Oumimoun, H. *Tetrahedron Lett.*, **2001**, *42*, 7953; (d) Daskiewicz, J.B.; Comte, G.; Barron, D.; Pietro, A.D.; Thomasson, F. *Tetrahedron Lett.*, **1999**, *40*, 7095; (e) Calloway, N.O.; Green, L.D. *J. Am. Chem. Soc.*, **1937**, *59*, 809; (f) Sz'ell, T.; Sohar, I. *Can. J. Chem.*, **1969**, *47*, 1254; (g) Mazza, L.; Guaram, A. *Synthesis*, **1980**, 41; (h) Narender, T.; Reddy, K.P. *Tetrahedron Lett.*, **2007**, *48*, 3177
54. (a) Li, J.T.; Yang, W.Z.; Wang, S.X.; Li, S.H.; Li, T.S. *Ultrason. Sonochem.*, **2002**, *9*, 237; (b) Le Gall, E.; Texier-Boullet, F.; Hamelin, J. *Synth. Commun.*, **1999**, *29*, 3651; (c) Stoyanov, E.T.; Champavier, Y.; Simon, A.; Basly, J.P. *Bioorg. Med. Chem. Lett.*, **2002**, *12*, 2685
55. Eddarir, S.; Cotello, N.; Bakkour, Y.; Rolando, C. *Tetrahedron Lett.*, **2003**, *44*, 5359
56. Fan, X.S.; Zhang, Y.M. *Chin. J. Chem.*, **2002**, *20*, 198
57. (a) Mann, J. *In Secondary Metabolism*, Clarendon Press, Oxford, **1987**; (b) Tietze, L.F.; Bell, H.P.; Chandrasekhar, S. *Angew. Chem. Int. Ed.*, **2003**, *42*, 3996
58. Walsh, J.J.; Coughlan, D.; Heneghan, N.; Gaynor, C.; Bell, A. *Bioorg. Med. Chem. Lett.*, **2007**, *17*, 3599
59. (a) Haynes, R.K. *Curr. Top. Med. Chem.*, **2006**, *6*, 509; (b) Foley, M.; Tilley, L. *Pharmacol. Ther.*, **1998**, *79*, 55
60. Gademan, K. *Chimia*, **2006**, *60*, 841
61. Sriram, D.; Yogeewari, P.; Gopal, G. *Eur. J. Med. Chem.*, **2005**, *40*, 1373
62. (a) Barbachyn, M.R. *in Annual Reports in Medicinal Chemistry*, Macor, J.E. (ed.), vol. 43, Elsevier Inc., U.S.A., **2008**, p. 281; (b) Robertson, G.T.; Bonventre, E.J.; Doyle, T.B.; Du, Q.; Duncan, L.; Morris, T.W.; Roche, E.D.; Yan, D.; Lynch, S.A. *Antimicrob. Agents Chemother.*, **2008**, *52*, 2313

## Chapter three

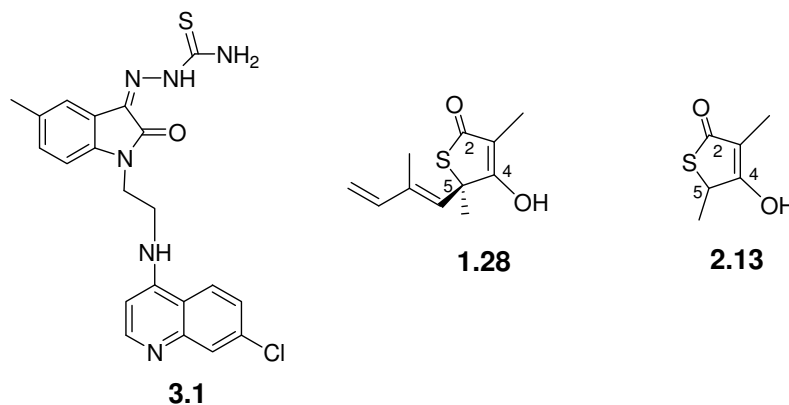
### DESIGN, SYNTHESIS AND CHARACTERIZATION OF THIOLACTONE-ISATIN HYBRIDS

#### 3.1. Introduction

The focus of this chapter is on the design, synthesis and characterization of an exploratory series of novel compounds obtained by hybridizing bioactive natural product scaffolds. These are the thiolactone and isatin ring systems of the naturally occurring compounds thiolactomycin and isatin. The synthetic strategy employed for these natural product-like hybrids involved simple standard chemistry, which led to the discovery of novel molecules bearing a novel tetracyclic ring system. These structurally intriguing molecules formed the basis of a more focused SAR study. All of the analogues synthesized were profiled for *in vitro* antiplasmodial activity against chloroquine resistant (W2) and chloroquine sensitive (D10) strains, inhibitory activity against the *P. falciparum* cysteine protease falcipain-2 and antimycobacterial activity against the sensitive H<sub>37</sub>Rv strain of *M. tuberculosis*.

#### 3.2. Rationale

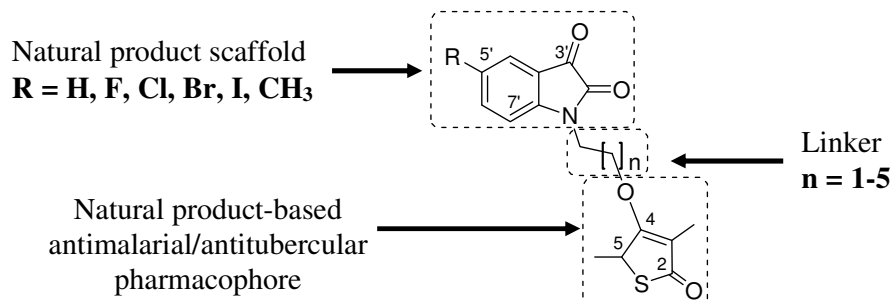
The term pharmacophore was first described by Paul Ehrlich in 1909 as "a molecular framework that carries (*phoros*) the essential features responsible for a drug's (*pharmakon's*) biological activity".<sup>1</sup> Pharmacophore hybridization is believed to be analogous to conventional combination therapy with the exception that the two drugs are covalently linked and available as a single entity<sup>2</sup>. This strategy renders further appeal in that it facilitates expeditious SAR studies since the library so generated is enriched in pharmacophores thus precluding the need for large libraries and concomitantly shortens the time needed to identify potential leads.<sup>2</sup> It has been successfully employed in our labs in the design of natural product-like hybrids modelled on isatin and the 7-chloroquinoline moiety. Identified from this study is the thiosemicarbazone derivative **3.1** (fig. 3.1) which showed promising *in vitro* activity against the CQR and CQS strains of *P. falciparum* with IC<sub>50</sub> values of 51nM and 79nM, respectively.<sup>3</sup>



**Figure 3.1:** Chemical structures of compound **3.1**, thiolactomycin **1.28** and thiolactone **2.13**

With regard to the selection of scaffolds, our first choice fell on thiolactomycin (**1.28**, fig. 3.1) which is known for its remarkable selectivity and more so its activity against a wide spectrum of pathogenic organisms as discussed in section 2.2.3.1. Additionally, the availability of detailed SAR studies on thiolactomycin analogues proved invaluable to our drug design effort. Another scaffold selected is the natural product, isatin which is present in a number of proprietary molecules and synthetic drugs.<sup>4</sup> Structurally, the isatin scaffold provides a hydrophobic aromatic ring which has the potential of binding to hydrophobic sites of target(s). Also, the wide spectrum of pharmacological activity exerted by isatin derivatives (section 2.2.3.2) confirms the privileged status of this scaffold and thus renders it worthy for inclusion in hybrid construction.

The need to consider cost-effectiveness in the design of drugs for infectious diseases is borne of the fact that these diseases are prevalent in poor developing countries.<sup>5</sup> Due consideration was given to the above in designing this series of compounds. The general structure of the compounds synthesized is shown in figure 3.2. Although modelled on **1.28** these compounds are devoid of the hydrophobic side chain at C-5 which is represented by an isoprenoid moiety in thiolactomycin. We hypothesized that these novel hybrids will display improved therapeutic properties. Some of the key structural and chemical features targeted for investigation through structure-activity relationship studies are summarized below.



**Figure 3.2:** Rationale for design of target compounds

- (i) It was envisaged that a non-hydrolyzable alkane linker would contribute towards enhancing the lipophilicity of the compounds to be synthesized. Lipophilicity, and therefore permeability, which refers to the ability of compounds to cross biological membranes is of particular importance in antimalarial<sup>6a</sup> and more so in antitubercular<sup>6b</sup> drug design and was therefore given due consideration in our drug design endeavour. SAR studies will probe the effect of the linker length on biological activity for this series.
- (ii) The thiolactone ring system (**2.13**, fig. 3.1) structurally resembles the transition state of the thiomalonate intermediate in the chain elongation step of fatty acid biosynthesis (section 1.4.2.2.3). As delineated from previous SAR studies, 4-*O*-alkylation of **2.13** was envisaged to afford analogues with enhanced antimalarial activity<sup>7</sup> and antitubercular<sup>8</sup> activity. The synthetic accessibility of C-4 derivatized analogues compared to C-5 analogues provides a further incentive, that is, despite the fact that synthesis of the latter has been the focus of intense research (section 2.2.3.1).
- (iii) By incorporating the isatin scaffold, we envisaged the transfer of drug-like properties to compounds synthesized. SAR will set out to investigate the substituent effect at C-5' (fig. 3.2) and would further explore *N*-alkylation of the scaffold which was shown to be a common and rewarding diversification route.<sup>2</sup>
- (iv) Overall it was expected that SAR would reveal regions of the target molecule important for retention or improvement of activity.

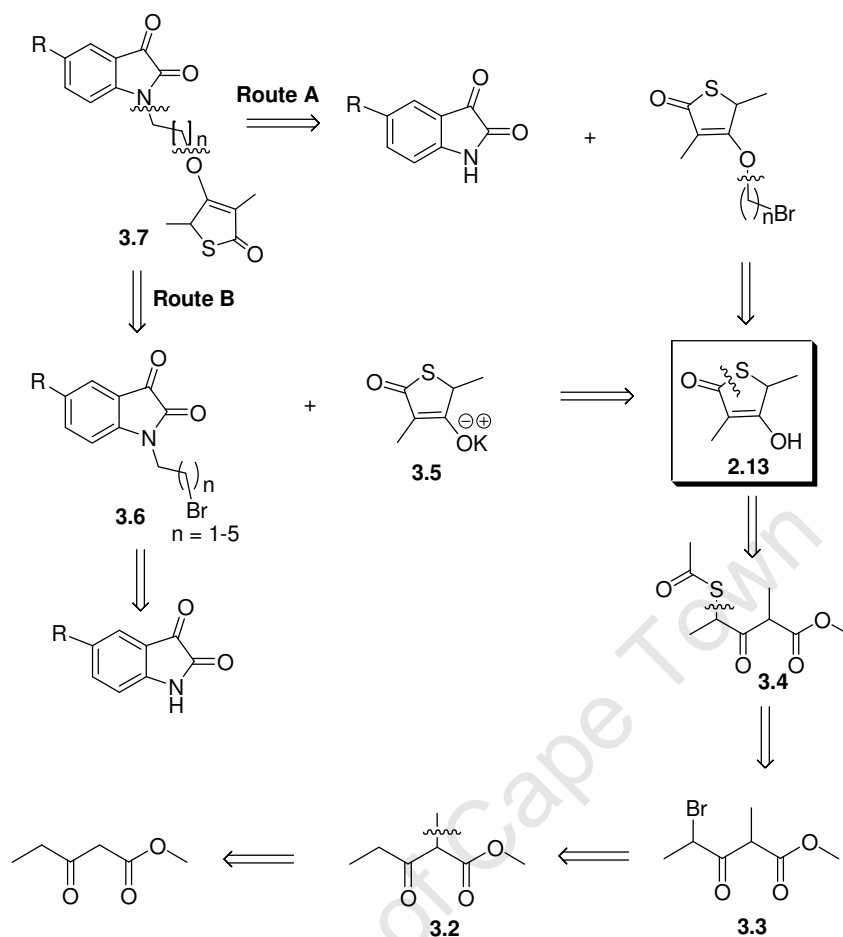
### 3.3. Chemical Synthesis

#### 3.3.1. Retrosynthetic Analysis

Scheme 3.1 shows that the target compounds can be obtained by two synthetic pathways. In route A synthesis of the target molecules is envisioned from an *O*-alkylated thiolactone intermediate and commercially available isatin/5-substituted isatin. Route B offers access to the desired hybrids through the reaction of the *N*-alkylated isatin/5-substituted isatin **3.6** with thiolactone **2.13**. The latter can be obtained from a base-mediated cyclization of the thioester **3.4** which in turn can be prepared from an  $\alpha$ -bromo compound **3.3**. Further analysis of **3.3** leads to methyl-2-methyl-3-oxopentanoate **3.2** which can be derived from the methylation of the commercially available methyl propionyl acetate.

Although both routes can afford the desired hybrids, route B offers several advantages such as straightforwardness and the ease of preparation of **3.6**. Also no additional base is needed in this approach, which is of particular importance considering that alkaline bases reportedly contribute towards the hydrolysis (*S*-acyl cleavage) of the thiolactone ring system.<sup>9a</sup> More importantly, with the benefit of hindsight, this route (B) led to the discovery of an unexpected and novel tetracyclic by-product and thus led to diverse thiolactomycin analogues. Further appeal to the use of route B resided in the fact that thiolactone **2.13**, which is obtained through a laborious 4-step reaction can participate as the limiting reagent and the easy to synthesize **3.6** as the excess reagent.



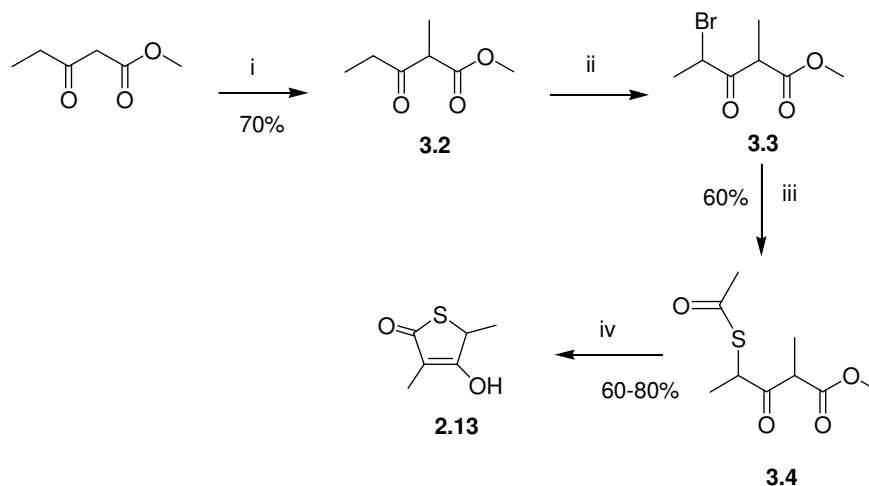


**Scheme 3.1:** Retrosynthesis of the isatin-thiolactone hybrids

### 3.3.2. Synthesis

#### 3.3.2.1. Synthesis of Thiolactone

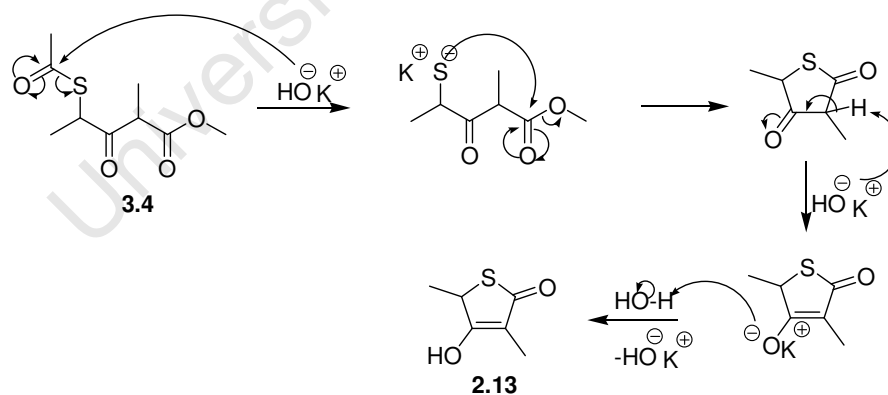
The thiolactone intermediate **2.13** was prepared using the synthesis methodology developed by Benary<sup>10</sup> and revised by Wang and Salvino.<sup>11</sup> As outlined in scheme 3.2, it starts with  $\alpha$ -methylation of commercially available methyl propionyl acetate to give 2-methyl-3-oxopentanoic acid methyl ester **3.2** in 70% yield after purification using column chromatography. Selective bromination of **3.2** afforded the bromo compound **3.3**, which was carried through to the next step without purification. The next step involved the treatment of **3.3** with thioacetic acid in the presence of triethylamine which afforded the thioester **3.4** in 60% yield. This was followed by the reaction of the **3.4** with 2 eq of KOH and afforded the key intermediate **2.13** as a racemic mixture in 60-80% yield after trituration with DCM, EtOAc and hexane. The melting point and spectroscopic data of **2.13** correlated well with that reported in literature.<sup>11</sup>



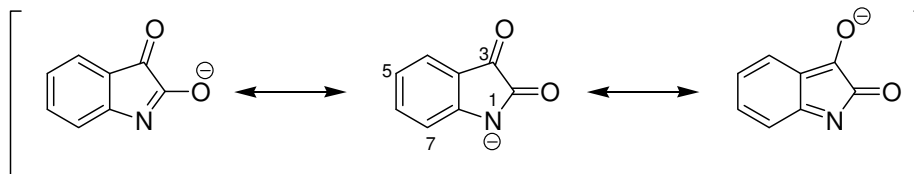
**Scheme 3.2:** Reagents and conditions: (i)  $\text{K}_2\text{CO}_3$ , THF, reflux, 4 h then  $\text{CH}_3\text{I}$ , 0-25°C, 15 h; (ii)  $\text{Br}_2$ ,  $\text{CHCl}_3$ , 1 h at 0°C and 15 h at 25°C; (iii)  $\text{CH}_3\text{COSH}$ ,  $\text{Et}_3\text{N}$ ,  $\text{CH}_2\text{Cl}_2$ , 0-25°C, 6 h; (iv)  $\text{KOH}$ ,  $\text{EtOH-H}_2\text{O}$ , 25°C, 4 h

### 3.3.2.1.1. Mechanistic Comments

The proposed mechanism for ring closure of the thioester intermediate **3.4** is shown in scheme 3.3. It involves the chemoselective hydrolysis of the thioester followed by an intramolecular acylation of the released thiolate to form the keto-lactone. Base-mediated enolization generates **2.13**.

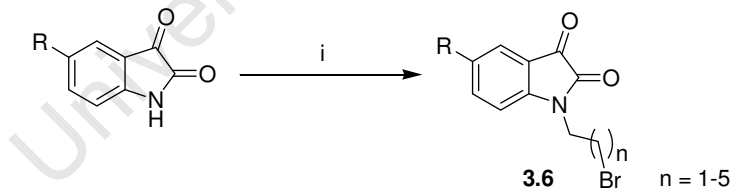


**Scheme 3.3:** Proposed mechanism for the base-mediated ring closure

3.3.2.2. Synthesis of *N*-alkylated Isatin/5-Substituted Isatin Intermediates

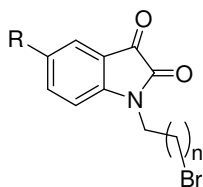
**Figure 3.4:** Resonance stabilization of the isatin anion

The synthetic versatility of the isatin scaffold has been the topic of several reviews. As mentioned earlier, alkylation and acylation appear to be common reactions and in most instances represent the first steps in the synthesis of more complex heterocycles.<sup>3,12</sup> Regarding the chemoselectivity of these reactions, it is reported that the treatment of the silver salts of isatin with alkylating agents yields the easily hydrolysable C-2 *O*-alkyl derivatives whereas the sodium and potassium salts of isatin generates the more stable *N*-alkyl derivatives.<sup>3,12b</sup> However, in another report the reaction of the sodium salt of isatin with the  $\gamma$ -butyrolactone<sup>13</sup> and allyl bromide<sup>14</sup>, respectively yielded the C-2 *O*-alkylated product as by-products. The resonance-stabilized isatide anion (fig. 3.4) can be prepared by basic removal of the acidic *N*-hydrogen in the pyrrole ring. One of the bases commonly used is NaH.<sup>15</sup> The use of potassium fluoride on alumina (KF-Al<sub>2</sub>O<sub>3</sub>)<sup>2b</sup>, K<sub>2</sub>CO<sub>3</sub><sup>16</sup> and CaH<sub>2</sub><sup>17</sup> in DMF has also been reported.



**Scheme 3.4:** Reagents and conditions: (i) NaH, DMF, dibromoalkane, 0-25°C for 1 h, then 60°C for 24 h

The synthesis of **3.6** involved simple chemistry (scheme 3.4). The sodium salt of the commercially available C-5-substituted/unsubstituted isatins was generated *in situ* using NaH in anhydrous DMF followed by treatment with a large excess of the appropriate dibromoalkane. Recrystallization from MeOH afforded the intermediates **3.6** in good to excellent yields as shown in table 3.1. The spectroscopic data of these intermediates correlate with literature data.

**Table 3.1:** Isolated yields and melting points of *N*-alkylated isatin/5-substituted isatin intermediates 3.6a-w

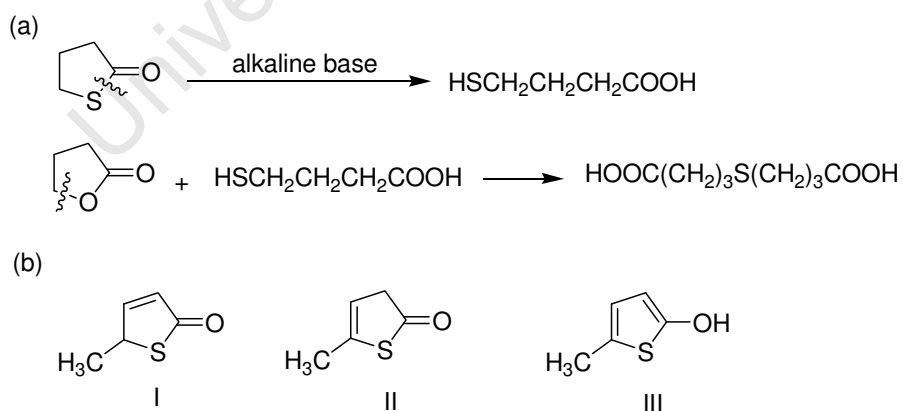
Compound	R	n	Yield(%)	m.p. (°C)
3.6a	H	1	95	128
3.6b	Br	1	92	143-144
3.6c	Cl	1	90	114-116
3.6d	F	1	83	102-104
3.6e	I	1	89	153-154
3.6f	CH <sub>3</sub>	1	98	117-119
3.6g	H	2	86	85
3.6h	H	3	100	52
3.6i	F	3	63	98
3.6j	Cl	3	76	93
3.6k	Br	3	72	87
3.6l	I	3	61	82-83
3.6m	CH <sub>3</sub>	3	63	114-115
3.6n	NO <sub>2</sub>	3	54	260
3.6o	H	4	97	- <sup>a</sup>
3.6p	Cl	4	62	93-95
3.6q	Br	4	78	103
3.6r	I	4	78	89
3.6s	H	5	100	42
3.6t	I	5	75	99-101
3.6u	Cl	5	70	57
3.6v	Br	5	66	64-65
3.6w	F	5	57	36

<sup>a</sup>Compound isolated as an oil

### 3.3.2.3. Thiolactones (Thiotetronic acids)

The chemical properties of the thiolactone ring system accounts for yields obtained for the target molecules synthesized. It was therefore deemed necessary to precede the discussion of the synthesis of the latter with a brief account on thiolactones and to extract from literature some of the challenges encountered in the synthesis of thiolactomycin analogues. Thiolactones (or thiotetronic acids) are described as “internal esters of mercapto-acids”.<sup>9</sup> Alpha - and  $\beta$ -thiolactones which represent the 3- and 4-membered rings, respectively, were discovered in the 1950s whereas the 5- and 6-membered (or  $\gamma$ - and  $\delta$ -thiolactones) were more extensively investigated by then. Various synthesis methods have been reported for the different thiolactones. For this project, however, we were interested in the reactivity and stability of  $\gamma$ -thiolactones.

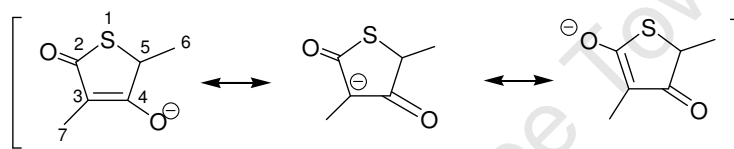
A review by Lin'kova and coworkers<sup>9a</sup> revealed that hydrolysis of  $\gamma$ - and  $\delta$ -thiolactones is most typically mediated by alkaline reagents such as sodium ethoxide, sodium hydride and sodium metal and proceed through cleavage of the *S*-acyl bond. Reportedly, the same cleavage is observed when  $\gamma$ - and  $\delta$ -thiolactones are subjected to aminolysis and alcoholysis. In demonstration, Reppe and coworkers<sup>9b,c</sup> reacted a saturated  $\gamma$ -thiolactone with a  $\gamma$ -lactones in the presence of an alkaline base [fig. 3.5(a)]. A correction of the erroneous interpretation made by the authors is that the salt of the mercapto acid generated by cleavage of the *S*-acyl bond of thiolactone causes cleavage of the C-O bond of the lactone.<sup>9a</sup>



**Figure 3.5:** (a) Alkaline hydrolysis of  $\gamma$ -thiolactone and  $\gamma$ -lactone<sup>9a</sup>; (b) Tautomeric forms of 5-methyl-2-hydroxythiophene<sup>18</sup>

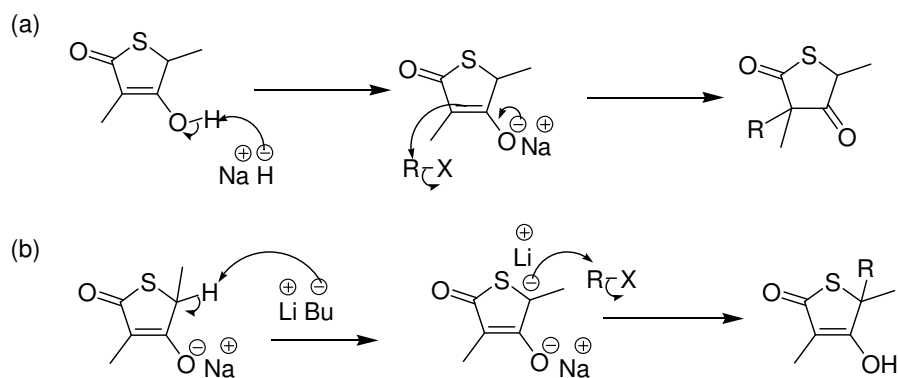
Substituted unsaturated  $\gamma$ -thiolactones are of particular relevance to the project. In the 1960s and early 1970s a series of investigative studies on unsaturated  $\gamma$ -thiolactones were undertaken and a bulk of the work focussed on their tautomeric properties.<sup>18,19</sup> Although three tautomeric forms are possible for 5-methyl-2-hydroxythiophene [III, fig. 3.5(b)] earlier studies confirmed the presence of only two forms being 5-methyl-3-thiolen-2-one [I, fig. 3.5(b)] and 5-methyl-4-thiolen-2-one [II, fig. 3.5(b)].<sup>18</sup> Research into 4-hydroxy-3,5-dimethylthiophen-2-(5*H*)-one ( $\gamma$ -thiolactone or thiolactone) **2.13** gained momentum with the discovery of its presence in pharmacologically important natural products. Alkylation of **2.13** appears to be the main synthesis route for the preparation of diverse analogues.

### 3.3.2.3.1. Alkylation of Thiolactone



**Figure 3.6:** Resonance stabilization of the thiolate anion

Cederlund and Hörnfeldt<sup>18</sup> were among the first to show that alkylation of unsaturated  $\gamma$ -thiolactone yields an isomeric mixture of compounds. This observation was attributed to the resonance stabilization of the thiolate anion as shown in figure 3.6. Douglas *et al* prepared racemic thiolactomycin analogues as potential antitubercular agents by utilizing different bases [NaH, lithium bis(trimethylsilyl)amide (LiHMDS), *t*-BuLi], and base combination (NaH/*t*-BuLi and NaHMDS/*t*-BuLi) for the deprotonation of **2.13**.<sup>20</sup> Intriguingly, reaction of the latter with an alkyl halide in the presence of 1 eq of NaH led to the formation of the 3-alkyl-3,5-dimethyl-thiophene-2,4-dione [fig. 3.7(a)]. With the base combination, NaH/*t*-BuLi it was found that whilst the relatively milder base NaH removed the more acidic enol proton, the stronger base, *t*-BuLi removed the proton at C-5 to generate the dianion [fig. 3.7 (b)]. The more nucleophilic center of the dianion reacted preferentially with appropriately substituted alkylating agents and afforded the desired 5-substituted analogues in 11-36% yield.<sup>20</sup> Kamal and coworkers, synthesized *O*-alkylated thiolactomycin derivatives with different alkane linkers using K<sub>2</sub>CO<sub>3</sub> in acetone.<sup>8</sup> No mention of yields was made in this paper.



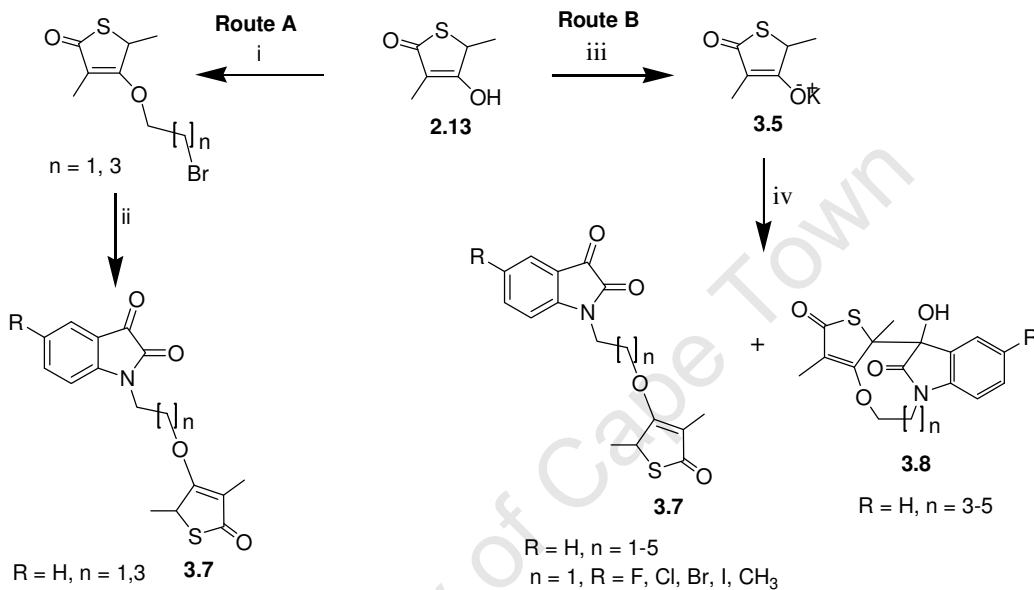
**Figure 3.7:** Base-catalyzed alkylation of **2.13** with (a) NaH and (b) n-BuLi<sup>20</sup>

For the synthesis of their first series of C-3 and C-5 thiolactomycin analogues, Jones and coworkers<sup>21</sup> treated the thiolactone dianion (prepared with the base combination NaH/n-BuLi) with different halides to afford the analogues in 4-22% yield. In recognition of the sensitivity of **2.13** to strongly basic conditions and the contribution thereof to the observed low yields, they decided to modify the alkylation procedure by constructing the appropriately substituted (thioester) carbon skeleton prior to cyclisation or ‘thiolactonization’.<sup>7</sup> In another study, Takabe and coworkers<sup>22</sup> attempted *O*-alkylation of **2.13** using NaH and CH<sub>3</sub>I at room temperature. Although the reaction proceeded regioselectively, only 9% of the product was obtained after 144 hours.

Alkylation procedures which yields the 4-alkoxy derivative of **2.13** exclusively, were developed by two independent research groups. Wengel and coworkers<sup>23</sup> reacted a derivative of **2.13** with tetrabutylammonium hydroxide (TBAH) and an appropriate dialkyl sulfate (phase transfer conditions) whereas Schobert and Siegfried<sup>24</sup> utilized appropriately substituted isourea. To synthesize a 4-alkoxy derivative of **2.13**, Shenoy and coworkers<sup>25</sup> used the abovementioned procedures. They however, obtained a mixture of 2- and 4-methoxy-3,5-dimethylthiotetronic acid in ratios of 1:6.4 (with the Wengel method) and 1:2.3 (with the Schobert method). The first step of the chemoenzymatic synthesis of **1.28** developed by Takabe and co-workers involved the protection of the C-4 hydroxyl group of **2.13**. They utilized the procedure developed by Wengel and Schobert for the methylation of **2.13**. The reaction proceeded chemoselectively - consistent with the findings of Shenoy - and afforded an isomeric mixture (4-methoxy:2-methoxy) in the ratio 82:18.<sup>22</sup> In another study, C-4 *O*-alkylated analogues of **1.28** were prepared by treating C-5 substituted thiolactone

derivatives with various alkylating agents in the presence of NaH in DMF.<sup>26</sup> In a few cases, depending on the alkylating agent used, a mixture of products was obtained; these included the C-3 C-alkylated, C-2 O-alkylated and the preferred C-4 O-alkylated product, as predicted by the resonance structures of the thiolate ion (fig. 3.6).<sup>26</sup>

### 3.3.2.3.2. Synthesis of Thiolactone-Isatin Hybrids



**Scheme 3.5:** Reagents and conditions: (i) Dibromoalkane, K<sub>2</sub>CO<sub>3</sub>, Acetone, reflux, 16 h, 40-65%, (ii) Isatin, NaH, DMF, 60°C, 10-13%, 48 h, (iii) KOH, MeOH-H<sub>2</sub>O, 25 °C, 4 h, 95-100%, (iv) **3.6**, DMF, 60°C, 48 h, 9-36%.

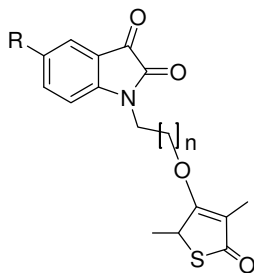
The two routes explored for the synthesis of **3.7** are shown in scheme 3.5. Our initial attempt (route A) involved the reaction of the colourless 4-O-alkylated thiolactone intermediates with isatin in the presence of NaH. The former was obtained by refluxing **2.13** and K<sub>2</sub>CO<sub>3</sub> and the appropriate dibromoalkane in acetone.<sup>8</sup> Because of the low-yields obtained for **3.7**, 11% for the ethyl linked hybrid and 13% for the butyl linked hybrid, route A was not pursued further. The alternative route (route B) differs from the former in that the desired hybrids **3.7** can be accessed by the reaction of the N-alkylated isatin intermediates **3.6** with **2.13**. Attempts to further optimize this route entailed exploring bases with varying strengths. The reaction of **3.6g** with **2.13** in the presence of NaH in anhydrous DMF afforded the desired hybrid but the yield obtained was still very poor. A second attempt involved replacing the relatively strong base NaH with the weak base, K<sub>2</sub>CO<sub>3</sub>. Refluxing **2.13** and **3.6g** in acetone in the presence of



4 eq of  $K_2CO_3$  did not produce the desired hybrid but instead an unidentified product was isolated. All efforts were then directed towards the method which utilizes the potassium salt of thiolactone **3.5**. The latter was obtained in excellent to quantitative yield from the reaction of **2.13** with an equimolar amount of aqueous potassium hydroxide in MeOH at room temperature. The hygroscopic nature of **3.5** required it to be dried thoroughly prior to its reaction with 1.5 eq of **3.6** in anhydrous DMF. The reaction mixture was stirred at room temperature under a nitrogen atmosphere, but no noticeable reaction was observed as inferred from the thin layer chromatography (tlc) of this red coloured mixture after 24 hours. The temperature was then increased to 60°C and tlc confirmed the consumption of the starting material after stirring at this temperature for 48 hours. The recrystallized yields obtained for the orange coloured products **3.7** (table 3.2) were still disappointingly low but are in agreement with previous publications in this area.<sup>7,11,20,27</sup> It is reasonable to assume that a likely contributor to these low yields is the ring-opening polymerization of the thiolactone ring under basic conditions (section 3.3.2.3). In fact a significant amount of unidentifiable baseline material was isolated during the purification of the crude product mixtures.

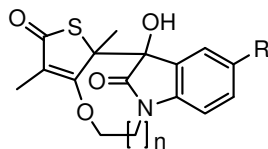
Another more likely cause of the low yields is the formation of by-products, as observed when the *N*-alkylated isatin intermediates bearing the  $n = 3-5$  linkers (**3.6h**, **3.6o** and **3.6s**) were reacted with **3.5**. The by-product had a slightly lower  $R_f$  than the desired hybrid and on tlc (40% EtOAc/Hex) appeared colourless to the naked eye compared to the orange spot observed for the desired hybrids. We initially suspected it to be a regioisomer (C-3-alkylated or C-2 *O*-alkylated product, fig. 3.6) because of similarities in the spectral and elemental analysis data. It was the single crystal X-ray structure which revealed the tetracyclic nature of the by-product **3.8**.

Purification of the isomeric mixture obtained for the  $n = 3-5$  linked hybrids presented an additional challenge. Repeated column chromatography and at times preparative tlc in combination with recrystallization were required to separate the isomers. The contribution of this elaborate purification towards the observed low yields can not be ruled out. The yields obtained for the yellow crystalline tetracycles **3.8** are shown in table 3.3. Despite the low yields sufficient amounts of both the desired hybrids **3.7** and by-product **3.8** were obtained for biological evaluation.

**Table 3.2:** Recrystallized yields and melting points of target molecules **3.7a-j**

Compound	R	n	Yield (%)	m.p. (°C)
<b>3.7a</b>	Br	1	9	168-169
<b>3.7b</b>	Cl	1	13	270-272
<b>3.7c</b>	F	1	14	132-133
<b>3.7d</b>	I	1	10	196
<b>3.7e</b>	CH <sub>3</sub>	1	26	130
<b>3.7f</b>	H	1	36	54
<b>3.7g</b>	H	2	18	150-151
<b>3.7h</b>	H	3	10	108
<b>3.7i</b>	H	4	22	77-79
<b>3.7j</b>	H	5	15	198

The product distribution for compounds **3.8a-c** is shown in Table 3.3. A combined yield of 45% was obtained for compounds **3.7h** and **3.8a**, with the latter being the major contributor. What is noticeable is the significant change in product distribution on going from the 4-carbon linker to the 5-carbon linker. A decrease in combined yields with increasing chain length was observed for **3.7i** and **3.8b** (31%) and **3.7j** and **3.8c** (21%). The lack of formation of **3.8** with the ethyl and propyl linked hybrids (**3.7f** and **3.7g**) and the low yields obtained for **3.8b** and **3.8c** will be discussed briefly in section 3.3.3.1.

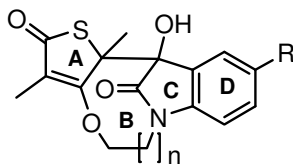
**Table 3.3:** Recrystallized yields and melting points of the tetracyclic by-products **3.8 a-c**

Compound	R	n	Ratio (D/T) <sup>a</sup>	Yield (%) <sup>b</sup>	m.p. (°C)
<b>3.8a</b>	H	3	22:78	35	184-185
<b>3.8b</b>	H	4	84:16	9	214-215
<b>3.8c</b>	H	5	71:29	6	198

<sup>a</sup>Ratio of desired hybrid (D) to tetracyclic by-product (T) as determined from recrystallized yield after 48 hour reaction. <sup>b</sup>Yields of the tetracyclic compounds only.

#### 3.3.2.4. Tetracyclic By-products

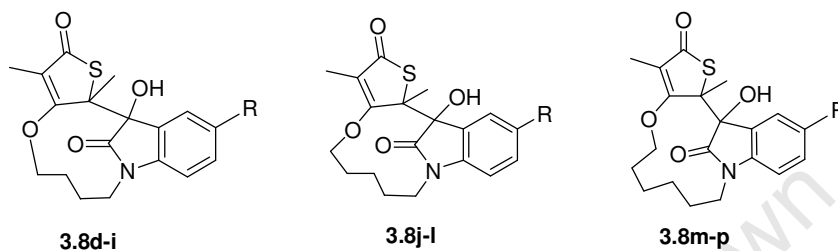
Interest in the tetracycles **3.8** was sparked by the ease and simplicity of their preparation and the novelty of their architecture. Attractive structural features include two vicinal quaternary stereogenic centers, the presence of various heteroatoms and a complex, bridged/bicyclic ring system (fig. 3.8). The difficulty and failure to name these unique structures according to the IUPAC nomenclature<sup>28</sup> is due to the complexity of the ring system. The bridge fused ring system is represented in figure 3.8 by the lactam rings B and C and the amidic carbonyl as bridge. Also present is the 3-substituted 3-hydroxyindolin-2-one moiety which features prominently in a number of biologically active natural products<sup>3,29</sup>

**Figure 3.8:** General structure of the tetracyclic compounds **3.8**

A preliminary biological screen showed that some of the tetracycles were more active than the corresponding desired hybrids. They therefore formed the basis of a more focussed SAR study. Synthesis of the tetracycles **3.8d-p** (table 3.4) was preceded by a model study with **3.5** and the *N*-alkylated isatin intermediate **3.6h**. This study moreover entailed monitoring the reaction progress and thus product distribution at regular intervals using <sup>1</sup>H NMR. An observation made is that prolonged reaction times led to an increase in tetracycle product

formation. Therefore to effect the synthesis of **3.8d-p** we employed the same reaction conditions shown in scheme 3.4, but increased the reaction duration from 48 hours to 120 hours. However, in spite of this modification we still obtained poor yields for these tetracycles (table 3.4).

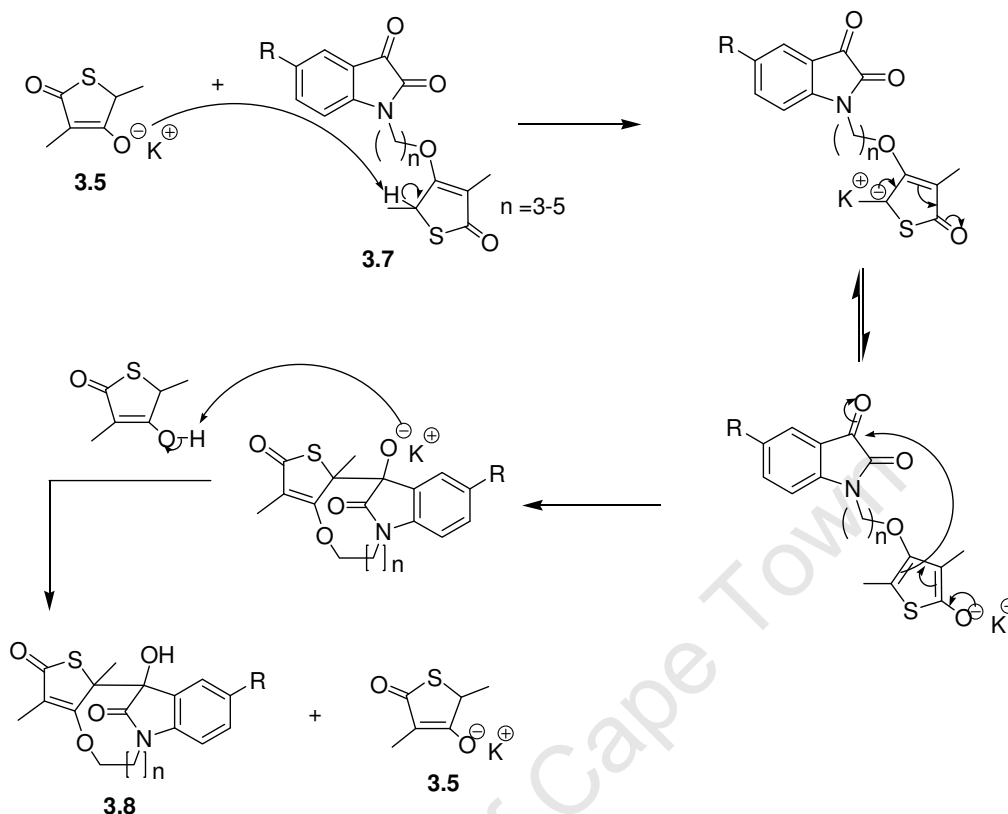
**Table 3.4:** Recrystallized yields and melting points of the tetracyclic compounds **3.8 d-p**



Compound	R	Ratio (D/T) <sup>a</sup>	Yield (%) <sup>b</sup>	m.p.(°C)
<b>3.8d</b>	Br	11:89	14	156-157
<b>3.8e</b>	I	10:90	15	205-206
<b>3.8f</b>	F	19:81	10	157
<b>3.8g</b>	Cl	15:85	14	208-210
<b>3.8h</b>	CH <sub>3</sub>	33:67	6	170-173
<b>3.8i</b>	NO <sub>2</sub>	30:70	8	215-217
<b>3.8j</b>	Cl	63:37 <sup>c</sup>	5	200-201
<b>3.8k</b>	Br	67:33 <sup>c</sup>	7	203-205
<b>3.8l</b>	I	60:40 <sup>c</sup>	4	189-192
<b>3.8m</b>	Cl	80:20	6	214-215
<b>3.8n</b>	F	69:31	7	212-213
<b>3.8o</b>	Br	73:27	6	211
<b>3.8p</b>	I	70:30	7	214-215

<sup>a</sup>Ratio of desired hybrid (D) to tetracyclic by-product (T) as determined from recrystallized yields after a 120 hour reaction. <sup>b</sup>Yields of the tetracyclic compounds only. <sup>c</sup>Ratio determined from the crude using <sup>1</sup>H NMR.

## 3.3.2.4.1. Proposed Mechanism for Tetracyclic Product Formation



Scheme 3.6: Proposed mechanism for the formation of the tetracycles 3.8

The proposed mechanism for the macrocyclization of 3.7 is shown in scheme 3.6. It involves an *in situ* deprotonation of the thiolactone ring in 3.7 at C-5, by the relatively basic 3.5. Carbanion formation destroys the original stereogenic center of 3.7. Moreover, this intermediate carbanion is stabilized by resonance and by the neighbouring sulfur. This stabilizing effect of sulfur on an adjacent carbanion is well known.<sup>30,31</sup> It reportedly involves the delocalization of the negative charge into the  $\sigma^*$  orbital of the C-S bond and is in contrast to the earlier belief that the negative charge is delocalized into the empty 3d orbital of sulfur.<sup>30a</sup> The flexibility of the alkane tether (for  $n = 3-5$ ) allows for the proper alignment of the orbitals of the reacting groups and thus facilitates the intramolecular addition of the nucleophile to the activated ketonic carbonyl of the isatin scaffold. The planar symmetry of the isatin scaffold or, more specifically, ketonic carbonyl allows for nucleophilic attack from above or below the plane leading to a racemic mixture of 3.8.

#### 3.3.2.4.2. Factors Controlling Macrocyclization

Medium (8-11 ring atoms) and large rings (12 or more ring atoms) are of particular interest in synthesis because of their appearance in natural products of biological importance. One of the drawbacks of macrocyclization reactions is poor yields which can be ascribed to several factors. The key factors which affect the ease of ring closure are ring strain, the entropy factor and stereoelectronic effects.<sup>31,32</sup>

Most of the information on strain energy originated from the study of cycloalkanes.<sup>31, 32b</sup> It reportedly arises from the combination of transannular, Pitzer and Baeyer strain. Transannular strain is commonly found in rings containing 7-13 carbons and arises from the repulsive interaction between ring atoms which as a consequence of cyclization are in close proximity to each other. Pitzer strain on the other hand, arises when substituents on adjacent carbon atoms are forced to adopt an eclipse or partial eclipse conformation to relieve transannular strain. Baeyer strain or large-angle strain occurs at the expense of transannular and Pitzer strain and is characterized by the distortion of ring bond angles.<sup>31</sup> Overall, the strain energy for medium size rings are reportedly fairly low whereas large rings experience little or no strain.<sup>31,32a</sup> It is conceivable that the lack of formation of **3.8** with the ethyl ( $n = 1$ ) and propyl ( $n = 2$ ) linked hybrids **3.7** is due to strain at the transition state.

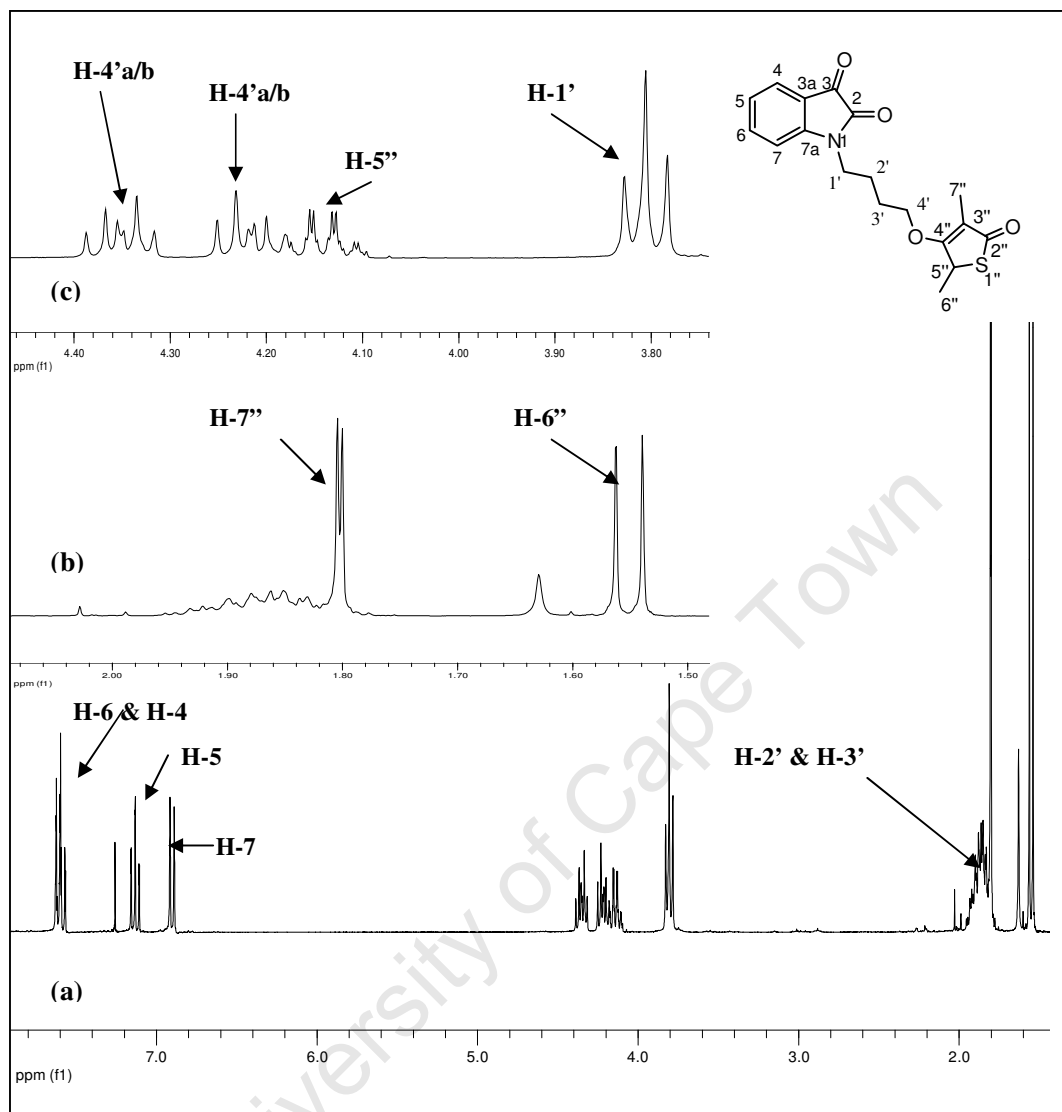
The entropy factor first postulated by Ruzicka in 1935,<sup>33</sup> refers to the probability of the reactive ends of a chain to coincide or merge. This probability reportedly decreases as the chain gets longer.<sup>32b</sup> The trade-off with large rings and to some extent medium rings, is that although they experience no or little ring strain the probability of the reactive ends coinciding is very poor. It is therefore reasonable to assume that the decrease in yield with increasing chain length as observed for the tetracycles **3.8** can be partly explained by the entropy effect.

### 3.4. Characterization

#### 3.4.1. Spectroscopic Analysis

The spectroscopic data obtained for the desired hybrids **3.7** is consistent with the proposed structures. The molecular ion peak observed in the mass spectra of all the compounds in this series correlated with their respective molecular weights. Common and characteristic infrared bands identified include the ketonic (C-3) and amidic (C-3) carbonyls of the isatin scaffold which appeared in the ranges 1732-1743  $\text{cm}^{-1}$  and 1600-1620  $\text{cm}^{-1}$ , respectively - consistent with literature data.<sup>2</sup> The infrared band at 1623-1675  $\text{cm}^{-1}$  is in agreement with the carbonyl absorption band of the thiolactone ring system.<sup>6</sup> Figures 3.9 and 3.10 show the  $^1\text{H}$  and  $^{13}\text{C}$  NMR spectra of the representative compound **3.7h**. The  $^1\text{H}$  NMR data [fig. 3.9(b)] showed some key signals which includes two doublets resonating at  $\delta$  1.55 and  $\delta$  1.80 each integrating for 3 protons which was assigned to the methyl protons H-6'' and H-7'', respectively. A pair of one-proton multiplets resonating at  $\delta$  4.22 and  $\delta$  4.35 was assigned to the methylene protons H-4'a/b [fig. 3.9(c)]. The observed splitting of these enantiotopic protons is most likely due to their proximity to the chiral center at C-5''. The protons of the isatin scaffold appeared in the aromatic region of the spectrum. Proton H-7 appeared as an unexpected doublet ( $J$  7.8) at  $\delta$  6.90 and H-5 as a triplet of doublets ( $J$  0.9, 7.8) at  $\delta$  7.13. The overlapping signals of H-6 and H-4 appeared at  $\delta$  7.60 [fig. 3.9(a)].

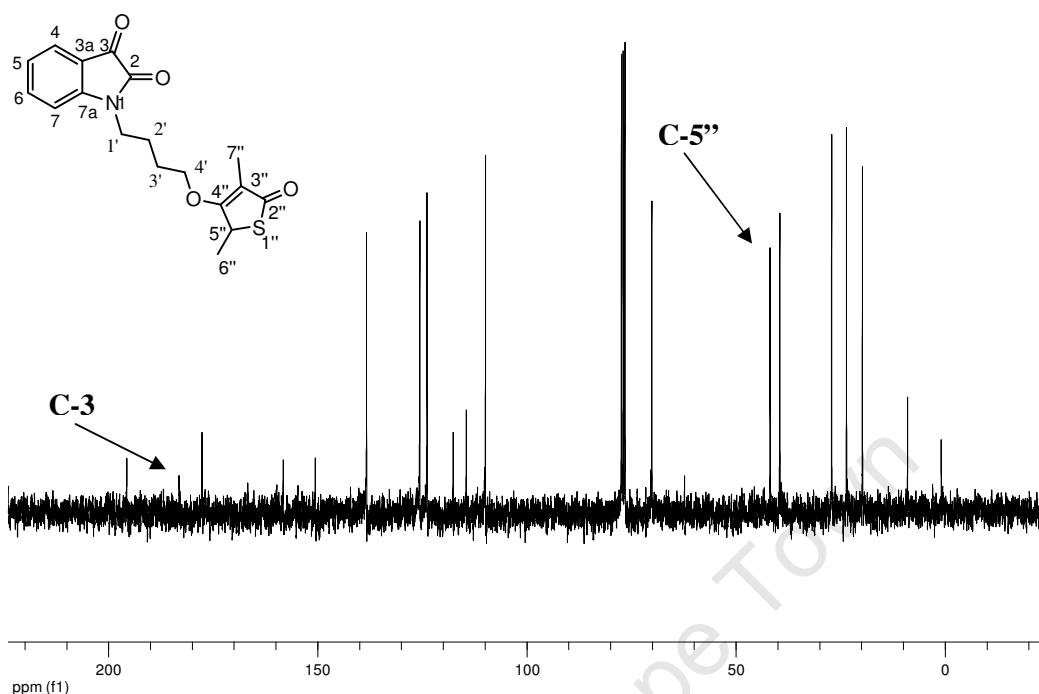
$^1\text{H}$  NMR data supplemented by  $^1\text{H}$ - $^1\text{H}$  COSY experiments revealed mutual coupling between the methyl protons H-7'' ( $J$  0.9) and the methine proton H-5'' at  $\delta$  4.14 which appeared as a quartet of doublets ( $J$  0.9 and  $J$  6.9). According to a recent publication this anomalous, mutual coupling which the authors erroneously referred to as an 'allylic' coupling is of diagnostic value and can be used to distinguish between the 2- and 4-enol (ethers) of the thiolactone.<sup>25</sup> This 'allylic' coupling is reportedly only observed with the 4-enol thiolactone derivatives and thus served as further confirmation of the proposed structure.



**Figure 3.9:** (a)  $^1\text{H}$  NMR spectrum of compound **3.7h** in  $\text{CDCl}_3$  at 300MHz with expanded regions (b) 1.50-2.00 ppm and (c) 3.74-4.40 ppm

The  $^{13}\text{C}$  NMR spectrum of compound **3.7h** showed 18 non-equivalent signals which correlates with the number of carbons expected. Key signals appeared at  $\delta$  195.7,  $\delta$  183.2 and  $\delta$  158.3 and were assigned to the carbonyl carbons at C-2'', C-3 and C-2 respectively. Another carbon signal of interest is the methine carbon C-5'' resonating at 41.9. Confirmation of assignment of the  $^{13}\text{C}$  NMR data was done by comparison with published data for natural occurring thiolactomycin<sup>11</sup> and *O*-alkylated thiolactomycin analogues.<sup>7</sup>

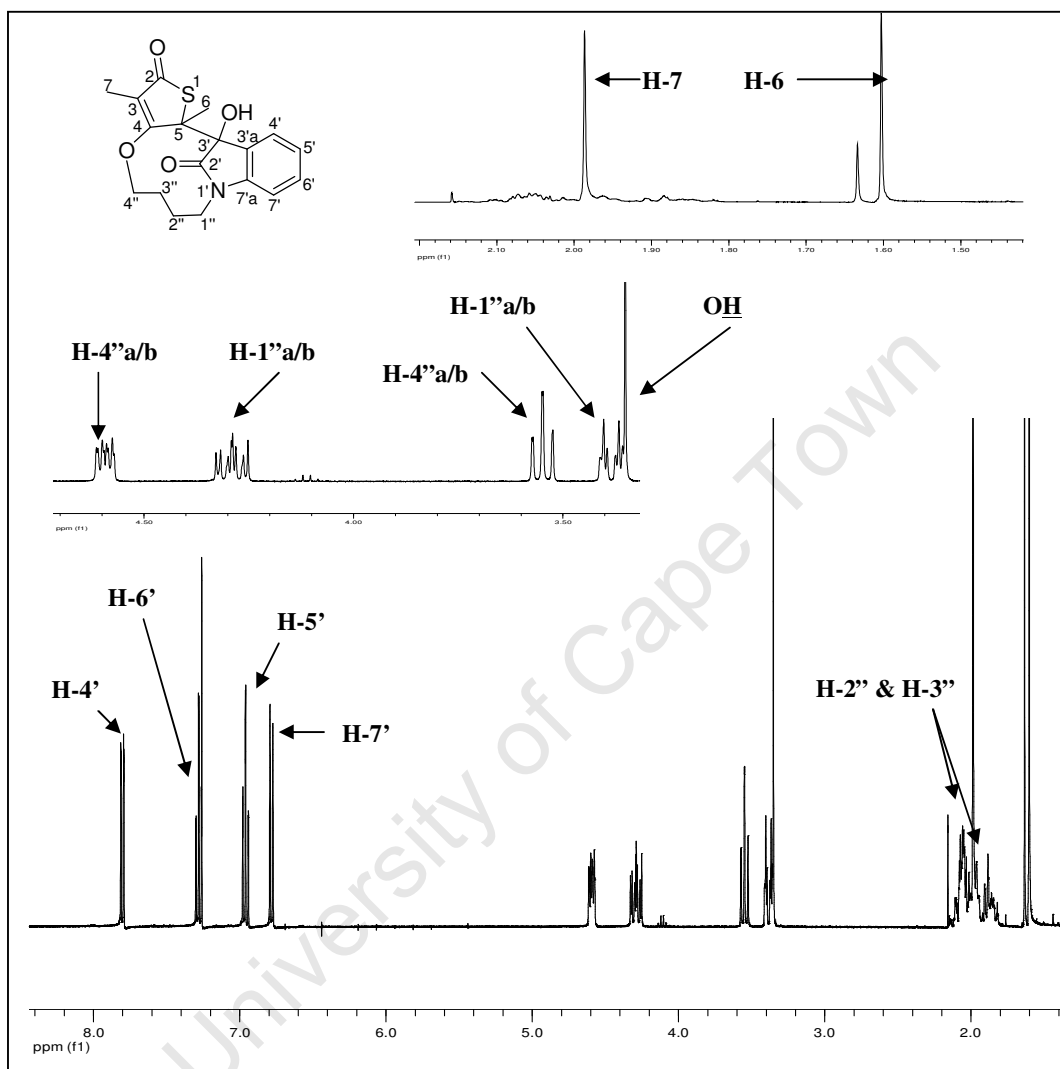




**Figure 3.10:**  $^{13}\text{C}$  NMR spectrum of compound **3.7h** in  $\text{CDCl}_3$  at 75MHz

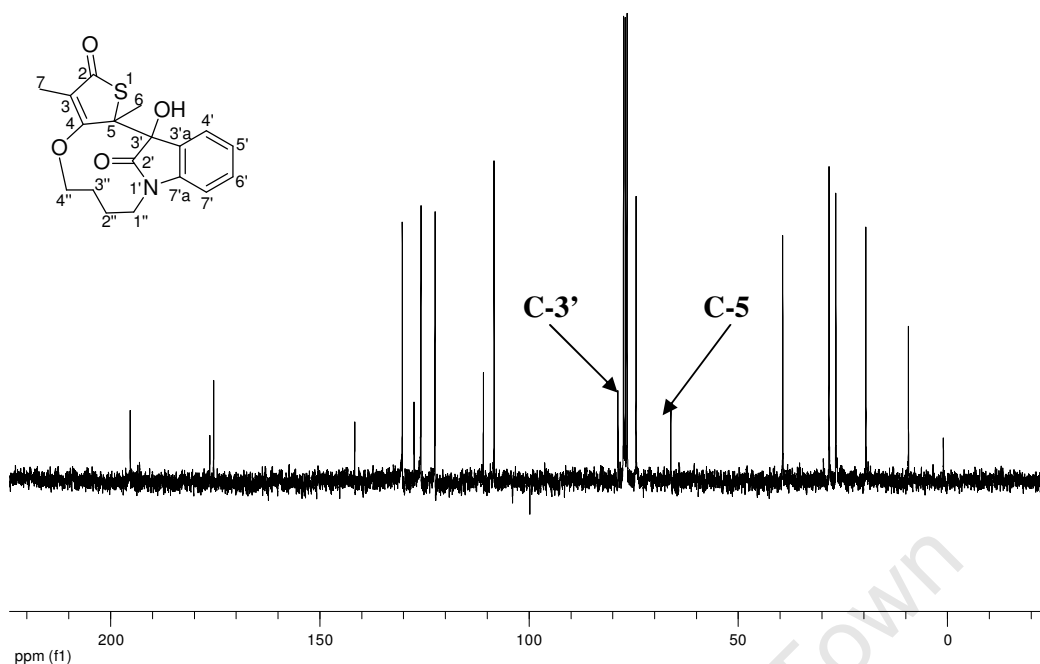
The unambiguous assignment of the structures for the tetracyclic by-products could not be done on the basis of spectroscopic data alone. The latter however, revealed some significant differences between compound **3.8a** and the previously discussed isomer **3.7h**. Characteristic IR absorptions for compound **3.8a** included the carbonyl of the thiolactone and the amidic carbonyl of the isatin scaffold observed at  $1700\text{ cm}^{-1}$  and  $1620\text{ cm}^{-1}$ , respectively. The presence of a hydroxyl group was inferred from the characteristic broad band at  $3311\text{ cm}^{-1}$ . Figure 3.11 shows the  $^1\text{H}$  NMR spectrum of the representative compound **3.8a**. The shielded end of the spectrum shows 2 three-proton singlets at  $\delta$  1.60 and  $\delta$  1.99 which was assigned to methyl protons H-6 and H-7, respectively. A signal of interest is the broad, one-proton singlet at  $\delta$  3.35 which disappeared after  $\text{D}_2\text{O}$ -exchange and was assigned to the hydroxyl proton. Also noteworthy is the observed splitting of the methylene protons of H-1'' and H-4'' which appeared as 4 one-proton multiplets. HSQC data aided the assignment of the geminal coupled protons at H-1'' which resonates at  $\delta$  3.38 and  $\delta$  4.29. In contrast to **3.7h** the signal in the aromatic region in the  $^1\text{H}$ -NMR spectrum of **3.8a**, assignable to the isatin scaffold, are well-resolved. The one-proton double doublet ( $J$  1.2 and 7.6) at  $\delta$  7.80 was assigned to H-4' which showed *ortho* ( $J$  7.6) and *meta* ( $J$  1.2) coupling to H-5' and H-6', respectively. The latter two both appeared as triplet of doublets with H-5' resonating at  $\delta$  6.96 ( $J$  0.8, 7.6) and

H-6' at  $\delta$  7.28 ( $J$  1.2, 8.0). The other signal in this region is the one-proton doublet ( $J$  8.0) at  $\delta$  6.78 assigned to H-7'.



**Figure 3.11:**  $^1\text{H}$  NMR spectrum of compound **3.8a** in  $\text{CDCl}_3$  at 400MHz

The  $^{13}\text{C}$  NMR spectrum of **3.8a** (fig. 3.12) supplemented with DEPT experiments was used to distinguish between methyl, methylene and quaternary carbons. Key signals are represented by the quaternary carbons C-5 and C-3' generated by the cyclization of **3.7h** which resonate at  $\delta$  66.1 and  $\delta$  78.8, respectively.



**Figure 3.12:**  $^{13}\text{C}$  NMR spectrum of compound **3.8a** in  $\text{CDCl}_3$  at 100MHz

### 3.4.2. Crystallographic Analysis

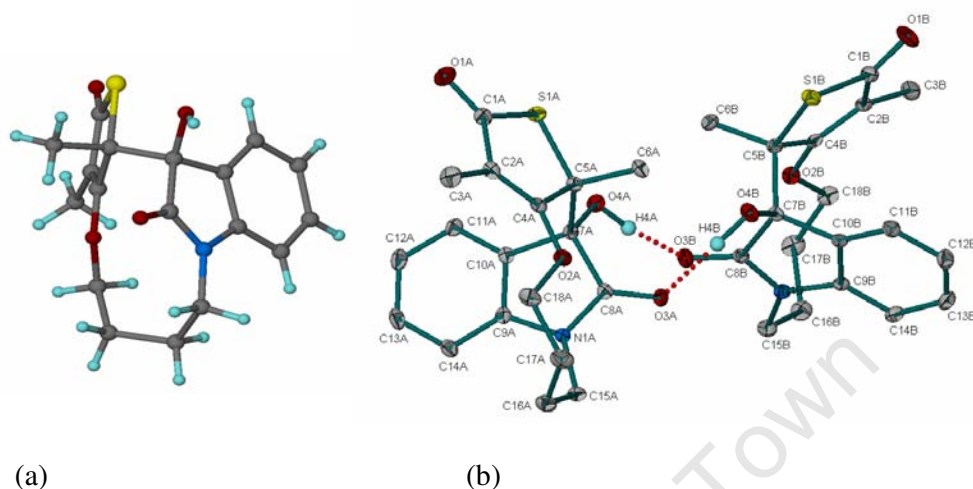
The single crystals obtained for compounds **3.8a-c** from MeOH at room temperature were subjected to crystallographic analysis. Crystal and refinement data obtained is summarized in table 3.5. For subsequent discussion we have used the atom numbering scheme of the molecular structure which differs from that used in the spectroscopic analysis section.

**Table 3.5:** Crystal data and structure refinement for compounds **3.8a-c** with estimated standard deviations in parentheses

Parameter	3.8a	3.8b	3.8c
<b>Molecular formula</b>	C <sub>18</sub> H <sub>19</sub> NO <sub>4</sub> S	C <sub>19</sub> H <sub>21</sub> NO <sub>4</sub> S	C <sub>20</sub> H <sub>23</sub> NO <sub>4</sub> S
<b>Formula weight (g/mol)</b>	345.40	359.43	373.45
<b>Crystal system</b>	triclinic	monoclinic	monoclinic
<b>Space group</b>	P-1	P2 <sub>1</sub> /n	P2 <sub>1</sub> /n
<b>Unit cell dimensions: a (Å)</b>	9.18680(10)	7.7577(2)	7.9966(3)
<b>b (Å)</b>	13.60830(10)	13.1299(5)	13.1259(4)
<b>c (Å)</b>	14.42970(10)	17.2540(6)	17.3410(7)
<b>α (°)</b>	67.7380(10)	90	90
<b>β (°)</b>	78.2150(10)	100.819(2)	97.644(2)
<b>γ (°)</b>	86.5250(10)	90	90
<b>Volume (Å<sup>3</sup>)</b>	1634.02(2)	1726.22(10)	1803.98(11)
<b>Z</b>	4	4	4
<b>Calc. density (Mg/m<sup>3</sup>)</b>	1.404	1.383	1.375
<b>μ(MoKa) (mm<sup>-1</sup>)</b>	0.220	0.212	0.205
<b>F (000)</b>	728	760	792
<b>Temperature (K)</b>	113	213	173
<b>Wavelength (Å)</b>	0.71073	0.71073	0.71073
<b>Crystal size (mm)</b>	0.35x0.33x0.25	0.24x0.22x0.21	0.33x0.26x0.22
<b>Range of θ scanned (°)</b>	3.62 to 25.39	3.09 to 26.72	2.68 to 25.69
<b>No. of measured reflections</b>	51402	7137	6705
<b>No. of unique reflections</b>	5962	3654	3432
<b>R(int)</b>	0.0439	0.0274	0.0330
<b>No. of parameters</b>	446	233	240
<b>Goodness-of-fit on F<sup>2</sup></b>	1.042	1.040	1.033
<b>R indices (all data)</b>	R1 = 0.0402 wR2 = 0.0768	R1 = 0.0531 wR2 = 0.0845	R1 = 0.0718 wR2 = 0.1066
<b>Extinction coefficient</b>	0.0016(9)	0.0026(8)	0.0027(9)
<b>Δρ excursions (e.Å<sup>-3</sup>)</b>	-0.270,0.271	-0.301, 0.217	-0.378, 0.367

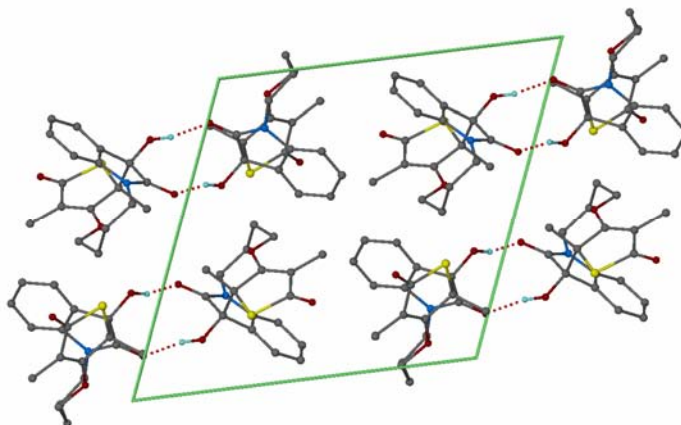
For compounds **3.8a** [fig. 3.13(a)], all non-hydrogen atoms were refined anisotropically and all hydrogen atoms except the hydroxyl hydrogens were fixed geometrically and were refined using a riding model, with C–H = 0.95 – 0.99 Å and  $U_{\text{iso}} = 1.2 - 1.5 \times U_{\text{eq}}(\text{C})$ . The hydroxyl hydrogens were located in the difference electron density maps and refined with simple bond length constraints. The structure was refined successfully with  $R = 0.0305$ . Compound **3.8a** crystallizes in the triclinic unit cell with space group P-1 and contains four molecules per unit cell. This centrosymmetric space group confirms the racemic nature of **3.8a**. The crystal structure furthermore revealed the presence of two non-equivalent molecules which is indicative of the presence of two enantiomeric pairs in the unit cell. These are differentiated

with the labels A and B in the atom numbering scheme [fig. 3.13 (b)]. Because of similar trends observed in the bond angles and lengths we have limited our discussion to diastereomer A.



**Figure 3.13:** (a) Molecular structure of **3.8a** with probability level = 40%, (b) structure of the dimer showing the atomic numbering scheme. All hydrogen atoms are omitted except the hydroxyl hydrogens.

In the structure of **3.8a** the indole ring is almost planar with C7A and C8A showing deviations of  $-0.0918(10)$  and  $+0.0902(11)$ , respectively from the least square plane. The *syn* relationship between the methyl at C5A and the hydroxyl group at C7A was inferred from the torsion angle of  $-65.86^\circ(14)$  for O4A-C7A-C5A-C6A. Also noted are the dihedral angles  $106.81^\circ(11)$  and  $113.29^\circ(11)$  for O4A-C7A-C5A and C6A-C5A-C7A, respectively which shows a distortion from the expected value ( $109.47^\circ$ ) for the tetrahedral  $sp^3$  carbon. It is conceivable that this distortion coupled with the bond lengthening of C7A-C5A [ $1.5783(19)$  Å] is due to the steric interaction between the relatively bulky hydroxyl group at C7A and the adjacent methyl group (C5A). Selected bond angles and lengths are given in table 3.6.



**Figure 3.14:** Packing diagram of **3.8a** with projection viewed along [100]. All hydrogen atoms are omitted except the hydroxyl hydrogens

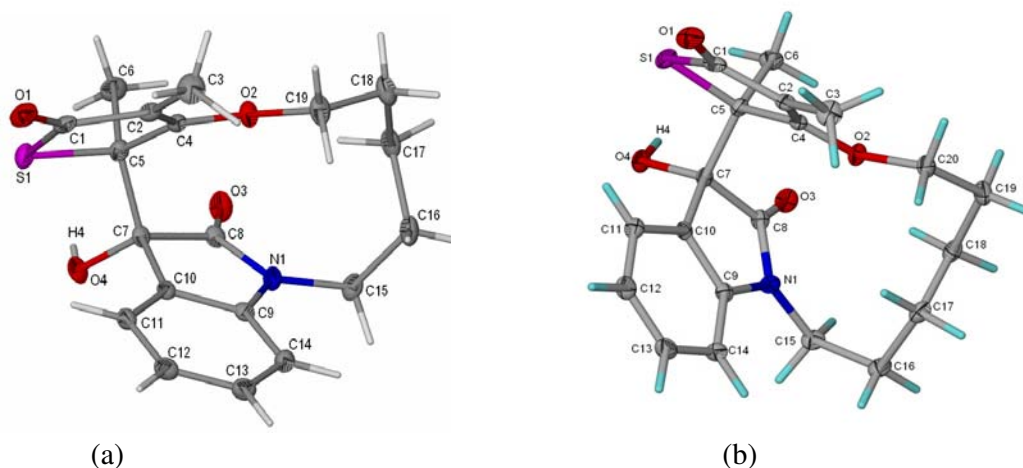
The stabilization effect of the hydrogen bonding for **3.8a** is illustrated in the packing diagram (fig. 3.14). Hydrogen bond lengths and bond angles are given in table 3.7.

**Table 3.6:** Selected bond angles (°) and bond lengths (Å) for compounds **3.8a-c**

	<b>3.8a<sup>a</sup></b>	<b>3.8b</b>	<b>3.8c</b>
C5A-C7A	1.5783(19)	1.5758(19)	1.572(3)
C8A-O3A	1.2271(17)	1.2229(16)	1.223(2)
C7A-O4A	1.4103(16)	1.4179(16)	1.425(2)
C6A-C5A-C7A	113.29(11)	113.96(12)	112.75(17)
O4A-C7A-C5A	106.81(11)	110.60(10)	109.39(16)
C6A-C5A-C7A-O4A	-65.86(14)	-70.17(15)	-65.3(2)
O3A-C8A-C7A-C5A	-73.33(17)	-66.50(17)	-64.0(3)
S1A-C5A-C7A-C8A	177.45(9)	172.63(9)	175.27(14)

<sup>a</sup> Parameters for diastereomer A

All the hydrogen atoms for compounds **3.8b-c** except the hydroxyl hydrogen H4 were included in idealised positions in a riding model with  $U_{iso}$  set at 1.2 or 1.5 times those of the parent atoms. The hydroxyl hydrogen H4 was located by difference Fourier methods and refined independently. The structure was refined successfully with  $R = 0.0335$  for **3.8b** and  $R = 0.0405$  for **3.8c**.



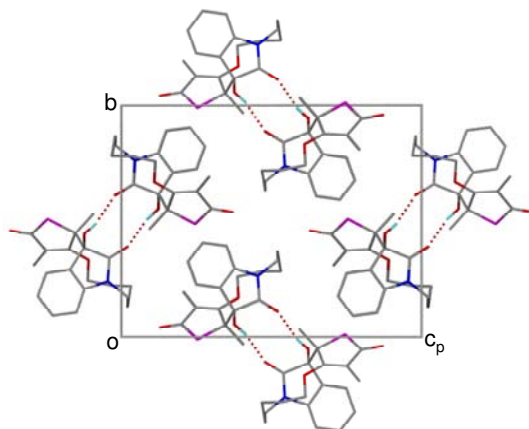
**Figure 3.15:** Molecular structure of (a) **3.8b** and (b) **3.8c** with ellipsoidal model of probability level = 35%.

Both compounds crystallized in the racemic monoclinic space group  $P2_1/n$  with 4 molecules in the asymmetric unit. Figure 3.15 shows the molecular structure and atom labelling for **3.8b** and **3.8c**. As observed for **3.8a** the indole moiety in these compounds is nearly planar with the maximum deviation from the least-squares plane for all the nine atoms in the ring at +0.045(1) and 0.0412(15) for C-8 in **3.8b** and **3.8c**, respectively. Important bond angles and lengths are summarized in table 3.6. Similar to **3.8a**, a stabilizing hydrogen bonding network was observed for both compounds exemplified by the packing diagram of **3.8b** (fig. 3.16).

**Table 3.7:** Hydrogen bond distances and bond angles for **3.8a-c**

Code	D-H...A	D-H(Å)	H...A(Å)	D...A(Å)	DHA(°)
<b>3.8a</b> <sup>a</sup>	O4A-H4A...O3B	0.9798(10)	1.808(6)	2.7638(14)	164.2(18)
<b>3.8b</b>	O4-H4...O3	0.8500(19)	1.947(19)	2.7723(15)	163.3(18)
<b>3.8c</b>	O4-H4...O3	0.9798(10)	1.894(9)	2.848(2)	164(3)

<sup>a</sup> Parameters for diastereomer A



**Figure 3.16:** Packing diagram of **3.8b** with projection viewed along [100]. All hydrogen atoms are omitted except the hydroxyl hydrogens H4. The hydrogen bond O4-H4...O3 is shown as a dotted line.

### 3.5. Biological Results and Discussion

Consistent with the objectives of this project compounds **3.7a-j**, **3.8a-p** and a few advanced intermediates **2.13** and **3.6**, were evaluated for *in vitro* growth inhibitory activity against chloroquine-resistant (W2) and chloroquine-sensitive (D10) strains of *P. falciparum*, for falcipain-2 inhibitory activity and for growth inhibitory activity of the H<sub>37</sub>Rv strain of *M. tuberculosis*.

Testing for antiplasmodial activity against the D10 strain of *P. falciparum* was conducted at the Department of Pharmacology, University of Cape Town (UCT). The evaluation of compounds for antiplasmodial activity against the W2 strain of *P. falciparum* and inhibitory activity against recombinant-falcipain-2 were conducted at the Department of Medicine, San Francisco General Hospital, University of California San Francisco (UCSF). Reference compounds used include chloroquine (CQ) for growth inhibition of the D10 and W2 strains and E64 for recombinant-falcipain 2 inhibition studies. Experimental details pertaining to the above assays are given in the experimental section. The antiplasmodial activities herein reported are expressed as a 50% inhibitory concentration (IC<sub>50</sub>) which describes the concentration of a drug necessary to bring about a therapeutic effect such as the inhibition of growth or activity in 50% of the test sample. Also determined is the resistance indices (RI) of compounds tested against both D10 and W2 strains. It serves as an indication of the difference in activity of the synthesized compounds in a sensitive and resistant strain of *P.*



*falciparum* and is used to evaluate whether novel antimalarials have potential activity against CQ resistant parasites.

$$RI = \frac{IC_{50}(W2)}{IC_{50}(D10)}$$

The calculated log P (c log P) is the physiochemical parameter that describes the lipophilicity of a compound and its ability to transverse membranes. The values herein reported were calculated using the ChemDraw Ultra 9.0 software.

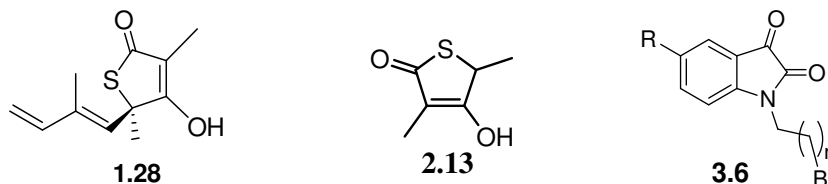
### 3.5.1. *In vitro* Antiplasmodial Activity

As can be seen from tables 3.8, 3.9 and 3.10, none of the compounds in this series exhibited any significant activity against the CQR resistant and CQS strains. In fact, the most active compound identified is **3.8p** ( $IC_{50} = 6.92 \mu\text{M}$ ) which is 100-fold less active than chloroquine against the W2 strain. Despite the low activity it is of interest to note that the target molecules, with the exception of **3.7e**, **3.7g-h** and **3.8b**, showed enhanced activity compared to natural occurring 5R-thiolactomycin **1.28** (Sigma-Aldrich, SA). The results also suggest that the tetracycles **3.8** are more active than their precursors **3.7**. None of the intermediates (table 3.8) were able to inhibit the W2 strain at the highest concentration ( $20\mu\text{M}$ ) tested. For the limited series **3.7**, compounds **3.7a**, **3.7i** and **3.7j** show poor growth inhibitory activity against the W2 strain with  $IC_{50}$ s of 17.40, 15.30 and  $12.30 \mu\text{M}$ , respectively whereas the remaining compounds in this series were inactive. A similar trend was observed for the tetracycles **3.8**. Compounds **3.8m**, **3.8o**, **3.8p** and **3.8c** showed moderate activity against the W2 strain.

In contrast to the tetracycles **3.8**, most of target molecules **3.7** (except **3.7e**, **3.7g**, **3.7h**) showed falcipain-2 inhibitory activity albeit weak compared to the control drug, E64. It is tempting to speculate that this is due to the presence and/or availability of the ketonic carbonyl on the isatin scaffold for interaction with the cysteine thiol of the enzyme. However, the results obtained for the tetracycle **3.8k** ( $IC_{50} = 10.10 \mu\text{M}$ ) argues against the importance of this functionality for enzyme inhibition. In fact, identified from this series is compound **3.8k** which displayed the best falcipain-2 inhibitory activity albeit 200-fold less active than the standard drug, E64. Furthermore, the inhibition of falcipain-2 activity despite the lack of growth inhibitory activity observed for **3.7d** may be an indication of the failure of the compound to reach the target site. This moreover implicates membrane impermeability

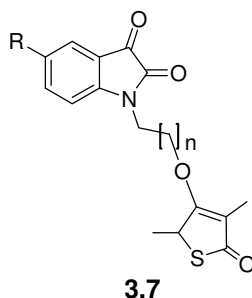
and/or inadequate solubility. It is therefore safe to assume that poor solubility is the main contributor towards the observed low activity for this series of compounds.

**Table 3.8:** *In vitro* antiplasmodial activity and falcipain-2 inhibitory activity of intermediates **2.13** and **3.6**



Compound Code	R	n	Rec-FP-2 IC <sub>50</sub> (μM)	W2 IC <sub>50</sub> (μM)	D10 IC <sub>50</sub> (μM)
Chloroquine	-	-	-	0.06938	0.03823
E64	-	-	0.04733	-	-
<b>1.28</b>	-	-	>100	>20	>100
<b>2.13</b>	-	-	>100	>20	>100
<b>3.6a</b>	H	1	>100	>20	>100
<b>3.6g</b>	H	2	>100	>20	78.29
<b>3.6h</b>	H	3	>100	>20	36.37
<b>3.6i</b>	I	3	>100	>20	<b>11.00</b>
<b>3.6j</b>	Cl	3	>100	>20	<b>9.03</b>
<b>3.6k</b>	Br	3	>100	>20	28.03
<b>3.6l</b>	F	3	>100	>20	63.21
<b>3.6m</b>	CH <sub>3</sub>	3	>100	>20	36.26
<b>3.6n</b>	NO <sub>2</sub>	3	11.35	>20	23.02
<b>3.6o</b>	H	4	>100	>20	38.22
<b>3.6s</b>	H	5	>100	>20	>100

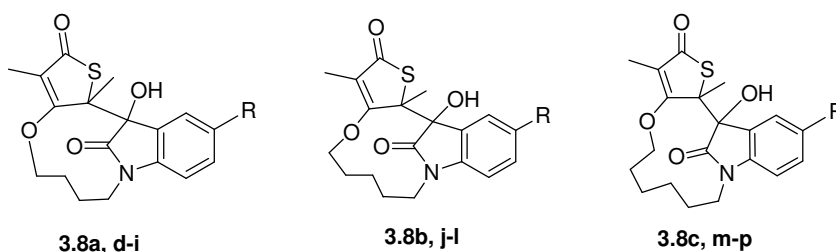
Regarding the effect of the substituent (R) at C-5 of the isatin scaffold on the antiplasmodial activity for the **3.7** series, the results obtained were inconclusive and no useful trends could be delineated. This is in contrast to the trends observed for tetracycles **3.8** which is graphically represented in figure 3.17(a). It shows that the unsubstituted isatin derivative (R = H) is the least active whereas the derivative bearing a nitro group- a known toxiphore<sup>34</sup> - is the most active for the butyl linked tetracycles. The preference for substitution at C-5 for the n = 5 linked hybrids is further demonstrated by the poor activity of unsubstituted isatin derivative **3.8c** relative to the substituted isatin derivatives (**3.8m**, **3.8n**, **3.8o**, **3.8p**).

**Table 3.9:** *In vitro* antiplasmodial activity and falcipain-2 inhibitory activity of compounds **3.7a-j**

Compound Code	R	n	<sup>a</sup> Rec-FP-2 IC <sub>50</sub> (μM)	W2 IC <sub>50</sub> (μM)	D10 IC <sub>50</sub> (μM)	RI	c log P
<b>CQ</b>	-	-	-	0.06938	0.03823	1.81	-
<b>E64</b>	-	-	0.04733	-	-	-	-
<b>1.28</b>	-	-	>100	>20	>100	-	-
<b>3.7a</b>	Br	1	31.70	17.40	42.65	0.41	2.88
<b>3.7b</b>	Cl	1	47.80	>20	32.12	-	2.73
<b>3.7c</b>	F	1	89.60	>20	99.30	-	2.16
<b>3.7d</b>	I	1	<b>17.80</b>	>20	>100	-	3.14
<b>3.7e</b>	CH <sub>3</sub>	1	>100	>20	66.99	-	2.34
<b>3.7f</b>	H	1	40.20	>20	31.95 <sup>b</sup>	-	1.84
<b>3.7g</b>	H	2	>100	>20	37.12	-	2.11
<b>3.7h</b>	H	3	>100	>20	61.09	-	2.16
<b>3.7i</b>	H	4	58.00	<b>15.30</b>	35.61	0.43	2.69
<b>3.7j</b>	H	5	32.00	<b>12.30</b>	26.00	0.47	3.22

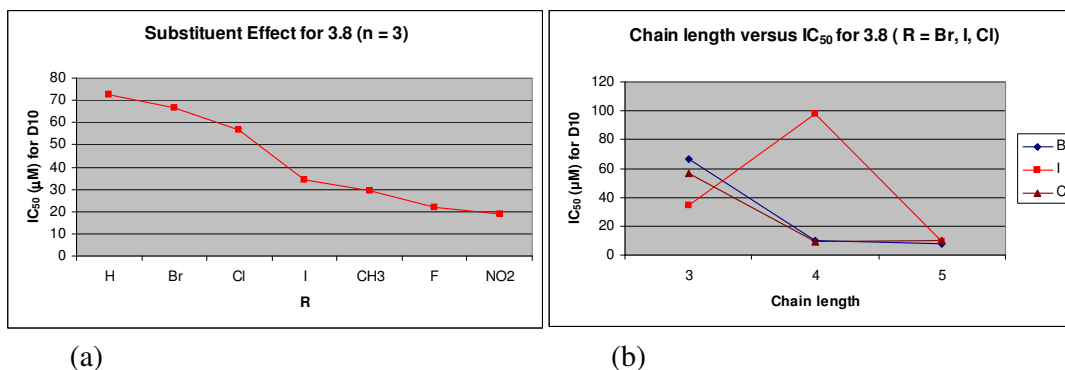
<sup>a</sup>Rec-FP-2 = Recombinant Falcipain-2; <sup>b</sup>Compound tested as a suspension

Lipophilicity is an important property for thiolactomycin analogues; considering that the former need to cross multiple membranes to reach their target site (section 1.4.2.2). The correlation between c log P and growth inhibitory activity against W2 is clearly evident for series **3.7** but is more pronounced for **3.8** [fig. 3.17(b)]. A comparison of the activity of unsubstituted isatins (R = H) with increasing chain length (n = 3-5) for the tetracycles (**3.8a-c**) reveal a decrease in the activity against the D10 strain whereas the activity against the W2 strain increase.

**Table 3.10:** *In vitro* antiplasmodial activity and falcipain-2 inhibitory activity of compounds **3.8a-p**

Compound	R	<sup>a</sup> Rec-FP-2 IC <sub>50</sub> (μM)	W2 IC <sub>50</sub> (μM)	D10 IC <sub>50</sub> (μM)	<sup>b</sup> RI	<sup>c</sup> log P
CQ	-	-	0.06938	0.03823	1.81	-
E64	-	0.04733	-	-	-	-
1.28	-	>100	>20	>100	-	-
3.8a	H	>100	>20	72.67	-	2.12
3.8b	H	>100	>20	>100	-	2.68
3.8c	H	>100	11.50	>100	-	3.24
3.8d	Br	>100	>20	66.41	-	3.12
3.8e	I	>100	>20	34.18 <sup>c</sup>	-	3.38
3.8f	F	>100	>20	21.96 <sup>c</sup>	-	2.40
3.8g	Cl	>100	>20	56.8	-	2.97
3.8h	CH <sub>3</sub>	>100	>20	29.46 <sup>c</sup>	-	2.62
3.8i	NO <sub>2</sub>	44.50	>20	18.85 <sup>c</sup>	-	2.16
3.8j	Cl	>100	>20	9.47	-	3.53
3.8k	Br	<b>10.10</b>	>20	9.93	-	3.68
3.8l	I	>100	>20	97.77	-	3.94
3.8m	Cl	>100	<b>7.41</b>	<b>9.86</b>	0.75	4.09
3.8n	F	>100	14.00	6.46 <sup>c</sup>	2.17	3.52
3.8o	Br	83.40	<b>6.93</b>	<b>7.44</b>	0.93	4.24
3.8p	I	48.30	<b>6.92</b>	<b>9.07<sup>c</sup></b>	0.76	4.50

<sup>a</sup>Rec-FP-2 = Recombinant Falcipain-2; <sup>b</sup>RI =Resistance Index; <sup>c</sup>Compound tested as a suspension



**Figure 3.17:** Graph showing (a) the substituent effect on activity against the D10 strain for the  $n = 3$  linked tetracycles **3.8**, and (b) the effect of chain length for the bromo-, iodo- and chloro-substituted tetracycle **3.8** on activity against the D10 strain of *P. falciparum*

### 3.5.2. *In vitro* Antitubercular Activity

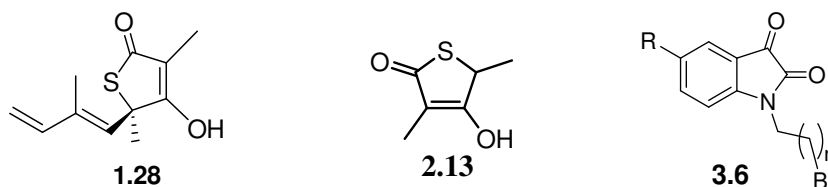
The synthesized compounds were evaluated for *in vitro* antitubercular activity in three different assays conducted at two independent laboratories. Testing for activity against the drug-sensitive strain of *M. tuberculosis*, H<sub>37</sub>Rv using the radiometric BACTEC method, was done locally at the Department of Biomedical Sciences, University of Stellenbosch (US). In brief, the BACTEC method involves the use of a broth medium containing <sup>14</sup>C-palmitic acid (substrate) as the only source of carbon. The growing mycobacteria consumes the substrate with subsequent release of radioactive <sup>14</sup>CO<sub>2</sub> during respiration, which is measured and recorded by the BACTEC TB 460 instrument (Becton Dickinson, Sparks, MD USA) as a growth index (GI) on a scale of 0-999.<sup>35</sup> The controls used were drug-free and undiluted with the same culture inoculum as used in the drug-containing vials. Because of the high cost of BACTEC and its limitation whereas large-scale testing is concerned only two intermediates were tested. Moreover, it served as a primary screening tool and as such the actual minimum inhibitory concentrations (MICs) of compounds were not determined; instead the percentage growth inhibition of the compounds relative to the control were determined and calculated at two concentrations (1 µg/mL, 10 µg/mL) using the formula:

$$\% \text{ Growth inhibition} = 100 - ((\text{GI of drug treated culture} / \text{GI of control culture}) \times 100).$$

All target molecules and advanced intermediates synthesized were evaluated for activity against the H<sub>37</sub>Rv strain of *M. tuberculosis* in a microplate Alamar Blue assay (MABA) with rifampin (RMP), streptomycin (SM), isoniazid (INH), moxifloxacin (MOX) and PA824 as controls. MABA is a nonradiometric assay which utilizes an Alamar blue dye as indicator of

cellular growth. The blue, oxidized non-fluorescent form undergoes reduction to the pink, fluorescent form, thus allowing for growth measurements to be made *via* a visual reading or by using either a fluorometer or spectrophotometer.<sup>36</sup> The challenges presented by the heterogeneous population of the bacilli in tuberculosis chemotherapy, has been alluded to in section 1.6.2. Of particular concern is the indirect contribution of the slow-growing sub-populations to the development of resistance and the fact that only a few of the established antitubercular agents are active against these nonreplicating persistent forms. Active compounds identified from the MABA assay, were therefore further evaluated for activity against non-replicating, persistent forms of *M. tuberculosis* in the luminescence-based low oxygen recovery assay (LORA). It is important to point out that the LORA assay was used as a secondary screening assay and thus the possibility of test compounds having activity only against the slow-growing mycobacteria cannot be ruled out. Both the MABA and the LORA assays were conducted at the Institute of Tuberculosis Research, University of Illinois at Chicago (UIC). Details pertaining to the above assays are given in the experimental section.

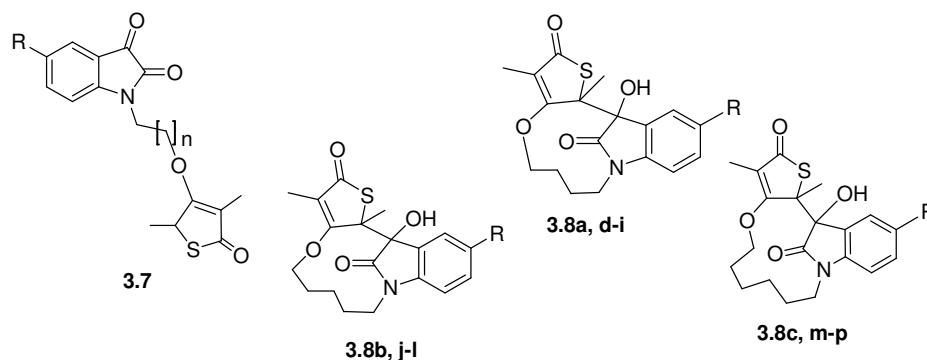
A comparative study of the BACTEC and MABA assays in the screening of 30 validated antimicrobial agents, was conducted by Franzblau and coworker. This study showed that the results obtained with the BACTEC assay showed a high degree of correlation with those obtained with the operational simple, rapid and inexpensive MABA assay.<sup>36</sup> Consistent with their findings, the results obtained for the synthesized compounds (tables 3.11 and 3.12) overall showed a reasonable agreement, despite the fact that tests were conducted in two independent laboratories and that MICs were determined only for the MABA assay. Moreover, in our study the results of both assays (BACTEC and MABA) showed that the intermediates (table 3.11) are more efficacious compared to the hybrids (table 3.12). Among the intermediates, compounds **3.6i** (R = I, n = 3) and **3.6o** (R = H, n = 4) are the most promising with MABA MICs of 15.5  $\mu\text{M}$  and 23.2  $\mu\text{M}$ , respectively. Also noted is the discrepancy in the result obtained for the parent drug thiolactomycin **1.28** - a known antimycobacterial (section 2.2.3.1). According to the BACTEC 460 system it exhibits moderate activity with a growth inhibition relative to the control of 58.3% at a concentration of 10  $\mu\text{g/mL}$  whereas it is practically inactive according to the MABA assay (MIC >128  $\mu\text{M}$ ).

**Table 3.11:** *In vitro* antitubercular activity of intermediates

Compound Code	R	n	BACTEC <sup>a</sup> 1.....10 μg/mL		MABA %Inh <sup>b</sup> ...MIC (μM)	LORA %Inh <sup>b</sup> MIC (μM)	c log P		
RMP					0.05	96.1	2.4		
INH					0.37	33.2	>128		
MOX					0.29	90.3	31.1		
SM					0.43	ND	ND		
PA824					0.16	98.7	3.3		
1.27	-	-	-3.8	58.6	35	>128	ND	ND	
2.13	-	-	-24.5	-32.8	0	>128	ND	ND	
3.6a	H	1	ND	ND	0	>128	ND	ND	1.30
3.6c	Cl	1	ND	ND	96	61.3	98.9	43.6	2.19
3.6f	CH <sub>3</sub>	1	ND	ND	32	>128	ND	ND	1.80
3.6g	H	2	ND	ND	57	>128	ND	ND	1.62
3.6h	H	3	ND	ND	93	96.5	99.7	28.8	2.00
3.6i	I	3	ND	ND	95	15.5	98.1	57.1	3.30
3.6j	Cl	3	ND	ND	94	30.5	99.1	30.5	2.90
3.6k	Br	3	ND	ND	94	30.5	99.2	37.7	3.04
3.6l	F	3	ND	ND	95	123	99.7	53.3	2.32
3.6m	CH <sub>3</sub>	3	ND	ND	96	62.7	101	53.2	2.50
3.6n	NO <sub>2</sub>	3	ND	ND	0	>128	ND	ND	2.13
3.6o	H	4	-22.1	34.8	91	23.2	99.6	44.4	2.53
3.6s	H	5	ND	ND	94	97.6	97.3	108	3.06
3.6u	Cl	5	ND	ND	93	29.7	101	31.0	3.95
3.6w	F	5	ND	ND	92	62.3	99.3	31.5	3.38

<sup>a</sup>Calculated using a control growth index of ~ 200; <sup>b</sup>Percentage inhibition at 128 μM

The MICs of all the target molecules (table 3.12) are greater than the highest concentration tested, with the exception of **3.7b** (MIC 63.7 μM). This lack of activity is also reflected by the BACTEC results. Also noticed is the negative results obtained with the BACTEC method, especially for the tetracycles **3.8**, which is indicative of the stimulation of mycobacterial growth.

**Table 3.12.** *In vitro* antitubercular activity of target molecules **3.7b-j** and **3.8a-p**

Compound Code	R	n	BACTEC <sup>a</sup> 1.....10 μg/mL		MABA %Inh <sup>b</sup> ...MIC (μM)		LORA %Inh <sup>b</sup> ...MIC (μM)		c log P
RMP						0.05	96.1	2.4	
INH						0.37	33.2	>128	
MOX						0.29	90.3	31.1	
SM						0.43	ND	ND	
PA824						0.16	98.7	3.3	
1.28			<b>-3.8</b>	<b>58.6</b>	35	>128	ND	ND	-
3.7b	Cl	1	-2.8	9.9	<b>92</b>	<b>63.7</b>	93.1	117.2	2.73
3.7c	F	1	-29	22	0	>128	ND	ND	2.16
3.7d	I	1	-3.2	42	82	>128	ND	ND	3.14
3.7e	CH <sub>3</sub>	1	-12	4	14	>128	ND	ND	2.34
3.7f	H	1	31.7	34.7	0	>128	ND	ND	1.84
3.7g	H	2	-18.5	14.9	0	>128	ND	ND	2.11
3.7h	H	3	33.4	42.6	0	>128	ND	ND	2.16
3.7i	H	4	34.9	34.0	3	>128	ND	ND	2.69
3.7j	H	5	27.7	36.4	14	>128	ND	ND	3.22
3.8a	H	3	17.0	18.1	0	>128	ND	ND	2.12
3.8b	H	4	21.9	32.6	45	>128	ND	ND	2.68
3.8c	H	5	9.4	18.5	2	>128	ND	ND	3.24
3.8d	Br	3	-16.2	5.1	3	>128	ND	ND	3.12
3.8e	I	3	4.9	19.6	4	>128	ND	ND	3.38
3.8f	F	3	4.9	1.3	5	>128	ND	ND	2.40
3.8g	Cl	3	13.4	15.3	0	>128	ND	ND	2.97
3.8h	CH <sub>3</sub>	3	-0.6	-4.0	0	>128	ND	ND	2.62
3.8i	NO <sub>2</sub>	3	-3.0	-5.1	11	>128	ND	ND	2.16
3.8j	Cl	4	0.0	-15.2	0	>128	ND	ND	3.53
3.8k	Br	4	-26.0	-21.1	0	>128	ND	ND	3.68
3.8l	I	4	-39.7	-40.7	0	>128	ND	ND	3.94
3.8m	Cl	5	-16	-20	19	>128	ND	ND	4.09
3.8n	F	5	-35	-28	0	>128	ND	ND	3.52
3.8o	Br	5	8.1	-0.5	4	>128	ND	ND	4.24
3.8p	I	5	-34	-40	7	>128	ND	ND	4.50

<sup>a</sup>Calculated using a control growth index of ~ 200; <sup>b</sup>Percentage inhibition at 128 μM



Of all the compounds tested with the LORA assay, the unsubstituted intermediate **3.6h** (R = H, n = 3) appears to be the most promising. With an MIC of 28.8  $\mu\text{M}$  it is more active than the standard drug moxifloxacin (MIC 31.1  $\mu\text{M}$ ). Other intermediates of interest are **3.6j**, **3.6u** and **3.6w**, which are almost equipotent to moxifloxacin. The hybrid **3.7b** appears to be less sensitive to the slow-growing mycobacteria with an MIC of 117.2  $\mu\text{M}$ .

### 3.7. Conclusion

The antiplasmodial results show that compounds in this series have poor activity compared to the control chloroquine but were more active than the parent natural products (**1.28** and isatin). Although the tetracycles **3.8** were not the compounds we initially envisaged, they turned out to be more active compared to their precursors **3.7**. Compound **3.8p** has been identified as the most active compound in this series which inhibits the CQR and CQS strains of *P. falciparum* with  $\text{IC}_{50}\text{s}$  of 6.92  $\mu\text{M}$  and 9.07  $\mu\text{M}$ , respectively. A structure-activity relationship derived for the tetracycles **3.8** is the need for substitution at C-5 of the isatin scaffold. Also concluded from the results is that falcipain-2 inhibition is not the primary mechanism of action for compounds **3.7** and **3.8**. It is evident that the alkyl linker contributed significantly towards lipophilicity. However, the observed activity for these compounds cannot be credited solely to their lipophilicity.

In contrast to the antiplasmodial results, the antitubercular results obtained clearly suggest that the intermediates are more efficacious than the hybrids. The lack of activity of the latter can most likely be ascribed to poor cell wall permeability or efflux of compounds. In general, from the results tabulated it is evident that the hybrids (except **3.7b**) do not merit further investigation as antitubercular agents.

### 3.7. References

1. Ehrlich, P.J. *Deutsch. Chem. Ges.*, **1909**, 42, 17
2. Biot, C.; Chibale, K. *Infect. Disord. Drug Targets*, **2006**, 6, 173
3. (a) Chiyanzu, I.; Clarkson, C.; Smith, P.J.; Lehman, J.; Gut, J.; Rosenthal, P.J.; Chibale, K. *Bioorg. Med. Chem.*; **2005**, 13, 3249; (b) Chiyanzu, I.; Hansell, E.; Gut, J.; Rosenthal, P.J.; McKerrow, J.H.; Chibale, K. *Bioorg. Med. Chem. Lett.*, **2003**, 13, 3527
4. Da Silva, J. F.M.; Garden, S.J.; Pinto, A.C. *J. Braz. Chem.*, **2001**, 12, 273
5. Chibale, K. *Pure Appl. Chem.*, **2005**, 77, 1957
6. (a) White, N.J. *J. Clin. Invest.*, **2004**, 113, 1084; (b) Budha, N.R.; Lee, R.E.; Meibohm, B. *Curr. Med. Chem.*, **2008**, 15, 809
7. Jones, S.M.; Urch, J.E.; Kaiser, M.; Brun, R.; Harwood, J.L.; Berry, C.; Gilbert, I.H. *J. Med. Chem.*, **2005**, 48, 5932
8. Kamal, A.; Shaik, A.A.; Sinha, R.; Yadav, J.S.; Arora, S.K. *Bioorg. Med. Chem. Lett.*, **2005**; 15; 1927
9. (a) Lin'kova, M.G.; Kuleshova, N.D.; Knunyants, I.L.; *Russian Chem. Rev.*; **1964**; 33; 493; (b) Reppe, W. *Annalen*, **1955**, 596, 170; (c) Reppe, W.; Friedrich, H.; Laib, H., *Chem. Zentr.*, **1955**, 3963
10. Benary, E. *Berichte*, **1913**, 46, 2103
11. Wang, C-L. J.; Salvino, J.M. *Tetrahedron Lett.*, **1984**, 25, 5246
12. (a) Shvekhgeimer, M.-G.A. *Chemistry of Heterocyclic Compounds*, **1996**, 32, 249; (b) Sumpter, W.C. *Chem. Rev.*, **1945**, 37, 443; (c) Tomchin, A.B.; Shirokii, G.A. *Zh. Org. Khim.*, **1977**, 13, 404
13. Aliev, N.A.; Ahmad-Hasan, E.I.; Abdusamatov, A. *Deposited Doc. VINITI 215- 78*, **1978** (CA 91:157670v)
14. Khuseinov, K. *Dokl. Akad. Nauk Tadzh. SSR.*, **1976**, 19, 30
15. (a) Muchowski, J. M.; Nelson, P.H. *Tetrahedron Lett.*, **1980**, 21, 4585; (b) Tatsugi, J.; Ikuma, K.; Izawa, Y. *Heterocycles*, **1996**, 43, 7
16. (a) Radul, O.M.; Zhungietu, G.I.; Rekhter, M.A.; Bukhanyuk, S.M. *Khim. Geterotsikl. Soedin.*, **1980**, 1562; (b) Radul, O.M.; Zhungietu, G.I.; Rekhter, M.A.; Bukhanyuk, S.M. *Khim. Geterotsikl. Soedin.*, **1983**, 353
17. Garden, S.J.; Torres, J.C.; Silva, L.E.; Pinto, A.C. *Synth. Commun.*, **1998**, 28, 1679
18. Cederlund, B.; Hörnfeldt, A.-B. *Acta Chemica Scandinavica*, **1971**, 25, 3324
19. (a) Hörnfeldt, A.-B. *Arkiv. Kemi*, **1968**, 29, 461; (b) Hörnfeldt, A.-B. *Arkiv. Kemi*, **1963**, 22, 211; (c) Hörnfeldt, A.-B. *Acta Chemica Scandinavica*, **1967**, 21, 673; (d) Hörnfeldt,

- A.-B.; Gronowitz, S. *Arkiv. Kemi*, **1963**, *21*, 239; (e) Hörnfeldt, A.-B. *Arkiv. Kemi*, **1968**, *29*, 427
20. Douglas, J.D.; Senior, S.J.; Morehouse, C.; Phetsukuri, B.; Campbell, I.B.; Besra, G.S.; Minnikin, D.E. *Microbiology*, **2002**, *148*, 3101
21. Jones, S.M.; Urch, J.E.; Brun, R.; Harwood, J.L.; Berry, C.; Gilbert, I.H. *Bioorg. Med. Chem.*, **2004**, *12*, 683
22. Toyama, K.; Tauchi, T.; Mase, N.; Yoda, H.; Takabe, K. *Tetrahedron Lett.*, **2006**, *47*, 7163
23. Wengel, A.S.; Reffstrup, T.; Boll, P.M. *Tetrahedron*, **1979**, *35*, 2181
24. Schobert, R.; Siegfried, S. *Synlett*, **2000**, *5*, 686
25. Shenoy, G.; Kim, P.; Goodwin, M.; Nguyen, Q.-A.; Barry, C.E. III; Dowd, C.S.; *Heterocycles*, **2004**, *63*, 519
26. McFadden, J.M.; Medghalchi, S.M.; Thupari, J.N.; Pinn, M.L.; Vadlamudi, A.; Miller, K.I.; Kuhajda, F.P.; Townsend, C.A. *J. Med. Chem.*, **2005**, *48*, 946
27. Sakya, S.M.; Suarez-Contreras, M.; Dirlam, J.P.; O'Connell, T.N.; Hayashi, S.F.; Santoro, S.L.; Kamicker, B.J.; George, D.M.; Ziegler, C.B. *Bioorg. Med. Chem. Lett.*, **2001**, *11*, 2751
28. Moss, G.P. IUPAC Organic Chemistry Division Commission on Nomenclature or Organic Chemistry (III.1), Nomenclature of Fused and Bridge Ring systems, *Pure & Appl. Chem.*, **1998**, *70*, 143
29. (a) Guo, X.; Huang, H.; Yang, L.; Hu, W. *Organic Lett.*, **2007**, *9*, 4721; (b) Chen, J.-R.; Liu, X.-P.; Zhu, X.-Y.; Li, L.; Qiao, Y.-F.; Zhang, J.-M.; Xiao, W.J. *Tetrahedron*, **2007**, *63*, 10437
30. (a) Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*, Oxford University Press, New York, **2001**, p. 1252; (b) Whitman, G.H. *Organosulfur Chemistry*, Oxford University Press Inc., New York, **1995**, p. 14
31. Smith, M.B.; March, J. *March's Advanced Organic Chemistry: Reactions, Mechanisms and Structure*, John Wiley and Sons Inc., U.S.A., **2007**, p. 223-254
32. (a) Knops, P.; Sendhoff, N.; Mekelburger, H.-B.; Vögtle, F. *Topics in Current Chemistry*, **1991**, *161*, 1; (b) Illuminati, G.; Mandolini, L. *Acc. Chem. Res.*, **1981**, *14*, 95; (c) Ziegler, K. In *Methoden der Organischen Chemie*, 4, Müller, E. (ed.), George Thieme Verlag, Stuttgart, **1955**; (d) Ruggli, P. *Liebigs Ann. Chem.*, **1912**, *392*, 92
33. Ruzicka, L. *Chem. Ind.*, **1935**, *54*, 2
34. Lagorce, D.; Speranio, O.; Gallons, H.; Miteva, M.A.; Villoutreix, B.O. *BMC*

*BioInformatics*, **2008**, 8, 396

35. (a) Zhanel, G.G.; Saunders, M.H.; Wolfe, J.N.; Hoban, D.J.; Karlowsky, J.A.; Kabani, A.M. *Antimicrob. Agents Chemother.*, **1998**, 42, 184; (b) Lall, N.; Meyer, J.J.M. *J.*

*Ethnopharm.*, **1999**, 66, 347

36. Collins L.A.; Franzblau, S.G. *Antimicrob. Agents Chemother.*, **1997**, 41, 1004

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## Chapter four

### DESIGN AND SYNTHESIS OF $\beta$ -AMINO ALCOHOL HYBRIDS

#### 4.1. Introduction

This chapter describes the design, synthesis and characterization of two novel series of natural-product-like hybrids. Incorporated in their design is the  $\beta$ -amino alcohol moiety, a known antimalarial pharmacophore, and the activity- and solubility enhancing 1,2,3-triazole ring system. Construction of this triazole library required the utilization of ‘click’ chemistry to aid the development of rapid SAR studies. All target compounds herein discussed, were tested for *in vitro* antiplasmodial activity against both CQR and CQS strains of *P. falciparum* as well as for anti-TB activity. In an attempt to elucidate the mechanism of action whereby these compounds exert their antimalarial activity, the cysteine proteases and in particular falcipain-2 inhibitory activity was examined.

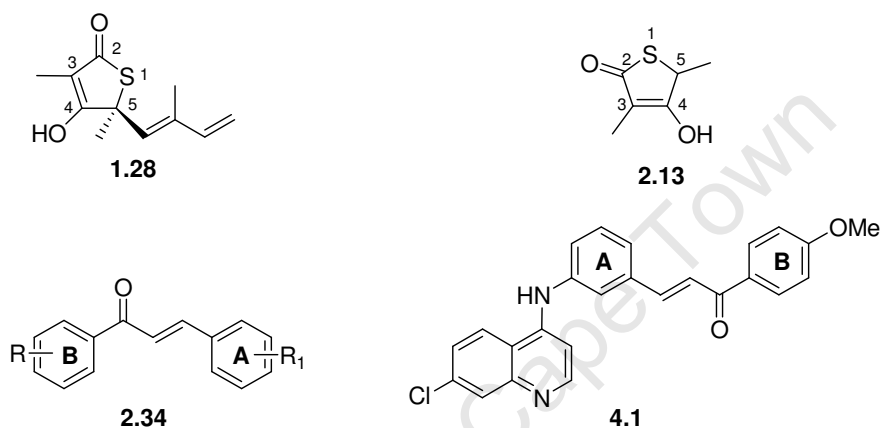
#### 4.2. $\beta$ -Amino Alcohol Thiolactone-Chalcone Hybrids

##### 4.2.1. Rationale

We relied on previous SAR studies and the TLM–FabB model (section 1.4.2.2.3) for guidance in analogue design and synthesis, because of the ‘uncertainty’ regarding the mechanism of action of thiolactomycin analogues (section 2.2.3.1). One of the key drug-receptor interactions revealed is the presence of an incompletely filled pantetheine pocket which houses the C-4 hydroxyl group. Stabilization of the latter is reportedly mediated *via* hydrogen bonding with water molecules present in this pocket.<sup>1</sup> As mentioned earlier, it has been demonstrated that effective antimalarial and antitubercular agents can be sourced from the C-4 derivatives of **1.28** and **2.13**.<sup>2</sup> With regard to tuberculosis, it is noteworthy that apart from the results reported by Kamal and coworkers,<sup>2a</sup> modification only of the C-4 hydroxyl of **2.13** is a relatively unexplored diversification route. Therefore in our study we set out to elaborate the C-4 substituent of **2.13** by introducing functionalities with hydrogen bond donors and acceptors to ensure potential increased and favourable interaction with the target site(s).

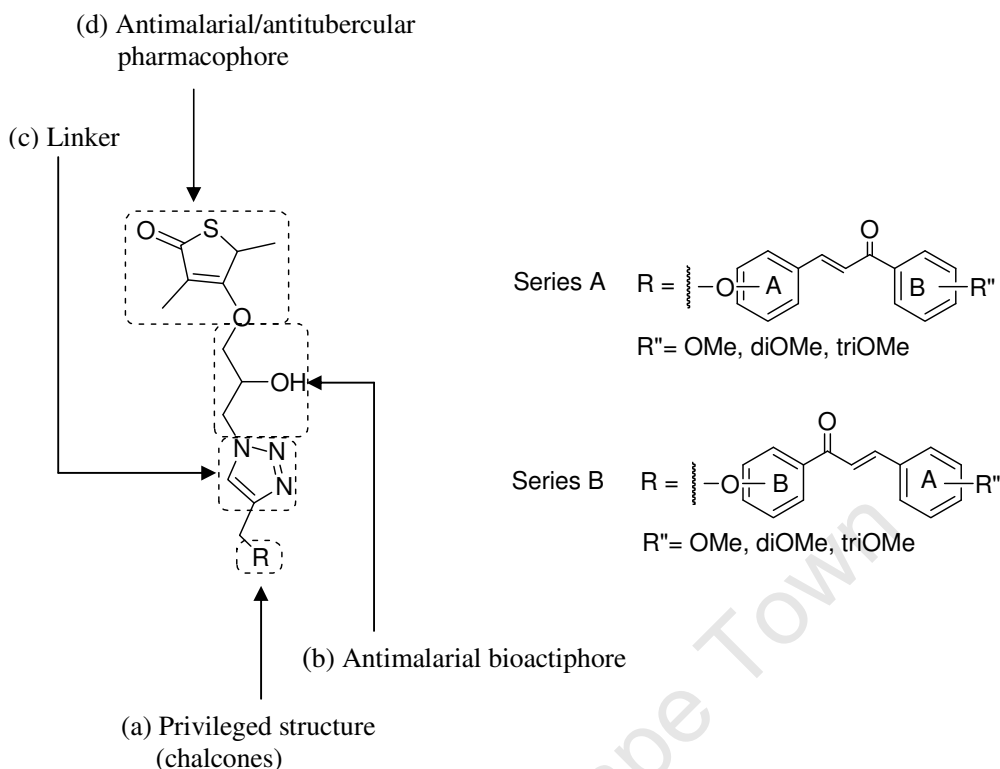
Chalcones **2.34** have been of considerable interest in drug discovery because of their diverse biological activities and in particular cysteine protease inhibition (section 2.2.3.3). Moreover,

this scaffold allows for the systematic variation of substituents and/or substitution patterns on the aromatic rings A and B for SAR investigation. Further appeal to the utilization of this scaffold stems from studies undertaken in our laboratories which involved the covalent linking of two privileged structures, the chalcone and 7-chloro-4-aminoquinoline moiety. Compound **4.1** (fig. 4.1) from this series showed *in vitro* growth inhibitory activity against two different CQR strains of *P. falciparum* with  $IC_{50}$  values of 0.87  $\mu$ M and 1.64  $\mu$ M for the FCM29 and W2 strains, respectively.<sup>3</sup>



**Figure 4.1:** Chemical structures of thiolactomyacin **1.28**, thiolactone **2.13**, the chalcone template **2.34** with rings A and B labelled and the chalcone-aminoquinoline hybrid **4.1**

By hybridizing thiolactone with chalcones and incorporating pharmacologically relevant moieties such as  $\beta$ -amino alcohol and 1,2,3-triazole ring system we envisaged stable analogues which could display enhanced therapeutic profiles. To our knowledge hybrids of this nature have not been reported in literature. Some of the key structural and chemical features targeted for investigation through structure-activity relationship studies are summarized in figure 4.2.

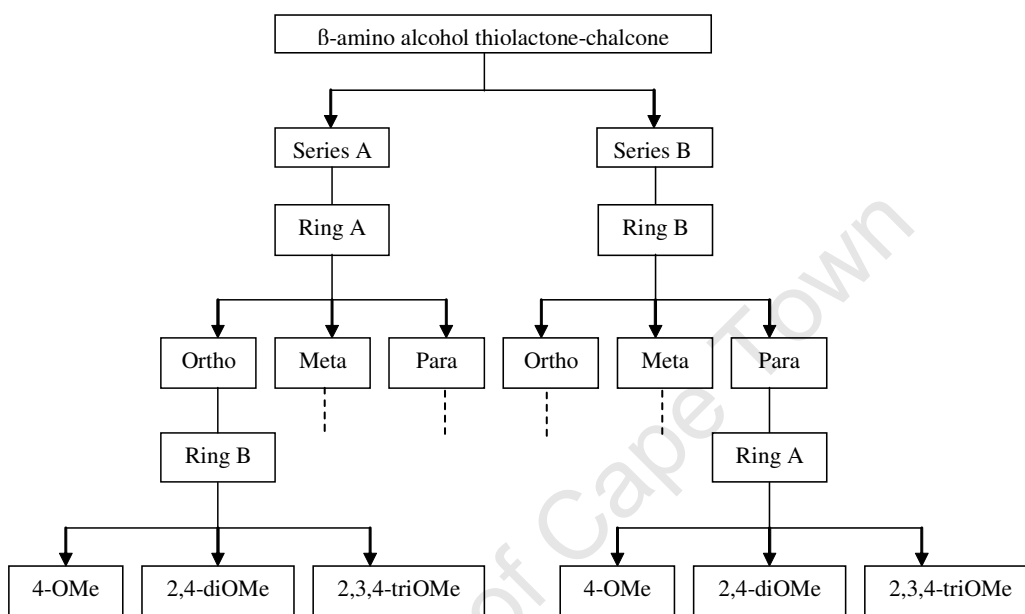


**Figure 4.2:** Rationale and general structures of the  $\beta$ -amino alcohol thiolactone-isatin hybrids

(a) Previous SAR studies on chalcones revealed that the minimum requirement for antimalarial activity is the presence of the enone linker<sup>4</sup> and for the double bond in this linker to have a *trans* (*E*)-configuration.<sup>5</sup> As shown in figure 4.2 these basic structural features were retained in the compounds synthesized. Our drug design effort was further guided by a study which showed that hydroxylated and alkoxyated chalcones are more important for antimalarial activity and that the latter are more potent.<sup>6,7</sup> With regard to cysteine protease inhibition, reports in the literature indicated a preference for electron-withdrawing substituents on ring A of the chalcone scaffold (fig. 4.2). This is believed to facilitate Michael addition at the  $\alpha,\beta$ -unsaturated carbonyl of the chalcone moiety by nucleophilic “cellular thiols”.<sup>4</sup> However, another study revealed that the *in vitro* antimalarial activity of chalcones was determined by the properties of (or substitution on) the B ring.<sup>6</sup> To add to the confusion, it is now generally accepted that cysteine protease inhibition is not the only mode of action by which chalcones exert their antimalarial activity (section 2.2.3.3).

What is noteworthy is that the number and extent of SAR studies exploring the antimalarial effect of chalcones is in stark contrast to those investigating their antimycobacterial activity.

In fact, only a few studies have been reported; one study was conducted on a 47-membered chalcone library which revealed that halogenated chalcones display superior antimycobacterial activity.<sup>8</sup> With the above considerations and in the interest of developing a focussed library from which meaningful SAR can be delineated, our choice of (additional) substituents on the chalcone scaffold was limited to the methoxy group.



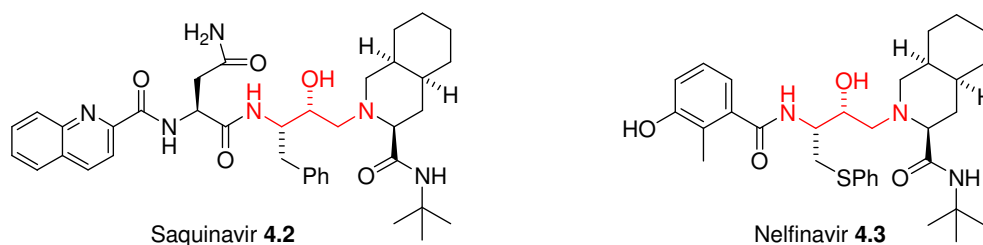
**Figure 4.3:** Organisation of  $\beta$ -amino alcohol thiolactone-chalcone hybrids

The envisaged 18-membered thiolactone-chalcone hybrid library is organized into two series labelled A and B as depicted in figure 4.3. The aromatic ring A of series A has the triazole moiety as a substituent attached at either the *ortho*-, *meta*- or *para*-position whereas ring B is methoxylated to varying degrees. A similar arrangement holds for series B the only difference is that the substituents on rings A and B are interchanged. SAR will set out to investigate the effect on antimalarial and antimycobacterial activity of (i) the number of methoxy substituents on ring B and ring A for series A and B, respectively, (ii) the position of attachment of the triazolic moiety on ring A and ring B for series A and B, respectively, and (iii) the importance of substitution on ring A/B for activity.

(b) The  $\beta$ -amino alcohol moiety is a pharmacophore which features prominently in antimalarial drugs in clinical use such as quinine (**1.1**, fig. 1.3), mefloquine (**1.4**, fig. 1.3) and halofantrine (**1.6**, fig. 1.3). A structural requirement for antimalarial activity for these amino alcohol antimalarials - which we have taken note of - is the presence of an aromatic and a  $\beta$ -



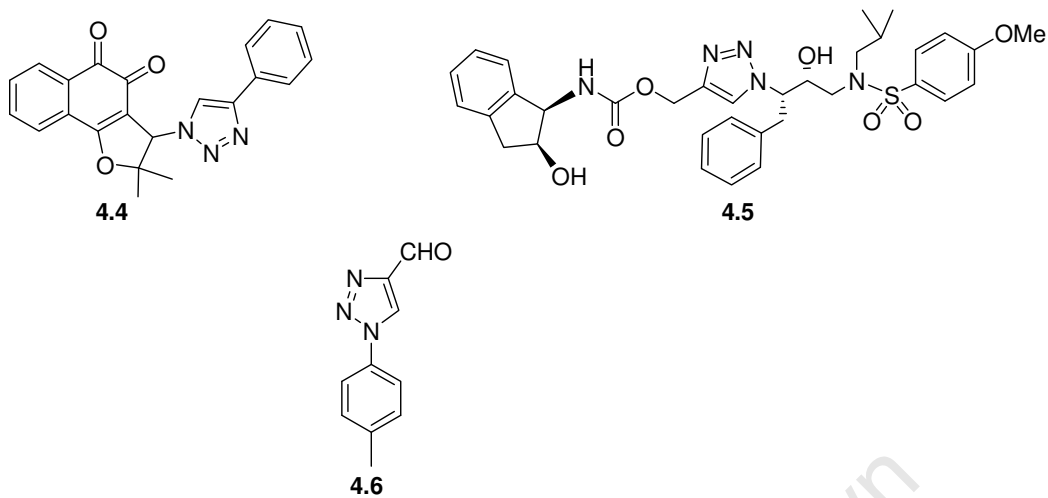
amino moiety separated by 2-3 carbon atoms.<sup>9</sup> This pharmacophore can also be found in clinically established anti-HIV drugs saquinavir **4.2** and nelfinavir **4.3** (fig. 4.4).



**Figure 4.4:** Chemical structures of  $\beta$ - amino alcohol containing anti HIV drugs saquinavir **4.2** and nelfinavir **4.3**

(c) The 1,2,3-triazole ring system obtained by ‘click’ chemistry has long shed the reputation of being a “passive linker”.<sup>10</sup> Among the attractive properties displayed by this moiety is its ability to associate with biological targets through hydrogen-bonding and dipole-dipole interactions. The latter is because of the strong dipole moment of this heterocyclic ring system.<sup>11</sup> It reportedly also mimics the atom arrangement and electronic properties of amide bonds but unlike the amide bond is not prone to hydrolytic cleavage.<sup>10-11a</sup>

Pharmacophore hybridization via the copper-catalyzed azide-acetylene cycloaddition (‘click’) reaction, offers a quick, reliable and efficient way for drug discovery and optimization. In demonstration, a 1,2,3-triazole library consisting of five nor-lapachone derivatives were screened for activity against infective bloodstream forms of *Trypanosoma cruzi*, the causative agent of Chagas’ disease. All of the derivatives were more active than the natural occurring progenitor, nor-lapachol with **4.4** (figure 4.5) being the most active.<sup>11b</sup> In another example, two focussed 1,2,3-triazole libraries of 50 compounds each based on the hydroxyethylamine peptide isostere, were synthesized with the aim of sourcing HIV protease inhibitors. Identified from this library is **4.5** (fig. 4.5) which showed inhibitory activity against four proteases (wild type HIV-1 and mutants G48V, V82F, V82A) in the low nanomolar range.<sup>12</sup> A more relevant example is the 24-membered library of *N*-substituted-phenyl-1,2,3-triazole-4-carbaldehydes which showed an impressive antimycobacterial profile. All compounds in this series showed growth inhibitory activity against *M. tuberculosis*. One of the active compounds in this series **4.6** (fig. 4.5) showed 100% growth inhibition at a MIC of 2.5  $\mu\text{g/mL}$ .<sup>13</sup>

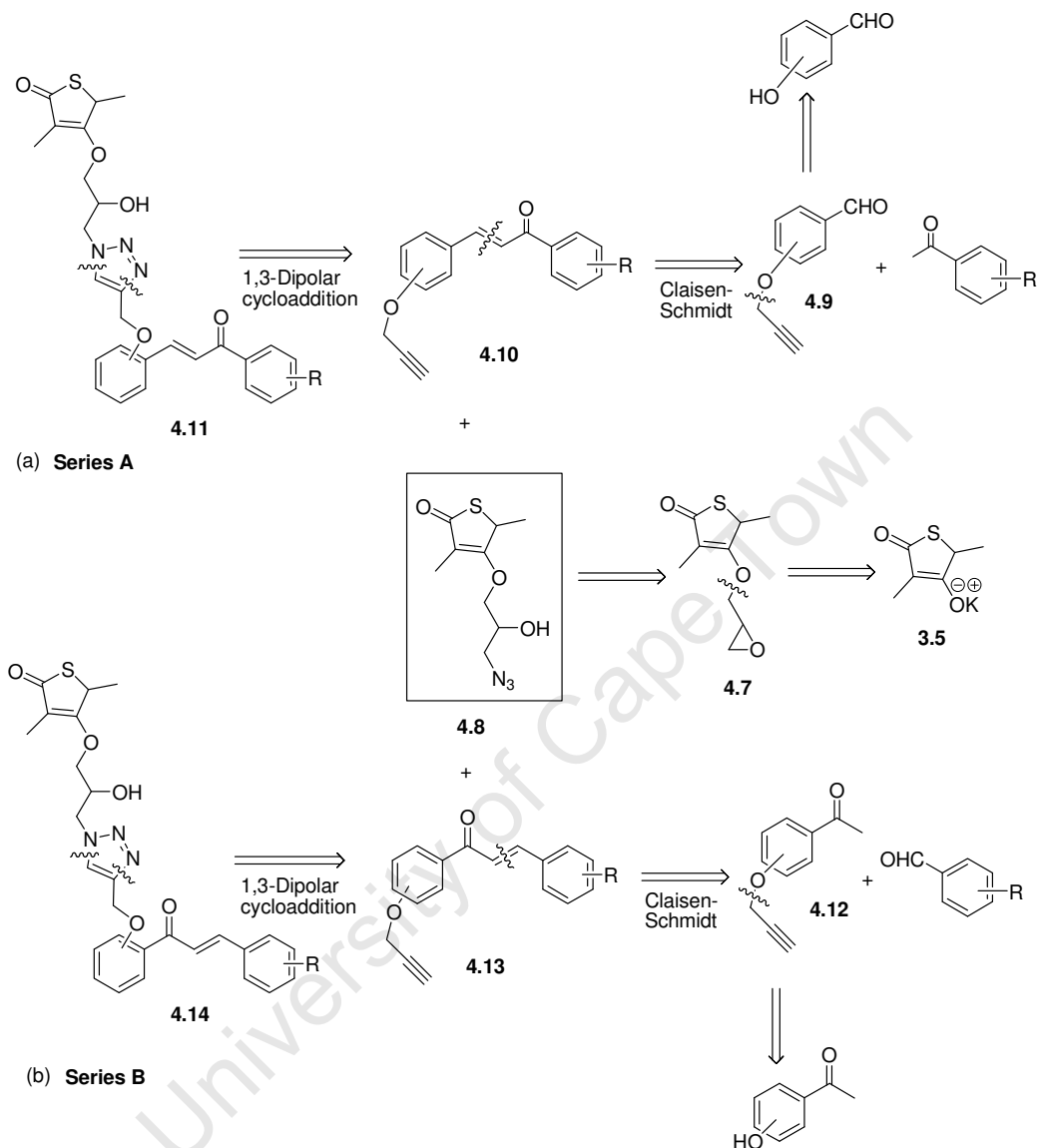


**Figure 4.5:** Chemical structures of compounds **4.4**, **4.5** and **4.6**

In addition to the activities mentioned above, 1,2,3-triazole-containing compounds are also reported to exhibit antibacterial,<sup>14</sup> anti-allergic<sup>15</sup> and fungicidal<sup>16</sup> activity.

## 4.2.2. Chemical Synthesis

## 4.2.2.1. Retrosynthetic Analysis

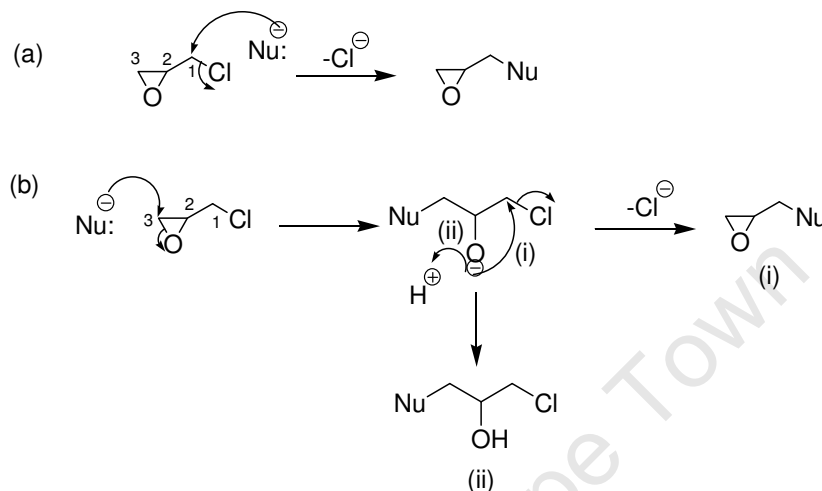


**Scheme 4.1:** Retrosynthesis of the  $\beta$ -amino alcohol thiolactone-isatin hybrid for (a) Series A and (b) Series B

The retrosynthesis of target molecules **4.11** and **4.14** is shown in scheme 4.1. Synthesis of **4.11** [scheme 4.1(a)] was envisaged from the 1,3-dipolar cycloaddition reaction of the thiolactone azide **4.8** and the acetylenic chalcone **4.10** (**4.13** for target molecule **4.14**). The latter can be obtained from the Claisen-Schmidt condensation of the acetylenic benzaldehyde **4.9** and commercially available methoxylated acetophenone. Synthesis of **4.8** in turn can be achieved *via* azidolysis of the epoxide **4.7** which can be accessed by *O*-alkylation of the

potassium salt of thiolactone **3.5** with commercially available epichlorohydrin. For series B [scheme 4.1(a)] synthesis of the acetylenic chalcone **4.13** was envisaged from the acetylenic acetophenone **4.12** and commercially available methoxylated benzaldehyde.

#### 4.2.2.2. Reactions of Epichlorohydrin with Nucleophiles



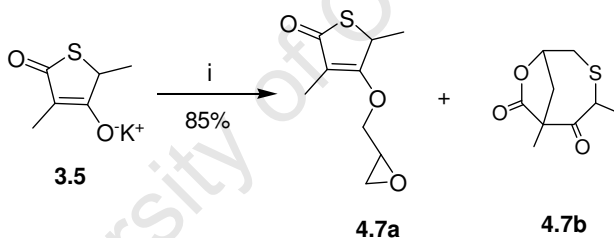
**Figure 4.6:** Reaction of epichlorohydrin with a nucleophile<sup>17</sup>

The versatility of epoxides as intermediates in organic synthesis stem from the polarity and strain of the three-membered ring; thus explaining their reactivity towards a wide range of nucleophiles, electrophiles, acids, bases as well as oxidizing and reducing agents.<sup>18</sup> Nucleophilic attack on epichlorohydrin generally leads to the formation of a new epoxide derivative. Moreover, depending on the reaction conditions there are two possible sites where nucleophilic attack can occur. Attack at C-1 [fig. 4.6 (a)] represents a direct displacement reaction. For an indirect displacement reaction nucleophilic attack occurs at C-3 followed by an intramolecular expulsion of the chloride ion by the alkoxide ion with subsequent generation of the epoxide (i) [fig. 4.6 (b)]. In the presence of a proton source or polar protic solvent the alkoxide intermediate becomes protonated leading to the formation of chloroalcohol (ii) in figure 4.6 (b).<sup>17,19a</sup> In a study conducted by McLure and coworkers the reactivity of epichlorohydrin and other 1-X-2,3-epoxy-propanes (X = MsO, TfO) were compared under different conditions, when reacted with *para*-substituted phenolates.<sup>19</sup> This study showed that the reaction of epichlorohydrin with phenolate in acetone gave the product resulting from C-3 attack preferentially, whereas the reaction in DMF led to a 1:1 mixture of products resulting from C-1 and C-3 attack. Also reported is the study by Ohishi and Nakanishi<sup>20</sup> which showed that for the reaction of epichlorohydrin with the potassium salt of

2-acetyl-7-hydroxy-benzo[*b*]furan in DMF; attack occurred predominantly at C-3 as reflected by the ratio (5:7).

#### 4.2.2.2.1. Synthesis of the Thiolactone Epoxide

The synthesis of **4.7** proved to be challenging and most of our earlier attempts were unsuccessful. One such attempt involved refluxing **2.13** with 1.2 eq of NaH and 3 eq of epichlorohydrin in dry THF in the presence of catalytic amount of *tert*-butylammonium iodide (Bu<sub>4</sub>NI). A second attempt entailed the reaction of **2.13** with 3 eq epichlorohydrin and 2 eq of KF-Al<sub>2</sub>O<sub>3</sub> in dry acetonitrile at room temperature. In a third, unsuccessful attempt, **2.13**, epichlorohydrin and 4 eq of K<sub>2</sub>CO<sub>3</sub> was stirred in dry DMF at room temperature for 16 hours and then for another 18 hours at 60°C. All of the above procedures gave a similar tlc profile and led to the isolation of an unknown product as well as unreacted starting material. A final and successful attempt was adopted from a procedure used in our group. It involved the synthesis of phthalimide epoxide by reaction of potassium phthalimide and epichlorohydrin in the neat at 160°C for 8 hours.<sup>21</sup>

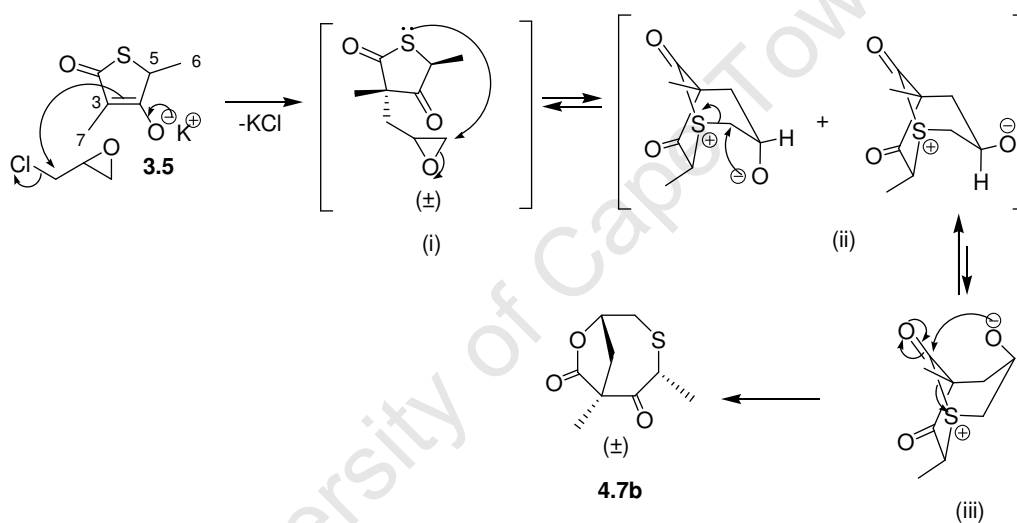


**Scheme 4.2:** Reagents and conditions: (i) ( $\pm$ )-Epichlorohydrin (neat), 130°C, 7 h

Compound **4.7** was therefore synthesized as shown in scheme 4.2. A slurry of 1.00 g of **3.5** and a large excess of ( $\pm$ )-epichlorohydrin (5 mL) was stirred at 130°C for 7 hours under an inert atmosphere. Isolated from the product mixture was a 50:50 diastereomeric mixture of the thiolactone epoxide **4.7a** (75% yield) - as inferred from <sup>1</sup>H-NMR and <sup>13</sup>C-NMR data - and a bicyclic by-product **4.7b** (10% yield). Interestingly enough, a reduction of the temperature from 130°C to 100°C did not yield the bicyclic byproduct **4.7b** and led to a concomitant increase in the yield from 75% to 90% for **4.7a**. However, the reaction under these conditions only went to completion after 12 hours.

We believe the formation of **4.7b** proceeds *via* the mechanism proposed in scheme 4.3. Guided by the relative stereochemistry of **4.7b** as inferred from its single crystal X-ray

structure (fig. 4.7); it appears that a resonance contributor of the thiolate ion (fig. 3.6) undergoes alkylation with racemic epichlorohydrin at C-3. The approach of the incoming electrophile is moreover guided by the methyl group at C-6 and the observed *syn* relationship of these methyls (C-6 and C-7) in the epoxide intermediate [scheme 4.3 (i)] is a direct result of this. The thermally enhanced nucleophilicity of sulfur allows it to - *via* its lone pair - attack the epoxide in a regioselective manner on the less hindered carbon with subsequent formation of the sulfonium ion intermediate [scheme 4.3 (ii)]. To effect lactonization, the alkoxide ion generated from the opening of the epoxide needs to have an equatorial-configuration in the chair transition state. Ring inversion to the high energy boat conformation [scheme 4.3 (iii)] brings the alkoxide ion in close proximity to the electrophilic carbonyl for intramolecular lactonization.

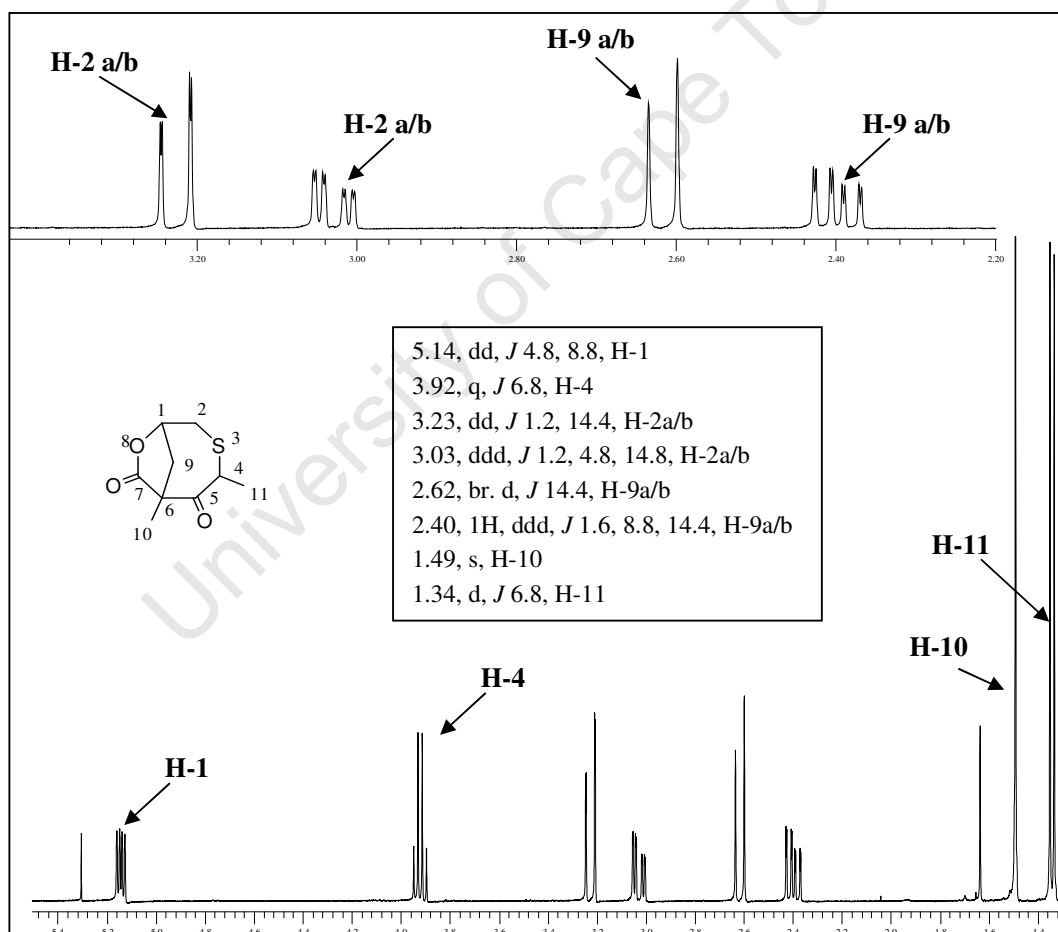


**Scheme 4.3:** Proposed mechanism for the formation of the racemic by-product **4.7b**

The observed formation of **4.7b** in low yield and the exclusive formation of **4.7a** at reduced temperatures led us to hypothesize the existence of a thermodynamic equilibrium between the two products, with **4.7a** being the thermodynamically stable product. However, in the absence of kinetic data and other supporting information the extent and influence of this proposed equilibrium on the product distribution cannot be further speculated on.

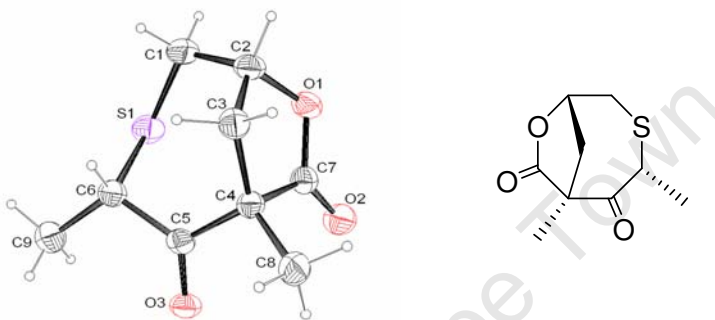
The proposed structure of the thiolactone epoxide **4.7a** was confirmed with spectroscopy data. HRMS(ESI) showed a pseudomolecular ion peak at  $m/z$  201.05809 ( $[M+H]^+$ ) for **4.7a** which corresponds to the molecular formula  $C_9H_{12}O_3S$ . Two characteristic absorption bands were identified in the IR spectra with the one at  $1677\text{cm}^{-1}$  characteristic for the carbonyl and

the strong absorption at  $1633\text{ cm}^{-1}$  for the vinyl ether functionality. In the  $^1\text{H-NMR}$  spectrum of **4.7a** the diastereotopic protons next to the oxygen of the thiolactone ring appeared as two, one-proton double doublets resonating at  $\delta$  4.06 ppm and  $\delta$  4.47 ppm. They appear significantly more downfield compared to the same protons in epichlorohydrin which appear as a two-proton doublet at  $\delta$  3.55 ppm. The presence of **4.7a** as a diastereomeric mixture was made evident by the duplication of signals in both the  $^1\text{H-NMR}$  and  $^{13}\text{C-NMR}$  spectra. Also, as mentioned in section 3.3.2.3.1, a common concern with the alkylation of  $\gamma$ -thiolactone is the regioselectivity of the reaction. The diagnostic value of the long range “allylic” coupling observed between the methyl protons H-7 and the methine protons H-5, has been discussed in the previous chapter (section 3.4.1). It provided additional confirmation of the proposed structure - on account of the small coupling constant observed for H-7 ( $J = 1.2$ ) at  $\delta$  1.83 ppm.



**Figure 4.6:**  $^1\text{H-NMR}$  spectra of compound **4.7b** in  $\text{CDCl}_3$  at 400 MHz with expanded region 2.20-3.40 ppm

The isomeric relationship between **4.7a** and **4.7b** was revealed by HR(ESI)MS which showed a pseudomolecular ion at  $m/z$  201.05803 ( $[M+H]^+$ ) for **4.7b**. X-ray crystal structure (fig. 4.7) provided the basis for the interpretation of the spectroscopic data obtained. The  $^1\text{H-NMR}$  spectrum of **4.7b** (fig. 4.5) shows the anomalous splitting patterns for the diastereotopic protons of H-9 and H-2, which is most likely due to through space coupling as a consequence of the bicyclic nature of **4.7b**.  $^1\text{H-}^1\text{H}$  COSY and other 2D experiments were invaluable in the assignment of these diastereotopic protons.



**Figure 4.7:** (a) Molecular structure of **4.7b** with the atomic numbering scheme and the displacement ellipsoids drawn at 40% probability level; (b) chemical structure of **4.7b** showing the relative stereochemistry inferred from the x-ray crystal structure

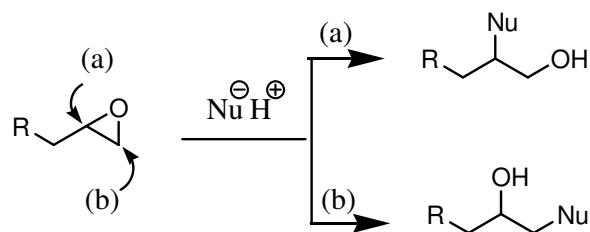
#### 4.2.2.3. Azidolysis

The regioselective opening of the epoxide ring can be performed under neutral, acidic or basic conditions. In the presence of an acid the reaction is under electronic control and proceeds *via* a  $\text{S}_{\text{N}}1$  or “borderline  $\text{S}_{\text{N}}2$ ” mechanism [fig. 4.8. (a)]; with the nucleophile attacking the more substituted carbon of the epoxide because of its ability to “accommodate” the developing positive charge in the transition state. The latter (i.e. the developing positive charge) arises from the coordination of the acid to the oxygen of the epoxide ring. Under basic and neutral conditions the reaction is under steric control and proceeds *via* an  $\text{S}_{\text{N}}2$  type reaction with the nucleophile attacking the less hindered carbon [fig. 4.8. (b)].<sup>18</sup>

In the case of monosubstituted epoxides, the substituents attached to the epoxide ring also govern the outcome of the reaction. For example when R is a phenyl or vinyl group (fig. 4.8) which are known for their ability to stabilize the developing positive charge through conjugation, nucleophilic attack at the more substituted carbon is favoured. However, when



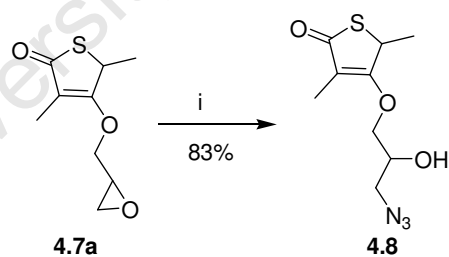
R is an electron withdrawing group, nucleophilic attack at the least hindered carbon is favoured.<sup>18</sup>



**Figure 4.8:** The mechanism of epoxide opening under different conditions

A common ring opening reaction of epoxides utilizes the azide anion as nucleophile (azidolysis) to form 2-azido alcohols. The classical azidolysis protocol employs sodium azide ( $\text{NaN}_3$ ) and  $\text{NH}_4\text{Cl}$  in aqueous alcohol at 65–80°C.<sup>22,23</sup> However, some of the drawbacks of this reaction are the long reaction times and the formation of side products resulting from isomerization, epimerization and other product rearrangements.<sup>23</sup> Various improvements on this method have since been reported with most of them involving the combination of  $\text{NaN}_3$  or  $\text{TMSN}_3$  as azide source, with a Lewis acid or transition-metal complex.<sup>24</sup>

#### 4.2.2.3.1. Synthesis of Thiolactone Azide



**Scheme 4.4:** Reagents and conditions: (i)  $\text{NaN}_3$ ,  $\text{NH}_4\text{Cl}$ , MeOH- $\text{H}_2\text{O}$  (8:1), 25°C, 48 h

In our study, the thiolactone azide **4.8** represented a key precursor. It was therefore deemed necessary to invest time in determining optimum conditions for its preparation. To this end, various reported procedures and modifications thereof have been attempted with varying success rates (table 4.1). The best procedure for the synthesis of **4.8** is a modification of the classical, non-chelating conditions (entry 1, table 4.1) which yielded the product in a regioselective manner, that is, one spot on tlc. We found that a reduction of the temperatures

from 80°C to room temperature 25°C resulted in a significant improvement of yield from 38% to 83%.

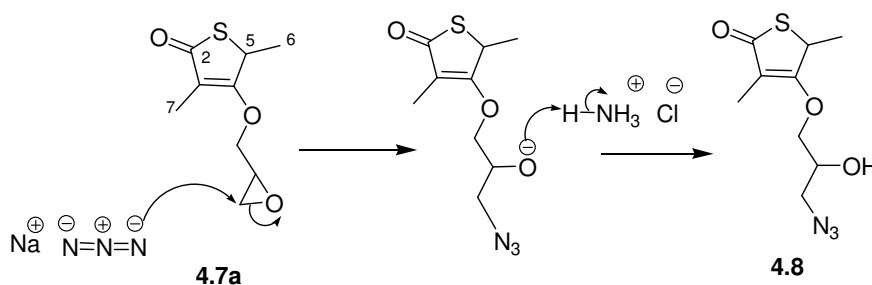
In agreement with the literature, the classical method is limited by the long reaction time but it proved far superior in terms of yield to other methods such as cerium(III) chloride in acetonitrile/H<sub>2</sub>O (9:1) (entry 2, table 4.1). Low yields (33%) were also obtained when **4.7a** was reacted with anhydrous LiClO<sub>4</sub> in dry acetonitrile at 60°C instead of the reported 80°C (entry 3, table 4.1). We were also attracted by the easy workup offered by the procedure which utilizes 4Å molecular sieves (entry 4, table 4.1), but recovered only starting material after a 24 hour reaction. With the combination of SnCl<sub>2</sub> and Mg in wet THF (entry 5, table 4.1) only traces of the product was detected on tlc after 24 hours.

**Table 4.1:** Isolated yield of **4.8** obtained under various reaction conditions

Entry	Conditions	Temperature	Duration	Yield
1	NaN <sub>3</sub> , NH <sub>4</sub> Cl, MeOH-H <sub>2</sub> O (8:1) <sup>22</sup>	80°C	24 h	38%
	NaN <sub>3</sub> , NH <sub>4</sub> Cl, MeOH-H <sub>2</sub> O (8:1)	25°C	48 h	83%
2	NaN <sub>3</sub> , CeCl <sub>3</sub> ·7H <sub>2</sub> O, CH <sub>3</sub> CN-H <sub>2</sub> O (9:1) <sup>25</sup>	80°C	24 h	29%
	NaN <sub>3</sub> , CeCl <sub>3</sub> ·7H <sub>2</sub> O, CH <sub>3</sub> CN-H <sub>2</sub> O (9:1)	25°C	24 h	0% <sup>a</sup>
3	NaN <sub>3</sub> , LiClO <sub>4</sub> , CH <sub>3</sub> CN <sup>26</sup>	60°C	19 h	33%
4	NaN <sub>3</sub> , 4Å molecular sieves, CH <sub>3</sub> CN <sup>27</sup>	25°C	24 h	0% <sup>a</sup>
5	NaN <sub>3</sub> , SnCl <sub>2</sub> , Mg, THF-H <sub>2</sub> O <sup>28</sup>	25°C	24 h	trace

<sup>a</sup> Recovered only starting material

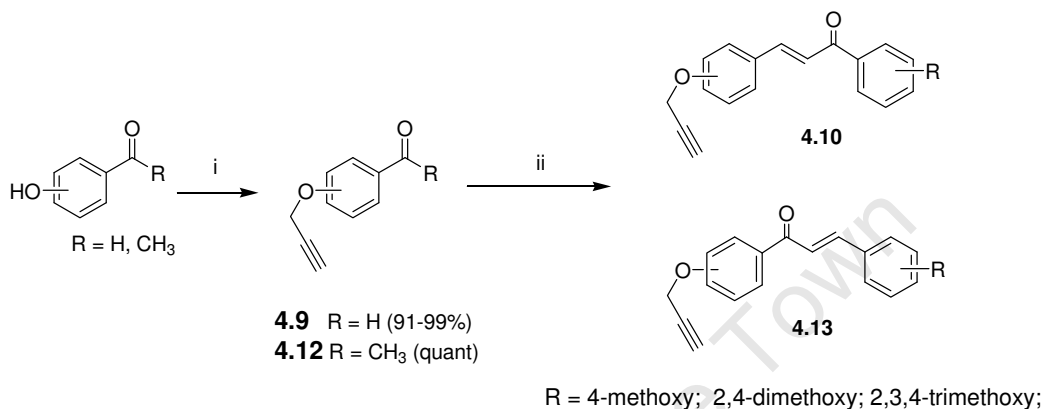
In the proposed azidolysis mechanism for **4.7a** as shown in scheme 4.5, NH<sub>4</sub>Cl acts as both buffer and proton source. The reaction is initiated by the nucleophilic attack of the azide ion on the less hindered carbon of the epoxide ring (section 4.2.2.3.1). The intermediate alkoxide so generated is protonated by NH<sub>4</sub>Cl to give the product **4.8**.



**Scheme 4.5:** Mechanism of the azidolysis of **4.7a**

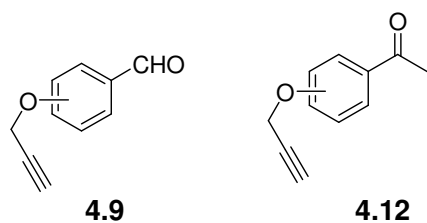
Compound **4.8** showed a strong absorption band in the IR spectrum at  $2103\text{ cm}^{-1}$  which is characteristic for the azide group. Also observed is a broad band at  $3428\text{ cm}^{-1}$  which confirms the presence of an OH group.  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$  and LRMS data correlated well with the proposed structure.

#### 4.2.2.4. Synthesis of Acetylenic Chalcones



**Scheme 4.6:** *Reagents and conditions* (i) Propargyl bromide,  $\text{K}_2\text{CO}_3$ , DMF,  $25^\circ\text{C}$ , 16 h for **4.9** and 24 h for **4.12**; (ii) Methoxylated acetophenone or benzaldehyde, 3% w/v NaOH, MeOH,  $25^\circ\text{C}$ , 16 h

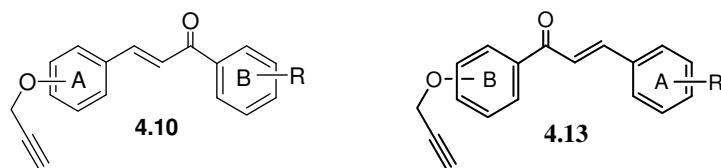
Synthesis of the acetylenic chalcones **4.10** and **4.13** is a two-step procedure as shown in scheme 4.6. The first step involved the reaction of variously substituted hydroxybenzaldehyde or hydroxyacetophenone with 1.2 eq of propargyl bromide in the presence of 1.5 eq of  $\text{K}_2\text{CO}_3$  in anhydrous DMF at room temperature. Acetylenes **4.9** and **4.12** were obtained in excellent to quantitative yields as shown in table 4.2.

**Table 4.2:** Isolated yields of acetylenes **4.9** and **4.12**

Compound	Position	Yield	m.p. (°C)
<b>4.9a</b>	ortho	quant	68
<b>4.9b</b>	meta	quant	- <sup>a</sup>
<b>4.9c</b>	para	91%	79-80
<b>4.12a</b>	ortho	quant.	38
<b>4.12b</b>	meta	quant.	29
<b>4.12c</b>	para	quant.	73

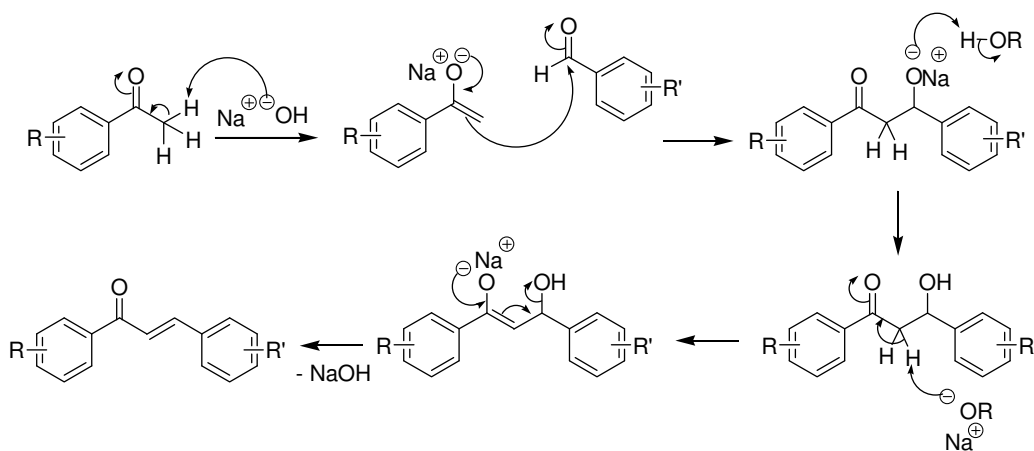
<sup>a</sup>Compound isolated as an oil

The second step of the synthesis known as the Claisen-Schmidt condensation, provided access to **4.10** and **4.13** using the method reported by Liu *et al.*<sup>6</sup> It involved the reaction of the acetylenes **4.9** and **4.12** with an equimolar amount of commercially available methoxylated acetophenones or benzaldehydes in methanol at room temperature, using methanolic NaOH as catalyst. The use of a minimal amount of solvent allowed for most of the chalcones to precipitate out of the reaction mixture as pale yellow solids. Workup and purification with column chromatography (0-40% EtOAc:Hex) was required for compounds **4.10c** and **4.13a-c** thus explaining the higher yields obtained for them (table 4.3). Recrystallized yields and melting points for compounds **4.10** and **4.13** are shown in table 4.3.

**Table 4.3:** Recrystallized yields and melting points of acetylenic chalcones

Compound	Position	R	Yield(%)	m.p.(°C)
4.10a	ortho	4-OMe	91	78-79
4.104b	ortho	2,4-diOMe	89	76
4.10c	ortho	2,3,4-triOMe	94 <sup>a</sup>	oil
4.10d	meta	4-OMe	78	68-69
4.10e	meta	2,4-diOMe	76	72-73
4.10f	meta	2,3,4-triOMe	76	61-62
4.10g	para	4-OMe	80	135-136
4.10h	para	2,4-diOMe	89	113
4.10i	para	2,3,4-triOMe	83	114
4.13a	ortho	4-OMe	85 <sup>a</sup>	oil
4.13b	ortho	2,4-diOMe	quant. <sup>a</sup>	oil
4.13c	ortho	2,3,4-triOMe	quant. <sup>a</sup>	oil
4.13d	meta	4-OMe	67	83
4.13e	meta	2,4-diOMe	88	132
4.13f	meta	2,3,4-triOMe	74	74
4.13g	para	4-OMe	87	112
4.13h	para	2,4-diOMe	78	76-77
4.13i	para	2,3,4-triOMe	71	97-98

<sup>a</sup>Isolated yield.



**Scheme 4.7:** Mechanism for the Claisen-Schmidt condensation

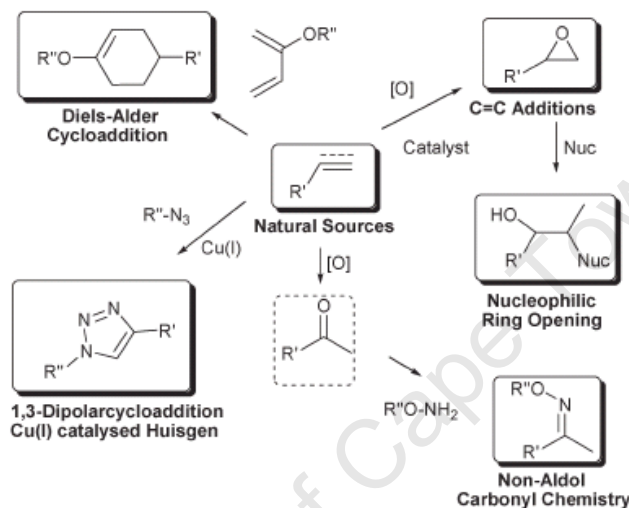
The mechanism for the Claisen-Schmidt condensation is depicted in scheme 4.7. It involves the base-catalyzed enolization of acetophenone followed by nucleophilic attack of the enolate on the benzaldehyde. The  $\beta$ -hydroxy ketone so formed undergoes base catalyzed elimination in a E1cB mechanism to yield the  $\alpha,\beta$ -unsaturated chalcone.

All of the chalcones were characterized with IR,  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR and low resolution mass spectroscopy. The presence of a triple bond was inferred from the weak absorption band appearing in the range  $2106\text{-}2125\text{ cm}^{-1}$  in the IR spectrum for **4.10** and **4.13**. In the aromatic region of the  $^1\text{H}$ -NMR spectrum the multiplicities or splitting patterns of the signals varied depending on the substitution pattern on the aromatic rings A and B. However, two characteristic one-proton doublets in the aromatic region were identified for all compounds in this series. The one resonating in the range  $\delta\ 7.26\text{-}7.67$  ppm was assigned to the alpha proton ( $\text{H}_\alpha$ ) of the enone linker whereas the beta proton ( $\text{H}_\beta$ ) proton appeared relatively more deshielded at  $\delta\ 7.63\text{-}8.07$  ppm. The coupling constant  $J$   $15.9\text{-}16.2$  noted for these protons is indicative of the *trans* geometry of the double bond. The remaining spectroscopic data compared well with that reported for other chalcone derivatives.<sup>6a</sup>

#### 4.2.2.5. 'Click' Chemistry

'Click' chemistry was coined by Sharpless and coworkers in 2001. It encompasses a series of reactions which involve carbon-heteroatom bond formation.<sup>29</sup> According to the criteria outline by Sharpless *et al*<sup>30</sup> these reactions are: modular, high yielding, wide in scope, insensitive to oxygen or water, stereospecific and form harmless by-products which can be removed without the need for chromatographic purification. Shown in figure 4.9 are

examples of 'click' reactions which include cycloaddition reactions such as the 1,3-dipolar and Diels-Alder reactions; the nucleophilic opening of strained rings for example epoxides and aziridines; additions to C=C double bonds for example epoxidation, dihydroxylation and some carbonyl reactions.<sup>30</sup> Within the class of cycloaddition reactions, the most popular - and of particular relevance to this project - is the 1,3-dipolar cycloaddition between azides and acetylenes which was first introduced by Huisgen in the 1960s.

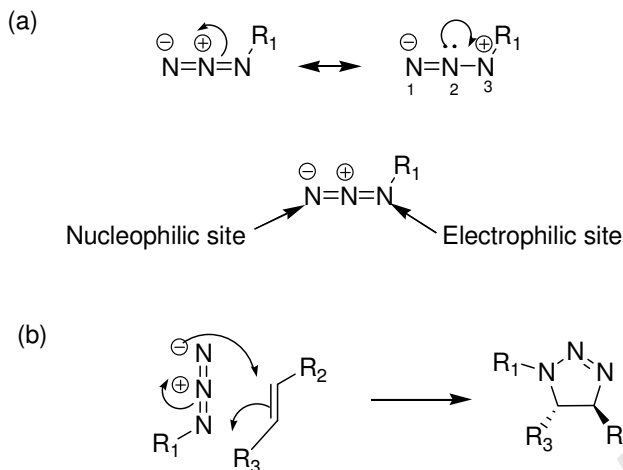


**Figure 4.9:** Examples of click reactions and their respective products<sup>31</sup>

#### 4.2.2.5.1. Huisgen 1,3-Dipolar Cycloaddition Reaction

The above is characterized by the reaction of a 1,3-dipole with a dipolarophile which leads to the formation of a 5-membered heterocyclic ring. Qualifying as 1,3-dipoles are molecules which contain one or more heteroatoms and can be described as having at least one mesomeric structure represented as a charged dipole (in a 1,3-relation) as shown for the azide functionality in figure 4.10(a). 1,3-Dipoles can be subdivided into two categories; the linear or propargyl anion-type which includes azides, nitrile oxides and diazoalkanes, whereas ozone, azomethine ylides and nitrones falls under the bent or allyl anion-type. The other reacting component is called a dipolarophile and contains a double or triple bond; examples therefore include alkenes, alkynes, carbonyls, nitriles and imines.<sup>32</sup> This pericyclic reaction moreover involves the concerted<sup>33</sup> movement of six electrons - 4  $\pi$ -electrons from the 1,3-dipole and 2  $\pi$ -electrons from the dipolarophile - in a suprafacial manner [fig. 4.10(b)] according to the Woodward-Hoffmann rules; hence the description thereof as a  $[2\pi_s+4\pi_s]$

cycloaddition [fig. 4.10(b)]. This notation moreover differs from the commonly and informally used [2+3] cycloaddition which refers to the number of atoms involved.<sup>33</sup>



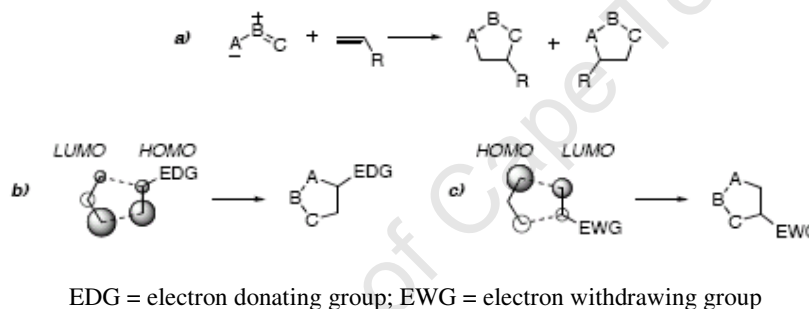
**Figure 4.10:** (a) Mesomeric forms of the azide 1,3-dipole; (b) Mechanism of the classical Huisgen cycloaddition between an azide and an alkene which demonstrates the suprafacial stereospecificity of the addition

Frontier molecular orbital (FMO) theory allows a prediction of the reactivity<sup>35</sup> and selectivity<sup>36</sup> of cycloaddition reaction and is based on the interaction of the HOMOs (Highest Occupied Molecular Orbital) and LUMOs (Lowest Unoccupied Molecular Orbital) of the 1,3-dipole and dipolarophile. Stabilization of the resulting complex (or transition state) is reportedly brought about by the closeness in energy of the interacting orbitals (HOMO and LUMO). The energy of the latter in turn is determined by the electron withdrawing or electron-donating ability of the substituent group attached.<sup>35a</sup> Moreover, the simultaneous presence of an electrophilic and nucleophilic site [fig. 4.10 (a)] allows 1,3-dipoles to react using either their HOMOs or LUMOs, that is, depending on whether the dipolarophile is electron-poor or electron-rich.<sup>32a</sup> For example, electron-withdrawing groups on the dipolarophile favour interaction of the LUMO of the dipolarophile with the HOMO of a 1,3-dipole and according to the Sustmann classification represents a type I or HOMO-controlled interaction which is most typical for nucleophilic 1,3-dipoles such as diazomethane. The reverse interaction, also known as a type III or LUMO-controlled interaction, will be dominant for electron-rich dipolarophiles and electrophilic 1,3-dipoles such as ozone. A type II or HOMO-LUMO-controlled interaction occurs with ambiphilic 1,3-dipoles. The interacting orbitals for this type of reaction display similar energies resulting in the reaction



being controlled by both  $\text{HOMO}_{\text{dipole}}\text{-LUMO}_{\text{dipolarophile}}$  and  $\text{LUMO}_{\text{dipole}}\text{-HOMO}_{\text{dipolarophile}}$  interactions.<sup>35,36a</sup>

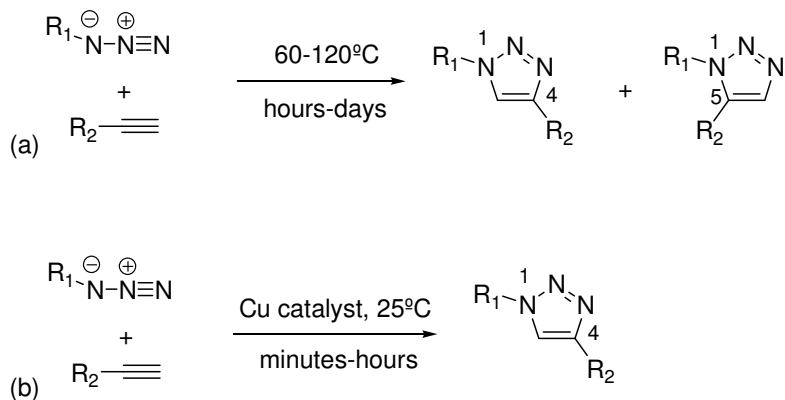
Unsymmetrical dipolarophiles may give rise to two possible regioisomers depending on the orientation of the dipole when attacking the dipolarophile as shown in figure 4.11(a). The preferred transition state will involve interaction of orbitals with large terminal coefficients as shown in figure 4.11(b). Accordingly, knowledge on the relative sizes (coefficients) of the interacting orbitals allows the prediction of the regioselectivity of the cycloadduct. It is important to note that the regio- and stereoselectivity of 1,3-dipolar cycloaddition reaction is influenced by both electronic and steric factors.<sup>36a,b</sup> Because of the limited scope of the project the latter will not be further elaborated on.



**Figure 4.11:** Regioselectivity in 1,3-dipolar cycloaddition reaction<sup>36c</sup>

#### 4.2.2.5.2. The Cu(I) Catalyzed Variant of Huisgen's 1,3-Dipolar Cycloaddition

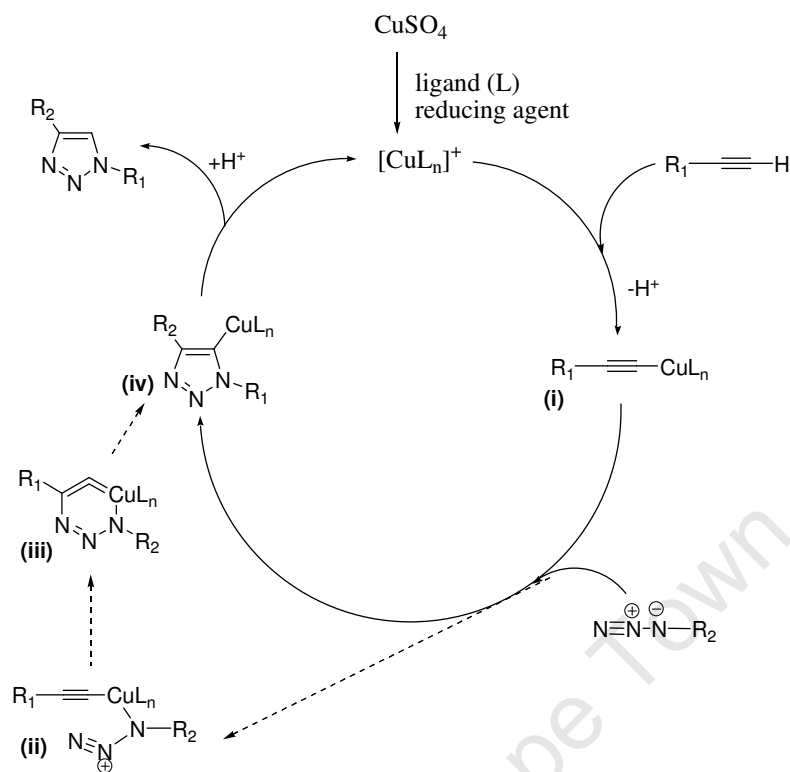
Under thermal conditions the Huisgen's cycloaddition reaction generates both 1,4- and 1,5-regioisomers [fig. 4.12 (a)]. The observed lack of selectivity was ascribed to similar activation energies for the concerted process which leads to these two regioisomers.<sup>37</sup> The research groups of Sharpless<sup>38</sup> and Meldal<sup>39</sup> showed in two independent studies that the addition of Cu(I) in catalytic amounts not only increased the reaction rate but also produced the 1,4-regioisomer exclusively [fig. 4.12 (b)]. Since this discovery the reaction has undergone further developments which resulted in a widening of its application in the fields of drug discovery,<sup>10</sup> bioconjugation<sup>40</sup> and materials science.<sup>40a,41</sup>



**Figure 4.12:** (a) Thermal and (b) Cu catalyzed azide-acetylene cycloaddition reaction<sup>42,43</sup>

The most common source of Cu(I) is from the *in situ* reduction of  $CuSO_4 \cdot 5H_2O$  or  $Cu(OAc)_2$  by sodium ascorbate.<sup>37,42,43</sup> Other sources of copper include the inorganic Cu(I) salts ( $CuI$  and  $CuBr$ ),<sup>44a</sup> organic Cu(I) complexes [ $Cu(PPh_3)_3Br$ ,<sup>44b</sup>  $(EtO)_3P^+CuI$  <sup>44c</sup>], the comproportionation of  $Cu(0)$  and  $Cu(II)$ <sup>40a,45</sup> and Cu(I) stabilized derivatives.<sup>46</sup> Another noteworthy development is the one-pot reaction which affords 1,4-disubstituted 1,2,3-triazoles derivatives from aromatic and activated aliphatic halides.<sup>47</sup> The synthetic usefulness of this procedure is that it avoids the isolation and handling of potentially explosive organic azides.

The mechanism proposed for the copper-catalyzed reaction differs from that depicted in figure 4.10(b) and is supported by kinetic studies and density functional theory (DFT) calculations.<sup>44a,48</sup> As shown in scheme 4.8, it is believed to proceed in a stepwise manner and is initiated by the formation of a copper-alkyne  $\pi$ -complex. Formation of the latter effectively lowers the  $pK_a$  of the alkyne thus making it acidic enough for deprotonation to occur in aqueous media with subsequent formation of the copper(I) acetylide [scheme 4.8 (i)]. In the next step a copper-acetylide-azide complex is formed [scheme 4.8 (ii)] which undergoes an intramolecular cyclization to generate an unusual six-membered Cu(III) metallocycle [scheme 4.8 (iii)]. Ring contraction of the latter generates the 1,2,3-triazole-copper intermediate [scheme 4.8 (iv)] which is protonated followed by the release of the 1,4-substituted 1,2,3-triazole and regeneration of the catalyst.

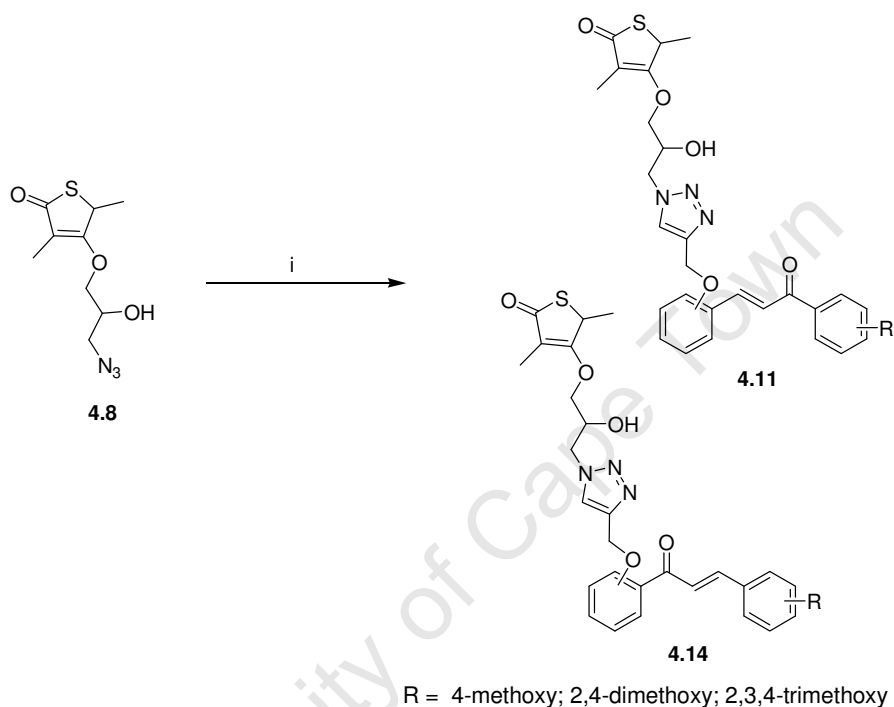


**Scheme 4.8:** Proposed mechanism for the Cu(I)-catalyzed azide-acetylene cycloaddition<sup>43,44</sup>

The advantages and scope of click chemistry outweigh its limitations. A common side reaction is the copper catalyzed homocoupling of acetylenes.<sup>49-51</sup> This stems from the similar conditions, also known as Hay's<sup>52</sup> conditions, employed in the Glaser reaction for the oxidative coupling of terminal acetylenes.<sup>49b,c</sup> To this end the use of nitrogen containing bases<sup>46a</sup> and the exclusion of oxygen from reaction mixtures is recommended whenever copper salts are employed as catalysts.<sup>39</sup> It is plausible that the oxidative dimers or bistriazoles identified as (minor) by-products in the copper catalyzed cycloadditions undertaken by Angell and coworker<sup>50</sup> originated from the *in situ* generated homocoupled acetylenes.

Also demonstrated are the structural limitations on acetylenes in the 'click' reaction. For example, a product mixture consisting of an enol lactone and its hydrolysis product were obtained when 4-pentynoic acid was reacted with benzyl azide under aqueous 'click' chemistry conditions.<sup>53a</sup> In a separate study, the reaction of sulfonyl substituted azides with acetylenes in the presence of Cu(I) and amines yielded only N-sulfonyl amines.<sup>53b</sup> With regard to yields, the promise of high yields is an important and attractive feature for these

type of reactions. However when Bhattacharjya and coworkers<sup>54</sup> employed the classical conditions (1 mol%  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , 10 mol% sodium ascorbate, in *t*-BuOH at 25°C) in the synthesis of medium to large ring triazolophanes, they recorded yields in the range 31-35% which could not be improved upon by changing solvents or increasing the amount of catalyst. Low yields as a result of the competing reaction discussed above has been reported by other research groups.<sup>49c</sup>



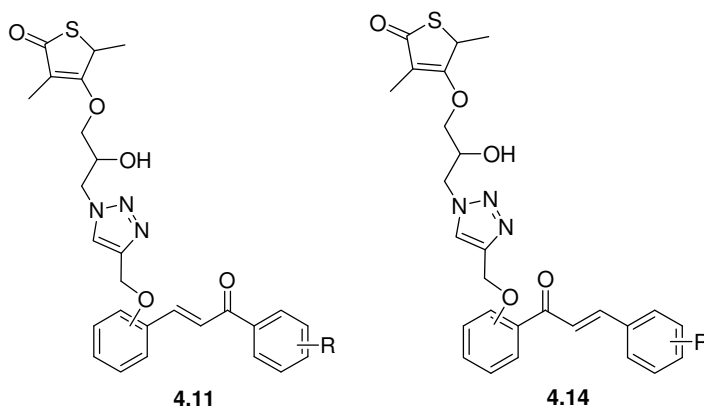
**Scheme 4.9:** Reagents and conditions: (i) **4.10** (for **4.11**) or **4.13** (for **4.14**),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , sodium ascorbate,  $\text{CH}_2\text{Cl}_2:\text{H}_2\text{O}(1:1)$ , 25°C, 16 h

#### 4.2.2.5.3. Synthesis of $\beta$ -Amino Alcohol Thiolactone-Chalcone Hybrids

As mentioned earlier, the classical ‘click’ conditions utilizes  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  or  $\text{Cu}(\text{OAc})_2$  in the presence of a reducing agent, sodium ascorbate, at ambient temperature; in water and an organic co-solvents such as ethanol, DMSO, THF, *tert*-butanol, acetonitrile and DCM.<sup>55</sup> The nonpolar nature of the acetylenic and azidic intermediates were considered in the selection of a suitable solvent system. Synthesis of target molecules **4.11** and **4.14** (scheme 4.9) was therefore achieved using the procedure reported by Lee *et al*<sup>55</sup> For the reaction, 1.0 eq of the azide **4.8**, 1.1 eq of the acetylene **4.10** (**4.13** for target molecules **4.14**), 5 mol % of  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$  and 10 mol % of sodium ascorbate in  $\text{CH}_2\text{Cl}_2/\text{H}_2\text{O}$  (1:1) was stirred overnight at 25°C. Because of the heterogeneous nature of the mixture vigorous stirring was maintained throughout. When the reaction was completed, as indicated by the consumption

of **4.8** on tlc, a blue colour was observed for the mother liquor and the precipitated product was covered with a thin layer of a brown residue. Speculatively, this observation concur with literature reports on the thermodynamic instability of Cu (I) and the ease with which it can be oxidized and/or disproportionated to Cu(II) (most likely responsible for the blue colour of the product mixture) and Cu(0) (the observed brown residue).<sup>46a,56</sup>

We anticipated the formation of mixture of diastereomers because of presence of two chiral centers in the target molecules. However, thin layer chromatography revealed two new spots; one for the product and the other for an unidentified by-product whose <sup>1</sup>H-NMR hinted at a homocoupled chalcone derivative (section 4.2.2.5.2). Purification by column chromatography was therefore required to separate the product from excess acetylene and by-product but also to remove the copper contaminants (brown residue).<sup>43,57</sup> It is possible that the chromatographic purification might have contributed towards product losses. The triazole products **4.11** and **4.14** were isolated either as yellow gums or foams and in some instances melting points could not be determined. Isolated yields are shown in table 4.4. The yields obtained for the target molecules are considerably lower than what is expected for reactions of this nature; this observation, however, cannot be explained.

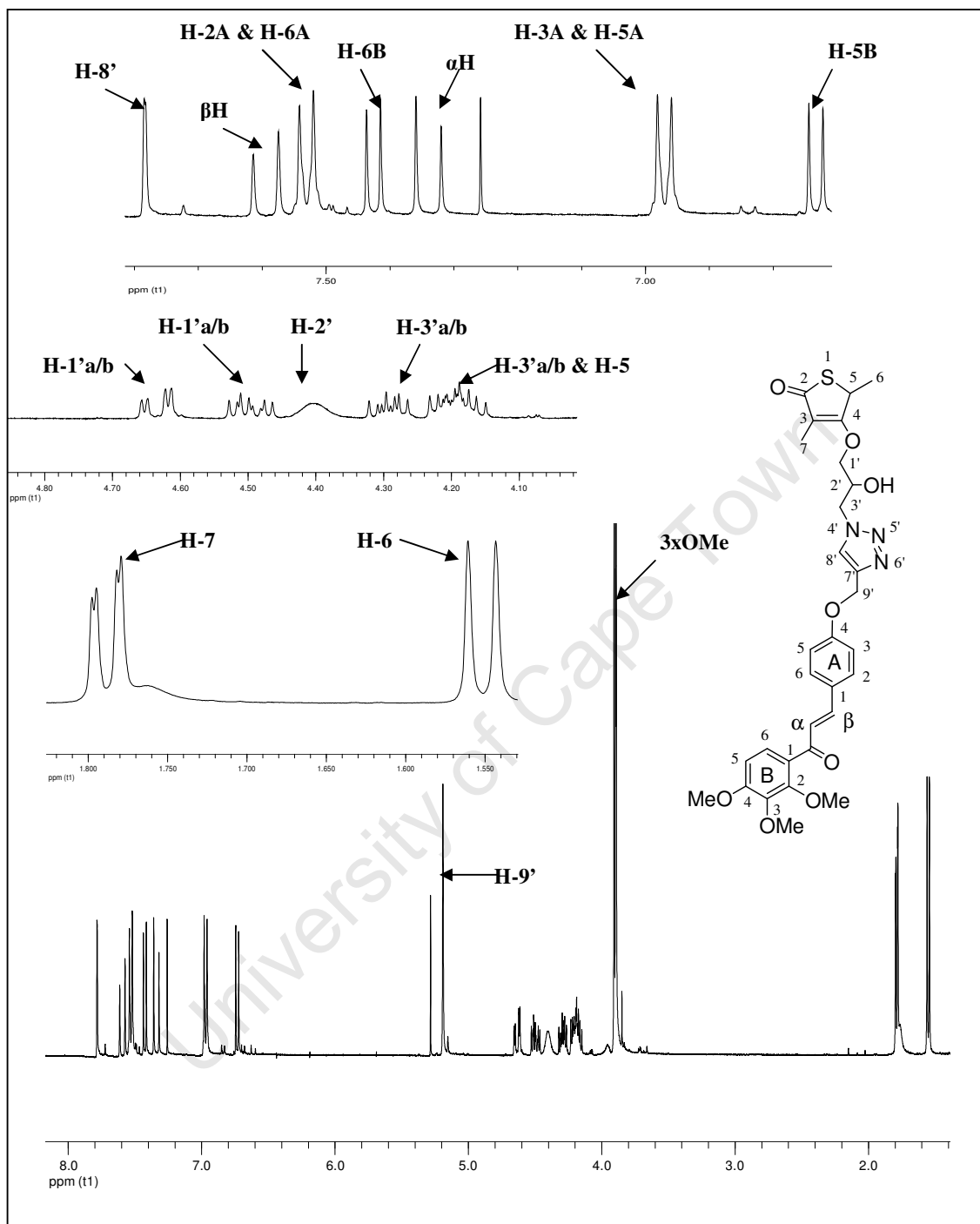
**Table 4.4:** Isolated yields of target molecules **4.11** and **4.14**

Compound	Position	R	Yield(%)
<b>4.11a</b>	ortho	4-OMe	19
<b>4.11b</b>	ortho	2,4-diOMe	38
<b>4.11c</b>	ortho	2,3,4-triOMe	32
<b>4.11d</b>	meta	4-OMe	20
<b>4.11e</b>	meta	2,4-diOMe	26
<b>4.11f</b>	meta	2,3,4-triOMe	22
<b>4.11g</b>	para	4-OMe	23
<b>4.11h</b>	para	2,4-diOMe	19
<b>4.11i</b>	para	2,3,4-triOMe	43
<b>4.14a</b>	ortho	4-OMe	32
<b>4.14b</b>	ortho	2,4-diOMe	36
<b>4.14c</b>	ortho	2,3,4-triOMe	33
<b>4.14d</b>	meta	4-OMe	23
<b>4.14e</b>	meta	2,4-diOMe	36
<b>4.14f</b>	meta	2,3,4-triOMe	18
<b>4.14g</b>	para	4-OMe	20
<b>4.14h</b>	para	2,4-diOMe	20
<b>4.14i</b>	para	2,3,4-triOMe	17

#### 4.2.2.5.3.1. Characterization

Target molecules **4.11** and **4.14** were fully characterized using  $^1\text{H-NMR}$ ,  $^{13}\text{C-NMR}$ , IR- and Mass spectroscopy. The experimentally determined elemental analysis results agreed within  $\pm 0.4\%$  with the calculated values. Identified from the infrared spectrum of these compounds are three characteristic bands; a broad band in the range  $3393\text{-}3418\text{ cm}^{-1}$  assigned to the OH group, a weak to medium band at  $1625\text{-}1633\text{ cm}^{-1}$  which corresponds to the carbonyl of the thiolactone ring and medium band at  $1600\text{-}1604\text{ cm}^{-1}$  for the  $\alpha,\beta$ -unsaturated carbonyl of the chalcone. In addition, confirmation of the molecular formula  $\text{C}_{30}\text{H}_{33}\text{O}_8\text{N}_3\text{S}$  for **4.11i** which is representative of compounds in this series was done using HREIMS which showed a molecular ion peak at  $m/z$  595.1993  $\text{M}^+$ .

Complete assignment of the  $^1\text{H-NMR}$  chemical shifts was done with the aid of  $^1\text{H-}^1\text{H-COSY}$ , HMQC and HMBC data. Among the key signal identified in the  $^1\text{H-NMR}$  spectrum (fig. 4.13) is a one proton singlet resonating downfield at  $\delta$  7.78 ppm which was assigned to the proton of the triazole ring system (H-8'). Further confirmation of the formation of the triazole ring system was presented by the change in multiplicity and the downfield shift of the methylenic protons H-9' from  $\delta$  4.72 ppm (*doublet*) for the acetylenic chalcone **4.10i** to  $\delta$  5.19 ppm (*singlet*) in **4.11i**. An anomalous splitting pattern was observed for the methyl protons H-7, which appeared as a double doublet (and for some compounds the signal collapsed to a broad doublet) resonating at  $\delta$  1.78 ppm for **4.11i**. Speculatively this may be due either to some through space coupling or could indicate the diastereomeric nature of **4.11i**. Also of interest was the methine proton at H-2' appearing as a one-proton multiplet sandwiched between the non-equivalent diastereotopic protons of H-1' and H-3', respectively. Compared to H-1' the H-3' protons appeared less deshielded as two multiplets at  $\delta$  4.29 ppm and  $\delta$  4.19 ppm, with the latter signal overlapping with that of the methine proton at H-5. The signals in the aromatic region of the spectra for these series of compounds, varied depending on the substitution pattern on rings A and B.



**Figure 4.13:**  $^1\text{H-NMR}$  spectrum of **4.11i** in  $\text{CDCl}_3$  at 400MHz



The  $^{13}\text{C}$ -NMR spectrum of **4.11i** is shown in figure 4.14. Characteristic signals include the two most deshielded quaternary carbons resonating at  $\delta$  195.7 and  $\delta$  191.1 assignable to C-2 and the  $\alpha,\beta$ -unsaturated carbonyl of the chalcone, respectively. Other carbons of the enone linker C $\alpha$  and C $\beta$  were found to resonate at  $\delta$  124.8 and  $\delta$  142.8, respectively. The formation of the triazole ring system was also inferred from the presence of a quaternary signal at  $\delta$  142.2 and a protonated carbon at  $\delta$  124.6 assigned to C-7' and C-8', respectively. No duplication of signals was observed thus ruling out the possibility of a mixture of diastereomers.

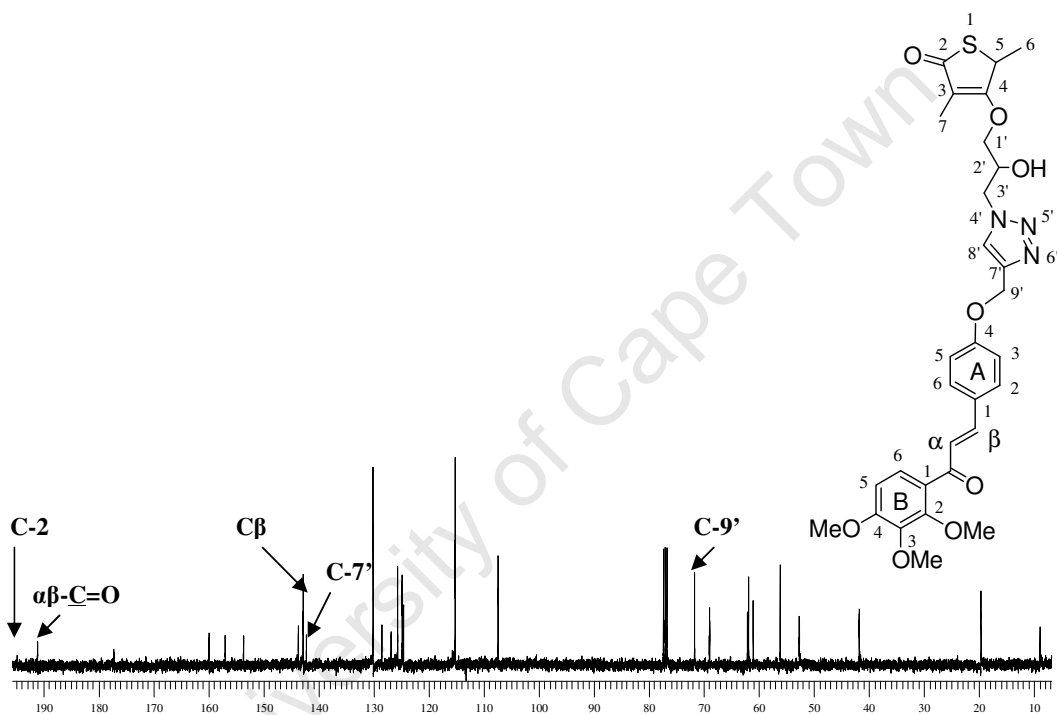


Figure 4.14:  $^{13}\text{C}$ -NMR spectrum of **4.11i** in  $\text{CDCl}_3$  at 100MHz

### 4.2.3. Biological Results and Discussion

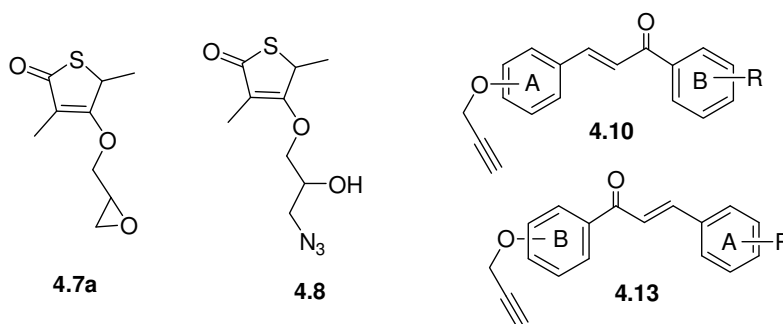
#### 4.2.3.1. *In vitro* Antiplasmodial Activity

Target molecules **4.11a-i**, **4.14a-i** and intermediates **4.7a**, **4.8**, **4.10a-i** and **4.13a-i** were tested for growth inhibitory activity against the CQR (W2) strain in the laboratories of Prof. P.J. Rosenthal at the Department of Medicine, San Francisco General Hospital, University of California San Francisco (UCSF); growth inhibitory activity against the CQS (D10) strain was conducted locally in the laboratories of Prof. P. Smith at the Department of Pharmacology (UCT). The results obtained are summarized in tables 4.5 and 4.6.

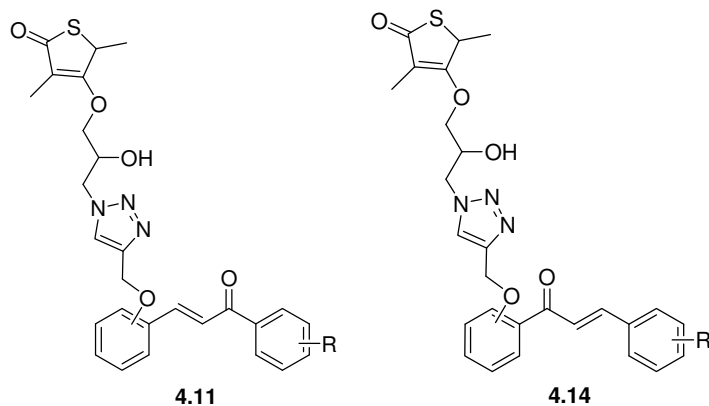
Of all the intermediates listed in table 4.5, the thiolactone epoxide **4.7a** with an  $IC_{50}$  of 2.29  $\mu$ M against the resistant (W2) strain and **4.10f** which displays enzyme inhibitory activity ( $IC_{50} = 19.0 \mu$ M) but no growth inhibitory activity, are worth pointing out. Also noticeable is the selective activity of some of the acetylenic chalcones against the sensitive (D10) strain. No useful trends with regard to degree of methoxylation and substitution pattern could be delineated.

Compared to the thiolactone-isatin hybrids discussed in the previous chapter, the  $\beta$ -amino alcohol thiolactone-chalcone hybrids **4.11** and **4.14** showed promising antiplasmodial activity. Indeed all of the target molecules showed activity against the W2 ( $IC_{50} = 0.68$ - $6.08 \mu$ M) and D10 ( $IC_{50} = 0.62$ - $54.6 \mu$ M) strain; although relative to chloroquine these activities are still poor. The most interesting compound identified is the *meta*-substituted, trimethoxylated derivative **4.14f** which is almost equipotent in the two strains [ $IC_{50} = 0.68$  (W2) and  $IC_{50} = 0.62$  (D10)]. The target molecules are more active against the CQR strain ( $IC_{50} < 7 \mu$ M) than the CQS strain as reflected by the RIs (table 4.6). It is also clear that the intermediates (except **4.11f**) and target molecules (except **4.14h**) do not show falcipain-2 inhibitory activity which is somewhat unexpected considering the association of chalcones with cysteine protease inhibition (section 1.4.2.1.4.2). However, reference has been made in section 2.2.3.3 to the inhibition of parasite-induced channels or other unknown targets by chalcone derivatives.

Furthermore, the results indicate that the growth inhibitory activity against the W2 strain increases with the number of methoxy substituents on the chalcones moiety as shown for compounds **4.11a-c** and **4.14a-i**. This is graphically represented in figure 4.15. It moreover confirms the importance of methoxy substituents on chalcones for antimalarial activity (section 4.2.1). Moreover, the greater inhibition observed for the series B derivatives as shown by **4.14f** and **4.14i** indicates a preference for methoxy substituents on ring A. Another interesting trend observed, is with the attachment of the triazole moiety to the chalcone scaffold. It is clear that a *meta* relationship is preferred as exemplified by compounds **4.11f** and **4.14f**. It is reasonable to conclude from the abovementioned trends, the low micromolar activity of the hybrids (**4.11**, **4.14**, table 4.6) against the W2 strain and the fact that the chalcone intermediates (**4.10**, **4.13**, table 4.5) are devoid of activity against the W2 strain, that thiolactone and chalcones moieties exert a synergistic or rather complementary antimalarial effect.

**Table 4.5:** *In vitro* antiplasmodial activity of intermediates

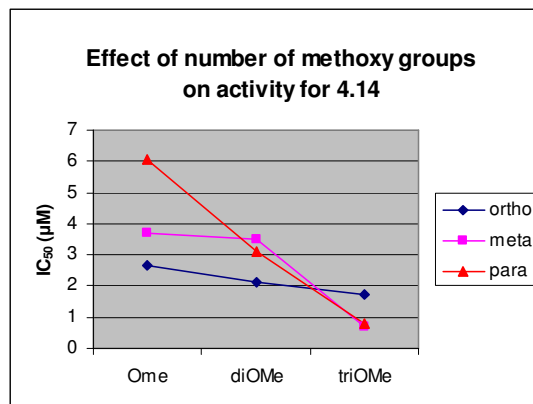
Compound Code	Position	R	rec-FP-2 IC <sub>50</sub> ( $\mu$ M)	W2 IC <sub>50</sub> ( $\mu$ M)	D10 IC <sub>50</sub> ( $\mu$ M)	c log P
CQ	-	-	-	0.06938	0.03823	-
E64	-	-	0.04733	-	-	-
1.28	-	-	>100	>20	>100	-
4.7a	-	-	>100	<b>2.29</b>	43.80	0.94
4.8	-	-	>100	>20	>100	1.50
4.10a	ortho	4-OMe	>100	>20	>100	4.36
4.10b	ortho	2,4-diOMe	>100	>20	<b>8.00</b>	4.45
4.10c	ortho	2,3,4-triOMe	>100	>20	50.57	3.74
4.10d	meta	4-OMe	>100	>20	11.60	4.36
4.10e	meta	2,4-diOMe	>100	>20	<b>7.57</b>	4.45
4.10f	meta	2,3,4-triOMe	<b>19.0</b>	>20	>100	3.74
4.10g	para	4-OMe	>100	>20	>100	4.36
4.10h	para	2,4-diOMe	>100	>20	54.78	4.45
4.10i	para	2,3,4-triOMe	>100	>20	13.34	3.74
4.13a	ortho	4-OMe	>100	>20	>100	4.36
4.13b	ortho	2,4-diOMe	>100	>20	18.86	4.45
4.13c	ortho	2,3,4-triOMe	>100	>20	>100	3.74
4.13d	meta	4-OMe	>100	>20	>100	4.36
4.13e	meta	2,4-diOMe	>100	>20	>100	4.45
4.13f	meta	2,3,4-triOMe	>100	>20	33.03	3.74
4.13g	para	4-OMe	>100	>20	16.52	4.36
4.13h	para	2,4-diOMe	>100	>20	>100	4.45
4.13i	para	2,3,4-triOMe	>100	>20	<b>4.43</b>	3.74

**Table 4.6:** *In vitro* antiplasmodial activity of target molecules **4.11** and **4.14**

Compound Code	Position	R	rec-FP-2 IC <sub>50</sub> (μM)	W2 IC <sub>50</sub> (μM)	D10 IC <sub>50</sub> (μM)	<sup>a</sup> RI	<sup>c</sup> log P
CQ	-	-	-	0.06938	0.03823	1.81	-
E64	-	-	0.04733	-	-	-	-
1.28	-	-	>100	>20	>100	-	-
4.11a	ortho	4-OMe	>100	2.31	8.57 <sup>c</sup>	0.27	3.32
4.11b	ortho	2,4-diOMe	>100	2.08	6.69 <sup>c</sup>	0.31	3.41
4.11c	ortho	2,3,4-triOMe	>100	<b>1.14</b>	<b>1.48</b>	0.77	2.71
4.11d	meta	4-OMe	>100	2.43	3.83	0.63	3.32
4.11e	meta	2,4-diOMe	>100	<b>1.64</b>	3.34 <sup>c</sup>	0.49	3.41
4.11f	meta	2,3,4-triOMe	>100	<b>2.00</b>	<b>1.53<sup>c</sup></b>	1.31	2.71
4.11g	para	4-OMe	>100	3.12	13.1 <sup>c</sup>	0.24	3.32
4.11h	para	2,4-diOMe	>100	<b>1.54</b>	11.8 <sup>c</sup>	0.13	3.41
4.11i	para	2,3,4-triOMe	>100	5.04	<b>1.90<sup>c</sup></b>	2.65	2.71
4.14a	ortho	4-OMe	>100	2.64	2.91 <sup>c</sup>	0.91	3.32
4.14b	ortho	2,4-diOMe	>100	2.14	4.26 <sup>c</sup>	0.50	3.41
4.14c	ortho	2,3,4-triOMe	>100	<b>1.72</b>	54.6	0.03	2.71
4.14d	meta	4-OMe	>100	3.69	16.2 <sup>c</sup>	0.23	3.32
4.14e	meta	2,4-diOMe	>100	3.51	4.38 <sup>c</sup>	0.80	3.41
4.14f	meta	2,3,4-triOMe	>100	<b>0.68</b>	<b>0.62</b>	1.10	2.71
4.14g	para	4-OMe	>100	6.08	6.93 <sup>c</sup>	0.88	3.32
4.14h	para	2,4-diOMe	28.5	3.10	2.63 <sup>c</sup>	1.18	3.41
4.14i	para	2,3,4-triOMe	>100	<b>0.81</b>	6.18 <sup>c</sup>	0.13	2.71

<sup>a</sup>Rec-FP-2 = recombinant falcipain-2; <sup>b</sup>RI = Resistance Index = [IC<sub>50</sub>(W2)]/[IC<sub>50</sub>(D10)]

<sup>c</sup>Compound tested as suspensions



**Figure 4.15:** Graph showing the effect of number of methoxy groups on activity against the W2 strain of *P. falciparum* for *ortho*-, *meta*- and *para*-substituted target molecules **4.14**

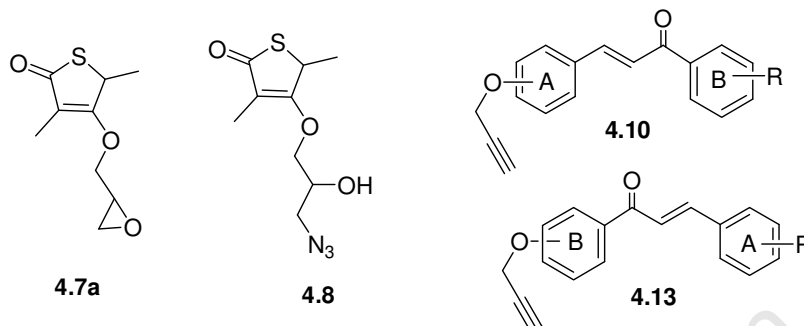
#### 4.2.3.2. *In vitro* Antitubercular Activity

Different *in vitro* assays were used to screen the synthesized compounds for antitubercular activity. The MABA and the LORA assays were conducted in the laboratories of Prof. S.G. Franzblau at the Institute of Tuberculosis, University of Illinois, Chicago (UIC) and the BACTEC assay at the Biomedical Research Department, University of Stellenbosch in the laboratories of Prof. P. Van Helden.

Previous studies indicated the preference for halogenated substituents on chalcones for antitubercular activity.<sup>8</sup> As shown in table 4.7, the BACTEC results showed that the combination of methoxy and acetylenic groups can yield chalcone derivatives with promising antitubercular activity. Also, from the good to modest activity of the acetylenic chalcones **4.10** and **4.13**, it is reasonable to conclude that they share a common target in *M. tuberculosis*. Activities worth highlighting are those for **4.13h** (MABA MIC 31.0  $\mu$ M), **4.13a** (MABA MIC 31.1  $\mu$ M) and the monomethoxylated derivatives **4.10a**, **4.10d** and **4.13d**. With the exception of the *para*-substituted derivatives **4.10g** and **4.13g** all monomethoxylated chalcones display superior antitubercular activity according to the MABA assay. More striking is the correlation with the BACTEC results, which show a clear bias towards the monomethoxylated chalcones **4.10a**, **4.10d**, **4.10g**, **4.13a**, **4.13d** and **4.13g**; they all showed more than 70% mycobacterial growth inhibition at 10  $\mu$ g/mL. The MABA MICs of all of the hybrids (table 4.8) are greater than the highest concentration (128 $\mu$ M) tested. The same trend in activity was reflected by the BACTEC results which showed no noticeable growth inhibition at 10  $\mu$ g/mL. According to the BACTEC results **4.11f** is the only compound which

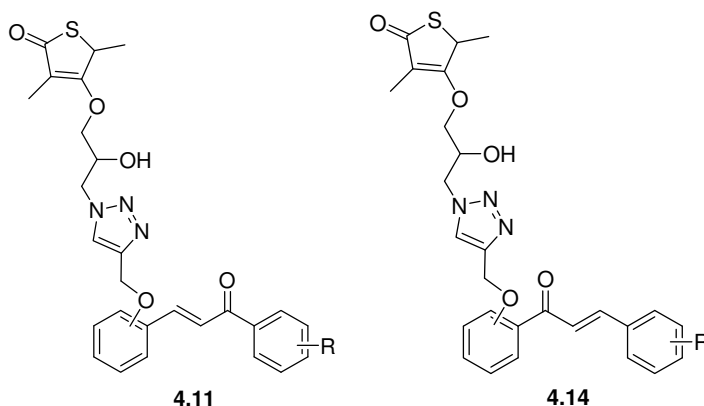
shows a higher percentage inhibition compared to the parent natural product, thiolactomycin 1.28.

**Table 4.7:** *In vitro* antitubercular activity of intermediates 4.7a, 4.8, 4.10 and 4.13



Code	Position	R	BACTEC <sup>a</sup> 1.....10 $\mu\text{g/mL}$		MABA %Inh <sup>b</sup> ..MIC ( $\mu\text{M}$ )		LORA %Inh <sup>b</sup> ..MIC ( $\mu\text{M}$ )		c log P
RMP						0.05	96.1	2.4	
INH						0.37	33.2	>128	
MOX						0.29	90.3	31.1	
SM						0.43	ND	ND	
PA 824						0.16	98.7	3.3	
1.28			-3.8	58.6	35	>128	ND	ND	
4.7a	-	-	-27.0	-13.2	ND	ND	ND	ND	0.94
4.8	-	-	-17.2	-23.5	0	>128	ND	ND	1.50
4.10a	ortho	4-OMe	20.1	78.9	99	47.5	99.5	50.7	4.36
4.10b	ortho	2,4-diOMe	4.4	45.6	99	59.1	99.1	<b>14.9</b>	4.45
4.10c	ortho	2,3,4-triOMe	ND	ND	98	61.9	99.2	<b>28.6</b>	3.74
4.10d	meta	4-OMe	25.5	70.1	100	40.0	94.0	31.7	4.36
4.10e	meta	2,4-diOMe	-12.7	13.2	99	54.9	101	<b>26.1</b>	4.45
4.10f	meta	2,3,4-triOMe	ND	ND	100	54.0	101	<b>13.1</b>	3.74
4.10g	para	4-OMe	17.2	70.6	28	>128	ND	ND	4.36
4.10h	para	2,4-diOMe	1.0	29.9	8	>128	ND	ND	4.45
4.10i	para	2,3,4-triOMe	ND	ND	0	>128	ND	ND	3.74
4.13a	ortho	4-OMe	28.9	76.0	95	<b>31.1</b>	99.3	56.7	4.36
4.13b	ortho	2,4-diOMe	-3.4	36.8	96	53.7	96.3	62.8	4.45
4.13c	ortho	2,3,4-triOMe	ND	ND	96	98.7	99.5	46.1	3.74
4.13d	meta	4-OMe	69.1	<b>92.6</b>	95	51.2	71.6	>128	4.36
4.13e	meta	2,4-diOMe	3.4	29.9	0	>128	ND	ND	4.45
4.13f	meta	2,3,4-triOMe	ND	ND	97	58.7	99.3	<b>29.7</b>	3.74
4.13g	para	4-OMe	40.2	<b>82.8</b>	43	>128	ND	ND	4.36
4.13h	para	2,4-diOMe	5.4	42.6	99	<b>31.0</b>	92.6	108	4.45
4.13i	para	2,3,4-triOMe	ND	ND	0	>128	ND	ND	3.74

<sup>a</sup>Calculated using a control growth index of  $\sim 200$ ; <sup>b</sup>Percentage inhibition at 128  $\mu\text{M}$

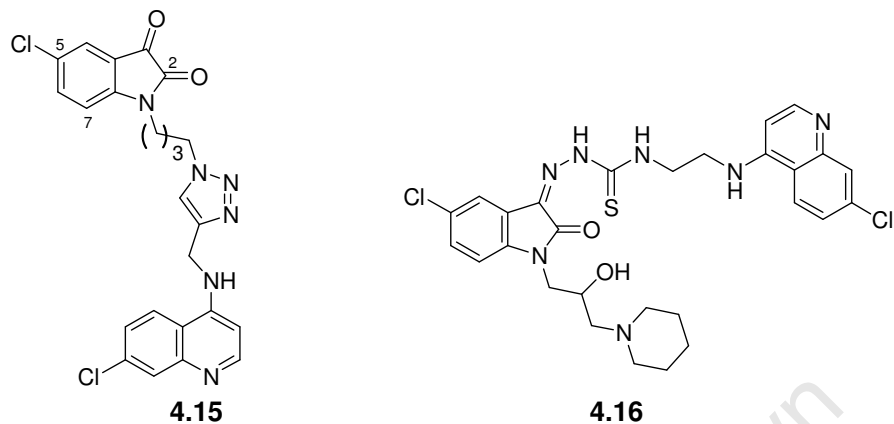
**Table 4.8:** Antitubercular activity of target molecules **4.11** and **4.14**

Code	Position	R	BACTEC <sup>a</sup> 1.....10 $\mu\text{g/mL}$		MABA % Inh <sup>b</sup> ...MIC ( $\mu\text{M}$ )		c log P
RMP						0.05	
INH						0.37	
MOX						0.29	
SM						0.43	
PA824						0.16	
1.28			-3.8	<b>58.6</b>	35	>128	
4.11a	ortho	4-OMe	11.9	18.6	62	>128	3.32
4.11b	ortho	2,4-diOMe	13.8	15.7	43	>128	3.36
4.11c	ortho	2,3,4-triOMe	-2.4	14.3	39	>128	2.61
4.11d	meta	4-OMe	-15	-34	57	>128	3.32
4.11e	meta	2,4-diOMe	-43	-17	54	>128	3.36
4.11f	meta	2,3,4-triOMe	-44	<b>73</b>	52	>128	2.61
4.11g	para	4-OMe	6.2	29.5	39	>128	3.32
4.11h	para	2,4-diOMe	-16	-12	<b>72</b>	>128	3.36
4.11i	para	2,3,4-triOMe	-4.3	14.8	60	>128	2.61
4.14a	ortho	4-OMe	-2.9	15.7	18	>128	3.32
4.14b	ortho	2,4-diOMe	5.2	10.0	59	>128	3.36
4.14c	ortho	2,3,4-triOMe	-2.4	16.7	60	>128	2.61
4.14d	meta	4-OMe	-29	10	57	>128	3.32
4.14e	meta	2,4-diOMe	20.5	12.9	67	>128	3.36
4.14f	meta	2,3,4-triOMe	-2.9	10.5	56	>128	2.61
4.14g	para	4-OMe	8.1	24.3	44	>128	3.32
4.14h	para	2,4-diOMe	5.2	13.8	61	>128	3.36
4.14i	para	2,3,4-triOMe	16.2	24.3	66	>128	2.61

<sup>a</sup>Calculated using a control growth index of  $\sim 200$ ; <sup>b</sup>Percentage inhibition at 128  $\mu\text{M}$

4.3.  $\beta$ -Amino Alcohol Isatin-Chalcone Hybrids

## 4.3.1. Rationale

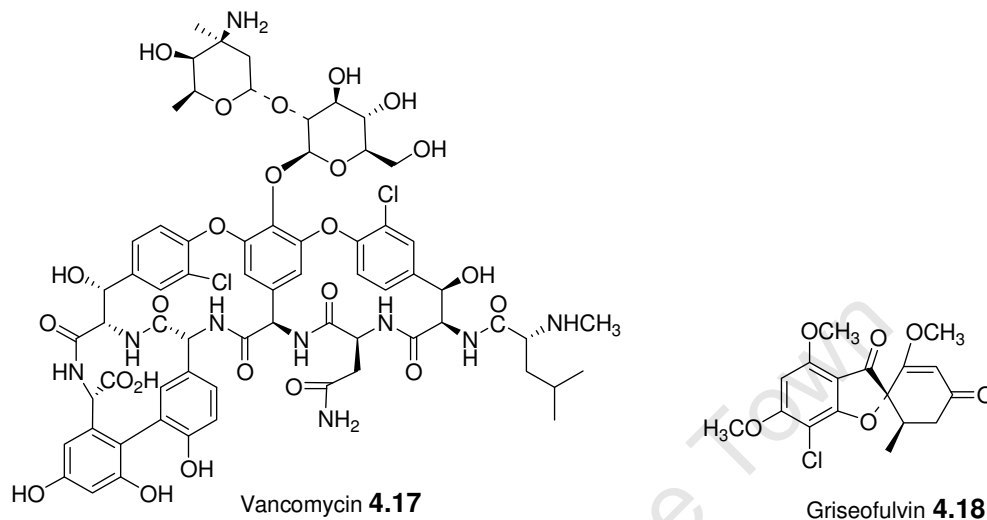


**Figure 4.16:** Chemical structures of compound **4.15** and **4.16**

The isatin scaffold is of pharmacological importance because of the association of its derivatives with diverse biological activity (section 2.2.3.2). Other attractive features include its non-peptidic nature and the ease with which it can be synthetically manipulated making it the ideal template onto which bioactiphores can be appended. In demonstration, a limited series of 1,2,3-triazole-isatin derivatives with varying substituents (H, Cl, CH<sub>3</sub>, F) at C-5 of the isatin scaffold were synthesized in our labs. Structure-activity relationship studies revealed compound **4.15** (fig 4.16) to be the most active, with activities against the W2 strain of *P. falciparum* in the low micromolar range (0.920  $\mu$ M).<sup>58a</sup> In another comparative study from our group the antiplasmodial activity of a  $\beta$ -amino alcohol isatin derivative **4.16** (fig. 4.16) was compared to a structural analogue devoid of the  $\beta$ -amino alcohol moiety. The aforementioned was 11 times more active with an IC<sub>50</sub> of 19.2 nM against the CQR of *P. falciparum*.<sup>58b</sup> Because of the presence of the 7-chloro-4-aminoquinoline moiety and other pharmacologically relevant moieties in these structures (fig. 4.16), the contribution of the 5-chloroisatin scaffold towards the observed activity may not be dominating, nevertheless these results collectively demonstrated; (i) the privileged nature of the isatin scaffold, (ii) the additive effect on activity exerted by the beta amino alcohol moiety and (iii) the superiority of the 5-chloroisatin compared to unsubstituted isatin and other 5-substituted isatins (F, CH<sub>3</sub>) derivatives with regard to antimalarial activity. Indeed, the modulating effect of the chlorine atom on the chemical and biological properties of compounds has been reported in literature.<sup>59</sup> Its presence in bioactive natural products and synthetic drugs for example vancomycin **4.17** (fig. 4.17), griesofulvin **4.18** (fig. 4.17), clindamycin (**1.20**, fig. 1.3) as well

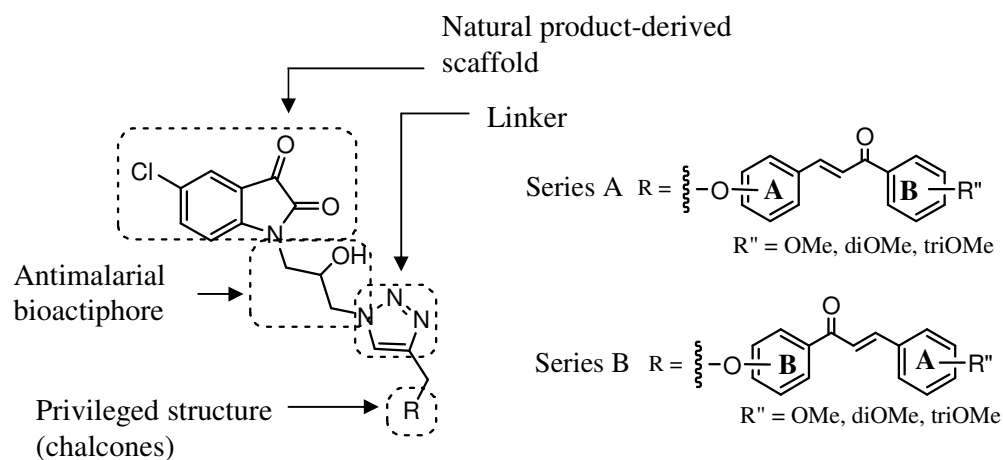


as chloroquine (**1.3**, fig. 1.3) and related aminoquinolines is noteworthy. In fact with regard to the latter, SAR studies showed that the chlorine group was important for inhibition of  $\beta$ -hematin formation and thus for antimalarial activity.<sup>60</sup>



**Figure 4.17:** Chemical structures of vancomycin **4.17** and griseofulvin **4.18**

The hybridization of two drugs or pharmacophores with a similar mode of action as is the case with the isatin and chalcone moieties may not be ideal (section 2.2.4). We were however hoping for these two privileged, drug-like moieties to exert a synergistic effect which we envisaged to be reinforced by the presence of the  $\beta$ -amino alcohol and the triazole functionalities. By employing click chemistry we envisaged a rapid and meaningful SAR for this novel series which will be comparable with the previously discussed thiolactone-chalcone hybrids (**4.11** and **4.14**). For this reason the same methoxylated, acetylenic chalcones **4.10a-i** and **4.13a-i** were used in the synthesis of these hybrids resulting in a similar organisation as shown in figure 4.3. The general structure of the target molecules is shown in figure 4.18.

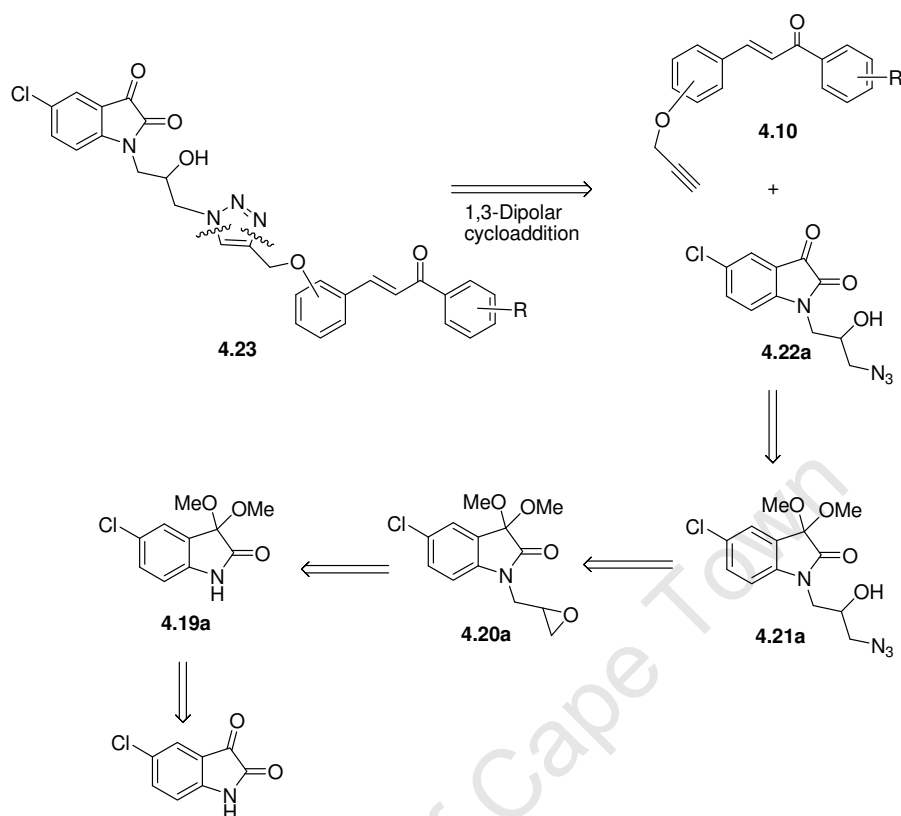


**Figure 4.18:** Rational and general structure of  $\beta$ -amino alcohol isatin-chalcone hybrids molecules

## 4.3.2. Chemical Synthesis

### 4.3.2.1. Retrosynthetic Analysis

Synthesis of target molecules **4.23** (and **4.24**) is a multi-step procedure which includes a protection/deprotection step. Synthesis was envisaged from acetylenic chalcone **4.10** (**4.13** for target molecules **4.24** and isatin azide **4.22a** (scheme 4.11) *via* a 1,3-dipolar cycloaddition reaction. Preparation of the latter is from a protected azide **4.21a** which in turn can be obtained by azidolysis of the epoxide **4.20a**. Compound **4.19a** the precursor of **4.20a** can be obtained by the ketalization of commercially available 5-chloroisatin.

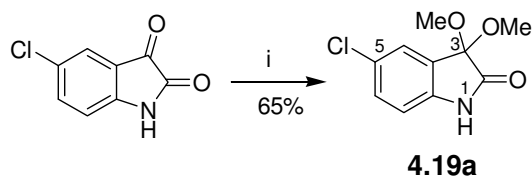


**Scheme 4.11:** Retrosynthesis of the  $\beta$ -amino alcohol isatin-chalcone hybrids represented by target molecule **4.23**

#### 4.3.2.2. Synthesis of Isatin Azide

The reactivity of the ketonic carbonyl C-3 of the isatin scaffold is reportedly enhanced by the presence of electron withdrawing substituents at C-5. However, to effect changes elsewhere in the molecule this carbonyl needs to be blocked. We were therefore compelled to introduce a protection/deprotection step in the synthesis of **4.22a**. The first step involved protection of the ketonic carbonyl as a ketal. Eco-friendly procedures using activated clays under microwave radiation<sup>61a</sup> and heteropolycompounds as catalysts<sup>61b</sup> for the ketalization of isatin and 5-haloisatins have recently been reported. The ketalization protocol we employed involved refluxing a mixture of 1 eq of 5-chloroisatin and 30 eq of anhydrous trimethyl orthoformate in anhydrous MeOH in the presence of a catalytic amount of *para*-toluene sulfonic acid for 48 hours under a nitrogen atmosphere. The reaction progress was monitored by thin layer chromatography (2:3 EtOAc:Hex) and the disappearance of the intense orange colour characteristic of isatins signalled the completion of the reaction. Column

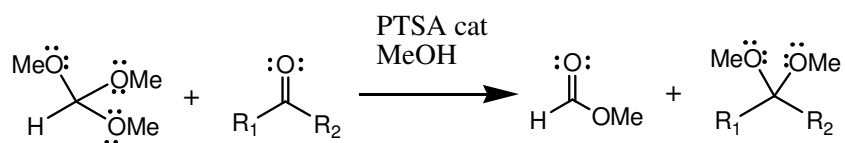
chromatography afforded the ketal **4.19a** in moderate yield (65%) and an unidentified yellow crystalline solid (most probable a hemiketal) as by-product.



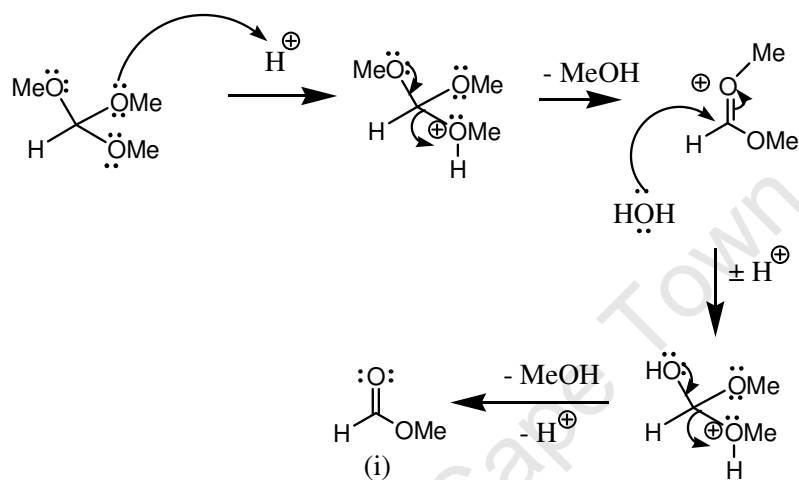
**Scheme 4.12:** *Reagents and conditions:* (i)  $\text{CH}(\text{OMe})_3$ , *p*-TsOH, MeOH, reflux, 48 h

Spectroscopic data for **4.19a** is in agreement with published data.<sup>61a</sup> The presence of NH was inferred from  $^1\text{H}$  NMR which showed a  $\text{D}_2\text{O}$ -exchangeable singlet at  $\delta$  8.66 ppm. Formation of the ketal was also confirmed by the presence of singlet at  $\delta$  3.58 ppm which integrated for 6 protons corresponding to the two methoxy groups. The  $^{13}\text{C}$ -NMR showed the disappearance of the ketonic carbonyl signal resonating at  $\delta$  185 ppm for 5-chloroisatin and the appearance of quaternary carbon signal at 97.2 ppm which was assigned to the ketal carbon C-3.

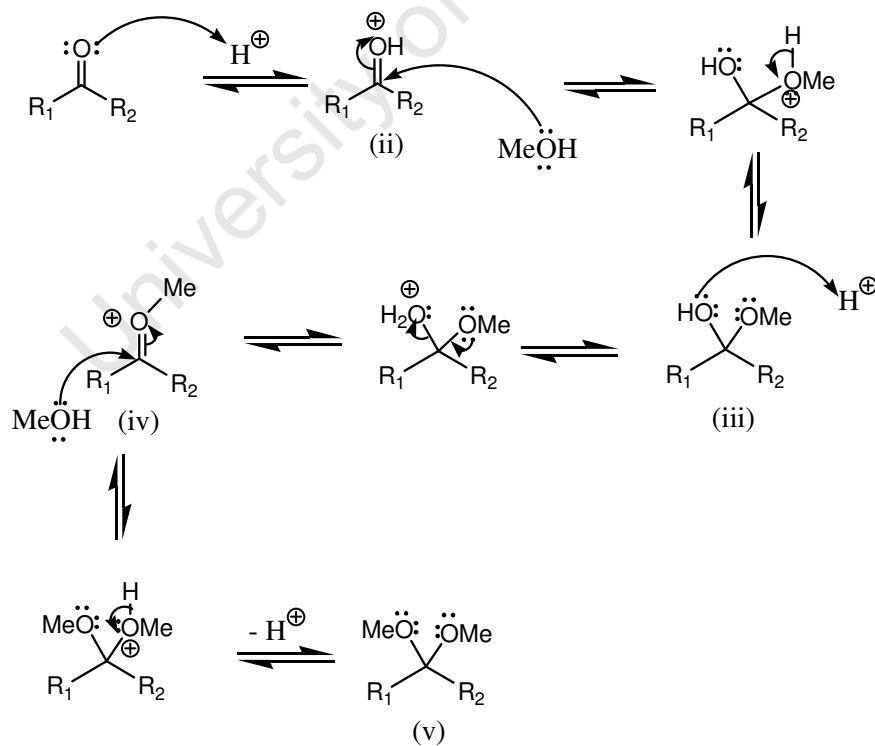
Overall reaction



Part A: Hydrolysis of trimethyl orthoformate



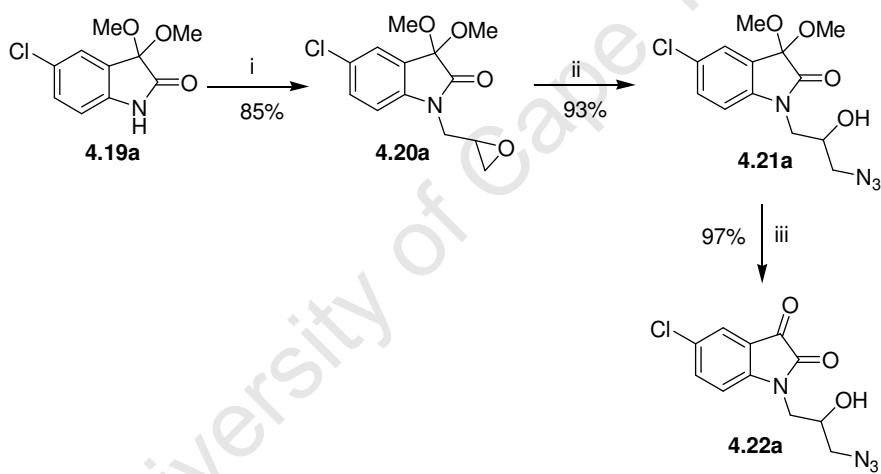
Part B: Ketalization



Scheme 4.13: Mechanism for ketalization

The mechanism for ketalization is shown in scheme 4.13. Essentially, ketalization is a concerted process but for clarification can be viewed as a two-step reaction with the first step involving acid-catalyzed hydrolysis of the trimethyl orthoformate by water which results in the formation of MeOH and methyl orthoformate [scheme 4.13 (i)]. The methanol generated reacts with the oxonium ion intermediate [scheme 4.13 (ii)] in the second, acid-catalyzed ketalization step. Deprotonation of the adduct so formed generates a hemiketal [scheme 4.13 (iii)] which undergoes protonation and subsequent expulsion of water to generate a second oxonium ion intermediate [scheme 4.13 (iv)]. The latter reacts with a second molecule of MeOH to form an intermediate which loses a proton to give the ketal [scheme 4.13 (v)].

The equilibrium of the hydrolysis step, effectively removes the water generated in the ketalization step thereby driving the reaction towards completion.



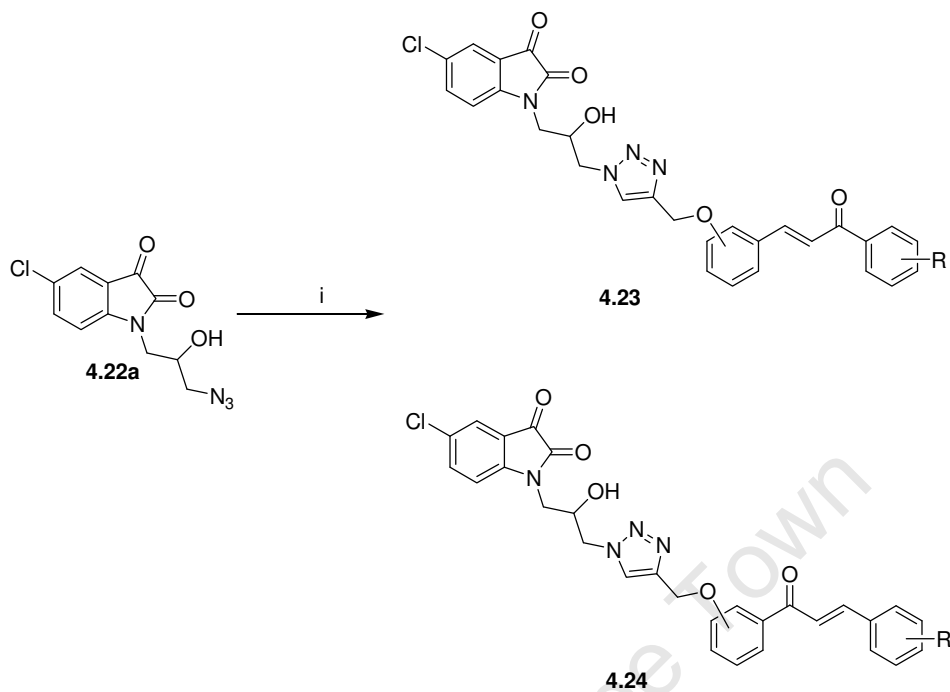
**Scheme 4.14:** Reagents and conditions: (i) (±)-Epichlorohydrin, KF/Al<sub>2</sub>O<sub>3</sub>, DCM, 25°C, 20 h; (ii) NaN<sub>3</sub>, NH<sub>4</sub>Cl, MeOH/H<sub>2</sub>O (8:1), 25°C, 16 h; (iii) 10% HCl, Acetone, 25°C, 3 h

With **4.19a** in hand, the next step involved synthesis of the epoxide **4.20a**. This was achieved by the reaction of **4.19a** with 5 eq of (±)-epichlorohydrin in the presence of KF-Al<sub>2</sub>O<sub>3</sub> in anhydrous DCM under nitrogen at 25°C. The advantage of using KF-Al<sub>2</sub>O<sub>3</sub> or other solid support reagents is the ease with which it can be removed from the product mixture. No elaborate workup is required and the reaction progress can be monitored using conventional methods, for example tlc.<sup>62</sup> Accordingly, the reaction proceeded smoothly and was completed after stirring for 20 hours to afford the product **4.20a** in 85% yield. Purification of

the product mixture by flash chromatography was required to remove the excess epichlorohydrin. Formation of isatin epoxide was inferred from the  $^1\text{H-NMR}$  spectrum of **4.20a** which showed the disappearance of the singlet at  $\delta$  8.66 ppm assigned to NH for the starting material **4.19a**.

For the next step in the synthesis (scheme 4.14) we used the azidolysis conditions employed for the synthesis of the thiolactone azide **4.8** (scheme 4.4). The epoxide **4.20a** was stirred for 16 hours with  $\text{NaN}_3$  and  $\text{NH}_4\text{Cl}$  in aqueous methanol at room temperature. A clean conversion to the azide was observed and the product **4.21a** was obtained in excellent yield (93%). The appearance of a strong absorption band in the IR spectrum of **4.21a** at  $2105\text{cm}^{-1}$  was characteristic for the azide group whereas the broad band at  $3457\text{cm}^{-1}$  indicated the presence of a hydroxyl group.

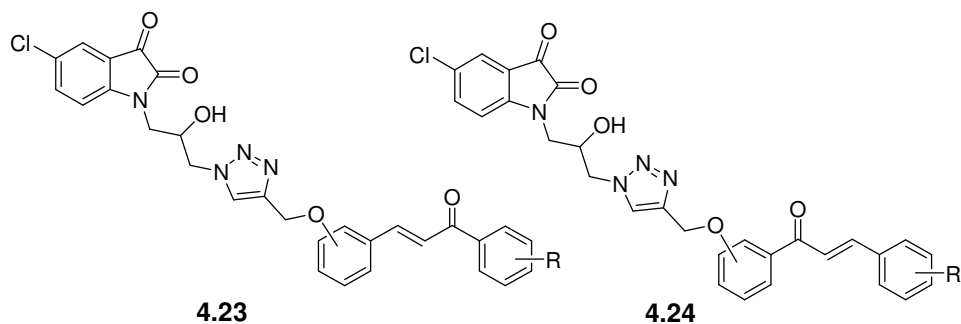
The final step toward the isatin azide **4.22a** involved an acid hydrolysis of **4.21a**. It was achieved by treatment of the latter with 10% HCl in acetone at room temperature. A noticeable change in colour, that is, from colourless to red was observed for the reaction mixture upon addition of HCl; this was evident of the restoration of conjugation in isatin scaffold. After workup the product was obtained as red needles in quantitative yield. Evidence for removal of the protecting group was obtained from the  $^1\text{H-NMR}$  which showed the disappearance of the methoxy signals at  $\delta$  3.54 - 3.55 ppm (in **4.21a**) and a noticeable downfield shift for the aromatic signals in particular those assigned to H-4 and H-6 for **4.22a**.  $^{13}\text{C-NMR}$  confirmed the disappearance of the quaternary ketal carbon and appearance of the carbonyl carbon at  $\delta$  182.0 ppm.

4.3.2.3. Synthesis of  $\beta$ -Amino Alcohol Isatin-Chalcone Hybrids

**Scheme 4.15:** Reagents and conditions: (i) **4.10** (for **4.23**) or **4.13** (for **4.24**),  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , sodium ascorbate,  $\text{CH}_2\text{Cl}_2:\text{H}_2\text{O}(1:1)$ ,  $25^\circ\text{C}$ , 3 h

The ligand-free Cu(I) catalyzed 1,3-cycloaddition protocol reported by Lee *et al*<sup>55</sup> was used in the synthesis of the target molecules **4.23** and **4.24**. As shown in scheme 4.15, the azide **4.22a** was treated with the acetylene **4.10** in the presence of 5 mol% of  $\text{CuSO}_4 \cdot \text{H}_2\text{O}$  and 10 mol % of sodium ascorbate in 4 mL  $\text{DCM}:\text{H}_2\text{O}$  (1:1) at room temperature. Synthesis of **4.24** proceeded in a similar fashion with the acetylenic chalcone **4.13**. The reactions proceeded cleanly and no by-products were detected on tlc. Additionally, for all reactions the product precipitated out after continuous stirring for 3 hours as a red or orange solid which was recovered from the reaction mixture *via* simple filtration. Consecutive washing of the filtrate with water and DCM was made to remove any adhering impurities. Target molecules were obtained in moderate to good yield (table 4.9). It is clear that the above reaction met the criteria of ‘click’ reaction (section 4.2.2.5); at least where high yields and the redundancy of chromatographic purification are concerned. Also the yields recorded for this series of compounds were significantly higher compared to those of the previously discussed thiolactone-chalcone hybrids. It is therefore reasonable to conclude that this discrepancy in yields for two different azide inputs (**4.8** and **4.22a**) is a further demonstration of the limitations of Cu(I) catalyzed variant of Huisgen’s 1,3-dipolar cycloaddition.



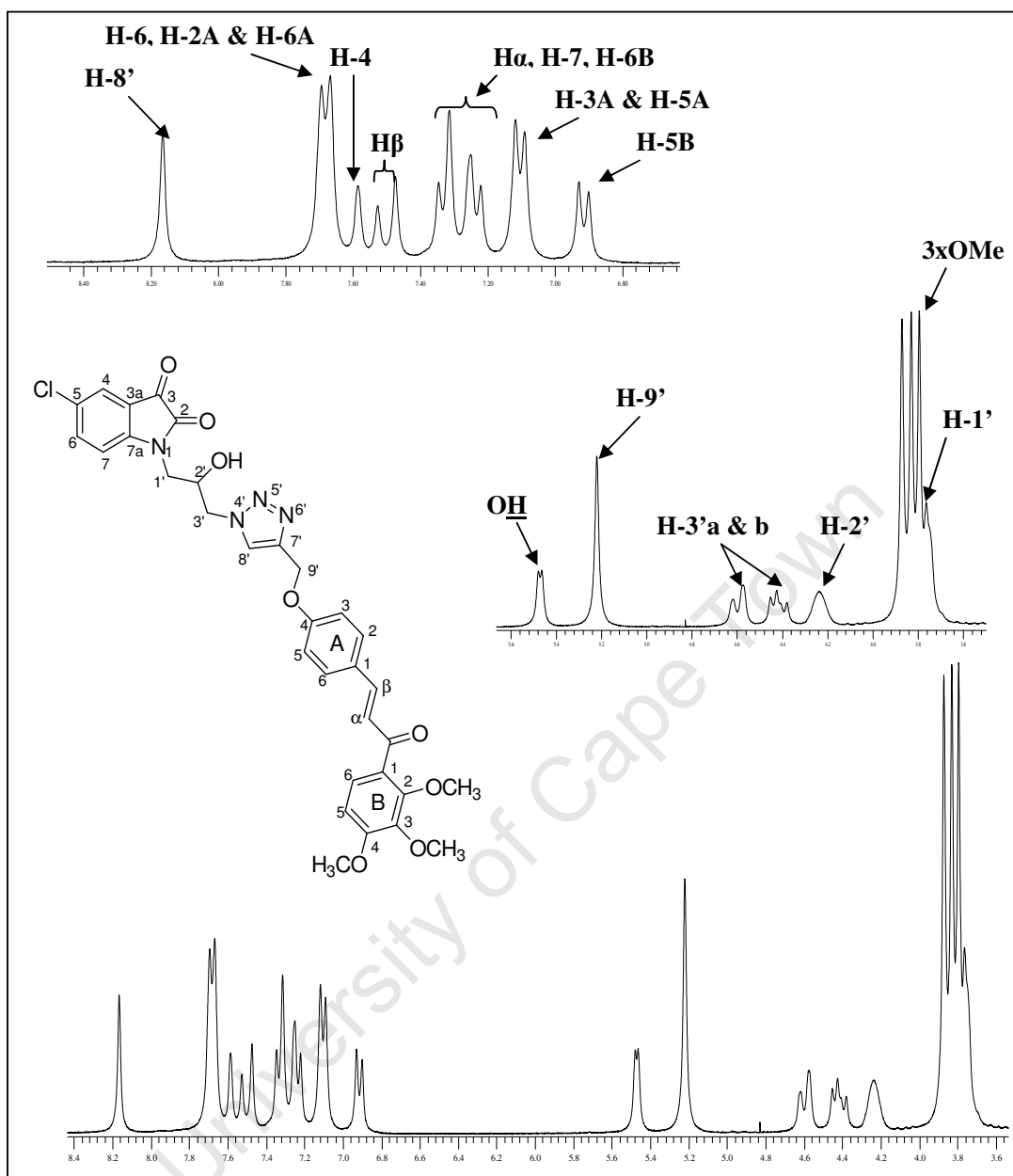
**Table 4.9:** Isolated yields obtained for target molecules **4.23** and **4.24**

Compound	Position	R	Yield (%)	m.p.(°C)
<b>4.23a</b>	ortho	4-OMe	58	178-180
<b>4.23b</b>	ortho	2,4-diOMe	61	189
<b>4.23c</b>	ortho	2,3,4-triOMe	64	93
<b>4.23d</b>	meta	4-OMe	60	186
<b>4.23e</b>	meta	2,4-diOMe	51	76
<b>4.23f</b>	meta	2,3,4-triOMe	54	127-128
<b>4.23g</b>	para	4-OMe	70	203-205
<b>4.23h</b>	para	2,4-diOMe	48	126-128
<b>4.23i</b>	para	2,3,4-triOMe	60	143-145
<b>4.24a</b>	ortho	4-OMe	84	93-94
<b>4.24b</b>	ortho	2,4-diOMe	54	97-98
<b>4.24c</b>	ortho	2,3,4-triOMe	83	96
<b>4.24d</b>	meta	4-OMe	70	180
<b>4.24e</b>	meta	2,4-diOMe	54	162
<b>4.24f</b>	meta	2,3,4-triOMe	52	149-150
<b>4.24g</b>	para	4-OMe	65	199-202
<b>4.24h</b>	para	2,4-diOMe	80	185-187
<b>4.24i</b>	para	2,3,4-triOMe	90	155-156

#### 4.3.2.3.1. Characterization

The IR spectra of the target molecules **4.23a-i** and **4.24a-i** displayed characteristic absorptions in the region  $3367\text{-}3489\text{ cm}^{-1}$  (OH),  $1744\text{-}1753\text{ cm}^{-1}$  ( $\text{C}_3=\text{O}$ ),  $1646\text{-}1662$  ( $\alpha,\beta$ -unsaturated  $\text{C}=\text{O}$ ) and  $1598\text{-}1611$  ( $\text{C}_2=\text{O}$ ). The molecular formula of **4.23i**, which is representative of other compounds in this series, was confirmed to be  $\text{C}_{32}\text{H}_{29}\text{ClN}_4\text{O}_8$  by LC(ESI)MS analysis which showed a pseudomolecular ion peak at  $m/z$  633.1747  $[\text{M}+\text{H}]^+$ .

The  $^1\text{H-NMR}$  spectrum of **4.23i** (fig. 4.19) was invaluable in confirming the proposed structure, despite the poor resolution. The intactness of the amino alcohol moiety was inferred from the presence of the methine proton H-2' appearing as a broad singlet at  $\delta$  4.23 ppm as oppose to a multiplet. The signal for the methylene protons at H-1' appear relatively more upfield at  $\delta$  3.76 ppm as a two-proton multiplet which overlaps partially with the methoxy signal at  $\delta$  3.79 ppm.  $^1\text{H-}^1\text{H-COSY}$  experiments aided the assignment of the two non-equivalent methylene protons of H-3' which appear as a geminal coupled ( $J = 13.2$ ) double doublet and a broad doublet at  $\delta$  4.40 ppm and  $\delta$  4.59 ppm, respectively. A signal appearing relatively downfield at  $\delta$  5.22 ppm as a two-proton singlet was assigned to H-9'. The proton of the hydroxyl group at C-2 resonates at  $\delta$  5.47 ppm (fig. 4.19). Key signals in the aromatic region include the one-proton singlet at  $\delta$  8.16 ppm assigned to H-8' of the triazole ring system. The characteristic  $\text{H}\beta$ , a one-proton doublet ( $J = 16.2$ ), appears at  $\delta$  7.50 ppm whereas  $\text{H}\alpha$  overlaps with aromatic signals of the isatin and chalcones moieties.



**Figure 4.19:**  $^1\text{H-NMR}$  spectrum of compound **4.23i** in  $\text{DMSO-d}_6$  at 300MHz

The  $^{13}\text{C-NMR}$  spectrum of **4.23i** (figure 4.20) shows 32 signals of which the most important are the quaternary signals at  $\delta$  190.1 ppm,  $\delta$  182.1 ppm and  $\delta$  159.9 ppm assignable to the  $\alpha,\beta$ -unsaturated carbonyl of the chalcone unit, C-3 and C-2 respectively. Also of interest are the  $\text{sp}^2$ -hybridized carbons of the triazole ring system with the quaternary C-7' appearing at  $\delta$  142.3 ppm and the protonated C-8' at  $\delta$  124.4 ppm.

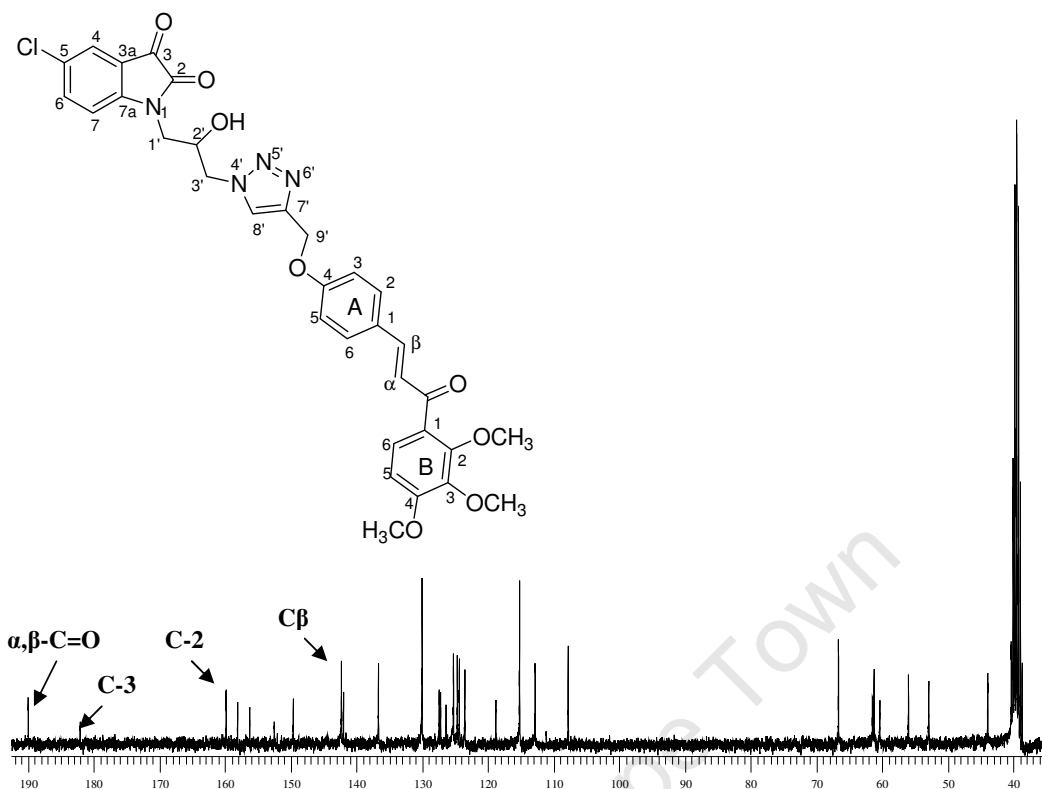


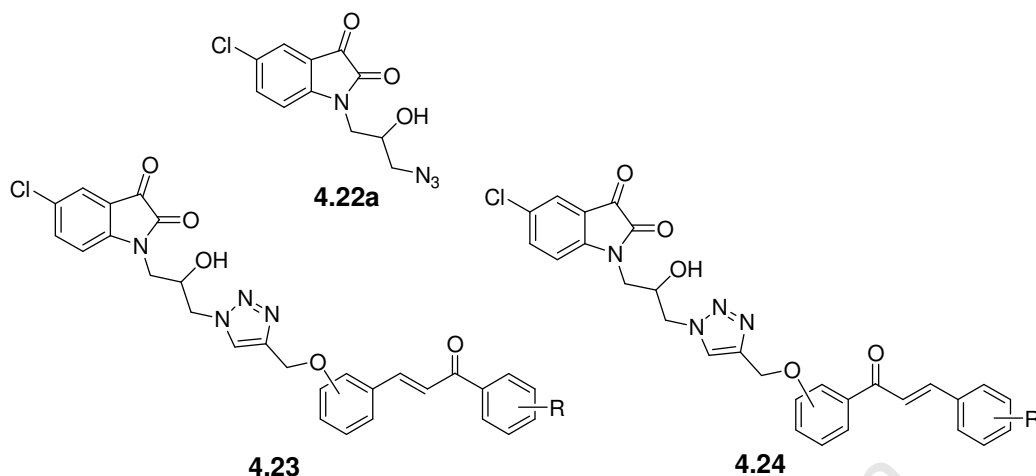
Figure 4.20:  $^{13}\text{C}$ -NMR spectrum of compound **4.23i** in  $\text{DMSO-d}_6$  at 75MHz

### 4.3.3. Biological Results and Discussion

Biological evaluation of compounds in this series were conducted in the same laboratories as mentioned in section 4.2.3.1 for antiplasmodial activity and section 4.2.3.2 for antitubercular activity.

#### 4.3.3.1. *In vitro* Antiplasmodial Activity

According to table 4.10 the most active compound in this series, represented by **4.24b** ( $\text{IC}_{50}$  2.09  $\mu\text{M}$ ) is 21 times less efficacious than chloroquine. Also, there are no noticeable trends regarding the effect of the number of methoxy groups or substitution patterns on activity. In addition, the compounds appear more active in the sensitive D10 strain than in the resistant W2 strain of *P. falciparum*. It is of interest to note that all compounds in this series show falcipain-2 inhibitory activity albeit weak. In fact a fairly good correlation between falcipain-2 inhibition and antimalarial activity against the D10 strain can be discerned when excluding the outlier **4.23c**.

**Table 4.10:** *In vitro* antiplasmodial activity of intermediate **4.22a** and target molecules **4.23** and **4.24**

Compound Code	Position	R	<sup>a</sup> rec-FP-2 IC <sub>50</sub> (μM)	W2 IC <sub>50</sub> (μM)	D10 IC <sub>50</sub> (μM)	<sup>b</sup> RI	<sup>c</sup> log P
CQ				0.0694	0.0382		
E64			0.04733				
<b>4.22a</b>			>100	>20	>20	-	
<b>4.23a</b>	ortho	4-OMe	25.44	>20	2.15	-	3.64
<b>4.23b</b>	ortho	2,4-diOMe	15.58	>20	2.11	-	3.67
<b>4.23c</b>	ortho	2,3,4-triOMe	90.47	6.10	3.27	1.86	2.93
<b>4.23d</b>	meta	4-OMe	10.29	5.75	1.97 <sup>c</sup>	2.92	3.64
<b>4.23e</b>	meta	2,4-diOMe	6.80	3.17	1.99 <sup>c</sup>	1.59	3.67
<b>4.23f</b>	meta	2,3,4-triOMe	11.49	<b>2.18</b>	<b>2.07<sup>c</sup></b>	1.05	2.93
<b>4.23g</b>	para	4-OMe	10.84	10.61	6.72	1.58	3.64
<b>4.23h</b>	para	2,4-diOMe	ND	9.73	4.10	2.37	3.67
<b>4.23i</b>	para	2,3,4-triOMe	16.62	10.98	2.53	4.34	2.93
<b>4.24a</b>	ortho	4-OMe	15.96	7.79	2.34	3.33	3.64
<b>4.24b</b>	ortho	2,4-diOMe	15.04	<b>2.09</b>	<b>1.92</b>	1.09	3.67
<b>4.24c</b>	ortho	2,3,4-triOMe	15.33	6.21	2.42	2.57	2.93
<b>4.24d</b>	meta	4-OMe	9.91	3.57	1.41 <sup>c</sup>	2.53	3.64
<b>4.24e</b>	meta	2,4-diOMe	10.61	2.95	0.99 <sup>c</sup>	2.98	3.67
<b>4.24f</b>	meta	2,3,4-triOMe	16.73	2.57	1.85 <sup>c</sup>	1.39	2.93
<b>4.24g</b>	para	4-OMe	15.89	14.90	5.62	2.65	3.64
<b>4.24h</b>	para	2,4-diOMe	18.28	14.52	5.14	2.82	3.67
<b>4.24i</b>	para	2,3,4-triOMe	25.06	>20	3.49	-	2.93

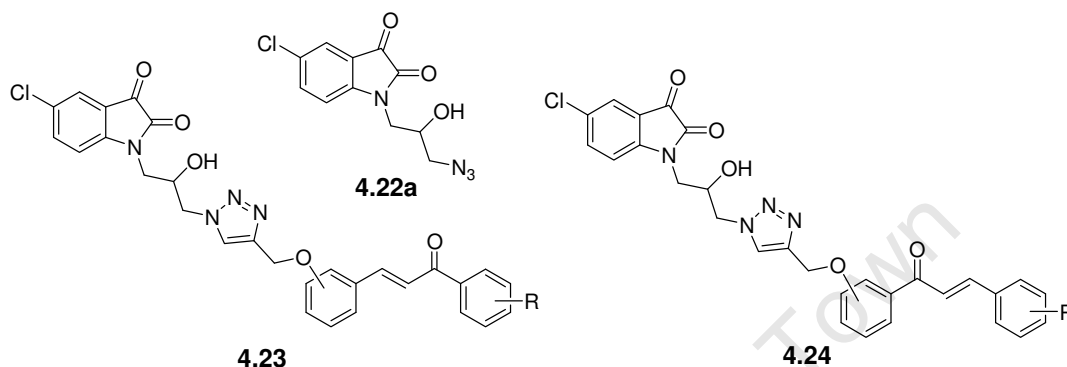
<sup>a</sup>rec-FP-2 = recombinant falcipain-2; <sup>b</sup>RI = Resistance Index = [IC<sub>50</sub>(W2)]/[IC<sub>50</sub>(D10)];

<sup>c</sup>Compound tested as suspensions; ND = not determined

4.3.3.2. *In vitro* antitubercular activity

Compounds in this series were not evaluated with the BACTEC method. The MABA assay revealed that all the hybrids are inactive, with the exception of compound **4.24a**, which showed 82% growth inhibition at 128  $\mu$ M,

**Table 4.11:** *In vitro* antitubercular activity of intermediate **4.22a** and target molecules **4.23** and **4.24**



Compound Code	Position	R	MABA %Inh <sup>a</sup> ...MIC ( $\mu$ M)	c log P	
RMP			0.05		
INH			0.37		
MOX			0.29		
SM			0.43		
PA824			0.16		
<b>4.22a</b>			0	>128	
<b>4.23a</b>	ortho	4-OMe	0	>128	3.64
<b>4.23b</b>	ortho	2,4-diOMe	23	>128	3.67
<b>4.23c</b>	ortho	2,3,4-triOMe	13	>128	2.93
<b>4.23d</b>	meta	4-OMe	6	>128	3.64
<b>4.23e</b>	meta	2,4-diOMe	2	>128	3.67
<b>4.23f</b>	meta	2,3,4-triOMe	11	>128	2.93
<b>4.23g</b>	para	4-OMe	5	>128	3.64
<b>4.23h</b>	para	2,4-diOMe	0	>128	3.67
<b>4.23i</b>	para	2,3,4-triOMe	0	>128	2.93
<b>4.24a</b>	ortho	4-OMe	<b>82</b>	>128	3.64
<b>4.24b</b>	ortho	2,4-diOMe	67	>128	3.67
<b>4.24c</b>	ortho	2,3,4-triOMe	68	>128	2.93
<b>4.24d</b>	meta	4-OMe	26	>128	3.64
<b>4.24e</b>	meta	2,4-diOMe	3	>128	3.67
<b>4.24f</b>	meta	2,3,4-triOMe	3	>128	2.93
<b>4.24g</b>	para	4-OMe	7	>128	3.64
<b>4.24h</b>	para	2,4-diOMe	6	>128	3.67
<b>4.24i</b>	para	2,3,4-triOMe	0	>128	2.93

<sup>a</sup>Percentage inhibition at 128  $\mu$ M

#### 4.4. Conclusions

When comparing the antiplasmodial activity of the thiolactone-chalcone and isatin –chalcone hybrids it is clear that the former is more efficacious compared to the latter. However, with regard to enzyme inhibitory activity, all of the isatin-derived hybrids are active whereas the thiolactone-chalcone hybrids are practically devoid of cysteine protease inhibitory activity. This demonstrates the contribution and importance of the isatin scaffold for falcipain-2 inhibition; considering that the acetylenic chalcones, with the exception of **4.10f** are devoid of enzyme inhibitory activity. Failure to correlate falcipain-2 inhibitory activity with growth inhibitory activity led us to conclude that the thiolactone-chalcone hybrids do not exert their parasitocidal effect *via* inhibition of falcipain-2. Despite the enzyme inhibitory activity of the isatin-chalcones, the aforementioned cannot be considered as their primary mode of action. The most active compound from this 36-membered library, is **4.14f** which showed submicromolar activity ( $IC_{50} = 0.62 \mu\text{M}$ ) in the resistant strain. Overall the hybrids are much more active than the intermediates which again demonstrate the potential of pharmacophore hybridization as drug discovery tool in this case.

In contrast to the antiplasmodial activity, the antitubercular activity of the hybrids was poor compared to the intermediates. In fact, of the 36 hybrids synthesized only compounds **4.11h** and **4.24a** showed significant activity at the highest concentration tested ( $128 \mu\text{M}$ ) according to the MABA assay. Identified among the intermediates is **4.13h** a para-substituted, dimethoxylated acetylenic chalcones which according to the MABA MIC ( $31.0 \mu\text{M}$ ) showed the highest efficacy against the fast growing, replicating mycobacteria; whereas **4.10f** a meta-substituted, trimethoxylated acetylenic chalcones is more efficacious against the slow-growing, persistent mycobacteria as shown by its LORA MIC ( $13.1 \mu\text{M}$ )

**4.5. References**

1. Price, A.C.; Choi, K.H.; Heath, R.J.; Li, Z.M.; White, S.W.; Rock, C.O. *J. Biol. Chem.*, **2001**, 276, 6551
2. (a) Kamal, A.; Shaik, A.A.; Sinha, R.; Yadav, J.S.; Arora, S.K. *Bioorg. Med. Chem. Lett.*, **2005**, 15, 1927; (b) Jones, S.M.; Urch, J.E.; Kaiser, M.; Brun, R.; Harwood, J.L.; Berrry, C.; Gilbert, I.H. *J. Med. Chem.*, **2005**, 48, 5932
3. De Melo, C.S. *M.Sc. Thesis*, University of Cape Town, **2006**
4. Li, R.; Kenyon, G.L.; Cohen, F.E.; Chen, X.; Gong, B.; Dominguez, J.N.; Davidson, E.; Kurzban, G.; Miller, R.E.; Nuzum, E. O.; Rosenthal, P.J., McKerrow, J.H. *J. Med. Chem.*, **1995**, 38, 5031
5. Larsen, M.; Kromann, H.; Kharazmi, A.; Nielsen, S.F. *Bioorg. Med. Chem. Lett.*, **2005**, 15, 4845
6. Liu, M.; Wilairat, P.; Go, M.-L. *J. Med. Chem.*, **2001**, 44, 4443
7. Xue, C.X.; Cui, S.Y.; Liu, M.C.; Hu, Z.D.; Fan, B.T. *Eur. J. Med. Chem.*, **2004**, 39, 745
8. Lin, Y.-M.; Zhou, Y.; Flavin, M.T.; Zhou, L.-M.; Nie, W.; Chen, F.-C. *Bioorg. Med. Chem.*, **2002**, 10, 2795
9. Bhattacharjee, A.K.; Karle, J.M. *J. Med. Chem.*, **1996**, 39, 4622
10. Kolb, H.C.; Sharpless, K.B. *Drug Discovery Today*, **2003**, 8, 1128
11. (a) Collin, M.-P.; Hobbie, S.N.; Böttger, E.C.; Vasella, A. *Helvetica Chimica Acta*, **2008**, 91, 1838; (b) Da Silva, E.N. Jr.; Menna-Barreto, R.F.S.; Pinto, M.C.F.R.; Silva, R.S.F.; Teixeira, D.V.; De Souza, M.C.B.V.; De Simone, C.A.; De Castro, S.L.; Ferreira, V.F.; Pinto, A.V. *Eur. J. Med. Chem.*, **2008**, 43, 1774
12. Brik, A.; Muldoon, J.; Lin, Y.-C.; Elder, J.H.; Goodsell, D.S.; Olson, A.J.; Fokin, V.V.; Sharpless, K.B.; Wong, C.-H. *ChemBioChem*, **2003**, 4, 1246
13. Costa, M.S.; Boechat, N.; Rangel, E.A.; Da Silva, F de C. De Sousa, A.M.T.; Rodrigues, C.R.; Castro, H.C.; Junior, I.N.; Lourenço, M.C. S.; Wardell, S. M.S.V.; Ferreira, V.T. *Bioorg. Med. Chem.*, **2006**, 14, 8644
14. Genin, M.J.; Allwine, D.A.; Anderson, D.J.; Barbachyn, M.R.; Emmet, D.E.; Garmon, S.A.; Graber, D.R.; Grega, K.C.; Hester, J.B.; Hutchinson, D.K.; Morris, J.; Reischer, R.J.; Ford, C.W, Zurenko, G.E.; Hamel, J.C.; Schaadt, R.D.; Stapert, D.; Yagi, B.H. *J. Med. Chem.*, **2000**, 43, 953
15. (a) Buckle, D.R.; Rockell, C.J.M.; Smith, H.; Spicer, B.A. *J. Med. Chem.*, **1984**, 27, 223; (b) Buckle, D.R.; Rockell, C.J.M.; Smith, H.; Spicer, B.A. *J. Med. Chem.*, **1986**, 29, 2262
16. Wamhoff, H., *In Comprehensive Heterocyclic Chemistry*, Katritzky, A.R., Rees, C.W.



- (eds.), Pergamon Press, Oxford, **1984**, 669-732
17. Chakraborti, A.K.; Kondaskar, A. *Tetrahedron Lett.*, **2003**, *44*, 8315
  18. Gorzynski-Smith, J. *Synthesis*, **1984**, 629
  19. (a) Merrill, G.N. *J. Phys. Org. Chem.*, **2007**, *20*, 19; (b) McClure, D.E.; Arison, B.H.; Baldwin, J.J. *J. Am. Chem. Soc.*, **1979**, *101*, 3666
  20. Oishi, Y.; Nakanishi, T. *Chem. Pharm. Bull.*, **1983**, *31*, 3418
  21. Musonda, C.C. *Ph.D. Thesis*, University of Cape Town, **2005**
  22. Swift, G.A.; Swern, D. *J. Org. Chem.*, **1967**, *32*, 511
  23. Scriven, E.F.V.; Turnbull, K. *Chem. Rev.*, **1988**, *88*, 297
  24. (a) Crotti, P.; Di Bussolo, V.; Favero, L.; Macchia, F.; Pineschi, M. *Eur. J. Org. Chem.*, **1998**, *8*, 1675; (b) Blandy, C.; Choukroun, R.; Gervais, D. *Tetrahedron Lett.*, **1983**, *24*, 4189; (c) Nugent, W.A. *J. Am. Chem. Soc.*, **1992**, *114*, 2768; (d) Das, B.; Saidi, V.S.; Fouzia, T.; Maddeboina, K. *Synthesis*, **2007**, 0666; (e) Fringuelli, F.; Pizzo, F.; Vaccaro, L. *J. Org. Chem.*, **2001**, *66*, 4719
  25. Sabitha, G.; Babu, S.R.; Rajkumar, M.; Yadav, J.S. *Org. Lett.*, **2002**, *4*, 343
  26. Chini, M.; Crotti, P.; Macchia, F. *Tetrahedron Lett.*, **1990**, *31*, 5641
  27. Boruwa, J.; Borah, J.C.; Kalita, B.; Barua, N.C. *Tetrahedron Lett.*, **2004**, *45*, 7355
  28. Sarangi, C.; Das, N.B.; Nanda, B.; Nayak, A.; Sharma, R.P. *J. Chem. Res. (S)*, **1997**, 378
  29. Gil, M.V.; Arévalo, M.J.; López, Ó. *Synthesis*, **2007**, *11*, 1589
  30. Kolb, H.C.; Finn, M.G.; Sharpless, K.B. *Angew. Chem. Int. Ed.*, **2001**, *40*, 2004
  31. Moses, J.E.; Moorhouse, A.D. *Chem. Soc. Rev.*, **2007**, *36*, 1249
  32. (a) Clayden, J.; Greeves, N.; Warren, S.; Wothers, P. *Organic Chemistry*, Oxford University Press, New York, **2001**, 932-933; (b) Smith, M.B.; March, J. *March's Advanced Organic Chemistry*, 6<sup>th</sup> Ed., John Wiley & Sons, Inc., New York, **2001**, 1187-1192
  33. Huisgen, R. In *1,3-Dipolar Cycloaddition Chemistry*, Padwa, A. (ed.), Vol. 1, New York, John Wiley & Sons, Inc., **1984**, 1-176
  34. Organic Chemistry Portal; <http://www.organic-chemistry.org/namedreactions/huisgen-1,3-dipolar-cycloaddition.shtm>;
  35. (a) Sustmann, R. *Tetrahedron Lett.*, **1971**, *12*, 2717; (b) Sustmann, R. *Pure Appl. Chem.*, **1974**, *40*, 569
  36. (a) Houk, K.N.; Yamaguchi, K. In *1,3-Dipolar Cycloaddition Chemistry*, Padwa, A. (ed.), Vol. 2, New York, John Wiley & Sons, Inc., **1984**, 407-450; (b) Houk, K.N.; Sims, J.; Watts, C.R.; Luskus, L.J. *J. Am. Chem. Soc.*, **1973**, *95*, 7301; (c) Torssell, S., *Ph.D.*

Thesis, Stockholm, 2007

37. Wang, Q.; Chittaboina, S.; Barnhill, H.N. *Lett. Org. Chem.*, **2005**, 2, 293
38. Rostovtsev, V.V.; Green, L.G.; Fokin, V.V.; Sharpless, K.B. *Angew. Chem. Int. Ed.*, **2002**, 41, 2596
39. (a) Tornøe, C.W.; Christensen, C.; Meldal, M. *J. Org. Chem.*, **2002**, 67, 3057
40. (a) Wang, Q.; Chan, T.R.; Hilgraf, R.; Fokin, V.V.; Sharpless, K.B.; Finn, M.G. *J. Am. Chem. Soc.*, **2003**, 125, 3192; (b) Speers, A.E.; Adam, G.C.; Cravatt, B.J. *J. Am. Chem. Soc.*, **2003**, 125, 4686
41. Diaz, D.D.; Punna, S.; Holzer, P.; McPherson, A.K.; Sharpless, K.B.; Fokin, V.V.; Finn, M.G. *J. Polym. Sci., Part A: Polym. Chem.*, **2004**, 42, 4392
42. Wu, P.; Fokin, V.V. *Aldrichimica Acta*, **2007**, 40, 7
43. Patton, G.C. *Development and Application of Click Chemistry*, **2004**,  
[http://chemistry.illinois.edu/research/organic/seminar\\_extracts/2004\\_2005/08\\_Patton\\_Abstract.pdf](http://chemistry.illinois.edu/research/organic/seminar_extracts/2004_2005/08_Patton_Abstract.pdf)
44. (a) Himo, F.; Lovell, T.; Hilgraf, R.; Rostovtsev, V.V.; Noodleman, L.; Sharpless, K.B.; Fokin, V.V. *J. Am. Chem. Soc.*, **2005**, 127, 210; (b) Malkoch, M.; Schleicher, K.; Drockenmuller, E.; Hawker, C.J.; Russell, T.P.; Wu, P.; Fokin, V.V. *Macromolecules*, **2005**, 38, 3663; (c) Perez-Balderas, F.; Ortega-Munoz, M.; Morales-Sanfrutos, J.; Hernandez-Mateo, F.; Calvo-Flores, F.G.; Calvo-Asin, J.A.; Isac-Garcia, J.; Santoyo-Gonzalez, F. *Org. Lett.*, **2003**, 5, 1951
45. Appukkutan, P.; Dehaen, W.; Fokin, V.V.; Van der Eycken, E. *Org. Lett.*, **2004**, 6, 4223
46. (a) Chan, T.R.; Hilgraf, R.; Sharpless, K.B.; Fokin, V.V. *Org. Lett.*, **2004**, 6, 2853; (b) Koradin, C.; Polborn, K.; Knochel, P. *Angew. Chem. Int. Ed.*, **2002**, 41, 2596
47. (a) Chan, T.R.; Hilgraf, R.; Sharpless, K.B.; Fokin, V.V. *Org. Lett.*, **2004**, 6, 2853; (b) Feldman, A.K.; Colasson, B.; Fokin, V.V. *Org. Lett.*, **2004**, 6, 3897
48. Rodinov, V.O.; Fokin, V.V.; Finn, M.G. *Angew. Chem. Int. Ed.*, **2005**, 44, 2210
49. (a) Cameron, M.D.; Bennett, G. *J. Org. Chem.*, **1957**, 22, 557; (b) Siemsen, P.; Livingston, R.C.; Diederich, F. *Angew. Chem. Int. Ed.*, **2000**, 39, 2632; (c) Gerard, B.; Ryan, J.; Beeler, A.B.; Porco J.A. Jr. *Tetrahedron*, **2006**, 62, 64051
50. Angell, Y.; Burgess, K. *Angew. Chem. Int. Ed.*, **2007**, 46, 3649
51. Bock V.D.; Hiemstra, H.; Van Maarseven, J.H. *Eur. J. Org. Chem.*, **2006**, 51
52. Hay, A.S. *J. Org. Chem.*, **1962**, 27, 3320
53. (a) Mindt, T.L.; Schibli, R. *J. Org. Chem.*, **2007**, 72, 10247; (b) Bae, I.; Han, H.; Chang, S. *J. Am. Chem. Soc.*, **2005**, 127, 2038

54. Ray, A.; Manoj, K.; Bhadbhade, M.M.; Mukhopadhyay, R.; Bhattacharjya, A. *Tetrahedron Lett.*, **2006**, 47, 2775
55. Lee, B.-Y.; Park, S.R.; Jeon, H.B.; Kim, K.S. *Tetraheron. Lett.*, **2006**, 47, 5105
56. Orgueira, H.A.; Fokas, D.; Isome, Y.; Chan, P.C.M.; Baldino, C.M. *Tetrahedron Lett.*, **2005**, 46, 2911
57. Lipshutz, B.; Taft, B.R. *Angew. Chem. In. Ed.*, **2006**, 45, 8235
58. (a) October, N. *Ph.D. Thesis*, University of Cape Town, **2007**; (b) Ntuli, N.A.; *M.Sc. Thesis*, University of Cape Town, **2005**
59. Naumann, K. *J. Prakt. Chem.*, **1999**, 341, 417
60. Kaschula, C.H.; Egan, T.J.; Hunter, R.; Basilico, N.; Parapini, S.; Taramelli, D.; Pasini, E.; Monti, D. *J. Med. Chem.*, **2002**, 45, 3531
61. (a) Ribeiro, N.M.; Pinto, A.C.; Da Silva, B.V.; Violante, F.A.; Dias, M.O. *Catal. Com.*, **2007**, 8, 2130; (b) Dos Santos, E.L.; Gomes W.A. Jr.; Ribeiro, N.M.; Andrade, H.M.C. *J. Mol. Catal. A: Chemical*, **2008**, 295, 18
62. Blass, B.E. *Tetrahedron*, **2002**, 58, 9301

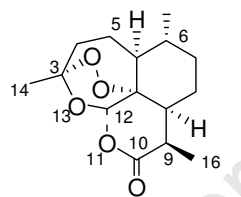
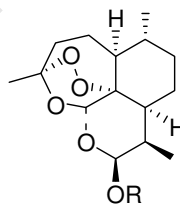
## Chapter five

## DESIGN, SYNTHESIS AND CHARACTERIZATION OF ARTEMISININ ANALOGUES

## 5.1. Introduction

This chapter describes the design, synthesis, characterization and biological evaluation of an exploratory library of semi-synthetic artemisinin analogues which are hybrid constructs of dihydroartemisinin with isatin, thiolactone, chalcone and other natural product derived scaffolds. With the focus still on malaria and tuberculosis as disease models, the hybrids synthesized were tested for growth inhibitory activity against (i) the CQR and CQS of *P. falciparum* and (ii) the drug-sensitive H<sub>37</sub>Rv strain of *M. tuberculosis*. Attempts to elucidate a possible antimalarial mode of action involved testing their cysteine protease inhibitory activity.

## 5.2. Background

Artemisinin **1.14**

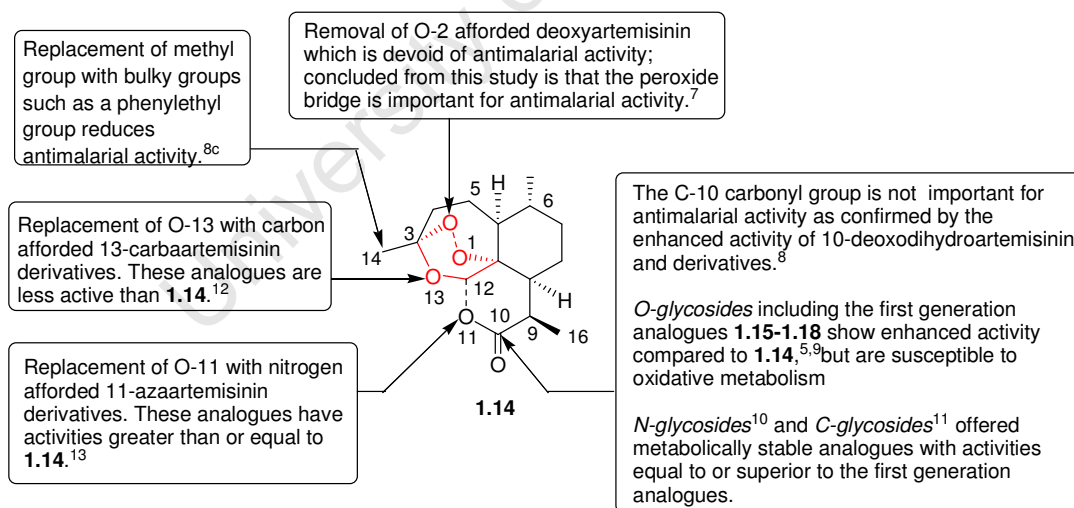
- R= H, Dihydroartemisinin **1.15**  
 R = CH<sub>3</sub>, Artemether **1.16**  
 R = CH<sub>2</sub>CH<sub>3</sub>, Arteether **1.17**  
 R= CO(CH<sub>2</sub>)<sub>2</sub>CO<sub>2</sub>Na, Artesunate **1.18**

**Figure 5.1:** Chemical structures of artemisinin **1.14** and the first generation analogues **1.15-1.18**

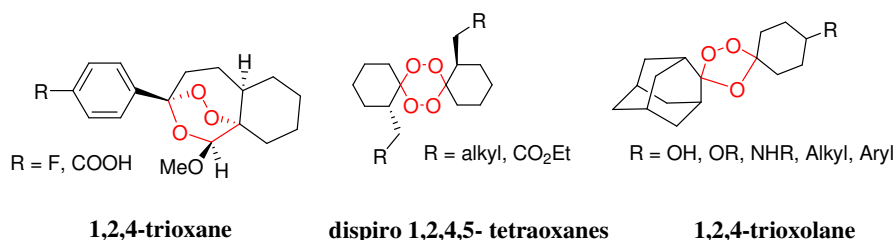
Artemisinin **1.14** (fig. 5.1) has since its discovery in the 1970s become the focus of a concerted research effort.<sup>1</sup> As mentioned in section 1.4.2.1.3, the mechanism of action of artemisinin and analogues differs from other antimalarials in clinical use. They are active against multidrug resistant forms of *P. falciparum* but are also reported to have anticancer<sup>2</sup> and immunosuppressive activity.<sup>3</sup> Also, the efficaciousness of artemisinins in parasitic diseases caused by *Toxoplasma gondii*,<sup>4a</sup> *Leishmania major*,<sup>4b</sup> *Schistosoma mansoni* and *Schistosoma japonicum*<sup>4c</sup> have been reported. However, the therapeutic utility of **1.14** is

impaired by its low solubility in water and oil,<sup>5a</sup> and its short half-life.<sup>5b</sup> With regard to the latter, two remedial strategies are currently employed one being the utilization of combination therapy which is advocated by WHO because of the pending risk of resistance development (section 1.5.2.). The other strategy ties in with our project objectives and involves the synthesis of analogues with enhanced activity.

Structural modification of **1.14** is an active field of research judging from the number of research papers, patents and reviews.<sup>1,6</sup> Moreover, there is a large volume of SAR data generated from semi-synthetic analogues obtained by the modifications of **1.14** at C-16, C-10, C-9, C-3, C-4, O-11 and O-13.<sup>1</sup> Some key structural-activity relations revealed by previous studies are summarized in figure 5.2. Despite the huge demand for **1.14** only a small amount can be extracted from the plant material. The preparation of synthetic peroxide analogues such as 1,2,4-trioxanes, 1,2,4,5-tetraoxanes and 1,2,4-trioxolanes (fig 5.3) was therefore explored. They are all modelled on the pharmacophore traced in red on the artemisinin scaffold (fig. 5.2). Some of these analogues showed exceptional activity profiles, a notable example being the 1,2,4-trioxolane derivative, **OZ277 2.8** (fig. 2.2) discussed in section 2.2.2.1.

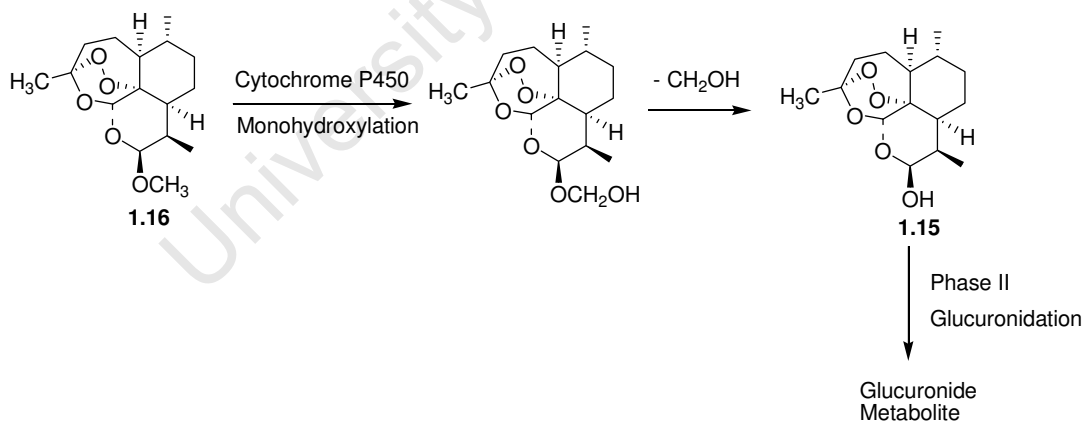


**Figure 5.2:** A few key structure-activity relationship pertaining to antimalarial activity of **1.14**



**Figure 5.3:** Chemical structures of synthetic analogues of artemisinin **1.14**

Of interest to this project are the C-10 semi-synthetic derivatives of dihydroartemisinin (**1.15**, DHA) which can be further subdivided into *O*-glycosides, *N*-glycosides and *C*-glycosides. Counted among the C-10 *O*-glycosides are the first generation analogues which were obtained by the sodium borohydride reduction of **1.14** to give **1.15** (fig. 5.1). The latter in turn served as a precursor for the oil-soluble artemether **1.16**, arteether **1.17** and the water-soluble artesunate **1.18** (fig. 5.1). Although they displayed enhanced activity *in vitro* compared to **1.14** these analogues are reported to be neurotoxic<sup>14</sup> and display poor bioavailability.<sup>15</sup> More specifically, it is reported that **1.16** and **1.17** undergo an oxidative dealkylation mediated by the action of cytochrome P450 (scheme 5.1). It is believed to proceed *via* a monohydroxylated derivative or hemiacetal. The latter decomposes to the neurotoxic **1.15** which in turn is glucuronidated and excreted in bile.<sup>16</sup>



**Scheme 5.1** Cytochrome P-450 –mediated oxidative dealkylation of **1.16**<sup>17</sup>

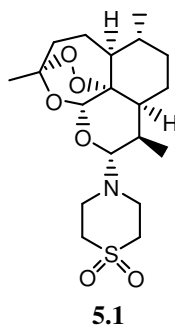
The introduction of minor structural changes at C-10 as observed with **1.16** and **1.17**, resulted in a significant enhancement in the antimalarial activity. This prompted many researchers to explore C-10 modification of **1.15**. However, the challenge was - and still is - to synthesize analogues which are not susceptible to oxidative dealkylation (scheme 5.1) and thus possess negligible neurotoxicity. The metabolic stabilization of C-10 ether derivatives was achieved by the introduction of the trifluoromethyl (CF<sub>3</sub>) group directly at C-10 as undertaken in the preparation of the trifluoromethyl analogues of **1.16-1.18** by Bonnet-Delpon and coworkers.<sup>18</sup> Another approach towards more stable analogues was explored by O'Neill and coworkers.<sup>17</sup> It entailed the replacement of the *O*-alkyl group in first generation analogues with an *O*-phenoxy group. They managed a further increase in stability for these analogues by introducing a fluoro- or trifluoromethyl group at the *para* position of the phenyl ring. Introduction of a carboxylic acid group at the *meta* position proved advantageous in enhancing the water solubility of these 10-β-aryl ethers of **1.15**.<sup>17,19</sup> A more common structural modification route explored, involves the replacement of the oxygen at C-10 with carbon to furnish the chemical and hydrolytic stable C-10 C-glycosides or deoxoartemisinins (fig. 5.2).<sup>11</sup> Hydrolytically stable analogues were also found among C-10 *N*-glycosides. These were prepared by the condensation of **1.15** or intermediates with aromatic and aliphatic amines.<sup>10</sup>

### 5.3 Rationale

Coartem™ is an example of a combination therapy where artemether **1.16** a fast acting antimalarial is partnered with the longer half lived, arylaminoalcohol lumefantrine **1.5** (fig. 1.3). As an alternative to this strategy, hybridization - the drug discovery 'tool' we have used throughout this project - has been applied in artemisinin analogue synthesis. In section 2.2.4 we cited the example of the artemisinin-quinine hybrid **2.25** (fig. 2.13) but other dimeric structures with activity more potent than that of **1.14** or its first generation analogues have been reported.<sup>2a</sup>

Also, the application of the ADME (absorption, distribution, metabolism and excretion) approach in artemisinin analogue design has come to the fore because of increasing concerns regarding the link between lipophilicity and neurotoxicity.<sup>10a,17,20</sup> Reportedly analogues with log P >3.5 are neurotoxic by virtue of their ability to cross the blood-brain-barrier.<sup>20b</sup> Utilizing the ADME approach, Haynes and coworkers developed artemisone **5.1** (fig 5.4) a 10-alkylaminoartemisinin which differ from current artemisinins in that it displays

favourable bioavailability and negligible neurotoxicity.<sup>21</sup> It should be pointed out that there are contradictory findings in the literature regarding the neurotoxicity of first generation alkyl ethers. For example, a study reported that the intramuscular administration of a high dose of artemether **1.16** showed a lack of neuronal death in patients.<sup>22</sup> However, a comparative study where a similar dosage is administered orally is still pending.<sup>22a</sup>



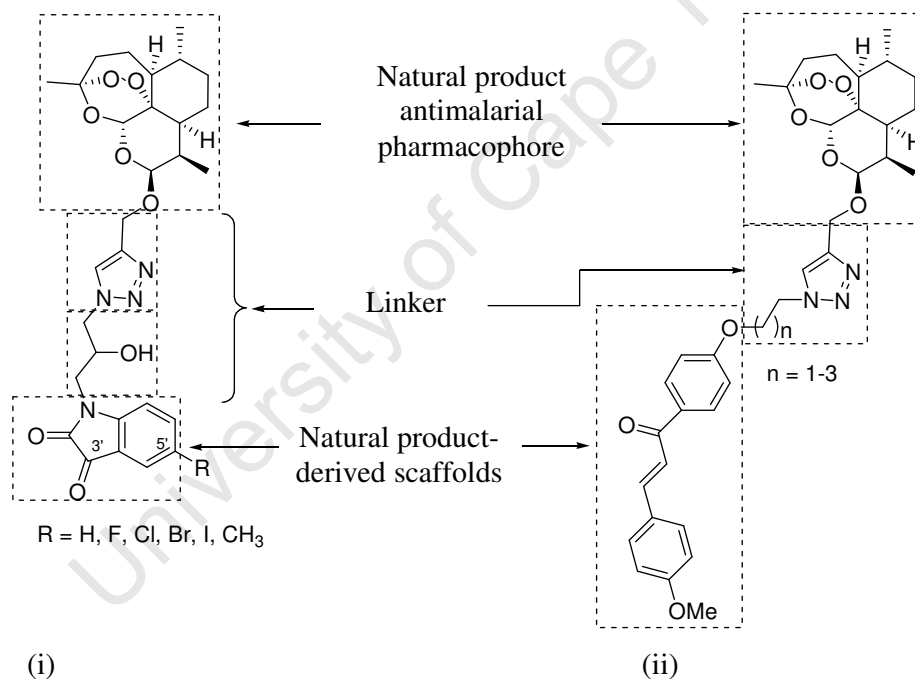
**Figure 5.4:** Chemical structure of artemisone **5.1**

The investigation of the antitubercular activity of artemisinins, to the best of our knowledge, is a relatively unexplored field. The publications we managed to trace, reported on the screening of mixed steroidal 1,2,4,5-tetraoxanes for activity against the *M. tuberculosis* H<sub>37</sub>Rv strain.<sup>23</sup> These analogues showed inhibitory activity with MICs as low as 4.73  $\mu$ M.<sup>23a</sup> On the basis of these considerations and the promising results obtained with the  $\beta$ -amino alcohol hybrids discussed in the previous chapter, we reasoned that the covalent linkage, *via* ‘click’ chemistry, of isatin, chalcone, zidovudine (AZT) and the 7-chloroquinoline moiety with **1.15** will lead to analogues with potentially enhanced therapeutic profiles. Incidentally, a recent paper reported on a 10-membered library of 1,4-substituted 1,2,3-triazole artemisinin analogues obtained *via* Huisgen’s 1,3-dipolar cycloaddition reaction. These compounds however were not tested for antimalarial activity but for cell proliferation inhibition activity against various cancer cell lines.<sup>24</sup>



The limited series of compounds designed and synthesized can be subdivided into three groups:

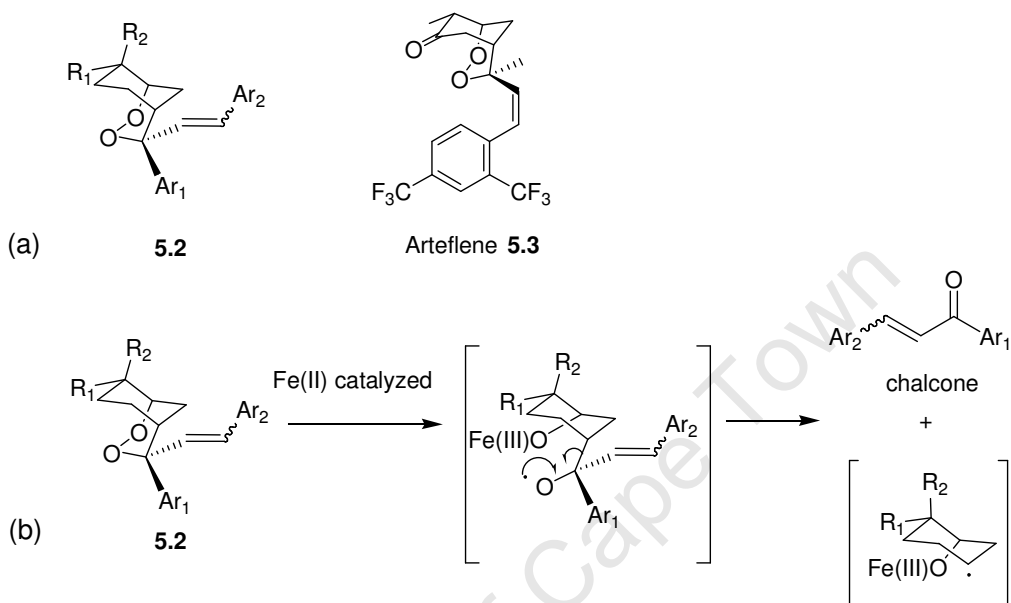
(i) The first group shown in figure 5.5 (i) represent the hybrid construct between **1.15** and a  $\beta$ -amino alcohol isatin derivative. We envisaged improved metabolic stability and aqueous solubility for these analogues which is to be facilitated by the  $\beta$ -amino alcohol group and the 1,2,3-triazole ring. Speculatively, the chemical stability and more so strong dipole moment of the latter (section 4.2.1) and its proximity to the acetal function (C-10) make these analogues electronically different from the easily hydrolysable acetyl-analogues. Also, the calculated log P values or lipophilicity for these compounds are relatively low ranging from 2.07 to 3.37. Structure-activity relationship studies will investigate the effect of different substituents at C-5' of the isatin moiety as well as the effect of lipophilicity on both antimalarial and antitubercular activity.



**Figure 5.5:** Rationale for design of target molecules

(ii) The design and synthesis of the second group of compounds [fig. 5.5 (ii)] was motivated in part, by the endoperoxide cysteine protease inhibitor (ECPI) prodrug model pioneered by O'Neil and coworkers.<sup>25</sup> Prodrugs, as defined by Adrien Albert, describes compounds which need to undergo biotransformation before they can elicit a pharmacological effect.<sup>26</sup> The masked prodrug **5.2** [(fig. 5.6 (a)], a structural analogue to the synthetic ART analogue, arteflene **5.3** [(fig. 5.6 (a)], was prepared with the intention to selectively deliver the chalcone

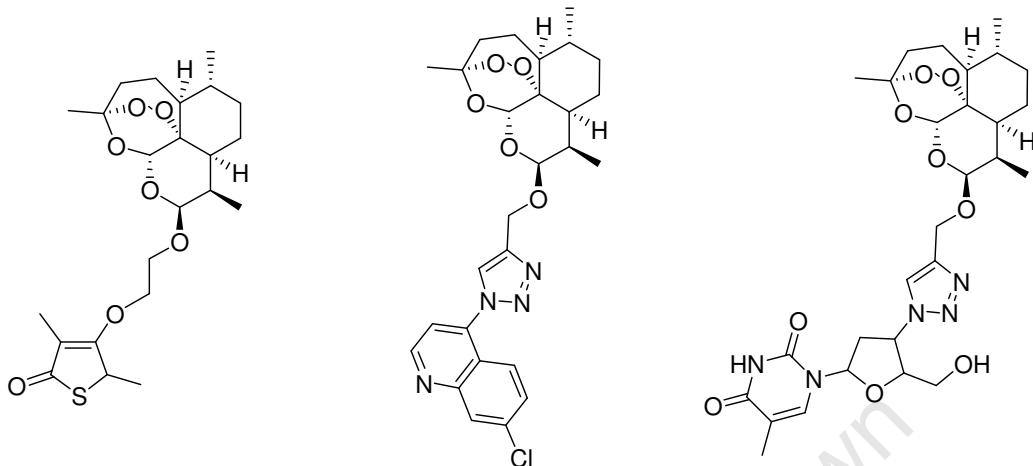
for cysteine protease inhibition (section 1.4.2.1.4.2) to the acidic food vacuole after a ferrous-mediated unmasking [fig. 5.6 (b)]. The active carbon centered radical species formed as a by-product in this reaction, is believe to exert a mechanism of action similar to that postulated for artemisinin and other related trioxanes.<sup>25</sup>



**Figure 5.6:** (a) Chemical structure of generic prodrug **5.2** and arteflene **5.3**; (b) Ferrous-catalyzed degradation of generic prodrug **5.2**<sup>19</sup>

Two recent independent studies utilized the isobologram method<sup>27</sup> to investigate the interaction of **1.14** and licochalcone A **1.22** (fig. 2.10)<sup>28a</sup> and the interaction of **1.14** with three synthetic chalconeazole derivatives,<sup>28b</sup> respectively. These studies collectively showed that fixed ratio combinations of **1.14** and chalcones displayed a synergistic or additive effect when evaluated for *in vitro* antiplasmodial activity. Some of the benefits of incorporating chalcones in hybrid constructs have been discussed in the previous chapter. The cost-effectiveness and simplicity of their preparation however, needs to be emphasized. Also because of the lack of stereogenic centers in the chalcone moiety there is no risk of hybrid formation leading to complex diastereomeric mixtures as was observed with some previously reported chimeras,<sup>19</sup> thereby ensuring interpretable SAR. The  $c \log P$  values (5.87 - 6.31) and molecular weights (645.7 g/mol to 673.8 g/mol) of the envisaged compounds are in clear violation of Lipinski's rules for oral drugs. It is however worthwhile investigating the effect

of the variable alkyl linker on activity; for this reason, substitution was restricted to a *para*-located methoxy group on ring A of the chalcone scaffold.



**Figure 5.7:** Rationale for design of target molecules

(iii) The third group of analogues (fig. 5.7) consists of structurally unrelated hybrids of **1.15** with thiolactone **2.13** (fig. 3.1), 7-chloro-4-aminoquinoline and AZT. The rationale for choosing **2.13** as a monomer in the construction of hybrids has been discussed in previous chapters. The nucleoside analogue, AZT is an antiretroviral drug which has found use in antimalarial drug design. There exist extensive reports on the efficacy and toxicity of AZT. Counted among the attractive properties is its ability to cross cell membranes via passive diffusion.<sup>29</sup> It is envisaged that the hybrid generated by the covalent linkage of AZT and **1.15** will display an improved toxicity profile whilst retaining nanomolar antimalarial activity.

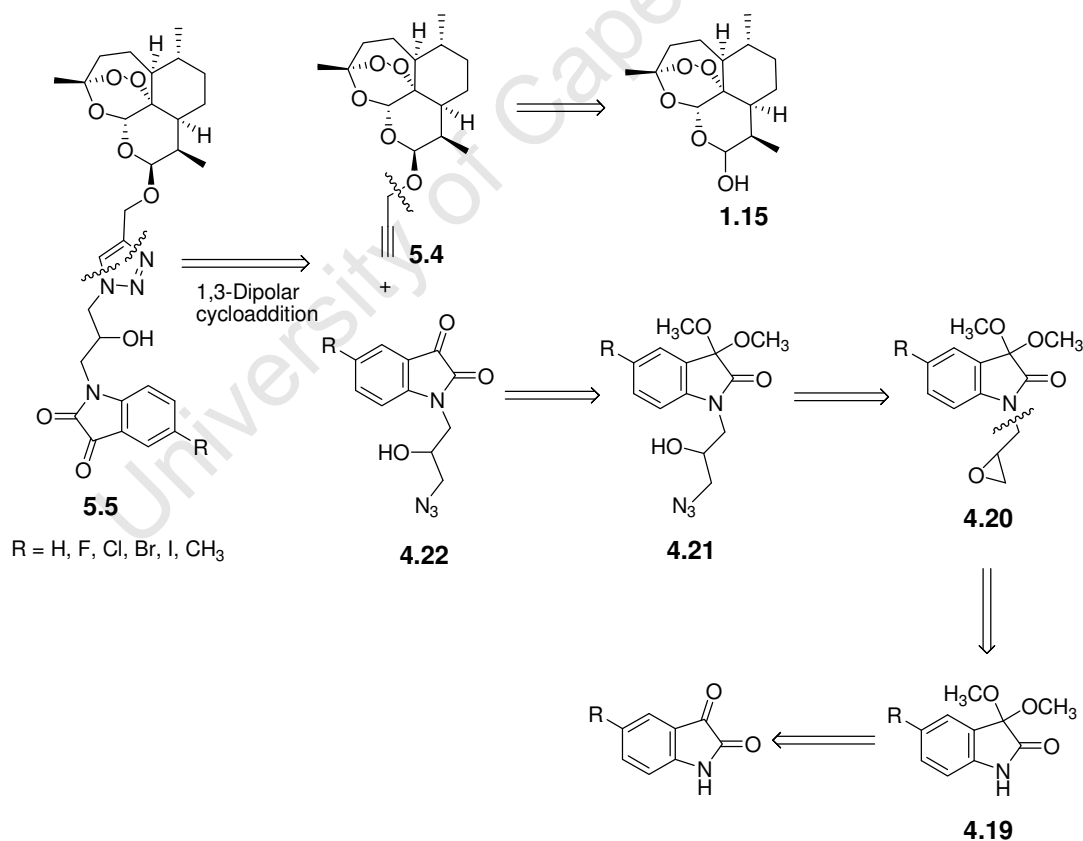
Reference has been made to the privileged status of the 7-chloro-4-aminoquinoline moiety. Previous studies identified this moiety as being important for the inhibition of haemozoin formation (section 1.4.2.1.2.1). The presence of a protonable nitrogen reportedly aids accumulation of the drug in the acidic food vacuole *via* pH trapping. This phenomenon describes the diffusion of the free base *via* a pH-gradient into the acidic food vacuole where the quinoline nitrogen gets protonated. In this way the compound (now positively charged) gets trapped and accumulates inside the food vacuole. It is expected that the aforementioned properties of the aminoquinoline moiety will complement those of the radical alkylating properties associated with **1.15** for the envisaged hybrid. Moreover, the utility of the 7-chloro-4-aminoquinoline moiety in hybrid construct is demonstrated by the trioxaquinones which are structurally characterized by the covalently bonded 7-chloro-4-aminoquinoline and

the synthetic artemisinin analogue, 1,2,4-trioxane. Identified from this series are derivatives with potent *in vitro* activity against the W2 strain of *P. falciparum* with IC<sub>50</sub> values of the three most active derivatives ranging between 2.26 – 12.44 nM.<sup>30</sup>

It is worth mentioning that we do not envisage the delineation of meaningful SAR from these miscellaneous hybrids. However, their synthesis and biological evaluation was deemed important in validating the strategy of hybridizing natural product derived scaffolds to source potential anti-infective agents and to make a contribution albeit mediocre, towards demonstrating the scope of ‘click’ chemistry.

## 5.4. Chemical Synthesis

### 5.4.1. Retrosynthesis, Synthesis and Characterization of Dihydroartemisinin-Isatin Hybrids



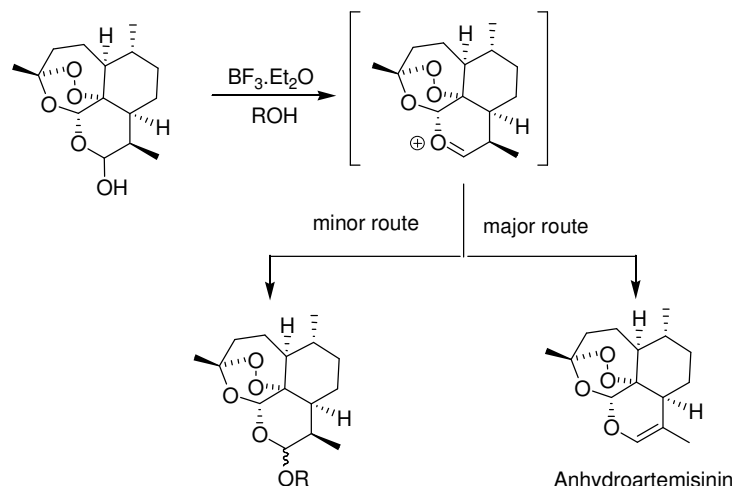
**Scheme 5.2:** Retrosynthetic analysis of **5.5**

As shown in scheme 5.2, synthesis of the target molecules **5.5** is envisaged from the Huisgen 1,3-dipolar cycloaddition reaction of the acetylenic intermediate **5.4** and the isatin azide **4.22** (scheme 5.2). The acetylene **5.4** can be easily obtained from condensation of dihydroartemisinin **1.15** and commercially available propargyl alcohol. The retrosynthetic analysis of **4.22** has been discussed in chapter 4 (section 4.3.2.1) and will therefore not be further elaborated on.

#### 5.4.1.1. Literature Review on the Synthesis of C-10 Ethers (*O*-glycosides) of **1.15**

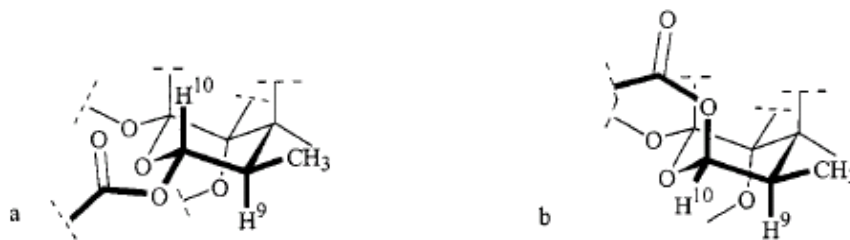
Dihydroartemisinin **1.15** is structurally characterized by the presence of ketal, acetal, and lactol functional groups. Each of these groups have varying sensitivities to or incompatibilities with acidic, alkaline or redox conditions. Also in view of the importance of the peroxide group for antimalarial activity, the preservation of this moiety is a priority in analogue synthesis and thus requires a judicious selection of reaction conditions. As alluded to earlier, the C-10 of **1.15** is comparable to the anomeric hydroxyl group of a pyranose ring in terms of chemical property and reactivity.<sup>31</sup> With this recognition, various synthetic methodologies have been developed for the synthesis of C-10 ether derivatives.

The most commonly used method affords the  $\beta$ -isomer of DHA ether derivatives predominantly and involves the reaction of **1.15** with alcohols in the presence of  $\text{BF}_3 \cdot \text{EtO}_2$  in anhydrous diethyl ether or DCM at room temperature.<sup>5a,9,32</sup> This reaction as shown in scheme 5.3, reportedly proceeds *via* an oxonium ion intermediate. Consequently, a common side reaction is the deprotonation of this reactive intermediate with subsequent formation of 9,10-dehydrodeoxoartemisinin (anhydroartemisinin). The latter is reported to be formed preferentially, when sterically hindered secondary or tertiary alcohols are used<sup>9a,32b</sup> or as the group of Bonnet-Delphon<sup>33</sup> discovered, when the alcohol displays poor nucleophilicity.



**Scheme 5.3:** Demonstration of the intermediacy of the oxonium ion in the aldol-type, acid catalyzed condensation reaction of **1.15** with alcohols.<sup>17</sup>

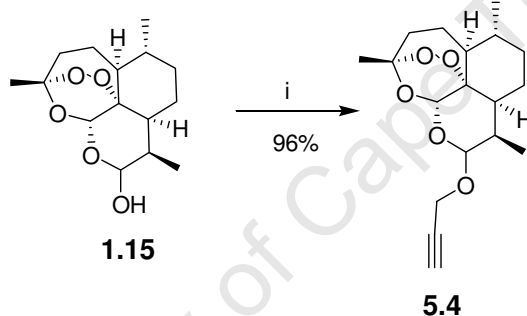
The formation of  $\alpha$ - and  $\beta$ -isomers is further proof of the intermediacy of the oxonium ion. More interesting however, is the preferential formation of the  $\beta$ -isomers under Lewis acid-catalyzed conditions, which reportedly stems from the anomeric effect. The latter describes the tendency of electronegative substituents on a pyranose ring to adopt the axial-configuration at the anomeric center (C-1 carbon) as opposed to the sterically less hindered equatorial-configuration.<sup>34</sup> In accordance, the axial configuration of the hemisuccinyl side chain in the chair pyranose ring for the  $\beta$ -isomer of **1.18** was confirmed by means of X-ray crystallographic analysis and <sup>1</sup>H-NMR data. A relative large coupling constant ( $J = 9.2$ ) confirmed the *trans*-diaxial relationship between the vicinal H-9 and H-10 for the  $\alpha$ -isomer [fig. 5.8 (a)] whereas a smaller coupling constant ( $J = 3.3$ ) was indicative of the *cis*-equatorial-axial relationship between these protons in the  $\beta$ -isomer [fig. 5.8 (b)].<sup>35</sup> Bulky substituents at C-10 reportedly cause the pyran ring of derivatives of **1.15** to adopt a twist-boat conformation thereby limiting the application of coupling constants in distinguishing between isomers.<sup>35</sup>



**Figure 5.8:** Partial structure of **1.18** showing the relationship between H-9 and H-10 in the (a)  $\alpha$ -isomer and (b)  $\beta$ -isomer<sup>35</sup>

The use of chlorotrimethylsilane (TMSCl) instead of  $\text{BF}_3 \cdot \text{OEt}_2$  for the synthesis of C-10 ethers of dihydroartemisinin has also been reported.<sup>36</sup> However, when the Bonnet-Delphon group used this method they isolated the ( $\alpha$ - and  $\beta$ ) epimers and a by-product which was identified as an isomerized ether. From this observation they concluded that under these conditions, the formation of anhydroartemisinin from the oxonium ion (scheme 5.3) is a reversible process with protonation at C-9 occurring either from the *re* or *si* face of the double bond.<sup>33</sup> Activation of the hydroxyl group of **1.15** followed by nucleophilic substitution reportedly also furnished the  $\beta$ -isomer under Schmidt–Hofmann<sup>37</sup> conditions (trichloroacetonitrile, DBU,  $\text{SnCl}_2$ ); the application of this synthesis protocol is discussed in detail in a review by Haynes *et al.*<sup>35</sup>

#### 5.4.1.1.1. Synthesis of the Acetylenic Intermediate of Dihydroartemisinin

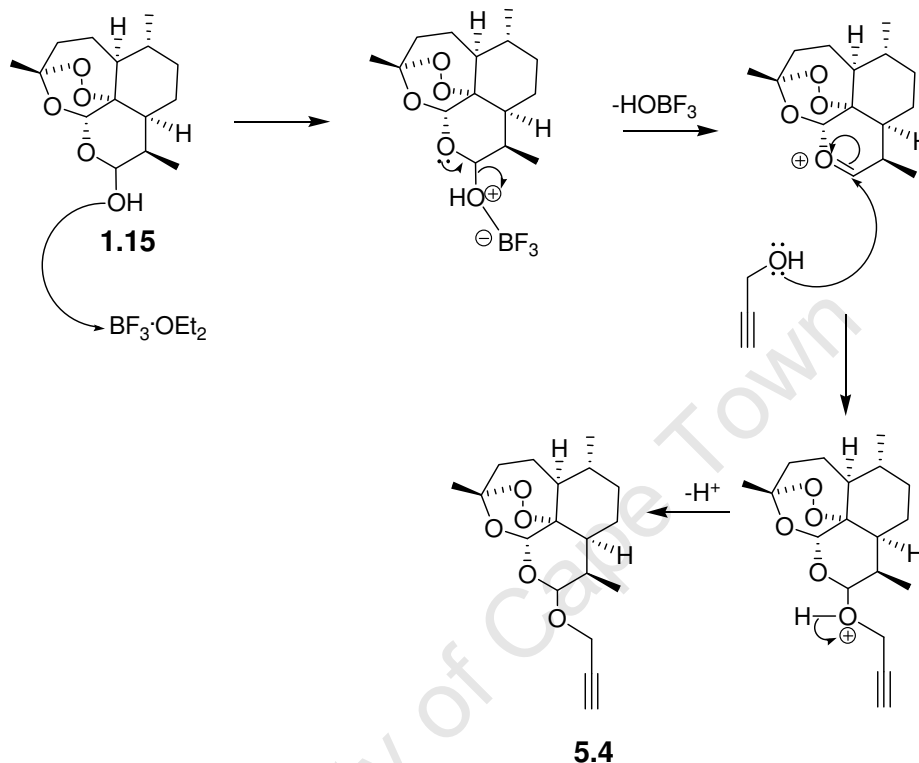


**Scheme 5.4.** Reagents and conditions: (i) Propargyl alcohol,  $\text{BF}_3 \cdot \text{OEt}_2$ , DCM, 25°C, 10 h

For the synthesis of **5.4** a procedure reported by Li *et al.*<sup>38</sup> was used (scheme 5.4). Dihydroartemisinin **1.15** was reacted with 4 eq of propargyl alcohol in 10 mL of dry DCM in the presence of 5 drops of  $\text{BF}_3 \cdot \text{Et}_2\text{O}$  at 25°C; the reaction progress was monitored by tlc with anisaldehyde spray reagent for detection. The product was obtained in excellent yield (96%) as a 21:79 mixture of the  $\alpha$ : $\beta$ -isomers which was separated using conventional column chromatography (EtOAc:Hex 0-20%). No dehydration product (anhydroartemisinin) was isolated and the reaction is very easy to scale-up.

As mentioned earlier, the  $\beta$ -isomer of *O*-glycosidic derivatives of **1.15** is formed preferentially under acid catalyzed conditions. It has also been reported that for some of these derivatives, the  $\alpha$ -isomers display superior antimalarial activity compared to the former.<sup>9c</sup> For this study however, we chose to prepare  $\beta$ -configured ether derivatives and therefore only the  $\beta$ -isomer of **5.4** was used in subsequent steps of the synthesis. The reason for separating the

isomers upstream, i.e. at the intermediate stage, as opposed to after the synthesis of the target compounds, was to avoid the formation of complex diastereomeric mixtures considering the chiral nature of some of the scaffolds the acetylene **5.4** was to be 'clicked' with.



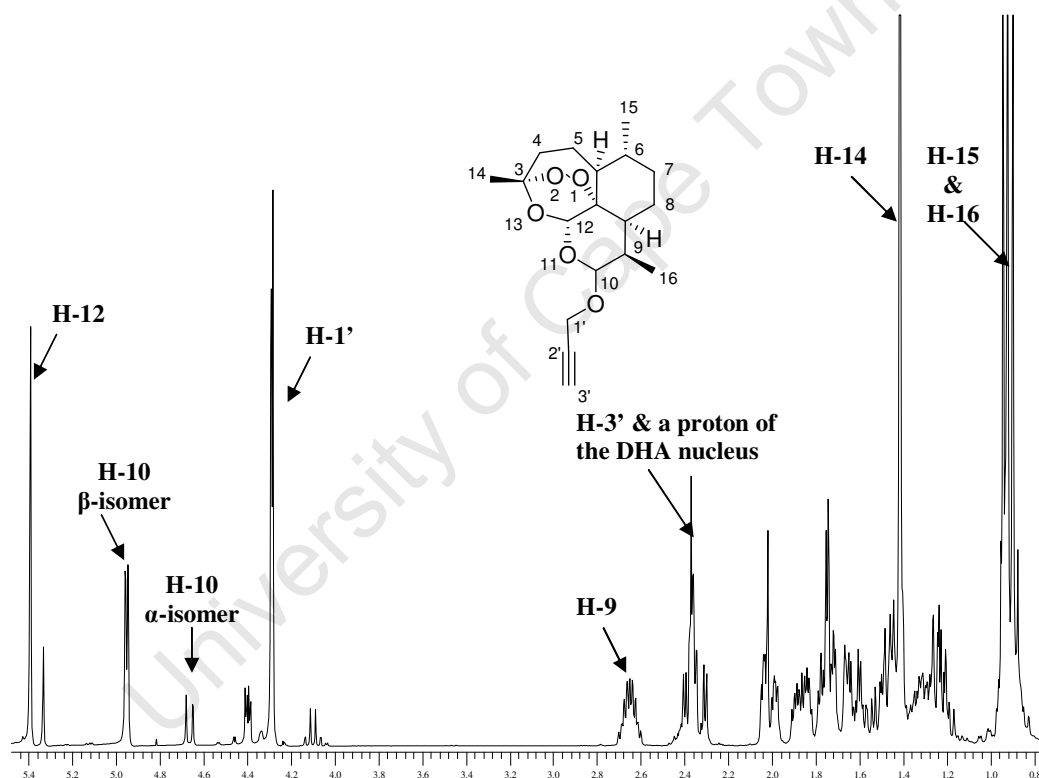
**Scheme 5.5.** Proposed mechanism for the condensation of **1.15** and propargyl alcohol

The postulated mechanism for the formation of **5.4** is shown in scheme 5.5. The condensation reaction is believed to proceed *via* an  $S_N1$  mechanism and starts with the coordination of the Lewis acid,  $BF_3$  to the hydroxyl group of **1.15** thereby generating a good leaving group. Expulsion of the leaving group is facilitated by the lone pair of electrons on the adjacent oxygen and leads to the reactive intermediate oxonium ion. The nucleophile (propargyl alcohol) attacks the oxonium ion preferentially on the  $\beta$ - face as suggested by the product distribution (21:79) for  $\alpha$ : $\beta$  isomers. Deprotonation of the adduct formed, leads to an epimeric mixture of **5.4**.

The  $^1H$ -NMR spectrum of the crude in  $CDCl_3$  (fig. 5.9) highlighted the key spectroscopic difference between the  $\alpha$ - and  $\beta$ -isomer for compound **5.4**. The anomeric proton (H-10) of the  $\alpha$ -isomer appear as a doublet resonating at  $\delta$  4.68 ppm ( $J = 9.3$ ).<sup>36</sup> A characteristic small coupling constant ( $J = 3.3$ ) was observed for the anomeric proton (H-10) of the  $\beta$ -isomer



which appeared as a doublet resonating at  $\delta$  4.95 ppm. This is ascribed to the gauche relationship between H-10 and H-9. Other key signals for **5.4** include the one assigned to H-12 - a one-proton singlet resonating downfield at  $\delta$  5.19 ppm. The assignment of the methyl protons at H-14, H-15 and H-16, were straightforward. In the shielded end of the  $^1\text{H-NMR}$  spectrum, H-14 appeared as a three-proton singlet at  $\delta$  1.41 ppm whereas H-15 and H-16 appeared as doublets resonating at  $\delta$  0.91 ppm ( $J = 7.2$ ) and  $\delta$  0.93 ppm ( $J = 6.0$ ), respectively. Protons of the acetylenic moiety such as H-1' appeared as a two-proton doublet at  $\delta$  4.29 ppm and H-3' overlapped with a peak assignable to a proton of the DHA nucleus in the region  $\delta$  2.40-2.30 ppm. Assignment of the remaining signals proved challenging and as evidence in the literature is not normally attempted.

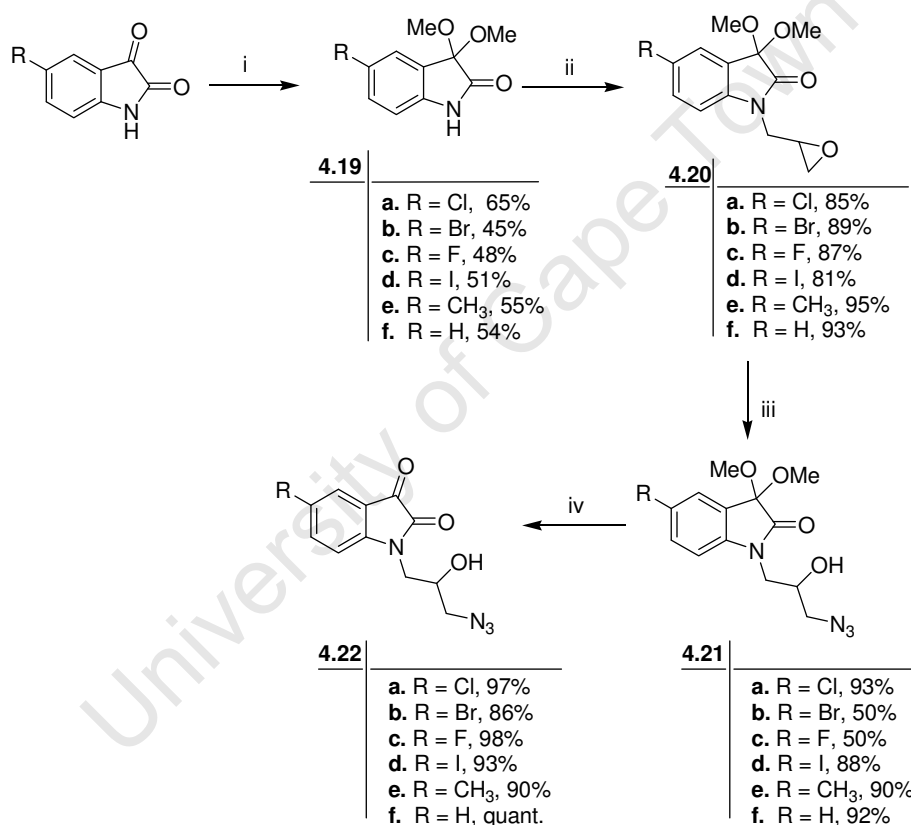


**Figure 5.9:**  $^1\text{H-NMR}$  spectrum of an epimeric mixture of **5.4** in  $\text{CDCl}_3$  at 300MHz

#### 5.4.1.1.2. Synthesis of the Isatin/5-substituted Isatin Azide Intermediates

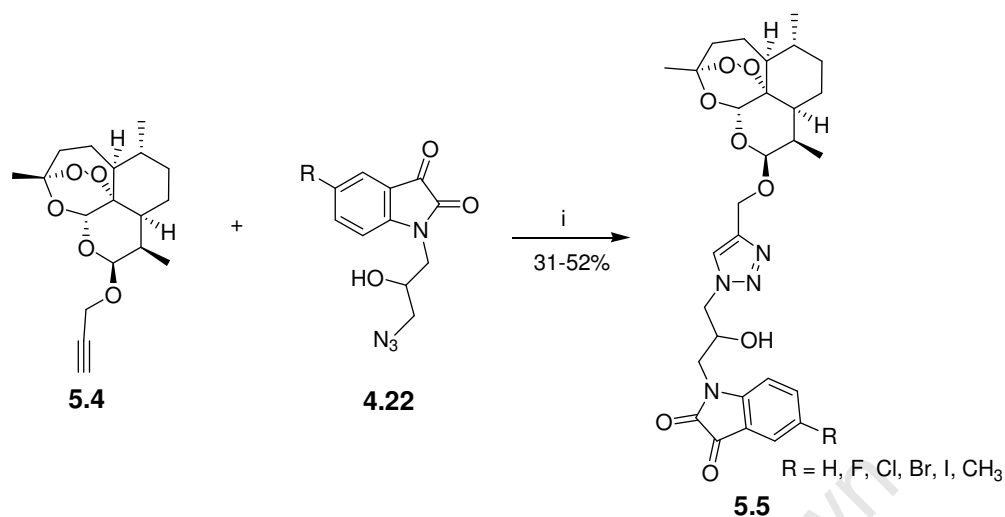
The synthesis and characterization of **4.22a** and its intermediates (**4.19a**, **4.20a**, **4.21a**) have been discussed in detail in section 4.3.2.2. For the synthesis of **4.22b-f** we employed the same reaction conditions and identified similar spectral features to confirm the proposed structures (section 4.3.2.2). Subsequent discussion will therefore focus on the yields obtained as indicated in scheme 5.6

The isolated yields for **4.19a-f** were moderate (48-65%, scheme 5.6) with the products obtained as variously coloured crystalline solids. In contrast, the epoxide intermediates **4.20** were isolated as colourless to light yellow oils in good to excellent yield (81-93%) after the chromatographic removal of excess epichlorohydrin. The azidohydrins **4.21** were obtained as colourless oils in moderate to excellent yield (50-93%) and the deketalization proceeded smoothly to afford the bright red coloured azide **4.22** in quantitative yield. It is worth mentioning that the deprotection step could have preceded the azidolysis as it is unlikely for the azide to react with the ketonic carbonyl in the following step. However, deprotection of the azide **4.21** proved just as feasible.



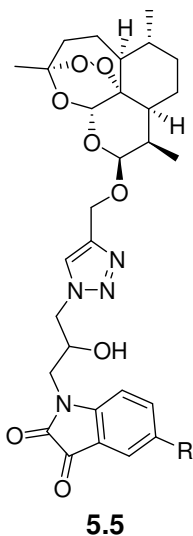
**Scheme 5.6:** Reagents and conditions: (i) CH(OMe)<sub>3</sub>, *p*-TsOH, MeOH, reflux, 48 h; (ii) (±)-Epichlorohydrin, KF-Al<sub>2</sub>O<sub>3</sub>, DCM, 25°C, 20 h; (iii) NaN<sub>3</sub>, NH<sub>4</sub>Cl, MeOH/H<sub>2</sub>O (8:1), 25°C, 16 h; (iv) 10% HCl, Acetone, 25°C, 3 h

## 5.4.1.1.3. Synthesis and Characterization of Dihydroartemisinin-Isatin Hybrids



**Scheme 5.7.** Reagents and conditions: (i) CuSO<sub>4</sub>·5H<sub>2</sub>O, sodium ascorbate, CH<sub>2</sub>Cl<sub>2</sub>:H<sub>2</sub>O(1:1), 25°C, 6 h

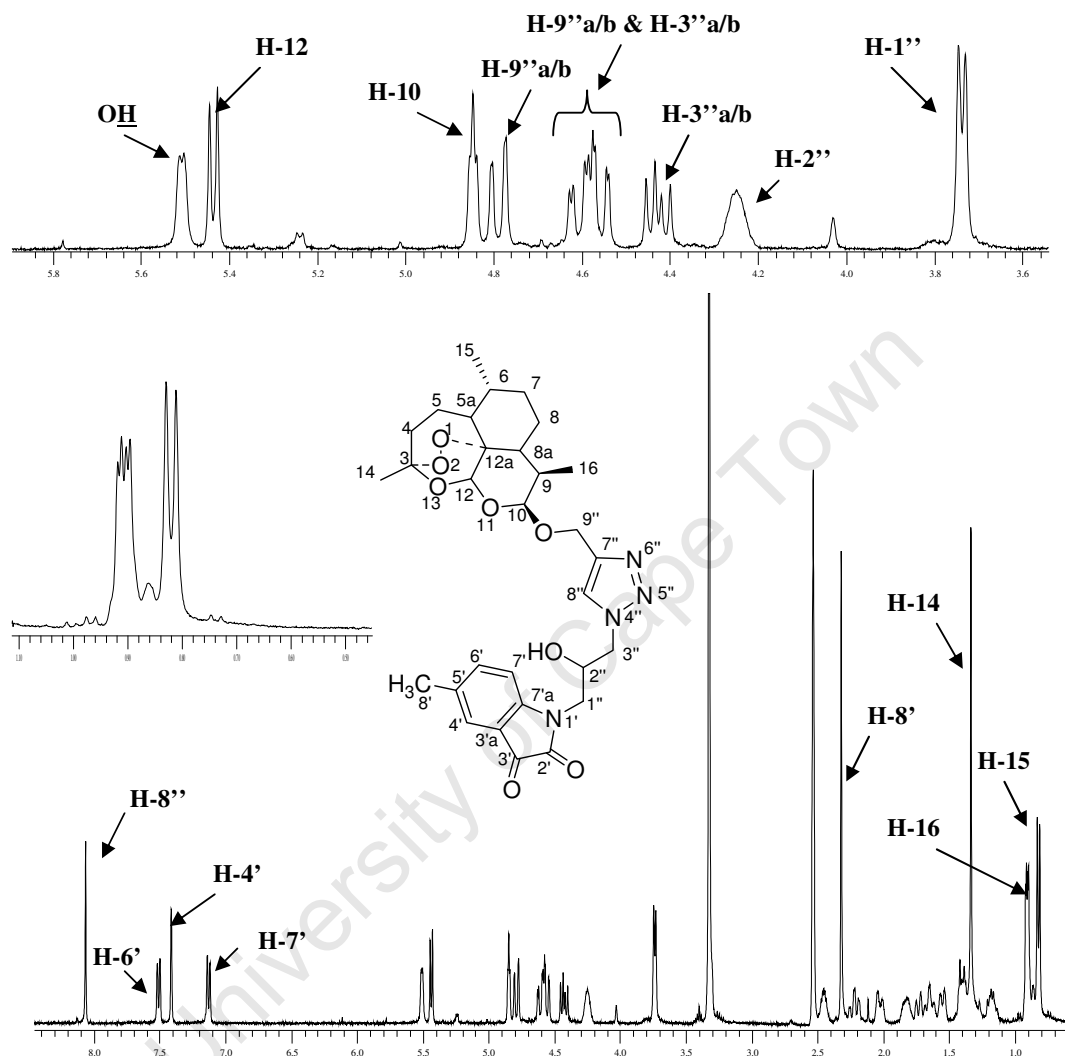
Because of the redox susceptibility of the peroxide bridge our initial concern was the stability of this functional group under ‘click’ chemistry conditions. This was fuelled by reports on the degradation of some biological scaffold mediated by copper or sodium ascorbate.<sup>39</sup> A small-scale reaction complemented by a published report on the synthesis of the 1,2,3-triazole artemisinin library using the CuSO<sub>4</sub>/sodium ascorbate catalyst system<sup>24</sup> proved these concerns to be invalid. Synthesis of target molecule **5.5** was therefore achieved using the conditions outlined in scheme 5.7. The reaction was completed in 6 hours as indicated by tlc with detection aided by the highly coloured nature of the isatins. Purification with column chromatography (EtOAc: Hex 20-80%) afforded the pure target molecules as orange foams in moderate yields as shown in table 5.1.

**Table 5.1.** Isolated yields and melting points of target molecules **5.5**

Compound	R	Yield (%)	m.p. (°C)
<b>5.5a</b>	Cl	52	99-101
<b>5.5b</b>	F	31	101-103
<b>5.5c</b>	H	48	92
<b>5.5d</b>	CH <sub>3</sub>	45	103-105
<b>5.5e</b>	I	42	112-115
<b>5.5f</b>	Br	52	104

A typical <sup>1</sup>H-NMR spectrum representative for compounds in this series, is shown for **5.5d** in figure 5.10. Due to the proximity of H-9'' to chiral centers of the dihydroartemisinin moiety, the two methylene protons are non-equivalent and appeared as one proton doublets at δ 4.79 ppm and δ 4.56 ppm; each with a large geminal coupling constant ( $J = 12.4$ - $12.8$ ). The signal at δ 4.85 ppm has the appearance of a broad doublet and was assigned to H-10. The coupling constant for the latter ( $J = 3.6$ ) confirmed the β-configuration of **5.5d**. Also, the anomalous splitting pattern observed for H-12 which appear as a doublet at δ 5.44 ppm is either due to through space coupling or may indicate that **5.5d** exists as a diastereomeric mixture which is expected because of the chiral center at C-2''. Further support of the latter stems from the multiplicity of the methyl protons H-16 at δ 0.91ppm, which appeared as a doublet of doublets ( $J = 2.8$  and  $6.0$ ) instead of the expected doublet. The signal at δ 5.51 ppm disappeared after being shaken with D<sub>2</sub>O and was therefore assigned to the proton of the hydroxyl group. As with the previous triazole products, the presence of the H-8'' proton

which appeared as a singlet resonating downfield at  $\delta$  8.06 ppm confirmed the formation of the triazole ring and thus the product **5.10d**.



**Figure 5.10:**  $^1\text{H}$ -NMR spectrum of **5.10d** in DMSO- $d_6$  at 400 MHz

Although the  $^{13}\text{C}$ -NMR spectrum (fig. 5.11) shows no duplication of signals the possibility that **5.5d** exists as a mixture of diastereomers cannot be ruled out. Nevertheless, 30 non-equivalent signals were detected. The carbon signals worth pointing out are the acetal carbons C-10 and C-3 resonating at  $\delta$  100.6 ppm and  $\delta$  125.3 ppm, respectively. Signals of interest in the  $\text{sp}^2$  region include those for C-7'' ( $\delta$  144.3 ppm) and C-8'' ( $\delta$  125.3 ppm) of the triazole ring.

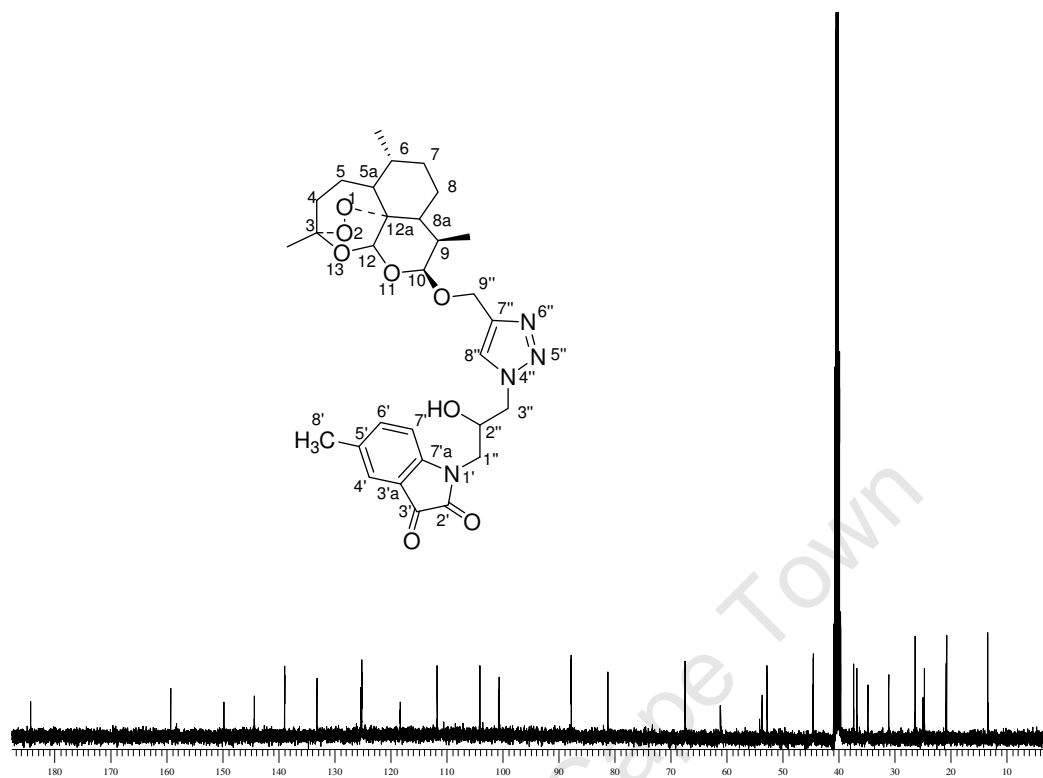
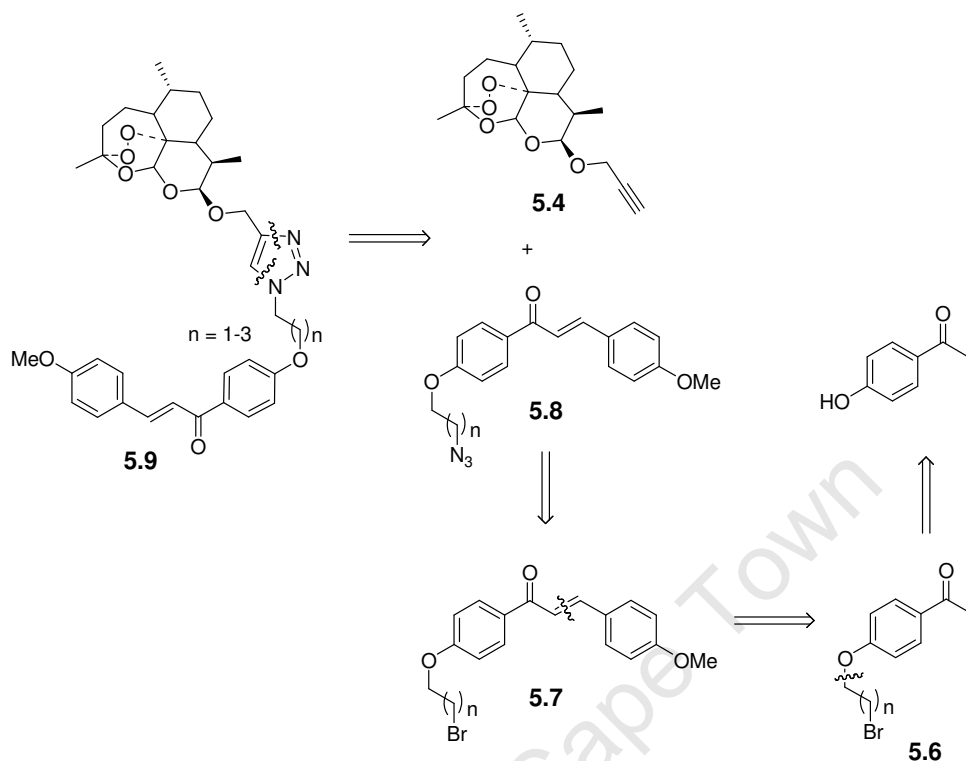


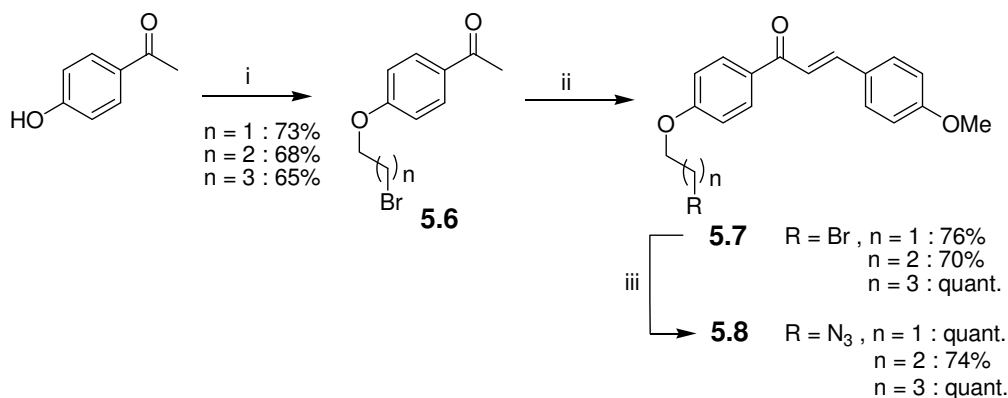
Figure 5.11:  $^{13}\text{C}$ -NMR spectrum of **5.5d** in DMSO- $d_6$  at 100 MHz

## 5.4.2. Retrosynthesis of Dihydroartemisinin–Chalcone Hybrids

Scheme 5.8: Retrosynthetic analysis of **5.9**

Synthesis of the triazole linked hybrid **5.9** is envisaged from the acetylene **5.4** and azidochalcone **5.8** as shown in scheme 5.8. The latter can be obtained by the azidation of **5.7**. Synthesis of **5.7** is similar to the previously discussed chalcones (chapter 4) and can be achieved by Claisen-Smith condensation of **5.6** with commercially available hydroxyacetophenone.

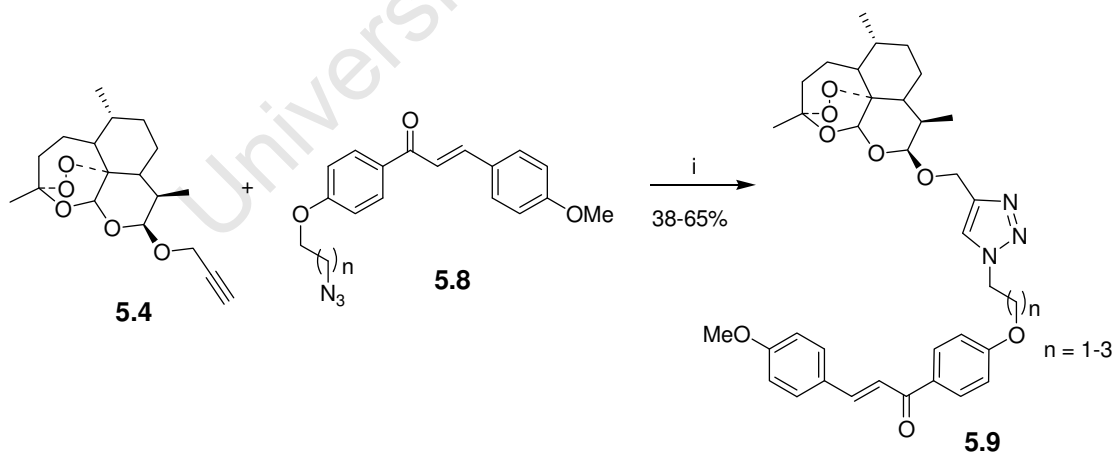
## 5.4.2.1. Synthesis of Azidochalcones



**Scheme 5.9:** Reagents and conditions: (i) Dibromoalkane,  $\text{K}_2\text{CO}_3$ , DMF,  $25^\circ\text{C}$ , 48 h; (ii) 4-Methoxybenzaldehyde, 3% w/v NaOH, MeOH,  $25^\circ\text{C}$ , 8 h; (iii)  $\text{NaN}_3$ , DMF,  $25^\circ\text{C}$ , 18 h

Synthesis of the azidochalcones **5.8** is a straightforward 3-step reaction, which starts with the *O*-alkylation of commercially available 4-hydroxyacetophenone with excess dibromoalkane in the presence of  $\text{K}_2\text{CO}_3$ . The product **5.6** was condensed with an equimolar amount of 4-methoxybenzaldehyde using the conditions shown in scheme 5.9 to generate **5.7**. Reaction of the latter with 2 eq of  $\text{NaN}_3$  in DMF at room temperature afforded **5.8** in good to quantitative yield. The  $^1\text{H}$  and  $^{13}\text{C}$ -NMR and IR data of **5.8** is consistent with the proposed structure.

## 5.4.2.2. Synthesis and Characterization of Dihydroartemisinin-Chalcone Hybrids



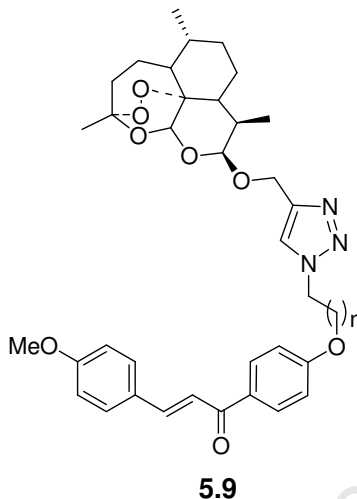
**Scheme 5.10.** Reagents and conditions (i)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , sodium ascorbate, DCM: $\text{H}_2\text{O}$  (1:1),  $25^\circ\text{C}$ , 6h

Synthesis of the target molecule **5.9** is depicted in scheme 5.10. Purification of the crude product mixtures using column chromatography (0-2.5% MeOH:DCM) afforded the pure products, **5.9b** as a light yellow solid and **5.9a,c** as light brown gels. The chimeras were



obtained in moderate yields as shown in table 5.2. A one-pot reaction of **5.7** and **5.4** to improve yields, may be worthwhile exploring for this series of compounds.

**Table 5.2:** Isolated yields and melting points of target molecules **5.9**



Compound	n	Yield (%)	m.p. (°C)
<b>5.9a</b>	1	38	- <sup>a</sup>
<b>5.9b</b>	2	65	145-146
<b>5.9c</b>	3	55	- <sup>a</sup>

<sup>a</sup>Isolated as a light brown gel

The <sup>1</sup>H-NMR spectrum of **5.9c** (fig. 5.12) displayed key signals such as the one assigned to H-12 which appear as a one proton singlet resonating at  $\delta$  5.39 ppm. The proton H-10 appeared as a doublet at  $\delta$  4.90 ppm ( $J = 4.8$ ) overlapping with one of the diastereotopic protons of H-10' ( $\delta$  4.67 ppm). The latter shows geminal coupling ( $J = 12.0$ ) with the signal at  $\delta$  4.91 ppm. Protons of the alkyl linker include H-4' and H-1' which appeared as two-proton triplets at  $\delta$  4.05 ppm and  $\delta$  4.44 ppm, respectively. The protons H-2' and H-3' overlapped with peaks assignable to protons of the dihydroartemisinin moiety, in the shielded region of the spectra. Identified in the aromatic region is the two AA'BB' systems for the aromatic rings of the chalcone moiety and the protons of the enone linker. The latter appeared as doublets at  $\delta$  7.40 ppm ( $J = 15.6$ ) and  $\delta$  7.75 ppm ( $J = 15.6$ ) assigned to H $\alpha$  and H $\beta$ , respectively. The number of carbon signals in the <sup>13</sup>C-NMR spectrum correlates with those indicated in the molecular formula of **5.9c**.

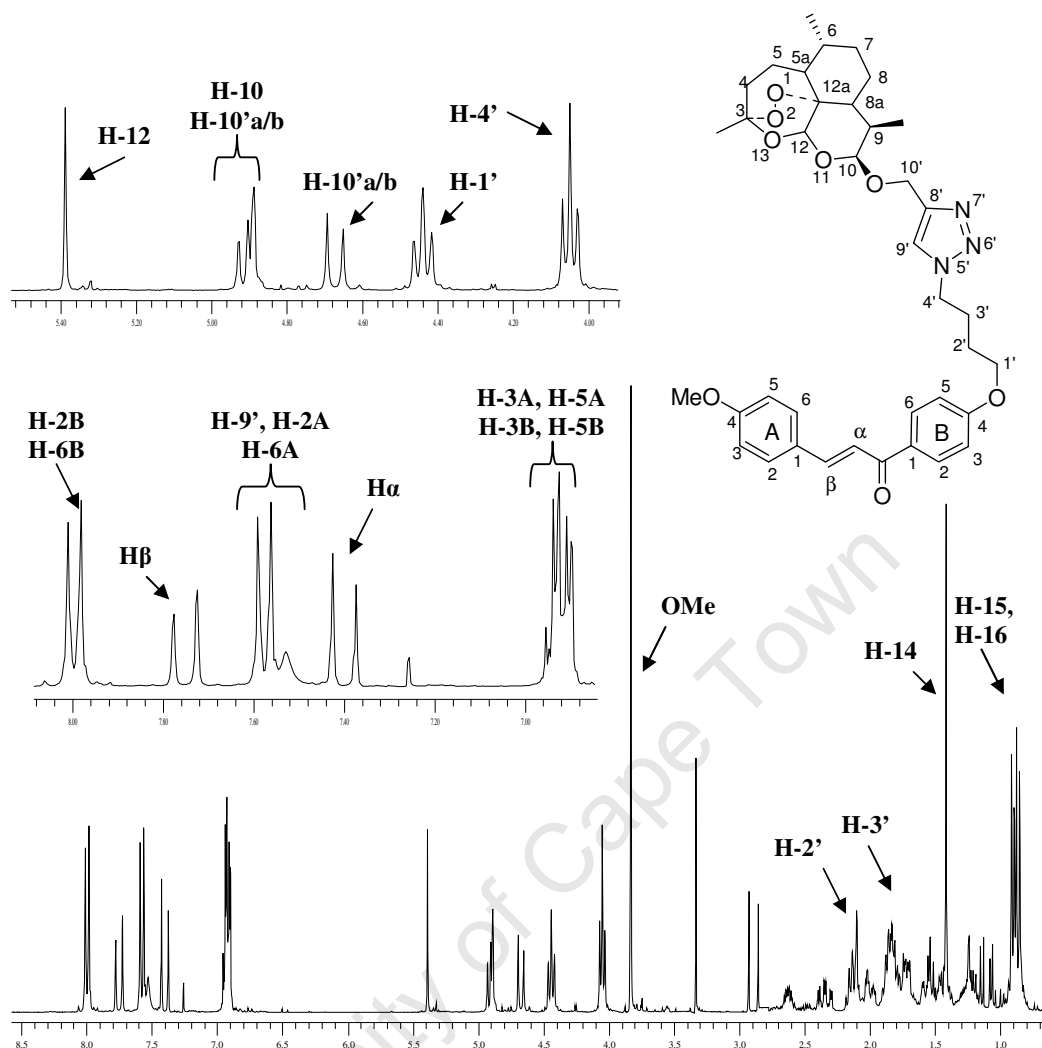
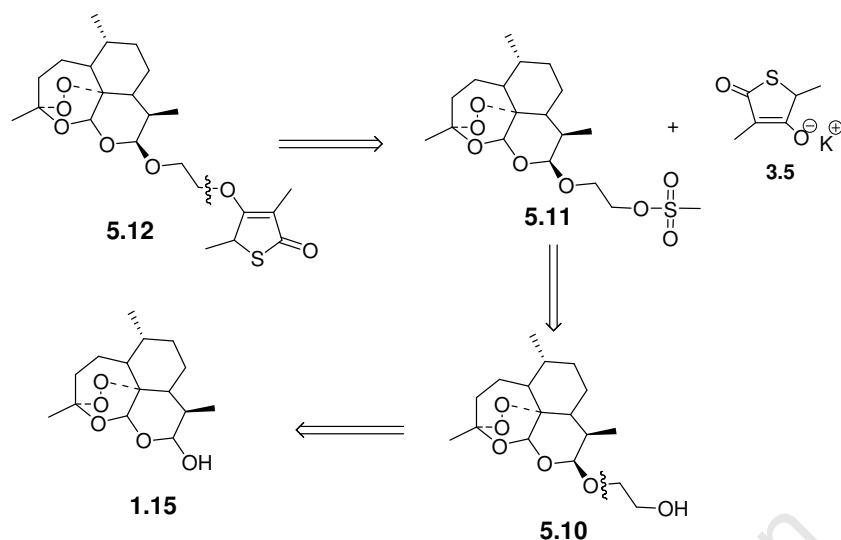


Figure 5.12:  $^1\text{H-NMR}$  spectrum of **5.9c** in  $\text{CDCl}_3$  at 300MHz

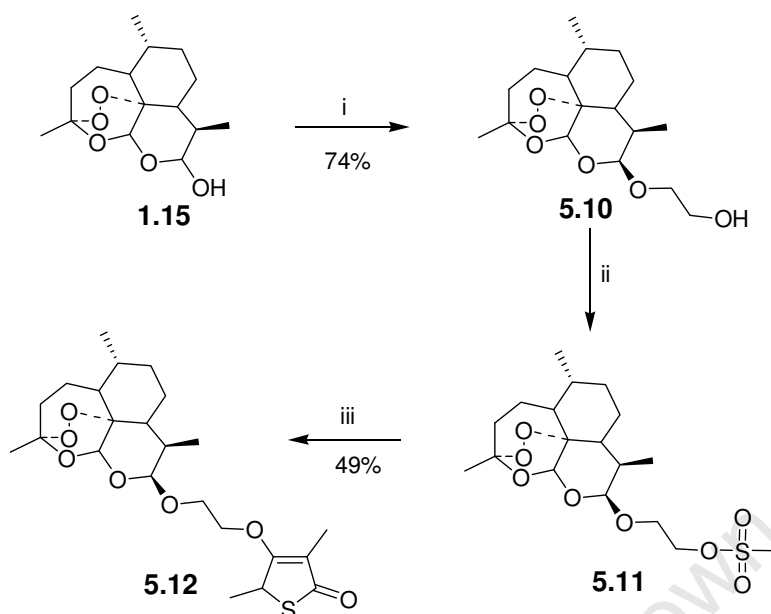
### 5.4.3. Retrosynthesis, Synthesis and Characterization of Dihydroartemisinin-Thiolactone Hybrid

As shown in scheme 5.11, compound **5.12** can be obtained by reaction of the potassium salt of thiolactone **3.5** with the mesylate **5.11**. Further analysis of **5.11** leads to the alcohol **5.10**, which in turn can be obtained from the condensation of **1.15** with commercially available ethylene glycol.



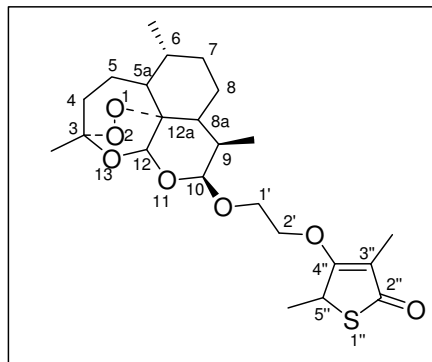
**Scheme 5.11:** Retrosynthetic analysis of **5.12**

Synthesis of **5.12** was initiated by the reaction of 1 eq of **1.15** with 2 eq of ethylene glycol in the presence of a catalytic amount of  $\text{BF}_3$  etherate in anhydrous DCM at room temperature (scheme 5.12).  $^1\text{H-NMR}$  analysis of the crude colourless gel obtained after workup revealed traces of the elimination product anhydroartemisinin in addition to a 10:90 ( $\alpha$ : $\beta$ ) isomeric mixture of **5.10**. Purification by column chromatography yielded the  $\beta$ -isomer **5.10** as light green prisms in 74% yield. For the mesylation, **5.10** was reacted with 1.5 eq of methanesulfonyl chloride in dry DCM in the presence of 1.1 eq of  $\text{Et}_3\text{N}$  at  $0^\circ\text{C}$  for 3 hours. The mesylate **5.11** was found to be unstable at room temperature as evidenced by a colour change from colourless to dark brown. The crude was therefore used in subsequent steps without purification. The potassium salt of thiolactone **3.5** was added to 1.2 eq of the crude mesylate **5.11** in anhydrous DMF under an inert atmosphere. After stirring at room temperature for 24 hours no reaction was detected (tlc). The reaction was therefore repeated with freshly prepared **5.11**, which and after stirring for 20 hours at  $60^\circ\text{C}$  followed by the purification of the crude product mixture with column chromatography (0-40% EtOAc:Hex), afforded **5.12** as a light yellow oil in 49% yield.



**Scheme 5.12:** Reagents and conditions: (i)  $\text{BF}_3 \cdot \text{OEt}_2$ , DCM, Ethylene glycol,  $25^\circ\text{C}$ , 19 h; (ii) methanesulfonyl chloride,  $\text{Et}_3\text{N}$ , DCM,  $0^\circ\text{C}$ , 2 h; (iii) **3.5**, DMF,  $60^\circ\text{C}$ , 20 h

We observed no changes in the physical appearance or  $^1\text{H-NMR}$  profile of **5.12**, after storing it undisturbed in the dark at ambient temperature over a period of 1 month. Following this period, we noticed the gel-like product **5.12** changed colour from light yellow to dark brown after repeated exposure to light. Further confirmation of the light sensitive nature of **5.12** was obtained from  $^1\text{H-NMR}$  which showed a complex spectrum for the decomposed product. It was for this reason that **5.12** was not submitted for biological evaluation. Moreover, the formation of “breakdown” products from chemically and thermally unstable artemisinin analogues has been reported.<sup>40</sup>

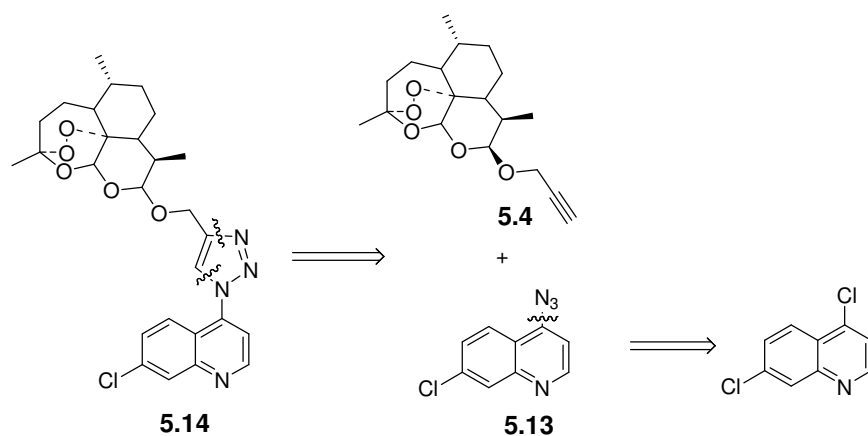


**Figure 5.13.** Chemical structure of **5.12**

Confirmation of the structure proposed for **5.12** (fig. 5.13) was done by means of  $^1\text{H}$ - and  $^{13}\text{C}$ -NMR analysis. Identified in the shielded end of the  $^1\text{H}$ -NMR spectrum are the methyl signals of the dihydroartemisinin nucleus, H-15 and H-16, which appeared as unresolved multiplets in the region  $\delta$  0.85-0.93 ppm. This observation coupled with anomalous splitting patterns of the methyl protons H-6'' and H-7'' at  $\delta$  1.57 ppm and  $\delta$  1.79 ppm, respectively indicated that **5.12** exist as a mixture of diastereomers. Other key signals include the methine proton H-5'' of the thiolactone ring system which appeared as a characteristic quartet of doublets ( $J = 1.2$  and  $6.8$ ) at  $\delta$  4.20 ppm. The coupling constant ( $J = 3.2$ ) of H-10 at  $\delta$  4.80 ppm indicated the  $\beta$ -isomer. Consistent with the  $^1\text{H}$ -NMR data, the  $^{13}\text{C}$ -NMR spectra of **5.12** showed a duplication of signals, and thus confirmed **5.12** to be a mixture.

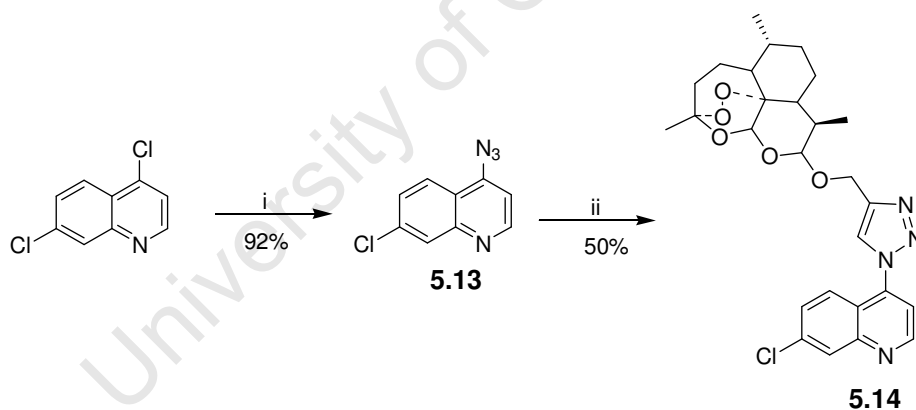
#### 5.4.4. Retrosynthesis, Synthesis and Characterization of Dihydroartemisinin-Aminoquinoline Hybrid

The retrosynthesis of **5.14** involves a two-step disconnection (scheme 5.13). Formation of the target molecule **5.14** is envisaged from **5.4** and the azido derivative **5.13** *via* a 1,3-dipolar cycloaddition. Compound **5.13** can be obtained from the azidation of 4,7-dichloroquinoline.



**Scheme 5.13:** Retrosynthetic analysis of **5.14**

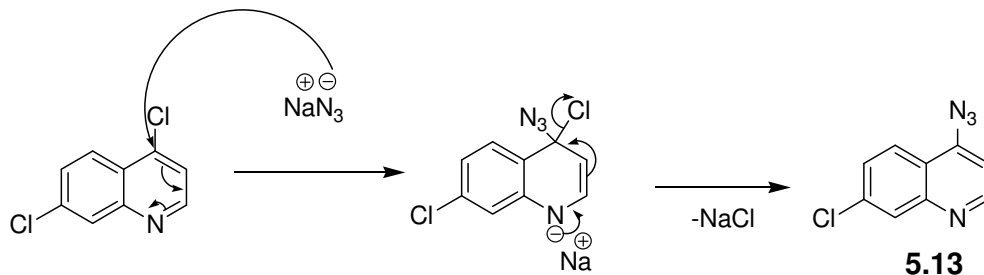
For the preparation of the azide **5.13**, commercially available 4,7 dichloroquinoline was stirred with  $\text{NaN}_3$  in DMF for 10 hours at  $60^\circ\text{C}$  (scheme 5.14). Ice-cold water was added to the reaction mixture which led to the precipitation of the crude product. Recrystallization of the crude product from DCM afforded **5.13** as light green needles in 92% yield. With **5.13** in hand, the ‘click’ reaction followed. Purification of the crude residue obtained after a 16 hour reaction afforded the product **5.14** as a brown gel in 50% yield.



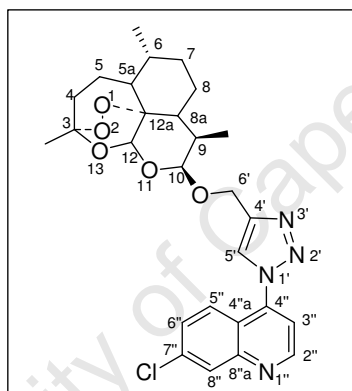
**Scheme 5.14:** Reagents and conditions: (i)  $\text{NaN}_3$ , DMF,  $60^\circ\text{C}$  10 h; (ii) **5.4**,  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , Sodium ascorbate, DCM/ $\text{H}_2\text{O}$  (1:1),  $25^\circ\text{C}$ , 16 h

Quinolines are structurally characterized as benzo-fused pyridine heterocycles. The reactivity of the latter reportedly matches that of the benzene and pyridine rings. For example, electrophilic substitution generally occurs at the electron rich benzene whereas nucleophilic substitution is favoured by the pyridine ring. The nitrogen in the quinoline ring enhances reactivity towards nucleophilic substitution of particularly, the C-2 and C-4 position by mesomeric and inductive effects. In the case of 4,7-dichloroquinoline the reactivity at the C-4

position is further enhanced by the presence of electron withdrawing (chloro) group and the close proximity of the nitrogen atom. As shown in scheme 5.15 the nucleophilic azide ion attacks the quinoline ring regioselectively at C-4 with the quinoline nitrogen acting as an electron sink. Rearomatization and expulsion of the chloride affords **5.13**.



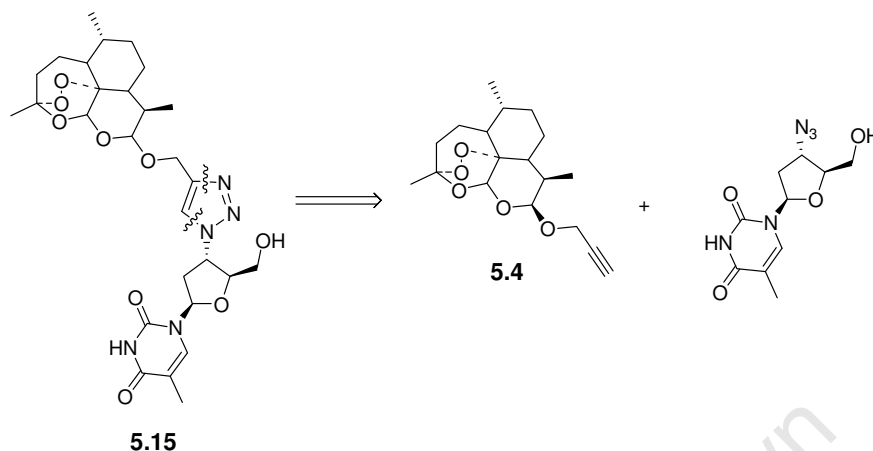
**Scheme 5.15:** Mechanism of formation of **5.13**



**Figure 5.14:** Chemical structures of **5.14**

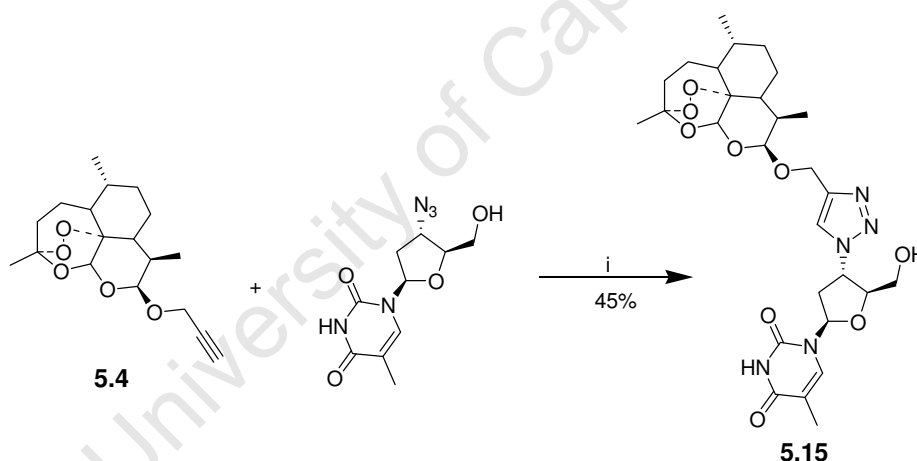
Identified in the aromatic region of the  $^1\text{H-NMR}$  spectrum of **5.14** (fig. 5.14) is the well-resolved signals of the quinoline ring system with their expected splitting patterns. The one-proton doublet resonating downfield at  $\delta$  9.03 ppm was assigned to H-2'' which couples ( $J = 4.2\text{-}4.5$ ) to H-3'' at  $\delta$  7.48 ppm. Also observed is the proton H-5'', a one-proton doublet ( $J = 9.3$ ) at  $\delta$  7.96 ppm which show *ortho*-coupling to H-6'' at  $\delta$  7.56 ppm. The latter in turn is *meta*-coupled to H-8'' at  $\delta$  8.22 ppm. The signal for the triazole proton, H-5' resonates at  $\delta$  7.96 ppm. Confirmation of the  $\beta$ -configuration of H-10 was obtained from the coupling constant ( $J = 4.5$ ) for the signal at  $\delta$  4.97 ppm. As observed with the previous triazole products, the methylene protons (H-6') on the carbon  $\alpha$  to the ether oxygen of the dihydroartemisinin moiety, are non-equivalent and appeared as two one-proton doublets with a geminal coupling constant ( $J = 13.2$ ) at  $\delta$  4.80 ppm and  $\delta$  5.04 ppm.

### 5.4.5. Retrosynthesis, Synthesis and Characterization of Dihydroartemisinin-AZT hybrid



**Scheme 5.16:** Retrosynthetic analysis of **5.19**

Scheme 5.16 shows that **5.15** can be accessed *via* a 1,3-dipolar cycloaddition reaction between **5.4** and commercially available AZT.



**Scheme 5.17:** Reagents and conditions: (i)  $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$ , Sodium ascorbate,  $\text{DCM}/\text{H}_2\text{O}$  (1:1),  $25^\circ\text{C}$ , 24 h

For the synthesis of **5.15**, as depicted in scheme 5.17, commercially available AZT was reacted with 1.1 eq of **5.4** under classical 'click' conditions. Purification of the crude afforded the desired product **5.15** as a light green foam in 45% yield.

Noticeable in the  $^1\text{H-NMR}$  spectrum of **5.15** (fig. 5.15) is the broadening of peaks in the non-polar  $\text{CDCl}_3$ . This is reflected in the splitting patterns of signals assigned to the furan ring; the anomeric proton H-1'' (triplet), H-3'' (doublet of triplets) and H-4'' (quartet) of which the



expected multiplicities are given in brackets. However, these signals appeared as broad one-proton singlets at  $\delta$  6.24 ppm,  $\delta$  4.43 ppm and  $\delta$  5.46 ppm, respectively. The unresolved signal at  $\delta$  3.00 ppm, a two-proton broad singlet, was assigned to the methylene protons at H-2''. The non-equivalent protons of H-5'' appeared as two geminal-coupled ( $J = 11.2$ - $11.6$ ), one-proton doublets instead of double doublets at  $\delta$  3.81 ppm and  $\delta$  4.02 ppm. Other peaks assignable to the AZT scaffold include the signal resonating downfield at  $\delta$  7.46 ppm which was assigned to H-6''' of the thymine ring.

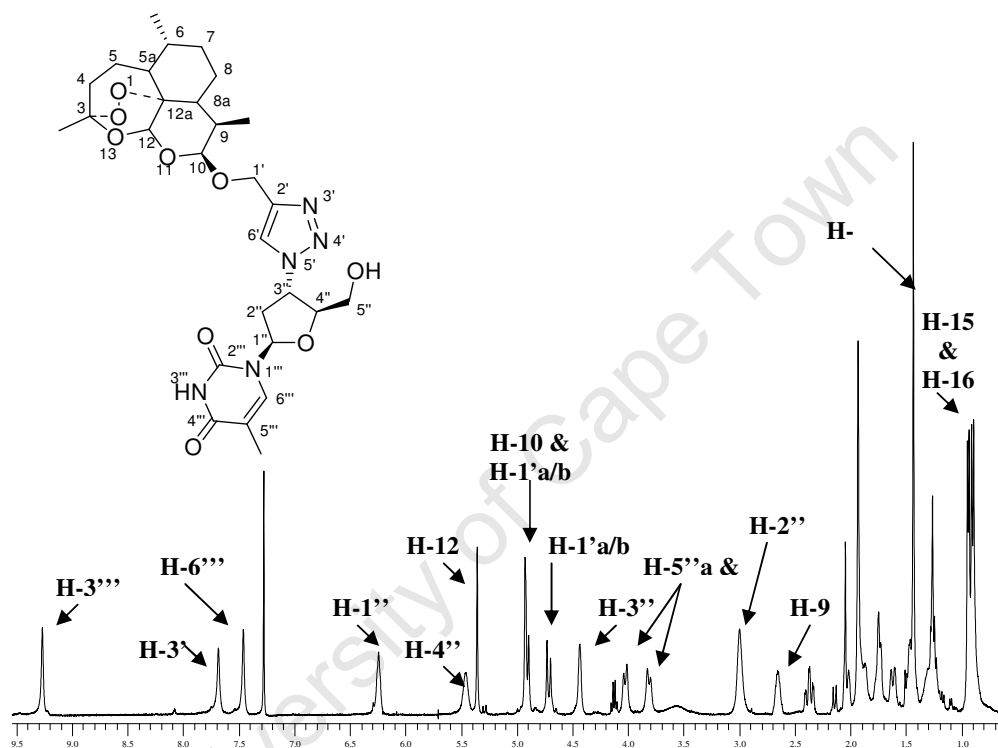


Figure 5.15:  $^1\text{H-NMR}$  spectrum of **5.15** in  $\text{CDCl}_3$  at 400 MHz

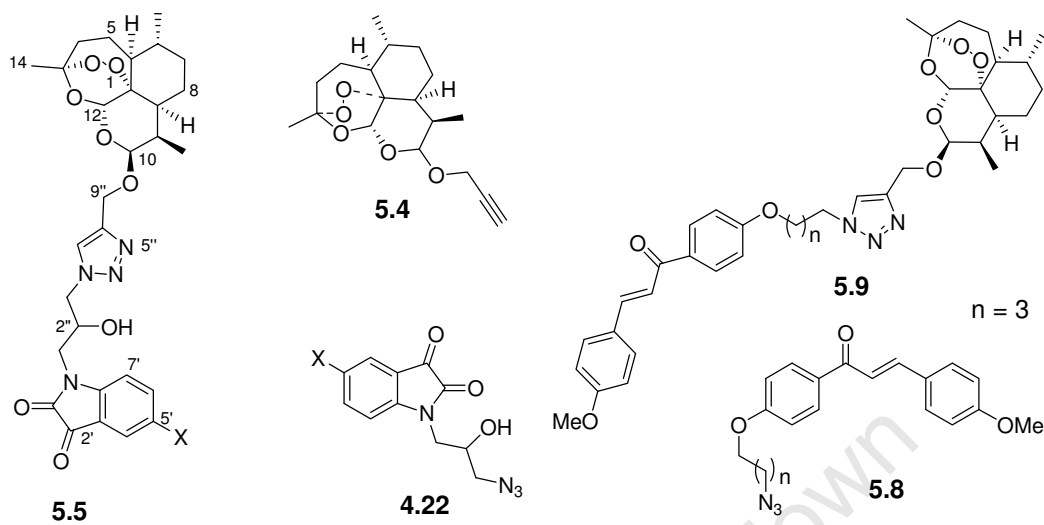
### 5.5. Biological Results and Discussion

As was the case with the previously discussed compounds, antiplasmodial testing against the W2 strain of *P. falciparum* and falcipain-2 inhibitory activity of the hybrids (**5.5**, **5.9**, **5.14**, **5.15**) and their advanced intermediates, were conducted in the laboratories of Prof. P. J. Rosenthal at the University of California San Francisco (UCSF). Evaluation of compounds for growth inhibitory activity against the CQS strain of *P. falciparum* was conducted locally in the laboratories of Prof. P. Smith at the Department of Pharmacology (UCT). The controls used include chloroquine (CQ), artemisinin (**1.14**, ART). A sample of the dihydroartemisinin (**1.15**, DHA), used as starting material in the synthesis of the abovementioned hybrids - kindly donated by Cipla (India) - was also submitted for testing.

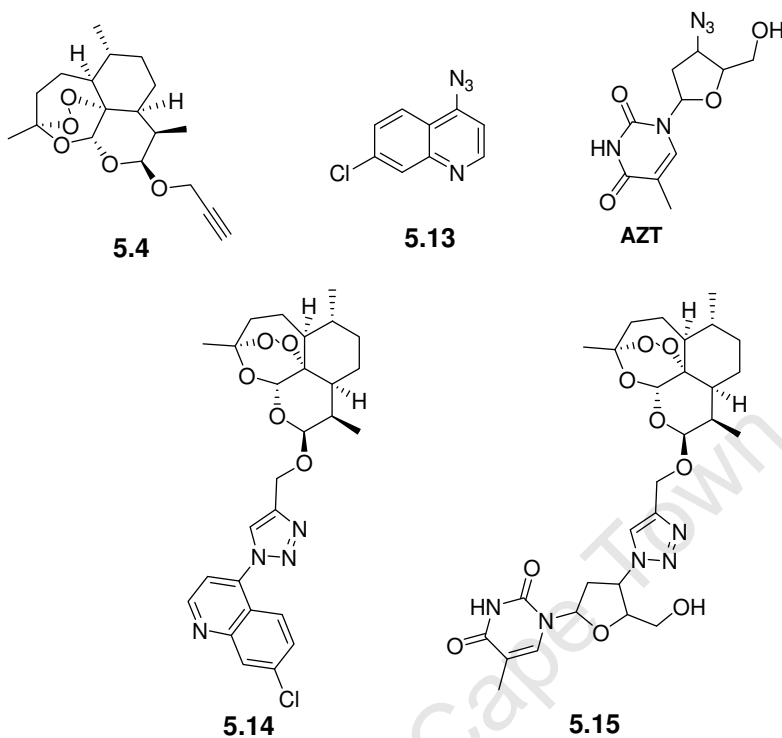
### 5.5.1. *In vitro* Antiplasmodial Activity

Although the hybrids **5.5** and **5.9** are less active than the natural product artemisinin (ART), they still retained nanomolar activity as shown in table 5.3. The most active compound identified from this series is the intermediate **5.4** ( $IC_{50}$  6.13 nM) which is more active than artemisinins ( $IC_{50}$  10.84 nM) against the CQR strain. It is also two-fold less active than the first generation analogue DHA, but 16 times more active than chloroquine. Results obtained for the limited isatin-derived series **5.5**, showed a need for substitution at C-5' of the isatin scaffold. This was inferred from the activity of the unsubstituted isatin derivative **5.5c** ( $IC_{50}$  73.68 nM) relative to the substituted derivatives **5.5a-b** and **5.5d-f**. The promising activities of **5.9a-c** against the W2 strain, lend support to earlier findings on the synergism between ART and chalcones derivatives (section 5.3). The most active compound of the three DHA-chalcone hybrids is the propyl-linked **5.9b** ( $n = 2$ ,  $IC_{50}$  33.00 nM).

The inhibition of falcipain-2 and falcipain-3 activity by artemisinin, as reported by Pandey and coworkers,<sup>41</sup> is comparable to the cysteine protease inhibitor E64. For this reason compounds **5.5** and **5.9** were evaluated for falcipain-2 inhibitory activity. The hybrids showed poor to moderate enzyme inhibitory activity which coupled with the lack of falcipain-2 inhibitory activity of the most active analogue **5.4**, is indicative that cysteine protease inhibition is not the primary, or the only mode of action for artemisinin analogues (section 1.4.2.1.3). The 7-chloro aminoquinoline unit is a validated antimalaria pharmacophore, as mentioned in section 5.3. However, the results in table 5.4 show that the quinoline based derivative **5.14** ( $IC_{50}$  286.5  $\mu$ M) is three fold less active than chloroquine ( $IC_{50}$  97.31 nM). Instead the AZT-derivative **5.15** ( $IC_{50}$  76.46 nM) appears to have a higher efficacy. The equipotency of ART and DHA against the CQR and CQS strains of *P. falciparum* has been reported.<sup>42</sup> Our results show that these two drugs are 4-fold more active against the resistant (W2) compared to the sensitive (D10) strain.

**Table 5.3:** *In vitro* antiplasmodial activity of intermediates **5.4**, **4.22**, **5.8** and target molecules **5.5** and **5.9**

Compound Code	n	X	Rec-FP-2 IC <sub>50</sub> (nM)	W2 IC <sub>50</sub> (nM)	D10 IC <sub>50</sub> (nM)	RI	c log P
ART		-	>100000	10.84	40.66	0.27	2.72
DHA		-	>100000	2.56	11.01	0.23	2.45
CQ		-	ND	97.31	29.48	3.30	-
E64		-	49.43	ND	ND	-	-
<b>5.4</b>		-	>100000	<b>6.13</b>	7.82	0.78	3.51
<b>4.22a</b>		Cl	ND	>20000	>20000	-	1.82
<b>4.22b</b>		Br	ND	>20000	ND	-	1.97
<b>4.22c</b>		F	ND	>20000	ND	-	1.25
<b>4.22d</b>		I	ND	>20000	ND	-	2.23
<b>4.22e</b>		CH <sub>3</sub>	ND	>20000	ND	-	1.42
<b>4.22f</b>		H	ND	>20000	ND	-	0.92
<b>5.5a</b>		Cl	80715	<b>25.24</b>	127.25	0.20	2.96
<b>5.5b</b>		F	57425	31.96	379.01	0.084	2.39
<b>5.5c</b>		H	96415	73.68	123.65	0.60	2.07
<b>5.5d</b>		CH <sub>3</sub>	27050	29.82	120.40	0.25	2.57
<b>5.5e</b>		I	17705	<b>25.04</b>	81.73	0.31	3.37
<b>5.5f</b>		Br	ND	26.23	134.21	0.20	3.11
<b>5.8a</b>	1		ND	>20000	>100000	-	4.96
<b>5.8b</b>	2		ND	9160	>100000	-	5.09
<b>5.8c</b>	3		ND	5951	>100000	-	5.62
<b>5.9a</b>	1		45450	85.46	467.68	0.18	5.87
<b>5.9b</b>	2		65805	33.00	91.55	0.36	6.18
<b>5.9c</b>	3		>100000	586.0	>100000	-	6.30

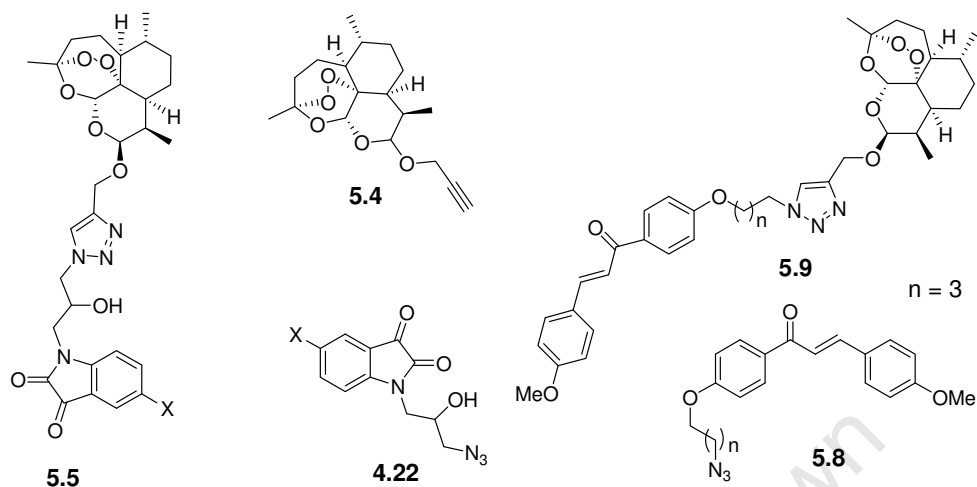
**Table 5.4:** *In vitro* antiplasmodial activity of intermediates **5.4**, **5.13**, **AZT** and target molecules **5.14** and **5.15**

Compound Code	Rec-FP-2 IC <sub>50</sub> (nM)	W2 IC <sub>50</sub> (nM)	D10 IC <sub>50</sub> (nM)	RI	c log P
ART	>100000	10.84	40.66	0.27	2.72
DHA	>100000	2.56	11.01	0.23	2.45
CQ	-	97.31	29.48	3.30	-
E64	49.43	-	-	-	-
<b>5.4</b>	>100000	<b>6.13</b>	7.82	0.78	3.51
AZT	ND	14615	>100000	-	0.0435
<b>5.13</b>	ND	1666	9970	0.17	3.98
<b>5.14</b>	85635	286.5	816.00	0.35	5.73
<b>5.15</b>	>100000	76.46	149.77	0.51	1.27

### 5.5.2. *In vitro* Antitubercular Activity

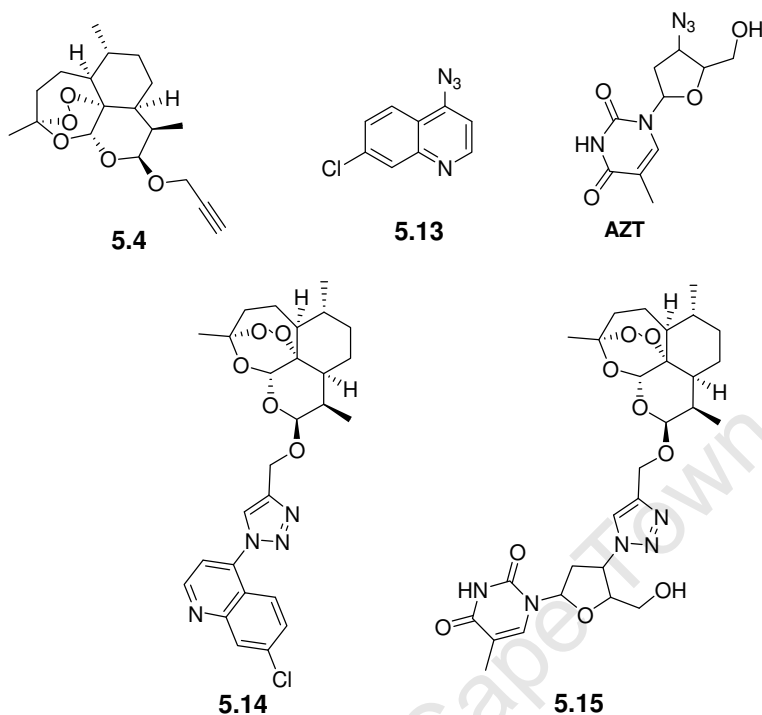
All target molecules and advanced intermediates synthesized were evaluated for antitubercular activity at the Institute of Tuberculosis Research, College Pharmacy, University of Illinois at Chicago (UIC); using the microplate Alamar Blue assay (MABA) and the low oxygen recovery assay (LORA)

The iron-mediated activation of the peroxide-bond of artemisinin analogues is now generally accepted. More so it has been postulated, as alluded to in section 1.4.2.1.3, that the peroxide bond requires activation either by haeme released during the breakdown of haemoglobin or by “free” iron in the infected red blood cell.<sup>19,43</sup> This iron-dependent mechanism of the artemisinins has been cited as the reason behind the antimalarial specificity of this class of compounds.<sup>44</sup> The antitubercular results obtained for the intermediates and hybrids as shown in tables 5.5. and 5.6, agrees with this finding. Most of the compounds tested, with the exception of **5.5e**, **5.5f** and **5.14**, are inactive at the highest concentration tested (128  $\mu\text{M}$ ). A comparison of the MABA MICs and LORA MICs of the active compounds show that they have a higher efficacy against fast growing replicating mycobacteria. The observed activity of **5.14** (MABA MIC 109.4  $\mu\text{M}$ ) deserves special mention. From published reports on the antitubercular activity of quinoline based compounds,<sup>45</sup> it is reasonable to conclude that the 7-chloro amininoquinoline moiety contributed significantly to the observed activity.

**Table 5.5:** *In vitro* antitubercular activity of intermediates **5.4**, **4.22**, **5.8** and target molecules **5.5** and **5.9**

Compound Code	n	X	MABA % Inh <sup>a</sup> ..MIC ( $\mu$ M)		LORA % Inh <sup>a</sup> ..MIC ( $\mu$ M)		c log P
RMP				0.05	96.1	2.4	
INH				0.37	33.2	>128	
MOX				0.29	90.3	31.1	
SM				0.43	ND	ND	
PA824				0.16	98.7	3.3	
ART			15	>128			2.72
DHA			41	>128			2.45
<b>5.4</b>			56	>128			3.51
<b>4.22a</b>		Cl	0	>128			1.82
<b>4.22b</b>		Br	0	>128			1.97
<b>4.22c</b>		F	0	>128			1.25
<b>4.22d</b>		I	24	>128			2.23
<b>4.22e</b>		CH <sub>3</sub>	0	>128			1.42
<b>4.22f</b>		H	0	>128			0.92
<b>5.5a</b>		Cl	87	>128			2.96
<b>5.5b</b>		F	62	>128			2.39
<b>5.5c</b>		H	51	>128			2.07
<b>5.5d</b>		CH <sub>3</sub>	77	>128			2.57
<b>5.5e</b>		I	<b>92</b>	<b>96.7</b>	64.2	>128	3.37
<b>5.5f</b>		Br	<b>90</b>	<b>62.4</b>	74.0	>128	3.11
<b>5.9a</b>	1		83	>128			4.96
<b>5.9b</b>	2		84	>128			5.09
<b>5.9c</b>	3		43	>128			5.62
<b>5.9a</b>	1		52	>128			5.87
<b>5.9b</b>	2		11	>128			6.18
<b>5.9c</b>	3		58	>128			6.30

<sup>a</sup>Percentage inhibition at 128  $\mu$ M

**Table 5.6:** *In vitro* antitubercular activity of intermediates **5.4**, **5.13**, **AZT** and target molecules **5.14** and **5.15**

Compound Code	MABA		LORA		c log P
	%Inh <sup>a</sup>	MIC (μM)	%Inh <sup>a</sup>	MIC (μM)	
<b>RMP</b>		0.05	96.1	2.4	
<b>INH</b>		0.37	33.2	>128	
<b>MOX</b>		0.29	90.3	31.1	
<b>SM</b>		0.43	ND	ND	
<b>PA824</b>		0.16	98.7	3.3	
<b>ART</b>	15	>128			2.72
<b>DHA</b>	41	>128			2.45
<b>5.4</b>	56	>128			3.51
<b>AZT</b>	1	>128			0.0435
<b>5.13</b>	42	>128			3.98
<b>5.14</b>	<b>95</b>	<b>109.4</b>	73.2	>128	5.73
<b>5.15</b>	2	>128			1.27

<sup>a</sup>Percentage inhibition at 128 μM

### 5.6. Conclusion

Of all the advanced intermediates and target molecules tested, **5.4** proved to be the most promising for this limited series of compounds. Most of the target molecules showed nanomolar antiplasmodial activity, which implicates the artemisinin moiety as the main contributor to the observed activity. From the lack of correlation of antiplasmodial activity and falcipain-2 inhibition activity as demonstrated by **5.4**, it is safe to assume that the inhibition of cysteine protease is not the primary target for this series of compounds. Moreover, the lack of, and in some cases poor antitubercular activity observed for the artemisinin analogues demonstrate their antimalarial specificity.

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## 5.7. References

1. Li, Y.; Huang, H.; Wu, Y.-L. In *Medicinal Chemistry of Bioactive Natural Products*, Liang, X.-T., Fang, W.-S (eds), John Wiley and Sons, New York, **2006**, 183-206
2. (a) Posner, G.H.; Paik, I.-H.; Sur, S.; McRiner, A.J.; Borstnik, K.; Xie, S.; Shapiro, T.A. *J. Med. Chem.*, **2003**, *46*, 1060; (b) Jung, M.; Lee, S.; Ham, J.; Lee, K.; Kim, H.; Kim, S.K. *J. Med. Chem.*, **2003**, *46*, 987
3. Lin, P. Y.; Pan, J.Q.; Feng, Z.M; Zhang, D.; Xiao, L.Y. In *Progress in Immunopharmacology*, Zhou, J.H.; Li, X.Y.; Rong, K.T.R. (eds), Chinese Science and Technique Press, Beijing, **1993**, 325-337
4. (a) Ou-Yang, K.; Krug, E.C.; Marr, J.J.; Berens, R.L. *Antimicrob. Agents Chemother.*, **1990**, *34*, 1961; (b) Yang, D.M.; Liew, F.Y. *Parasitology*, **1993**, *106*, 7; (c) Meshnick, S.R.; Taylor, T.E.; Kamchonwongpaisan, S. *Microbiol. Rev.*, **1996**, *60*, 301
5. (a) China Cooperative Research Group *J. Trad. Chin. Med.*, **1982**, *2*, 9; (b) China Cooperative Research Group *J. Trad. Chin. Med.*, **1982**, *2*, 45
6. Mital, A. *Curr. Med. Chem.*, **2007**, *14*, 759
7. Klayman, D.L. *Qinghaosu Science*, **1985**, 228, 1049
8. (a) Jung, M.; Li, X.; Bustos, D.A.; El-Sohly, H.N.; McChesney, J.D.; Milhous, W.K. *J. Med. Chem.*, **1990**, *33*, 1516; (b) Ye, B.; Wu, Y.-L. *J. Chem. Soc. Chem. Commun.*, **1990**, 726; (c) Avery, M.A.; Mehrotra, S.; Johnson, T.L.; Bonk, J.D.; Vroman, J.A., Miller, R. *J. Med. Chem.*, **1996**, *39*, 4149
9. (a) Brossi, A.; Venugopalan, B.; Gerpe, D.L.; Yeh, H.J.C.; Flippen-Anderson, J.L.; Buchs, P.; Luo, X.D.; Milhous, W.; Peters, W. *J. Med. Chem.*, **1988**, *31*, 645; (b) Li, Y.; Zhu, Y.M.; Jiang, H.J.; Pan, J.P.; Wu, G.S.; Wu, J.M. *J. Med. Chem.*, **2000**, *43*, 1635; (c) Singh, C.; Chaudhary, S.; Puri, S.K. *J. Med. Chem.*, **2006**, *49*, 7227
10. (a) Haynes, R.K. *Curr. Opin. Infect. Dis.*, **2001**, *14*, 719; (b) Yang, Y.H., Li, Y.; Shi, Y.-L.; Yang, J.-D.; Wu, B.-A. *Bioorg. Med. Chem. Lett.*, **1995**, *5*, 1791; (c) Lin, A.J.; Li, L.-Q.; Klayman, D.L.; George, C.F.; Flippen-Anderson, J.L. *J. Med. Chem.*, **1990**, *33*, 2610
11. (a) Haynes, R.K.; Vonwiller, S.C. *Synlett*, **1992**, 481; (b) Jung, M.; Bae, J. *Heterocycles*, **2000**, *53*, 261; (c) Pu, Y.M.; Katz, E.; Ziffer, H. *J. Med. Chem.*, **1995**, *38*, 613; (d) O'Neil, P.M.; Searle, N.L.; Kan, K.W.; Storr, R.C.; Maggs, J.L.; Ward, S.A.; Raynes, K.; Park, B.K. *J. Med. Chem.*, **1999**, *42*, 5487; (e) Hindley, S.; Ward, S.A.; Storr, R.C.; Searle, N.L.; Bray, P.G.; Park, B.K.; Davies, J.; O'Neill, P.M. *J. Med. Chem.*, **2002**, *45*, 1052; (f) Woo, S.H.; Parker, M.H.; Ploypradith, P.; Nosten, F.; Posner, G.H. *Tetrahed. Lett.*, **1998**, *39*, 1533; (g) O'Dowd, H.; Ploypradith, P.; Xie, S.; Shapiro, T.A.; Posner,

- G.H. *Tetrahedron*, **1999**, *55*, 3625; (h) Jung, M.; Yu, D.; Bustos, D.; El-Sohly, H.N.; McChesney, J.D. *Bioorg. Med. Chem. Lett.*, **1991**, *1*, 741; (i) Haynes, R.K.; Chan, H.-W.; Cheung, M.-K.; Chung, S.T.; Lam, W.-L.; Tsang, H.-W.; Voertse, A.; Williams, I.A. *Eur. J. Org. Chem.*, **2003**, 2098
12. Avery, M.A.; Fan, P.; Karle, J.M.; Bonk, J.D.; Miller, R.; Goins, K.D. *J. Med. Chem.*, **1996**, *39*, 1885
13. Torok, D.J.; Ziffer, H.; Meshnick, S.R.; Pan, Q.-X.; Ager, A. *J. Med. Chem.*, **1995**, *38*, 5054
14. Fishwick, J.; McClean, W.G.; Edwards, G.; Ward, S.A. *Chem. Biol. Interact.*, **1995**, *96*, 263
15. Maggs, J.L.; Bishop, L.P.D.; Edwards, G.; O'Neill, P.M.; Ward, S.A.; Winstanley, P.A.; Park, B.K. *Drug. Metab. Dispos.*, **2000**, *28*, 209
16. Maggs, J.L.; Batty, K.T.; Ilett, K.F.; Edwards, G.; Park, B.K. *Br. J. Clin. Pharmacol.*, **1998**, *46*, 293P
17. O'Neill P.M.; Miller, A.; Bishop, L.P.D.; Hindley, S.; Maggs, J.L.; Ward, S.A.; Roberts, S.M.; Scheinmann, F.; Stachulski, A.V.; Posner, G.H.; Park, B.K. *J. Med. Chem.*, **2001**, *44*, 58
18. Magueur, G.; Crousse, B.; Charneau, S.; Grellier, P.; Bgu, J.-P.; Bonnet-Delpon, D. *J. Med. Chem.*, **2004**, *47*, 2694
19. O'Neill, P.M.; Posner, G.H. *J. Med. Chem.*, **2004**, *47*, 2945
20. (a) Battachacharjee, A.K., Karle, J.M. *Chem. Res. Toxicol.*, **1999**, *12*, 422; (b) Ramu, K.; Baker, J.K. *J. Med. Chem.*, **1995**, *38*, 1911
21. Haynes, R.K.; Fugmann, B.; Stetter, J.; Rieckmann, K.; Heilmann, H.-D.; Chan, H.-W.; Cheung, M.-K.; Lam, W.L.; Wong, H.N.; Croft, S.L.; Vivas, L.; Rattray, L.; Stewart, L.; Peters, W.; Robinson, B.L.; Edstein, M.D.; Kotecka, B.; Kyle, D.E.; Beckermann, B.; Gerisch, M.; Radtke, M.; Schmuck, G.; Steinke, W.; Wollborn, U.; Schmeer, K.; Römer, A. *Angew. Chem. Int. Ed.*, **2006**, *45*, 2082
22. (a) Hien, T.; Turner, G.D.H.; Mai, N.T.H.; Phu, N.H.; Bethell, D.; Blakemore, W.F.; Cavanagh, J.B.; Dayan, A.; Medana, I.; Weller, R.O.; Day, N.P.J.; White, N.J. *Lancet*, **2003**, *362*, 295; (b) Gordi, T.; Lepist, E.-I. *Toxicology Lett.*, **2004**, *147*, 99
23. (a) Šolaja, B.A.; Terzić, N.; Pocsfalvi, G.; Gerena, L.; Tinant, B.; Opsenica, D.; Milhous, W.K. *J. Med. Chem.*, **2002**, *45*, 3331; (b) Opsenica, D.; Kyle, D.E.; Milhous, W.K.; Šolaja, B.A. *J. Serbian Chem. Soc.*, **2003**, *68*, 291
24. Cho, S.; Oh, S.; Um, Y.; Jung, J. -H.; Ham, J.; Shin, W.-S.; Lee, S. *Bioorg. Med. Chem.*

- Lett.*, **2009**, *19*; 382
25. O'Neill, P.M.; Stocks, P.A.; Pugh, M.D.; Araujo, N.C.; Korshin, E.E.; Bickley, J.F.; Ward, S.A.; Bray, P.G.; Pasini, E.; Davies, J.; Verissimo, E.; Bachi, M.D. *Angew. Chem. Int. Ed.*, **2004**, *43*, 4193
26. Albert, A. *Nature*, **1958**, *182*, 421
27. Fivelman, Q.L.; Adagu, I.S.; Warhurst, D.C. *Antimicrob. Agents Chemother.*, **2004**, *48*, 4097
28. (a) Mishra, L.C.; Bhattacharya, A.; Bhasin, V.K. *Acta Tropica*, **2009**, *109*, 194;  
(b) Bhattacharya, A.; Mishra, L.C.; Sharma, M.; Awasthi, S.K.; Bhasin, V.K. *Eur. J. Med. Chem.*, **2009**, doi:10.1016/j.ejmech.2009.02.008
29. Masereeuw, R.; Jaehde, U.; Langemeijer, M. W.; De Boer, A. G.; Breimer, D. D. *Pharmaceutical Research*, **1994**, *11*, 324
30. Opsenica, I.; Opsenica, D.; Lanteri, C.A.; Anova, L.; Milhous, W.K.; Smith, K.S.; Šolaja, B.A. *J. Med. Chem.*, **2008**, *51*, 6216
31. Posner, G.H.; Ploypradith, P.; Parker, M.H.; O'Dowd, H.; Woo, S.H.; Northrop, J.; Krasavin, M.; Dolan, P.; Kensler, T.W.; Xie, S.J.; Shapiro, T.A. *J. Med. Chem.*, **1999**, *42*, 4275
32. (a) Li, Y.; Yu, P.-L.; Chen, Y. -X.; Li, L.-Q.; Gai, Y.-Z.; Wang, D.-S.; Zheng, Y.-P. *Acta. Pharm. Sin.*, **1981**, *16*, 429; (b) Lin, A.; Klayman, D.L.; Milhous, W.K. *J. Med. Chem.*, **1987**, *30*, 2147
33. Nga, T.T.T.; Ménage, C.; Bégué, J.-P.; Bonnet-Delpon, D.; Pradines J.-C.G.; Thac, J.-C.D.D. *J. Med. Chem.*, **1998**, *41*, 4101
34. Edward, J.T. *Chem. Ind.*, **1955**, 1102
35. Haynes, R.K.; Chan, H.-W.; Cheung, M.-K.; Lam, W.-L.; Soo, M.-K.; Tsang, H.-W.; Voerste, A.; Williams, I.D. *Eur. J. Org. Chem.*, **2002**, *2002*, 113
36. Bhakuni, R. S.; Jain, D.C.; Sharma, R.P. *Indian J. Chem.*, **1995**, *34B*, 529
37. Schmidt, R.R.; Hoffman, M. *Tetrahedron Lett.*, **1982**, *23*, 409
38. Li, Y.; Yang, Z.-S.; Zhang, H.; Cao, B.-J.; Wang, F.-D.; Zhang, Y.; Shi, Y.-L.; Yang, J.-D.; Wu, B.-A. *Bioorg. Med. Chem.*, **2003**, *11*, 4363
39. Wu, P.; Fokin, V.V. *Aldrichimica Acta*, **2007**, *40*, 7
40. Jansen, F.H.; Soomro, S.A. *Curr. Med. Chem.*, **2007**, *14*, 3243
41. Pandey, A.V.; Tekwani, B.L.; Singh, R.L.; Chauhan, V.S. *J. Biol. Chem.*, **1999**, *274*, 19383
42. Golenser, J.; Waknine, J.H.; Krugliak, M.; Hunt, N.H.; Grau, G.E. *Int. J. Parasitol.*, **2006**,

36, 1427

43. (a) Jefford, C.W. *Curr. Med. Chem.*, **2001**, 8, 1803; (b) Meshnick, S.R. *Int. J. Parasitol.*, **2002**, 32, 1655
44. Kaiser, M.; Wittlin, S.; Nehrbass-Stuedli, A.; Dong, X.; Wang, X.; Hemphill, A.; Matile, H.; Brun, R.; Vennerstrom, J.L. *Antimicrob. Agents Chemother.*, **2007**, 51, 2991
45. Lilienkampf, A.; Mao, J.; Wan, B.; Wang, Y.; Franzblau, S.G.; Kozikowski, A. P. *J. Med. Chem.*, **2009**, 52, 2109

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## Chapter 6

### SUMMARY, CONCLUSION AND RECOMMENDATIONS FOR FUTURE WORK

#### 6.1. General

We have successfully synthesized novel hybrids modelled on the isatin, chalcone and thiolactone scaffolds with promising activity profiles against the disease models under investigation. More importantly, the results obtained reiterate the importance and benefits of using natural products in drug discovery. This is to be seen in the context of the number of active compounds identified from the relatively small library of compounds synthesized and the SAR delineated from this study. As mentioned earlier, the hybrids showed superior antiplasmodial activity compared to the (advanced) intermediates thus lending support to the potential of hybridization as a drug discovery tool. Of particular interest was the observed antimalarial specificity of the synthesized hybrids, that is, they displayed moderate to good antimalarial activity but showed relatively poor, and in some cases were devoid of, antitubercular activity. This lack of antitubercular activity may be an indication of the inability of the hybrids to penetrate the unusual lipid-rich cell wall of mycobacteria, but may also be indicative of a lack of broad range activity which is an attractive feature for any potential drug insofar as limiting off-target activity is concerned. Confirmation of the latter will require further testing of the synthesized compounds in other disease models.

#### 6.2. Click chemistry

One of the aims of this project was to further demonstrate the utility of ‘click’ chemistry in drug discovery. To this end, different types of ‘click’ reactions were employed in the course of this project. These include the nucleophilic ring opening of epoxides **4.7** and **4.20** to yield the corresponding  $\beta$ -amino alcohol derivatives **4.8** and **4.21**. Also listed among the ‘click’ reactions is the “protecting group” or carbonyl group chemistry which we employed in the ketalization of isatin and its derivatives **4.23**. The Huisgen 1,3-dipolar cycloaddition reaction was used in chapters 4 and 5 for the synthesis of the  $\beta$ -amino alcohol hybrids. The reaction which led to the triazole-linked  $\beta$ -amino alcohol thiolactone-chalcone hybrids, however, deserves special mention. The product mixture of this reaction required elaborate chromatographic purification and afforded the product in low yields (section 4.2.2.5.3) and therefore does not fulfil the requirements of a ‘click’ reaction (section 4.2.2.5). Nonetheless, it provided access to a novel series of potential antimalarials with an, as yet, undetermined

mechanism of action. Also demonstrated, is the tolerability of the classical ‘click’ conditions for various functional groups in the artemisinin hybridization reaction; the combined presence of relatively sensitive functional groups, that is, the peroxy, ketal, acetal and lactone moieties was an initial concern (section 5.4.1.1.3). Overall the results obtained are in full support of the fact that ‘click’ chemistry is a powerful and indispensable synthesis tool in drug discovery.

### 6.3. Thiolactone-Isatin Hybrids

As mentioned earlier, the advancement of thiolactomycin analogues to drug candidates is hindered by the difficulty of developing viable synthesis routes. The less than desirable yields obtained for the hybrids **3.7** and **3.8**, albeit *via* a simple and straightforward synthesis route, attest to this. Nonetheless a novel tetracyclic ring system **3.8** was discovered, whose bioactive ‘calling’ is still to be revealed. Contrary to our expectations, the antiplasmodial activity of compounds **3.7** and **3.8** ranged from poor to moderate when compared to chloroquine which incidentally, exert its antimalarial effect *via* a different mode of action. Also, the antitubercular activity of the hybrids were not very promising, instead the intermediates and in particular compounds **3.6i** and **3.6o** were more active.

Speculatively, the fact that the tetracycles **3.8** are more active than their precursors **3.7**, hint at the need for substitution at C-5 of the thiolactone ring for antimalarial and more so antitubercular activity. Further studies are however needed, to make a meaningful contribution to the ongoing debate on the importance of the isoprenoid moiety (or substitution at C-5), or more specifically, to identify the essential antimalarial/antitubercular pharmacophore of thiolactomycin (section 2.2.3.1). Future studies may also explore viable synthesis routes for thiolactomycin analogues. In this regard, one of the optimization conditions which we did not explore in the synthesis of the tetracycles **3.8** is the high dilution technique. The dilution principle advocates the use of low concentration of reactants in ring formation reactions so that ring closure is favoured over polymerization or oligomerization. The rationale is that by isolating the reacting molecules, as in the case of a diluted solution, intramolecular cyclization (as opposed to the intermolecular reaction) can take place preferentially. The application of this principle in cyclization reactions has been reported<sup>1</sup> and may be worthwhile exploring in future.

The discovery that type II fatty acid synthesis is non-essential in the symptomatic blood stage but important for the development of the late liver stage forms of the malaria parasite was discussed in section 1.4.2.2.1. It is therefore reasonable to assume, that the hybrids (**3.7**, **3.8**) may display an improved activity profile when tested in *in vitro* assays on the liver stage forms of the malaria parasite. These results will not only complement the *in vitro* data on the blood stage forms of the parasite presented in this report, but may also contribute towards elucidating the mode of action of the hybrids.

#### **6.4. $\beta$ -Amino Alcohol Hybrids**

As mentioned earlier, for the synthesis of the  $\beta$ -amino alcohol hybrids we employed the click reaction which afforded the thiolactone-chalcones (**4.11** and **4.14**) in low yields and the isatin-chalcones (**4.23** and **4.24**) in moderate to high yields. In essence, compounds in this series consist of four structural units: 1,2,3-triazole ring,  $\beta$ -amino alcohol unit, chalcone moiety and the thiolactone or isatin moiety; with the triazole and  $\beta$ -amino alcohol unit functioning as a linker. It may be worthwhile to investigate the contribution of each of these units to the observed antiplasmodial activity. Moreover, the poor antiplasmodial activity of the acetylenic chalcones (**4.10** and **4.13**, table 4.4) and the moderate to good activity of the hybrids (**4.11**, **4.14**, table 4.5 and **4.23**, **4.24**, table 4.9) support the strategy of hybridization in antimalarial drug discovery for this series of compounds

Reference has also been made to the low solubility of compounds in this series and the fact that the enhancement thereof may lead to improved activity (section 4.3.3.1). Future studies on the isatin-chalcone hybrids may include the condensation of the ketonic carbonyl on the isatin scaffold with (thio)semicarbazide. We envisaged that the Schiff-base derivatives so generated will have enhanced solubility and activity profiles.

#### **6.5. Semi-synthetic Artemisinin Analogues**

Of all the compounds synthesized in this project, the most active were found among the semi-synthetic analogues of artemisinins. Our efforts towards the synthesis of hydrolytically stable acetyl type analogues of artemisinin entailed the introduction of polar groups such as the hydroxyl group and the triazole ring system. Metabolic studies on the hybrids **5.5**, **5.9**, **5.14** and **5.15** are required, to confirm their hydrolytic stability.

### 6.6. References

1. (a) Knops, P., Sendhoff, N., Mekelburger, H.-B., Vögtle, F., *Topics Curr. Chem.*, **1991**, 161, 1; (b) Illuminati, G., Mandolini, L., *Acc. Chem. Res.*, **1981**, 14, 95; (c) Ziegler, K. in *Methoden der Organischen Chemie*, 4, Müller, E. (ed.), George Thieme Verlag, Stuttgart, **1955**; (d) Ruggli, P., *Liebigs Ann. Chem.*, **1912**, 392, 92

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## Chapter 7

### EXPERIMENTAL

#### 7.1. General

All commercially available chemicals used in this project were purchased either from Sigma-Aldrich or Merck in South Africa. With the exception of DMF and MeOH, which were purchased as anhydrous solvents, all commonly used solvents were purified and dried as described in literature.<sup>1</sup> Tetrahydrofuran (THF) and diethyl ether were dried over and distilled from sodium wire with benzophenone as indicator. Triethylamine was dried over and distilled from CaH<sub>2</sub> and stored over potassium hydroxide pellets whereas dichloromethane (DCM) was distilled from phosphorous pentoxide prior to use. Reactions were monitored by thin layer chromatography (tlc) using Merck F<sub>254</sub> aluminium-backed silica gel 60 coated plates. Detection and visualization of spots were done using (a) the naked eye (visible region) especially for the highly coloured isatin derivatives, (b) ultraviolet light (254 nm/ 366 nm), (c) iodine vapours and (d) anisaldehyde spray reagent. Column chromatography and preparative layer chromatography were carried out on silica gel (Merck Kieselgel 60) and used in the purification of samples.

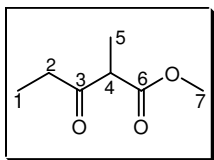
Proton nuclear magnetic resonance (<sup>1</sup>H NMR) spectra were recorded in deuteriochloroform (CDCl<sub>3</sub>), deuteromethanol (CD<sub>3</sub>OD) or deuterodimethylsulfoxide ((CD<sub>3</sub>)<sub>2</sub>SO) on a Varian Gemini (300 MHz) or Varian Unity Spectrometer (400 MHz) with tetramethylsilane (TMS) as internal standard. All chemical shifts (δ) are given in ppm and coupling constants (*J*), rounded off to one decimal place, are given in Hz. Carbon-13 nuclear magnetic resonance (<sup>13</sup>C NMR) spectra were recorded on the same instruments at 75 MHz or 100 MHz and in the same deuterated solvents with TMS as internal standard. The format used for recording <sup>13</sup>C NMR data is accepted by most international journals. In this format chemical shift values are listed without specific assignment to carbon atoms.

Melting points were determined using a Reichert-Jung Thermovar hot stage microscope and are uncorrected. Infrared (IR) spectra were recorded on a Thermo Nicolette FTIR instrument in the 3800 cm<sup>-1</sup> – 900 cm<sup>-1</sup> range as chloroform solutions, KBr pellets or as thin films on NaCl discs. Microanalyses were performed on a Fisons EA 1108 CHNS-O instrument. Low resolution mass spectrometry (LRMS) analysis was conducted at the Department of Pharmacology (UCT) on an API 2000 from Applied Biosystems. High resolution mass

spectrometry (HRMS) was performed on a VG70-SEQ (in EI and ESI mode) at the University of Witwatersrand (SA) and on a Waters API QTOF Ultima apparatus with a Waters UPLC system (Waters, Milford, MA, USA) at the University of Stellenbosch.

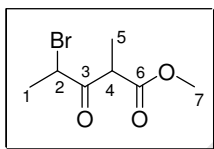
## 7.2. Experimental details pertaining to chapter 3

### 2-Methyl-3-oxo-pentanoic acid methyl ester, **3.2**

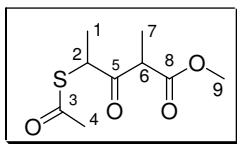


Anhydrous  $K_2CO_3$  (17.0 g, 122.9 mmol, 4.0 eq) was added to a solution of commercially available methyl propionyl acetate (4.0 g, 30.7 mmol, 1.0 eq) in dry THF (50 mL). The mixture was refluxed for 4 hours under nitrogen and thereafter cooled to  $0^\circ C$ . MeI (6.54 g, 46.1 mmol, 1.5 eq) was added and stirring continued for a further 2 hours at the same temperature ( $0^\circ C$ ). The reaction mixture was then allowed to warm to  $25^\circ C$  and left to stir at this temperature for 15 hours. Upon completion, the mixture was filtered on a celite pad. Evaporation of the solvent *in vacuo* followed by chromatography (eluent EtOAc:Hex 1:9) afforded a colourless oil (2.8 g, 70%);  $R_f$  (EtOAc:Hex 1:20) 0.19;  $\delta_H$  (300MHz,  $CDCl_3$ ) 3.70 (3H, s, H-7), 3.45 (1H, q,  $J$  7.3, H-4), 2.54 (2H, q,  $J$  7.3, H-2), 1.32 (3H, d,  $J$  7.3, H-5), 1.05 (3H, t,  $J$  7.1, H-1);  $\delta_C$  (75MHz,  $CDCl_3$ ) 206.3, 171.1, 52.4, 52.3, 34.6, 12.8, 7.6.

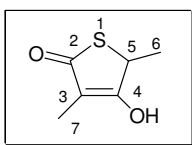
### 4-Bromo-2-Methyl-3-oxo-pentanoic acid methyl ester, **3.3**



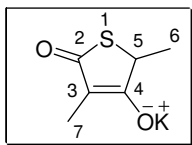
Bromine (2.4 g, 15.1 mmol, 1.0 eq) in chloroform was added dropwise over a period of 1 hour, to a solution of compound **3.2** (2.2 g, 15.1 mmol, 1.0 eq) in chloroform at  $0^\circ C$ . The resulting mixture was left to stir at  $25^\circ C$  for a period of 15 hours. Upon completion, a stream of air was passed through the reaction mixture. The latter was then dried over anhydrous  $Na_2SO_4$  and the solvent evaporated under reduced pressure to afford a light orange oil. This crude bromo compound was used without further purification.

**4-Acetylsufanyl-2-methyl-3-oxo-pentanoic acid methyl ester, 3.4**

Dry dichloromethane was added to the crude bromo compound **3.3** (3.0 g, 13.4 mmol, 1.0 eq). The resulting mixture was added dropwise over a period of 30 minutes to a mixture of thiolacetic acid (1.22 g, 16.0 mmol, 1.2 eq) and dry Et<sub>3</sub>N (2.03 g, 20.0 mmol, 1.5 eq) in dry dichloromethane at 0°C. The mixture so obtained was stirred at room temperature (25°C) under an inert atmosphere for a period of 6 hours. Upon completion, water was added to the reaction mass. The resulting mixture was extracted with DCM and the combined organic layer washed with saturated NaCl solution. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>, concentrated and the resulting crude residue purified by column chromatography (eluent EtOAc:Hex 1:4) to afford **3.4** as an orange oil (1.8 g, 60%); R<sub>f</sub> (EtOAc:Hex 1:5) 0.49; δ<sub>H</sub> (300MHz, CDCl<sub>3</sub>) 4.52 (1H, q, *J* 7.2, H-2), 3.84 (1H, q, *J* 7.5, H-6), 3.71 (3H, s, H-9), 2.35 (3H, s, H-4), 1.41 (3H, d, *J* 7.1, H-1), 1.32 (3H, d, *J* 7.1, H-7); δ<sub>C</sub> (75MHz, CDCl<sub>3</sub>) 202.9, 193.9, 170.6, 52.4, 49.7, 46.1, 30.1, 16.0, 12.9

**4-Hydroxy-3,5-dimethylthiophen-2(5H)-one, 2.13**

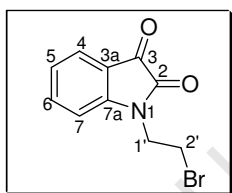
A 10% KOH solution (1.4 g, 25.6 mmol, 2.0 eq) was added to a stirred solution of thioester **3.4** (2.8 g, 12.8 mmol, 1.0 eq) in 16 mL ethanol at 0°C. The reaction mixture was stirred at room temperature for 4 hours. The solvent was then evaporated under reduced pressure, and the residue taken up in water. The resulting mixture was extracted with diethyl ether and the ether layer discarded. The aqueous layer was acidified to pH 1 with 10% HCl and extracted with EtOAc. The combined organic layer was washed with saturated brine solution and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure gave a crude product mixture. Recrystallization from DCM gave the thiolactone as an off-white solid (1.7 g, 60%); R<sub>f</sub> (EtOAc:Hex 1:10) 0.36; m.p. 130-131°C (lit. m. p. 128-130°C)<sup>2</sup>; δ<sub>H</sub> (300MHz, CD<sub>3</sub>OD) 4.12 (1H, q, *J* 7.1, H-5), 1.66 (3H, s, H-7), 1.56 (3H, d, *J* 7.1, H-6); δ<sub>C</sub> (75 MHz, CD<sub>3</sub>OD) 198.6, 181.2, 110.9, 44.1, 19.6, 7.6

**4-Hydroxy-3,5-dimethyl-5H-thiophen-2-one, potassium derivative, 3.5**

A 2.5% KOH solution (390 mg, 6.94 mmol, 1.0 eq) was added to **2.13** (1.00 g, 6.94 mmol, 1.0 eq) in 10 mL MeOH. The resulting mixture was stirred at 25°C for 4 hours. Evaporation of the solvent under reduced pressure afforded an off-white hygroscopic solid (954 mg, 95%); m.p. >300°C;  $\delta_{\text{H}}$  (300MHz, CD<sub>3</sub>OD) 4.12 (1H, q,  $J$  7.1, H-5), 1.66 (3H, s, H-7), 1.56 (3H, d,  $J$  7.1, H-6);  $\delta_{\text{C}}$  (75 MHz, CD<sub>3</sub>OD) 194.9, 188.6, 95.2, 45.4, 20.0, 9.2

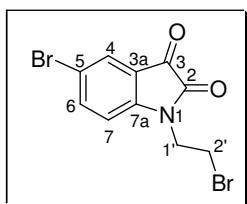
**A. General procedure for preparation of compounds 3.6a-w**

Sodium hydride, 60% suspended in mineral oil (10.2 mmol, 1.5 eq) was added to commercially available isatin/5-substituted isatin (6.8 mmol, 1.0 eq) in 10 mL of anhydrous DMF at 0°C. The dibromoalkane (27.2 mmol, 4.0 eq) was added and the resulting mixture slowly warmed to 25°C. Stirring was continued for 1 hour at this temperature. The temperature was then increased to 60°C and the reaction mixture stirred for 24 hours at this temperature. Ice-cold water was added to the orange coloured reaction mixture and the precipitate that formed was filtered, washed with water and recrystallized from MeOH.

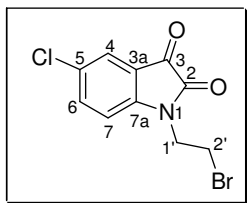
**1-(2-Bromo-ethyl)- 1H-indole-2,3-dione, 3.6a**

The conditions employed for the preparation of this compound were those described in general procedure A. However 6.80 mmol of isatin and 27.2 mmol of 1,2-dibromoethane was used. The product was obtained as an orange crystalline solid (1.64 g, 95%); m.p. 128°C;  $R_{\text{f}}$  (EtOAc:Hex 1:1) 0.61;  $\text{IR}_{\text{vmax}}(\text{KBr})/\text{cm}^{-1}$  1735 (C<sub>3</sub>=O), 1612 (C<sub>2</sub>=O), 1472 (Ar C=C);  $\delta_{\text{H}}$  (300MHz, CDCl<sub>3</sub>) 7.61 (2H, m, H-4 and H-6), 7.14 (1H, td,  $J$  0.9 and 7.2, H-5), 6.99 (1H, d\*,  $J$  7.8, H-7), 4.14 (2H, t,  $J$  6.9, H-2'), 3.61 (2H, t,  $J$  6.9, H-1');  $\delta_{\text{C}}$  (75MHz, CDCl<sub>3</sub>) 183.0, 158.2, 150.4, 138.4, 125.6, 124.0, 117.6, 110.2, 27.0, 41.9; LRMS(ESI) found  $m/z$  254.0 (54%, [M+H]<sup>+</sup>) requires 253.0 for C<sub>10</sub>H<sub>8</sub>O<sub>2</sub>NBr.

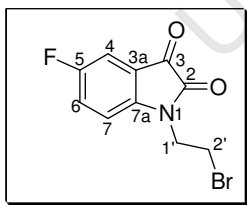
\* H-7 of the isatin scaffold consistently appeared as a doublet instead of the expected doublet of doublet for all unsubstituted isatin derivatives (fig. 3.8).

**5-Bromo-1-(2-bromo-ethyl)-1H-indole-2,3-dione, 3.6b**

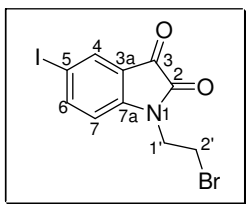
The conditions employed for the preparation of this compound were those described in general procedure A. However 4.42 mmol of 5-bromoisatin and 17.7 mmol of 1,2-dibromoethane was used. The product was obtained as an orange solid (1.35 g, 92%); m.p. 143-144°C;  $R_f$  (EtOAc:Hex 1:1) 0.78;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  1736 ( $\text{C}_3=\text{O}$ ), 1603 ( $\text{C}_2=\text{O}$ ), 1470 (Ar C=C);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.72 (2H, m, H-4 and H-6), 6.93 (1H, d,  $J$  7.5, H-7), 4.14 (2H, t,  $J$  6.9, H-2'), 3.61 (2H, t,  $J$  6.9, H-1');  $\delta_C$  (75MHz,  $\text{CDCl}_3$ ) 183.1, 157.5, 149.2, 140.6, 128.4, 118.8, 116.9, 112.0, 42.2, 27.1; LRMS(ESI) found  $m/z$  331.4 (28%,  $[\text{M}+\text{H}]^+$ ) requires 330.9 for  $\text{C}_{10}\text{H}_7\text{O}_2\text{NBr}_2$ .

**1-(2-Bromo-ethyl)-5-chloro-1H-indole-2,3-dione, 3.6c**

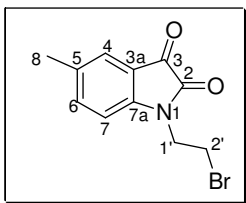
The conditions employed for the preparation of this compound were those described in general procedure A. However 5.51 mmol of the 5-chloroisatin and 22.0 mmol of 1,2-dibromoethane was used. The product was obtained as an orange solid (1.43 g, 90%); m.p. 114-116°C;  $R_f$  (EtOAc:Hex 1:1) 0.74;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  1738 ( $\text{C}_3=\text{O}$ ), 1605 ( $\text{C}_2=\text{O}$ ), 1471 (Ar C=C);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.57 (2H, m, H-4 and H-6), 6.98 (1H, d,  $J$  7.8, H-7), 4.14 (2H, t,  $J$  6.9, H-2'), 3.61 (2H, t,  $J$  6.9, H-1');  $\delta_C$  (75MHz,  $\text{CDCl}_3$ ) 183.6, 158.2, 150.4, 137.7, 129.9, 125.5, 117.6, 111.6, 42.2, 27.1; LRMS(ESI) found  $m/z$  288.0 (26%,  $[\text{M}+\text{H}]^+$ ) requires 286.9 for  $\text{C}_{10}\text{H}_7\text{O}_2\text{NBrCl}$ .

**1-(2-Bromo-ethyl)-5-fluoro-1H-indole-2,3-dione, 3.6d**

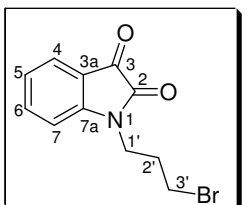
The conditions employed for the preparation of this compound were those described in general procedure A. However 6.06 mmol of 5-fluoroisatin and 24.2 mmol of 1,2-dibromoethane was used. The product was obtained as an orange-red crystalline solid (1.37 g, 83%); m.p. 102-104°C;  $R_f$  (EtOAc:Hex 1:1) 0.54;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  1736 ( $\text{C}_3=\text{O}$ ), 1617 ( $\text{C}_2=\text{O}$ ), 1485 (Ar C=C);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.32 (2H, m, H-4 and H-6), 6.99 (1H, dd,  $J$  3.9 and 9.6, H-7), 4.14 (2H, t,  $J$  6.9, H-2'), 3.61 (2H, t,  $J$  6.9, H-1');  $\delta_C$  (75MHz,  $\text{CDCl}_3$ ) 183.8, 161.0, 157.7, 146.6, 129.9, 124.7, 112.6, 111.5, 42.1, 27.1; LRMS(ESI) found  $m/z$  272.2 (42%,  $[\text{M}+\text{H}]^+$ ) requires 270.9 for  $\text{C}_{10}\text{H}_7\text{O}_2\text{NBrF}$ .

**1-(2-Bromo-ethyl)-5-iodo-1*H*-indole-2,3-dione, 3.6e**

The conditions employed for the preparation of this compound were those described in general procedure A. However 3.66 mmol of the 5-iodoisatin and 14.7 mmol of 1,2-dibromoethane was used. The product was obtained as an orange solid (1.24 g, 89%); m.p. 153-154°C;  $R_f$  (EtOAc:Hex 1:1) 0.84;  $IR_{\max}$ (KBr)/ $\text{cm}^{-1}$  1733 ( $\text{C}_3=\text{O}$ ), 1600 ( $\text{C}_2=\text{O}$ ), 1467 (Ar C=C);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.90 (2H, m, H-4 and H-6), 6.82 (1H, d,  $J$  8.7, H-7), 4.13 (2H, t,  $J$  6.9, H-2'), 3.60 (2H, t,  $J$  6.9, H-1');  $\delta_C$  (75MHz,  $\text{CDCl}_3$ ) 183.8, 160.0, 150.0, 146.3, 134.1, 119.1, 112.4, 86.2, 42.1, 27.1; LRMS(ESI) found  $m/z$  380.4 (26%,  $[\text{M}+\text{H}]^+$ ) requires 378.9 for  $\text{C}_{10}\text{H}_7\text{O}_2\text{NBrI}$ .

**1-(2-Bromo-ethyl)-5-methyl-1*H*-indole-2,3-dione, 3.6f**

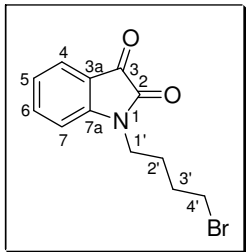
The conditions employed for the preparation of this compound were those described in general procedure A. However 6.21 mmol of the 5-methylisatin and 24.8 mmol of 1,2-dibromoethane was used. The product was obtained as an orange solid (1.63 g, 98%); m.p. 117-119°C;  $R_f$  (EtOAc:Hex 1:1) 0.80;  $IR_{\max}$ (KBr)/ $\text{cm}^{-1}$  1738 ( $\text{C}_3=\text{O}$ ), 1621 (amidic  $\text{C}_2=\text{O}$ ), 1490 (Ar C=C);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.40 (2H, m, H-4 and H-6), 6.88 (1H, d,  $J$  8.1, H-7), 4.11 (2H, t,  $J$  6.9, H-2'), 3.59 (2H, t,  $J$  6.9, H-1'), 2.34 (3H, s, H-8);  $\delta_C$  (75MHz,  $\text{CDCl}_3$ ) 183.0, 158.3, 150.0, 138.8, 133.9, 126.0, 117.6, 110.0, 41.9, 27.1, 20.6; LRMS(ESI) found  $m/z$  268.0 (75%,  $[\text{M}+\text{H}]^+$ ) requires 267.0 for  $\text{C}_{10}\text{H}_7\text{O}_2\text{NBrI}$ .

**1-(3-Bromo-propyl)-1*H*-indole-2,3-dione, 3.6g**

The conditions employed for the preparation of this compound were those described in general procedure A. However 6.80 mmol of isatin and 27.2 mmol of 1,3-dibromopropane was used. The product was obtained as an orange crystalline solid (1.57 g, 86%); m.p. 85°C;  $R_f$  (EtOAc:Hex 1:1) 0.66;  $IR_{\max}$ (KBr)/ $\text{cm}^{-1}$  1740 ( $\text{C}_3=\text{O}$ ), 1615 ( $\text{C}_2=\text{O}$ ), 1470 (Ar C=C);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.60 (2H, m, H-4 and H-6), 7.12 (1H, td,  $J$  1.2 and 7.8, H-5), 7.01 (1H, d,  $J$  8.4, H-7), 3.89 (2H, t,  $J$  6.9, H-3'), 3.47 (2H, t,  $J$  6.3, H-1'), 2.28 (2H, quint,  $J$  6.3, H-2');

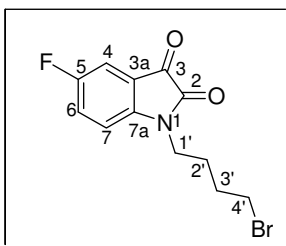
$\delta_C$  (75MHz,  $CDCl_3$ ) 183.0, 158.3, 150.7, 138.5, 125.6, 123.9, 117.6, 110.0, 38.8, 30.3, 30.0; LRMS(ESI) found  $m/z$  268.0 (69%,  $[M+H]^+$ ) requires 267.0 for  $C_{11}H_{10}O_2NBr$ .

### 1-(4-Bromo-butyl)-1*H*-indole-2,3-dione, 3.6h



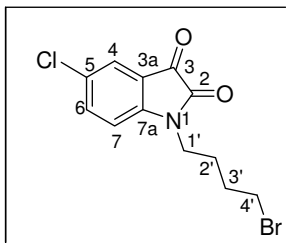
The conditions employed for the preparation of this compound were those described in general procedure A. However 6.80 mmol of isatin and 27.2 mmol of 1,4-dibromobutane was used. The product was obtained as an orange solid (1.92 g, quant.); m.p. 52°C;  $R_f$  (EtOAc:Hex 1:1) 0.75;  $IR_{v_{max}}$ (KBr)/ $cm^{-1}$  1727 ( $C_3=O$ ), 1609 ( $C_2=O$ ), 1472 (Ar  $C=C$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.58 (2H, m, H-4 and H-6), 7.10 (1H, td,  $J$  0.9 and 7.2, H-5), 6.92 (1H, d,  $J$  7.8, H-7), 3.74 (2H, t,  $J$  6.9, H-4'), 3.44 (2H, t,  $J$  6.9, H-1'), 1.97-1.86 (4H, m, H-2' and H-3');  $\delta_C$  (75MHz,  $CDCl_3$ ) 183.2, 158.1, 150.6, 138.4, 125.4, 123.7, 117.5, 110.1, 39.1, 32.7, 29.5, 25.6; LRMS(ESI) found  $m/z$  282.3  $[M+H]^+$  requires 281.0 for  $C_{12}H_{12}O_2NBr$ .

### 1-(4-Bromobutyl)-5-fluoroindoline-2,3-dione, 3.6i



The conditions employed for the preparation of this compound were those described in general procedure A. However 12.1 mmol of 5-fluoroisatin and 48.4 mmol of 1,4-dibromobutane was used. The product was obtained as an orange solid (2.30 g, 63%); m.p. 98°C;  $R_f$  (EtOAc:Hex 1:1) 0.70;  $IR_{v_{max}}$ (KBr)/ $cm^{-1}$  1727 ( $C_3=O$ ), 1621 ( $C_2=O$ ), 1486 (Ar  $C=C$ );  $\delta_H$  (400MHz,  $CDCl_3$ ) 7.33 (2H, m, H-4 and H-6), 6.91 (1H, m, H-7), 3.77 (2H, t,  $J$  6.8, H-4'), 3.47 (2H, t,  $J$  6.4, H-1'), 1.98-1.88 (4H, m, H-2' and H-3');  $\delta_C$  (100MHz,  $CDCl_3$ ) 182.3, 160.6, 158.1, 146.7, 124.7, 118.3, 112.6, 111.3, 39.4, 32.7, 29.5, 25.6; LRMS(ESI) found  $m/z$  300.4 (89%,  $[M+H]^+$ ) requires 299.0 for  $C_{12}H_{11}O_2NBrF$ .

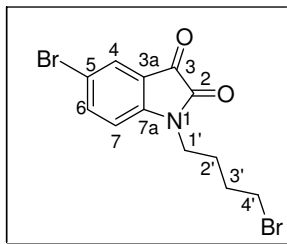
### 1-(4-Bromobutyl)-5-chloroindoline-2,3-dione, 3.6j



The conditions employed for the preparation of this compound were those described in general procedure A. However 11.0 mmol of 5-chloroisatin and 44.1 mmol of 1,4-dibromobutane was used. The product was obtained as an orange solid (2.66 g, 76%); m.p.

93°C;  $R_f$  (EtOAc:Hex, 1:1) 0.75;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  1735 ( $\text{C}_3=\text{O}$ ), 1604 ( $\text{C}_2=\text{O}$ ), 1474 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.58 (2H, m, H-4 and H-6), 6.91 (1H, d,  $J$  8.8, H-7), 3.78 (2H, t,  $J$  6.8, H-4'), 3.47 (2H, t,  $J$  6.4, H-1'), 1.97-1.88 (4H, m, H-2' and H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 182.3, 157.7, 149.0, 137.7, 129.7, 125.5, 118.5, 111.4, 39.4, 32.6, 29.5, 25.6; LRMS(ESI) found  $m/z$  316.2 (66%,  $[\text{M}+\text{H}]^+$ ) requires 315.0 for  $\text{C}_{12}\text{H}_{11}\text{O}_2\text{NBrCl}$ .

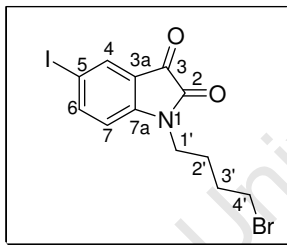
### 5-Bromo-1-(4-bromobutyl)indoline-2,3-dione, 3.6k



The conditions employed for the preparation of this compound were those described in general procedure A. However 8.85 mmol of 5-bromoisatin and 35.4 mmol of 1,4-dibromobutane was used. The product was obtained as an orange solid (2.30 g, 72%); m.p.

87°C;  $R_f$  (EtOAc:Hex 1:1) 0.73;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  1735 ( $\text{C}_3=\text{O}$ ), 1601 ( $\text{C}_2=\text{O}$ ), 1471 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.72 (2H, m, H-4 and H-6), 6.86 (1H, d,  $J$  8.8, H-7), 3.77 (2H, t,  $J$  6.8, H-4'), 3.47 (2H, t,  $J$  6.0, H-1'), 1.97-1.89 (4H, m, H-2' and H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 182.1, 157.5, 149.4, 140.6, 128.3, 118.8, 116.7, 111.8, 39.2, 32.6, 29.5, 25.6; LRMS(ESI) found  $m/z$  360.0 (59%,  $[\text{M}+\text{H}]^+$ ) requires 358.9 for  $\text{C}_{12}\text{H}_{11}\text{O}_2\text{NBr}_2$ .

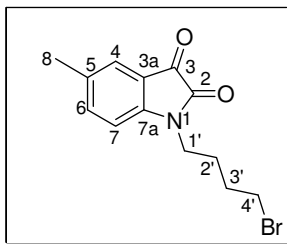
### 1-(4-Bromobutyl)-5-iodoindoline-2,3-dione, 3.6l



The conditions employed for the preparation of this compound were those described in general procedure A. However 7.33 mmol of 5-iodoisatin and 29.3 mmol of 1,4-dibromobutane was used. The product was obtained as an orange solid (1.81 g, 61%); m.p.

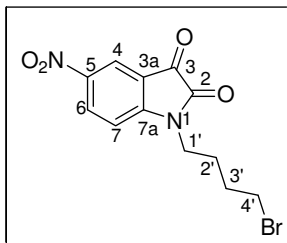
82-83°C;  $R_f$  (EtOAc:Hex 1:1) 0.82;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  1726 ( $\text{C}_3=\text{O}$ ), 1600 ( $\text{C}_2=\text{O}$ ), 1469 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.90 (2H, m, H-4 and H-6), 6.76 (1H, d,  $J$  8.8, H-7), 3.76 (2H, t,  $J$  6.8, H-4'), 3.47 (2H, t,  $J$  6.4, H-1'), 1.976-1.86 (4H, m, H-2' and H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 181.9, 157.2, 150.0, 146.5, 134.0, 119.2, 112.2, 86.0, 39.4, 32.6, 29.5, 25.6; LRMS(ESI) found  $m/z$  408.1 (89%,  $[\text{M}+\text{H}]^+$ ) requires 406.9 for  $\text{C}_{12}\text{H}_{11}\text{O}_2\text{NBrI}$ .



**1-(4-Bromobutyl)-5-methylindoline-2,3-dione, 3.6m**

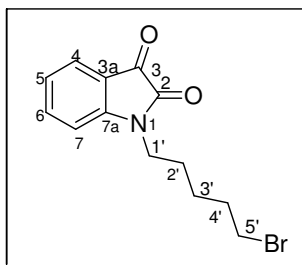
The conditions employed for the preparation of this compound were those described in general procedure A. However 12.4 mmol of 5-methylisatin and 49.6 mmol of 1,4-dibromobutane was used. The product was obtained as an orange solid (2.31 g, 63%); m.p.

114-115°C;  $R_f$  (EtOAc:Hex 1:1) 0.69;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  1738 ( $\text{C}_3=\text{O}$ ), 1621 ( $\text{C}_2=\text{O}$ ), 1491 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.42 (2H, m, H-4 and H-6), 6.83 (1H, d,  $J$  8.0, H-7), 3.76 (2H, t,  $J$  6.8, H-4'), 3.47 (2H, t,  $J$  6.4, H-1'), 2.35 (3H, s, H-8), 1.98-1.87 (4H, m, H-2' and H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 183.7, 156.8, 148.5, 138.8, 133.7, 125.9, 117.7, 109.9, 39.2, 32.7, 29.6, 25.7, 20.6; LRMS(ESI) found  $m/z$  296.3 (84%,  $[\text{M}+\text{H}]^+$ ) requires 295.0 for  $\text{C}_{13}\text{H}_{14}\text{O}_2\text{NBr}$ .

**1-(4-Bromobutyl)-5-nitroindoline-2,3-dione, 3.6n**

The conditions employed for the preparation of this compound were those described in general procedure A. However 10.4 mmol of 5-nitroisatin and 41.6 mmol of 1,4-dibromobutane was used. The product was obtained as a yellow solid (1.85 g, 54%); m.p.

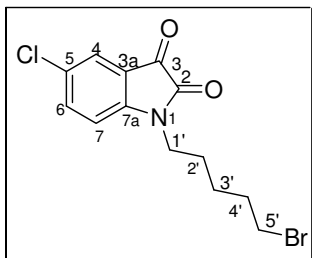
260°C;  $R_f$  (EtOAc:Hex 1:1) 0.55;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  1746 ( $\text{C}_3=\text{O}$ ), 1610 ( $\text{C}_2=\text{O}$ ), 1477 (Ar C=C), 1520/1336 (Asym./sym.  $\text{NO}_2$  str.);  $\delta_{\text{H}}$  (400MHz,  $\text{DMSO-d}_6$ ) 8.49 (1H, dd,  $J$  2.4 and 8.5, H-6), 8.22 (1H, d,  $J$  2.4, H-4), 7.42 (1H, d,  $J$  8.4, H-7), 3.78 (2H, t,  $J$  6.4, H-4'), 3.56 (2H, t,  $J$  6.4, H-1'), 1.76-1.68 (4H, m, H-2' and H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{DMSO-d}_6$ ) 181.1, 158.8, 154.9, 142.8, 132.8, 119.0, 117.9, 111.0, 39.6, 34.5, 29.3, 25.5; LRMS(ESI) found  $m/z$  327.0  $[\text{M}+\text{H}]^+$  requires 326.0 for  $\text{C}_{12}\text{H}_{11}\text{O}_4\text{N}_2\text{Br}$ .

**1-(5-Bromo-pentyl)-1H-indole-2,3-dione, 3.6o**

The conditions employed for the preparation of this compound were those described in general procedure A. However 6.80 mmol of isatin and 27.2 mmol of 1,5-dibromopentane was used. The product was obtained as an orange oil (1.95 g, 97%);  $R_f$  (EtOAc:Hex 1:1) 0.78;  $IR_{\text{max}}(\text{film})/\text{cm}^{-1}$  1740 ( $\text{C}_3=\text{O}$ ), 1611

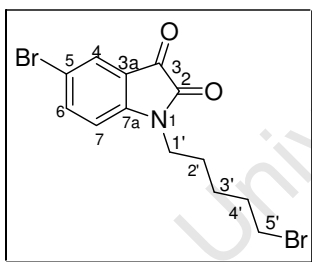
(C<sub>2</sub>=O), 1470 (Ar C=C);  $\delta_{\text{H}}$  (300MHz, CDCl<sub>3</sub>) 7.56 (2H, m, H-4 and H-6), 7.08 (1H, td, *J* 0.9 and 7.5, H-5), 6.88 (1H, d, *J* 8.4, H-7), 3.70 (2H, t, *J* 6.9, H-5'), 3.36 (2H, t, *J* 6.9, H-1'), 1.86 (2H, quint, *J* 6.9, H-4'), 1.63 (2H, quint, *J* 6.9, H-2'), 1.51 (2H, quint, *J* 7.2, H-3');  $\delta_{\text{C}}$  (75 MHz, CDCl<sub>3</sub>) 183.3, 158.1, 150.7, 138.3, 125.3, 123.6, 117.4, 110.0, 39.8, 33.1, 32.0, 26.3, 25.3; LRMS(ESI) found *m/z* 296.1 (100%, [M+H]<sup>+</sup>) requires 295.0 for C<sub>13</sub>H<sub>14</sub>O<sub>2</sub>NBr.

### 1-(5-Bromopentyl)-5-chloroindoline-2,3-dione, 3.6p

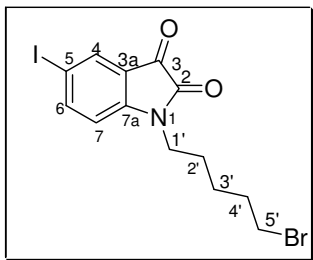


The conditions employed for the preparation of this compound were those described in general procedure A. However 11.0 mmol of 5-chloroisatin and 44.1 mmol of 1,5-dibromopentane was used. The product was obtained as a red crystalline solid (2.27 g, 62%); m.p. 93-95°C; *R<sub>f</sub>* (EtOAc:Hex 2:3) 0.69;  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>) 7.58-7.55 (2H, m, H-4 and H-6), 6.87 (1H, d, *J* 8.0, H-7), 3.75 (2H, t, *J* 7.6, H-5'), 3.41 (2H, t, *J* 6.4, H-1'), 1.93 (2H, quint, *J* 7.2, H-4'), 1.74 (2H, quint, *J* 7.2, H-2'), 1.57 (2H, quint, *J* 7.6, H-3');  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>) 182.1, 158.0, 149.2, 137.8, 129.9, 125.2, 118.0, 111.3, 40.1, 33.1, 32.0, 26.4, 25.4; LRMS(ESI) found *m/z* 330.2 (74%, [M+H]<sup>+</sup>) requires 329.0 for C<sub>13</sub>H<sub>13</sub>O<sub>2</sub>NBrCl.

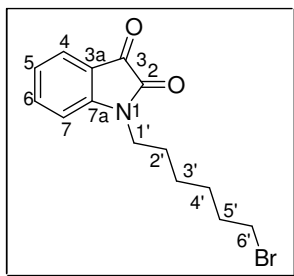
### 5-Bromo-1-(5-bromopentyl)indoline-2,3-dione, 3.6q



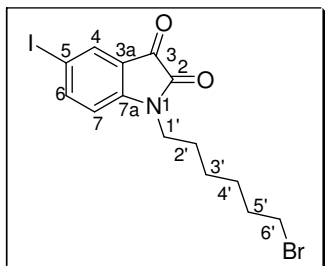
The conditions employed for the preparation of this compound were those described in general procedure A. However 8.85 mmol of 5-bromoisatin and 35.4 mmol of 1,5-dibromopentane was used. The product was obtained as an orange crystalline solid (2.58 g, 78%); m.p. 103°C; *R<sub>f</sub>* (EtOAc:Hex 2:3) 0.65;  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>) 7.73-7.70 (2H, m, H-4 and H-6), 6.82 (1H, d, *J* 8.8, H-7), 3.74 (2H, t, *J* 7.2, H-5'), 3.41 (2H, t, *J* 6.8, H-1'), 1.93 (2H, quint, *J* 7.2, H-4'), 1.74 (2H, quint, *J* 7.2, H-2'), 1.57 (2H, quint, *J* 7.6, H-3');  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>) 182.2, 157.5, 149.6, 140.6, 128.3, 118.8, 116.6, 111.7, 40.1, 33.1, 32.0, 26.4, 25.4; LRMS(ESI) found *m/z* 374.1 (58%, [M+H]<sup>+</sup>) requires 372.9 for C<sub>13</sub>H<sub>13</sub>O<sub>2</sub>NBr<sub>2</sub>.

**1-(5-Bromopentyl)-5-iodoindoline-2,3-dione, 3.6r**

The conditions employed for the preparation of this compound were those described in general procedure A. However 7.32 mmol of 5-iodoisatin and 29.3 mmol of 1,5-dibromopentane was used. The product was obtained as a red crystalline solid (2.41 g, 78%); m.p. 89°C;  $R_f$  (EtOAc:Hex 2:3) 0.68;  $\delta_H$  (400MHz,  $CDCl_3$ ) 7.91-7.89 (2H, m, H-4 and H-6), 6.72 (1H, d,  $J$  8.8, H-7), 3.73 (2H, t,  $J$  7.2, H-5'), 3.41 (2H, t,  $J$  6.4, H-1'), 1.92 (2H, quint,  $J$  7.2, H-4'), 1.73 (2H, quint,  $J$  7.2, H-2'), 1.54 (2H, quint,  $J$  7.6, H-3');  $\delta_C$  (100MHz,  $CDCl_3$ ) 182.2, 157.2, 150.2, 146.4, 134.0, 119.2, 112.2, 85.9, 40.1, 33.1, 32.0, 26.4, 25.4; LRMS(ESI) found  $m/z$  422.3 (100%,  $[M+H]^+$ ) requires 420.9 for  $C_{13}H_{13}O_2NBrI$ .

**1-(6-Bromo-hexyl)-1H-indole-2,3-dione, 3.6s**

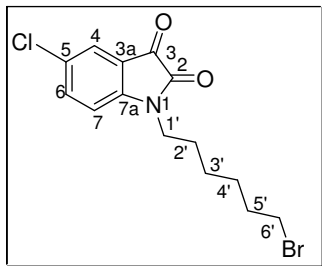
The conditions employed for the preparation of this compound were those described in general procedure A. However 6.80 mmol of isatin and 27.2 mmol of 1,6-dibromohexane was used. The product was obtained as a red crystalline solid (2.11 g, quant); m.p. 42°C;  $R_f$  (EtOAc:Hex 1:1) 0.77;  $IR_{v_{max}}$ (KBr)/ $cm^{-1}$  1740 ( $C_3=O$ ), 1611 ( $C_2=O$ ), 1471 (Ar  $C=C$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.57 (2H, m, H-4 and H-6), 7.10 (1H, td,  $J$  0.9 and 7.8, H-5), 6.88 (1H, d,  $J$  8.4, H-7), 3.71 (2H, t,  $J$  6.9, H-6'), 3.38 (2H, t,  $J$  7.2, H-1'), 1.82 (2H, quint,  $J$  6.9, H-2'), 1.65 (2H, quint,  $J$  7.2, H-5'), 1.54-1.36 (4H, m, H-3' and H-4');  $\delta_C$  (75 MHz,  $CDCl_3$ ) 183.4, 158.1, 150.9, 138.3, 125.4, 123.6, 117.6, 110.0, 40.0, 33.6, 32.4, 27.6, 27.0, 26.0; LRMS(ESI) found  $m/z$  310.0 (95%,  $[M+H]^+$ ) requires 309.0 for  $C_{14}H_{16}O_2NBr$ .

**1-(6-Bromohexyl)-5-iodoindoline-2,3-dione, 3.6t**

The conditions employed for the preparation of this compound were those described in general procedure A. However 7.32 mmol of 5-iodoisatin and 29.3 mmol of 1,6-dibromohexane was used. The product was obtained as an orange crystalline solid (2.41 g, 75%); m.p. 99-101°C;  $R_f$  (EtOAc:Hex 1:1) 0.82;  $IR_{v_{max}}$ (KBr)/ $cm^{-1}$  1751 ( $C_3=O$ ),

1601 (C<sub>2</sub>=O), 1468 (Ar C=C);  $\delta_{\text{H}}$  (300MHz, CDCl<sub>3</sub>) 7.87 (2H, m, H-4, H-6), 6.70 (1H, d, *J* 8.7, H-7), 3.70 (2H, t, *J* 7.2, H-6'), 3.39 (2H, t, *J* 6.6, H-1'), 1.85 (2H, quint, *J* 6.9, H-5'), 1.70 (2H, quint, *J* 7.2, H-2'), 1.55-1.36 (4H, m, H-3', H-4');  $\delta_{\text{C}}$  (75MHz, CDCl<sub>3</sub>) 182.2, 157.1, 150.2, 146.3, 133.9, 119.2, 112.2, 85.8, 40.1, 33.5, 32.4, 27.6, 27.0, 26.0; LRMS(ESI) found *m/z* 436.2 (96%, [M+H]<sup>+</sup>) requires 434.9 for C<sub>14</sub>H<sub>15</sub>O<sub>2</sub>NBrI.

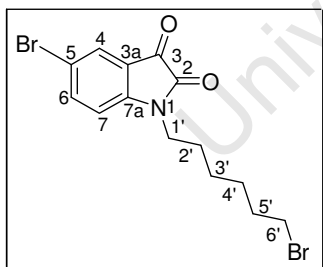
### 1-(6-Bromohexyl)-5-chloroindoline-2,3-dione, 3.6u



The conditions employed for the preparation of this compound were those described in general procedure A. However 11.0 mmol of 5-chloroisatin and 44.1 mmol of 1,6-dibromohexane was used. The product was obtained as an orange crystalline solid (2.66 g, 70%); m.p. 57°C; R<sub>f</sub> (EtOAc:Hex 1:1) 0.75;

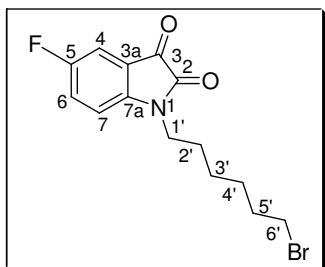
IR<sub>v</sub>max(KBr)/cm<sup>-1</sup> 1731 (C<sub>3</sub>=O), 1606 (C<sub>2</sub>=O), 1474 (Ar C=C);  $\delta_{\text{H}}$  (300MHz, CDCl<sub>3</sub>) 7.54 (2H, m, H-4 and H-6), 6.85 (1H, d, *J* 8.7, H-7), 3.71 (2H, t, *J* 7.2, H-6'), 3.38 (2H, t, *J* 6.9, H-1'), 1.84 (2H, quint, *J* 6.9, H-5'), 1.70 (2H, quint, *J* 7.2, H-2'), 1.54-1.36 (4H, m, H-3', H-4');  $\delta_{\text{C}}$  (75MHz, CDCl<sub>3</sub>) 182.4, 157.6, 149.1, 137.6, 129.5, 125.3, 118.4, 111.3, 40.2, 33.5, 32.4, 27.6, 27.0, 26.0; LRMS(ESI) found *m/z* 344.4 (75%, [M+H]<sup>+</sup>) requires 343.0 for C<sub>14</sub>H<sub>15</sub>O<sub>2</sub>NCIBr.

### 5-Bromo-1-(6-bromohexyl)indoline-2,3-dione, 3.6v



The conditions employed for the preparation of this compound were those described in general procedure A. However 8.84 mmol of 5-bromoisatin and 35.4 mmol of 1,6-dibromohexane was used. The product was obtained as an orange crystalline solid (2.26 g, 66%); m.p. 64-65°C; R<sub>f</sub> (EtOAc:Hex 1:1) 0.85;

IR<sub>v</sub>max(KBr)/cm<sup>-1</sup> 1753 (C<sub>3</sub>=O), 1606 (C<sub>2</sub>=O), 1472 (Ar C=C);  $\delta_{\text{H}}$  (300MHz, CDCl<sub>3</sub>) 7.68 (2H, m, H-4, H-6), 6.80 (1H, d, *J* 9.3, H-7), 3.71 (2H, t, *J* 7.2, H-6'), 3.38 (2H, t, *J* 6.9, H-1'), 1.84 (2H, quint, *J* 6.9, H-5'), 1.70 (2H, quint, *J* 7.2, H-2'), 1.52-1.36 (4H, m, H-3', H-4');  $\delta_{\text{C}}$  (75MHz, CDCl<sub>3</sub>) 182.3, 157.4, 149.6, 140.5, 128.2, 118.8, 116.4, 111.8, 40.2, 33.5, 32.4, 27.6, 27.0, 26.0; LRMS(ESI) found *m/z* 388.1 (58%, [M+H]<sup>+</sup>) requires 386.9 for C<sub>14</sub>H<sub>15</sub>O<sub>2</sub>NBr<sub>2</sub>.

**1-(6-Bromohexyl)-5-fluoroindoline-2,3-dione, 3.6w**

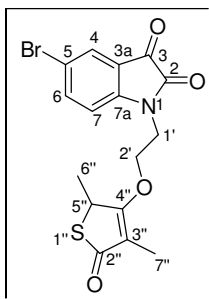
The conditions employed for the preparation of this compound were those described in general procedure A. However 12.1 mmol of 5-fluoroisatin and 48.4 mmol of 1,6-dibromohexane was used. The product was obtained as an orange crystalline solid (2.25 g, 57%); m.p. 36°C;  $R_f$  (EtOAc:Hex 1:1) 0.74;

$IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  1736 ( $\text{C}_3=\text{O}$ ), 1620 ( $\text{C}_2=\text{O}$ ), 1485 (Ar C=C);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.26-7.32 (2H, m, H-4 and H-6), 6.85 (1H, dd,  $J$  3.3 and 9.0, H-7), 3.71 (2H, t,  $J$  7.5, H-6'), 3.39 (2H, t,  $J$  6.9, H-1'), 1.84 (2H, quint,  $J$  7.2, H-5'), 1.71 (2H, quint,  $J$  6.9, H-2'), 1.53-1.37 (4H, m, H-3', H-4');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 182.0, 157.6, 146.9, 124.6, 118.2, 112.5, 111.2, 40.2, 33.5, 32.4, 27.6, 27.0, 26.0; LRMS(ESI) found  $m/z$  328.3 (11%,  $[\text{M}+\text{H}]^+$ ) requires 327.0 for  $\text{C}_{14}\text{H}_{15}\text{O}_2\text{NFB}$ .

**B. General procedure for preparation of compounds 3.7 a-j**

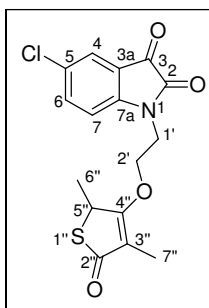
The appropriate *N*-alkylated isatin/5-substituted isatin **3.6a-h,o,s** (4.11 mmol, 1.5 eq) and **3.5** (2.74 mmol, 1.0 eq) was dissolved in 3 ml anhydrous DMF. The resulting mixture was stirred at 60°C for 48 hours under a  $\text{N}_2$  atmosphere. When the reaction was completed, ice-cold water was added to the dark red product mixture. The precipitate so obtained was filtered, washed with water and purified using column chromatography (eluent EtOAc:Hex 3:2). When no precipitate formed upon the addition of ice-cold water, the product mixture was extracted with EtOAc. The combined organic layer was then washed with deionized water to remove DMF and subsequently dried over anhydrous  $\text{Na}_2\text{SO}_4$ . Concentration under reduced pressure afforded the crude product mixture which was subjected to column chromatography to yield the pure product.

**5-Bromo-1-[2-(2,4-dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-ethyl]-indole-2,3-dione, 3.7a (RBrn2O)**



Orange solid (97.7 mg, 9%), m.p. 168-169°C;  $R_f$  (EtOAc:Hex 1:1) 0.28;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1748 ( $\text{C}_3=\text{O}$ ), 1624 ( $\text{C}_2=\text{O}$ ), 1603 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.73 (2H, m, H-4 and H-6), 6.95 (1H, d,  $J$  7.5, H-7), 4.57 (1H, m, H-2'a/b), 4.46 (1H, m, H-2'a/b), 4.08 (3H, m, H-5'' and H-1'), 1.76 (3H, d<sup>†</sup>,  $J$  1.6, H-7''), 1.49 (3H, d,  $J$  6.8, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.5, 182.0, 176.4, 157.7, 149.5, 140.5, 128.5, 118.8, 117.1, 115.3, 112.0, 68.2, 41.7, 40.5, 19.7, 9.1; HRMS(EI) found  $m/z$  394.98277 for  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSBr}$ , requires 394.98269; Anal. Calc. for  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSBr}$ : C, 48.50%; H, 3.56%; N, 3.53%; S, 8.09%. Found: C, 48.40%; H, 3.45%; N, 3.70%; S, 7.70%.

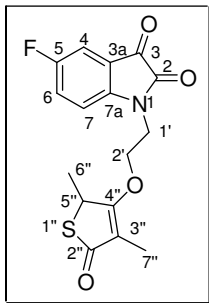
**5-Chloro-1-[2-(2,4-dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-ethyl]-1H-indole-2,3-dione, 3.7b (RCln2O)**



Orange solid (125 mg, 13%); m.p. 270-272°C;  $R_f$  (EtOAc:Hex 1:1) 0.23;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1743 ( $\text{C}_3=\text{O}$ ), 1633 ( $\text{C}_2=\text{O}$ ), 1610 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.58 (2H, m, H-6 and H-4), 7.00 (1H, d,  $J$  8.0, H-7), 4.56 (1H, m, H-2'a/b), 4.46 (1H, m, H-2'a/b), 4.08 (3H, m, H-5'' and H-1'), 1.76 (3H, d,  $J$  1.2, H-7''), 1.25 (3H, d,  $J$  7.2, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.1, 181.5, 176.4, 157.8, 149.1, 137.7, 130.1, 125.6, 118.4, 115.3, 111.7, 68.2, 41.7, 40.5, 19.7, 9.1; HRMS(EI) found  $m/z$  350.99468,  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSCl}$ , requires 351.03321; Anal. Calc. for  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSCl}$ : C, 54.62%; H, 4.01%; N, 3.98%; S, 9.11%. Found: C, 54.41%; H, 3.88; N, 3.80%; S, 8.89%.

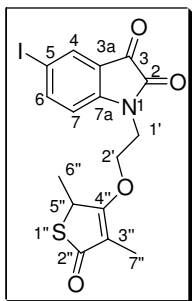
<sup>†</sup> The anomalous coupling of the methyl protons, H-7'' to the methine proton, H-5'' is discussed in section 3.4.1.

**1-[2-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-ethyl]-5-fluoro-1*H*-indole-2,3-dione, 3.7c** (RFn2O)



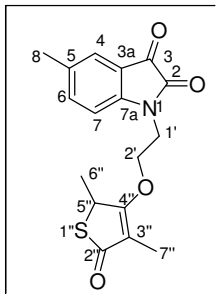
Orange solid (129 mg, 14%), m.p. 132-133°C;  $R_f$  (EtOAc:Hex 1:1) 0.19;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1740 ( $\text{C}_3=\text{O}$ ), 1641 ( $\text{C}_2''=\text{O}$ ), 1620 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.34 (2H, m, H-4 and H-6), 7.02 (1H, dd,  $J$  4.0 and 9.6, H-7), 4.57 (1H, m, H-2'a/b), 4.46 (1H, m, H-2'a/b), 4.09 (3H, m, H-5'' and H-1'), 1.76 (3H, d,  $J$  1.2, H-7''), 1.49 (3H, d,  $J$  6.8, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.1, 181.9, 176.5, 160.7, 158.2, 146.8, 124.7, 118.3, 115.3, 112.7, 111.6, 68.2, 41.7, 40.5, 19.7, 9.0; HRMS(EI) found  $m/z$  335.06248 for  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSF}$ , requires 335.06276; Anal. Calc. for  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NF}$ : C, 57.30%; H, 4.21%; N, 4.18%; S, 9.56%. Found: C, 57.13%; H, 4.06%; N, 3.72%; S, 9.22%.

**1-[2-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-ethyl]-5-iodo-1*H*-indole-2,3-dione, 3.7d** (RIn2O)



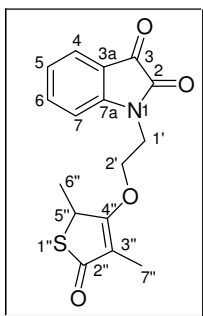
Orange solid (121 mg, 10%); m.p. 196°C;  $R_f$  (EtOAc:Hex 1:1) 0.26;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1740 ( $\text{C}_3=\text{O}$ ), 1623 ( $\text{C}_2''=\text{O}$ ), 1600 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.92 (2H, m, H-4 and H-6), 6.84 (1H, d,  $J$  8.8, H-7), 4.56 (1H, m, H-2'a/b), 4.45 (1H, m, H-2'a/b), 4.08 (3H, m, H-5'' and H-1'), 1.77 (3H, d,  $J$  1.2, H-7''), 1.49 (3H, d,  $J$  7.2, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.1, 182.5, 178.4, 169.2, 157.4, 150.1, 146.3, 134.2, 119.1, 112.4, 86.4, 68.2, 41.7, 40.4, 19.7, 9.1; HRMS(EI) found  $m/z$  442.97039,  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSI}$ , requires 442.96883; Anal. Calc. for  $\text{C}_{16}\text{H}_{14}\text{O}_4\text{NSI}$ : C, 43.35%; H, 3.18%; N, 3.16%; S, 7.23%. Found: C, 42.95%; H, 3.29%; N, 2.82%; S, 6.70%.

**1-[2-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-ethyl]-5-methyl-1H-indole-2,3-dione, 3.7e (RCH<sub>3</sub>n2O)**



Orange solid (236 mg, 26%), m.p. 130°C;  $R_f$  (EtOAc:Hex 1:1) 0.23;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1740 ( $\text{C}_3=\text{O}$ ), 1643 ( $\text{C}_2''=\text{O}$ ), 1619 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.41 (2H, m, H-6 and H-4), 6.91 (1H, d,  $J$  8.0, H-7), 4.56 (1H, m, H-2'a/b), 4.46 (1H, m, H-2'a/b), 4.07 (3H, m, H-5'' and H-1'), 2.35 (3H, s, H-8), 1.76 (3H, d,  $J$  1.6, H-7''), 1.48 (3H, d,  $J$  6.8, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.3, 182.8, 176.6, 158.6, 148.6, 138.7, 134.1, 126.0, 117.6, 115.0, 110.1, 68.2, 41.7, 40.3, 20.6, 19.6, 9.0; HRMS(EI) found  $m/z$  331.08822 for  $\text{C}_{17}\text{H}_{17}\text{O}_4\text{NS}$  requires 331.08783; Anal. Calc. for  $\text{C}_{17}\text{H}_{17}\text{O}_4\text{NS}$ : C, 61.61%; H, 5.17%; N, 4.23%; S, 9.68%. Found: C, 61.51%; H, 5.20%; N, 4.04%; S, 9.35%.

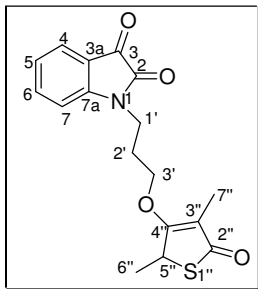
**1-[2-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-ethyl]-1H-indole-2,3-dione, 3.7f (RHn2O)**



Orange solid (258 mg, 36%); m.p. 54°C;  $R_f$  (EtOAc:Hex 1:1) 0.18;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1740 ( $\text{C}_3=\text{O}$ ), 1644 ( $\text{C}_2''=\text{O}$ ), 1611 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.62 (2H, m, H-4 and H-6), 7.16 (1H, td,  $J$  0.9 and 7.8, H-5), 7.03 (1H, d,  $J$  7.8, H-7), 4.58 (1H, m, H-2'a/b), 4.47 (1H, m, H-2'a/b), 4.09 (3H, m, H-5'' and H-1'), 1.75 (3H, d,  $J$  1.5, H-7''), 1.48 (3H, d,  $J$  6.6, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.2, 182.5, 176.6, 158.4, 150.8, 138.3, 125.7, 124.2, 117.6, 115.1, 110.3, 68.2, 41.8, 40.3, 19.6, 9.0; HRMS(EI) found  $m/z$  317.0722 for  $\text{C}_{16}\text{H}_{15}\text{O}_4\text{NS}$  requires 317.07218; Anal Calc. for  $\text{C}_{16}\text{H}_{15}\text{O}_4\text{NS}$ : C, 60.55%; H, 4.76%; N, 4.41%; S, 10.10%. Found: C, 60.38%; H, 4.75%; N, 4.01%; S, 9.73%.

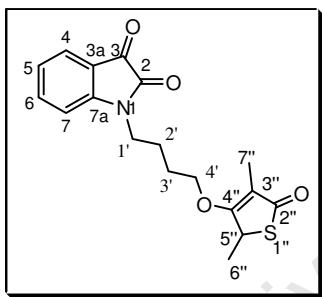


**1-[3-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-propyl]-1H-indole-2,3-dione, 3.7g (RHn3O)**



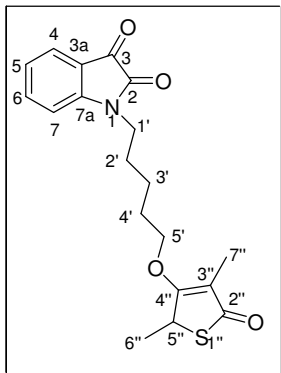
Orange solid (161 mg, 18%); m.p. 150-151°C;  $R_f$  (EtOAc:Hex 1:1) 0.24;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  1736 ( $\text{C}_3=\text{O}$ ), 1663 ( $\text{C}_2''=\text{O}$ ), 1612 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.62 (2H, m, H-6 and H-4), 7.15 (1H, td,  $J$  0.9 and 7.6, H-5), 6.92 (1H, d,  $J$  7.6, H-7), 4.39 (1H, m, H-3'a/b), 4.27 (1H, m, H-3'a/b), 4.15 (1H, qd,  $J$  1.2 and 7.2, H-5''), 3.93 (2H, t,  $J$  6.8, H-1'), 2.18 (2H, m, H-2'), 1.82 (3H, d,  $J$  1.2, H-7''), 1.57 (3H, d,  $J$  7.2, H-6'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.5, 183.0, 177.3, 158.4, 150.6, 138.5, 125.7, 124.0, 117.7, 114.8, 109.7, 68.3, 41.9, 37.0, 28.0, 19.8, 9.0; HRMS(EI) found  $m/z$  331.08695 for  $\text{C}_{17}\text{H}_{17}\text{O}_4\text{NS}$  requires 331.08783; Anal Calc. for  $\text{C}_{17}\text{H}_{17}\text{O}_4\text{NS}$ : C, 61.67%; H, 5.17%; N, 4.23%; S, 9.68%. Found: C, 61.63%; H, 5.05%; N, 3.99%; S, 9.19%.

**1-[4-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-butyl]-1H-indole-2,3-dione, 3.7h (RHn4O)**



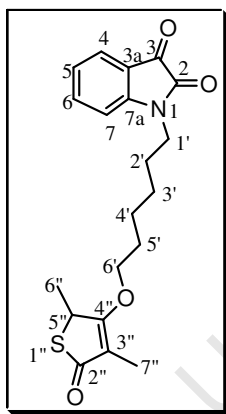
Orange solid (91.6 mg, 10%); m.p. 108°C;  $R_f$  (EtOAc:Hex 1:1) 0.18;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  1733 ( $\text{C}_3=\text{O}$ ), 1639 ( $\text{C}_2''=\text{O}$ ), 1606 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.60 (2H, m, H-6 and H-4), 7.13 (1H, td,  $J$  0.9 and 7.8, H-5), 6.90 (1H, d,  $J$  7.8, H-7), 4.35 (1H, m, H-4'a/b), 4.22 (1H, m, H-4'a/b), 4.14 (1H, qd,  $J$  0.9 and 6.9, H-5''), 3.81 (2H, t,  $J$  6.9, H-1'), 1.86 (4H, m, H-2' and H-3'), 1.80 (3H, d,  $J$  0.9, H-7''), 1.55 (3H, d,  $J$  6.9, H-6'');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.7, 183.2, 177.7, 158.3, 150.6, 138.3, 125.6, 123.9, 117.6, 114.5, 109.9, 70.2, 41.9, 39.5, 27.1, 23.6, 19.8, 9.0; HRMS(EI) found  $m/z$  345.1029 for  $\text{C}_{18}\text{H}_{19}\text{O}_4\text{NS}$  requires 345.10348; Anal Calc. for  $\text{C}_{18}\text{H}_{19}\text{O}_4\text{NS}$ : C, 62.59%; H, 5.54%; N, 4.06%; S, 9.28%. Found: C, 62.20%; H, 5.52%; N, 4.27%; S, 8.96%.

**1-[5-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-pentyl]-1*H*-indole-2,3-dione, 3.7i (RHn5O)**



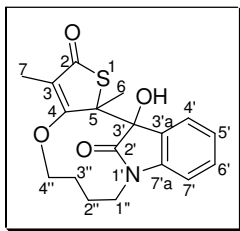
Orange solid (217 mg, 22%); m.p. 77-79°C;  $R_f$  (EtOAc:Hex 1:1) 0.36;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  1732 ( $\text{C}_3=\text{O}$ ), 1670 ( $\text{C}_2''=\text{O}$ ), 1620 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.59 (2H, m, H-6 and H-4), 7.12 (1H, td,  $J$  0.9 and 7.6, H-5), 6.89 (1H, d,  $J$  8, H-7), 4.30 (1H, m, H-5'a/b), 4.13 (2H, m, H-5'a/b and H-5''), 3.77 (2H, t,  $J$  7.2, H-1'), 1.80 (7H, m, H-7'', H-2' and H-4'), 1.54, (5H, m, H-6'' and H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.4, 183.3, 177.9, 158.2, 150.8, 138.3, 125.6, 123.8, 117.6, 114.3, 110.0, 70.8, 41.9, 39.8, 29.4, 26.9, 23.1, 19.8, 9.0; HRMS(EI) found  $m/z$  359.11842 for  $\text{C}_{19}\text{H}_{21}\text{O}_4\text{NS}$  requires 359.11913; Anal. Calc. for  $\text{C}_{19}\text{H}_{21}\text{O}_4\text{NS}$ : C, 63.49%; H, 5.89%; N, 3.90%; S, 8.92%. Found: C, 63.43%; H, 5.94%; N, 3.40%; S, 7.86%.

**1-[6-(2,4-Dimethyl-5-oxo-2,5-dihydro-thiophen-3-yloxy)-hexyl]-1*H*-indole-2,3-dione, 3.7j (RHn6O)**

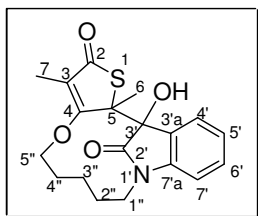


Orange oil (154 mg, 15%);  $R_f$  (EtOAc:Hex 1:1) 0.31;  $IR_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  1739 ( $\text{C}_3=\text{O}$ ), 1675 ( $\text{C}_2''=\text{O}$ ), 1611 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.57 (2H, m, H-4 and H-6), 7.09 (1H, td,  $J$  0.9 and 7.4, H-5), 6.88 (1H, d,  $J$  7.8, H-7), 4.27 (1H, m, H-6'a/b), 4.13 (2H, m, H-6'a/b and H-5''), 3.72 (2H, t,  $J$  7.5, H-1'), 1.79 (3H, d,  $J$  0.9, H-7''), 1.76-1.68 (4H, m, H-2' and H-5'), 1.54 (3H, d,  $J$  6.6, H-6''), 1.41-1.50 (4H, m, H-3' and H-4');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.7, 183.4, 178.0, 158.1, 150.8, 138.2, 125.4, 123.6, 117.5, 114.0, 110.0, 71.0, 41.9, 39.9, 29.7, 27.1, 26.4, 25.3, 19.8, 9.0; HRMS(EI) found  $m/z$  373.1346 for  $\text{C}_{20}\text{H}_{23}\text{O}_4\text{NS}$  requires 373.13478; Anal. Calc. for  $\text{C}_{20}\text{H}_{23}\text{O}_4\text{NS}$ : C, 64.32%; H, 6.21%; N, 3.75%; S, 8.59%. Found: C, 63.64%; H, 6.46%; N, 3.65%; S, 7.24%.

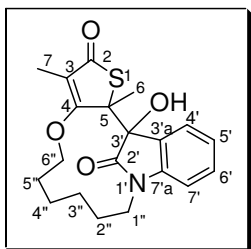
The conditions employed for the preparation of compound **3.8a-p** are those described in general procedure B. However, the reaction duration for compounds **3.8d-p** was increased from 48 hours to 120 hours.

**Compound 3.8a (RHn4Y)**

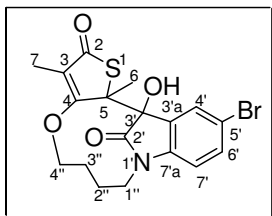
Yellow crystalline solid (333 mg, 35%); m.p. 184-185°C;  $R_f$  (EtOAc:Hex 1:1) 0.29;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3311 (OH), 1700 ( $\text{C}_2=\text{O}$ ), 1620 ( $\text{C}_2=\text{O}$ ), 1468 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.80 (1H, dd,  $J$  1.2 and 7.6, H-4'), 7.28 (1H, td,  $J$  1.2 and 8.0, H-6'), 6.96 (1H, td,  $J$  0.8 and 7.6, H-5'), 6.78 (1H, d,  $J$  8.0, H-7'), 4.59 (1H, m, H-4''a/b), 4.29 (1H, m, H-1''a/b), 3.55 (1H, m, H-4''a/b), 3.38 (1H, dt,  $J$  3.6 and 14.4, H-1''a/b), 3.35 (1H, s, OH), 2.08-1.86 (4H, m, H-2'' and H-3''), 1.99 (3H, s, H-7), 1.60 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.3, 176.3, 175.4, 141.7, 130.3, 127.5, 125.8, 122.5, 110.9, 108.4, 78.8, 74.4, 66.1, 39.4, 28.3, 26.7, 19.5, 9.3; HRMS(EI) found  $m/z$  345.1030 for  $\text{C}_{18}\text{H}_{19}\text{O}_4\text{NS}$  requires 345.10348; Anal Calc. for  $\text{C}_{18}\text{H}_{19}\text{O}_4\text{NS}$ : C, 62.59%; H, 5.54%; N, 4.06%; S, 9.28%. Found: C, 62.48%; H, 5.09%; N, 3.70%; S, 9.01%.

**Compound 3.8b (RHn5Y)**

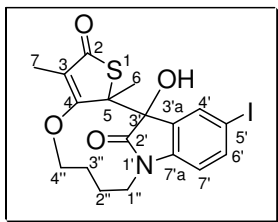
Light yellow crystalline solid (85.3 mg, 9%); m.p. 214-215°C;  $R_f$  (EtOAc:Hex 1:1) 0.38;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3380 (OH), 1695 ( $\text{C}_2=\text{O}$ ), 1622 ( $\text{C}_2=\text{O}$ ), 1471 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.80 (1H, dd,  $J$  1.2 and 7.6, H-4'), 7.28 (1H, td,  $J$  1.2 and 8.0, H-6'), 6.96 (1H, td,  $J$  0.8 and 7.6, H-5'), 6.78 (1H, d,  $J$  8.0, H-7'), 4.59 (1H, m, H-5''a/b), 4.29 (1H, m, H-1''a/b), 3.45 (1H, m, H-5''a/b), 3.29 (1H, br. s, OH), 3.26 (1H, dt,  $J$  3.2 and 14.0, H-1''a/b), 1.96 (3H, s, H-7), 1.91-1.72 (4H, m, H-2'' and H-4''), 1.60 (3H, s, H-6), 1.44 (2H, m, H-3'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.7, 176.3, 175.2, 142.3, 130.5, 127.8, 125.4, 122.9, 110.8, 107.9, 78.3, 67.7, 66.0, 40.8, 28.5, 23.2, 19.7, 19.5, 9.7; HRMS(EI) found  $m/z$  359.1186 for  $\text{C}_{19}\text{H}_{21}\text{O}_4\text{NS}$  requires 359.11913; Anal Calc. for  $\text{C}_{19}\text{H}_{21}\text{O}_4\text{NS}$ : C, 63.49%; H, 5.89%; N, 3.90%; S, 8.92%. Found: C, 63.02%; H, 6.08%; N, 2.98%; S, 9.05%.

**Compound 3.8c** (RHn6Y)

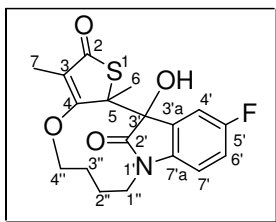
Yellow crystalline solid (61.4 mg, 6%); m.p. 198°C;  $R_f$  (EtOAc:Hex 1:1) 0.40; IR  $\nu_{\max}$ (KBr)/ $\text{cm}^{-1}$  3412 (OH), 1697 ( $\text{C}_2=\text{O}$ ), 1630 ( $\text{C}_2=\text{O}$ ), 1471 (Ar C=C);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.86 (1H, dd,  $J$  1.5 and 7.8, H-4'), 7.28 (1H, td,  $J$  1.2 and 7.8, H-6'), 6.94 (1H, td,  $J$  0.9 and 7.8, H-5'), 6.83 (1H, d,  $J$  7.8, H-7'), 4.26-4.10 (3H, m, H-1''a/b and H-6''), 3.28 (1H, m, H-1''a/b), 3.18 (1H, br. s, OH), 2.04 (3H, s, H-7), 1.98-1.81 (3H, m, H-2''a/b and H-5''), 1.67 (3H, s, H-6), 1.62-1.30 (5H, m, H-2''a/b, H-3'' and H-4'');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.6, 176.2, 175.2, 144.0, 130.5, 127.2, 125.1, 122.6, 111.7, 108.4, 77.4, 72.5, 65.4, 39.2, 26.0, 24.1, 23.8, 22.6, 21.2, 9.7; HRMS(EI) found  $m/z$  373.1341 for  $\text{C}_{20}\text{H}_{23}\text{O}_4\text{NS}$  requires 373.13478; Anal Calc. for  $\text{C}_{20}\text{H}_{23}\text{O}_4\text{NS}$ : C, 64.32%; H, 6.21%; N, 3.75%; S, 8.59%. Found: C, 64.31%; H, 6.13%; N, 3.70%; S, 8.66%.

**Compound 3.8d** (RBrn4Y)

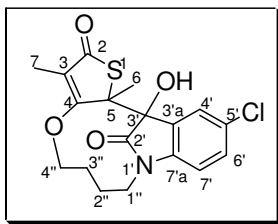
Off-white amorphous powder (158 mg, 14%); m. p. 156-157°C;  $R_f$  (EtOAc:Hex, 1:1) 0.27; IR  $\nu_{\max}$ (KBr)/ $\text{cm}^{-1}$  3416 (OH), 1705 (ester C=O), 1618 (amidic C=O), 1482 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.93 (1H, d,  $J$  1.8, H-4'), 7.43 (1H, dd,  $J$  2.1 and 8.4, H-6'), 6.67 (1H, d,  $J$  8.4, H-7'), 4.63 (1H, m, H-4''a/b), 4.32 (1H, m, H-1''a/b), 3.59 (1H, m, H-4''a/b), 3.40 (1H, dt,  $J$  3.6 and 14.4, H-1''a/b), 3.18 (1H, br. s, OH), 2.08-1.87 (4H, m, H-2'' and H-3''), 2.00 (3H, s, H-7), 1.68 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.4, 185.1, 175.0, 156.0, 133.2, 129.4, 129.2, 115.2, 111.1, 109.7, 78.7, 74.5, 65.9, 39.5, 28.3, 26.7, 19.8, 9.5; HRMS(ESI) found  $m/z$  424.02123  $[\text{M}+\text{H}]^+$  for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSBr}$  requires 423.01399; Anal Calc. for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSBr}$ : C, 50.95%; H, 4.28%; N, 3.30%; S, 7.56%. Found: C, 50.36%; H, 4.45%; N, 2.96%; S, 7.77%.

**Compound 3.8e** (RIn4Y)

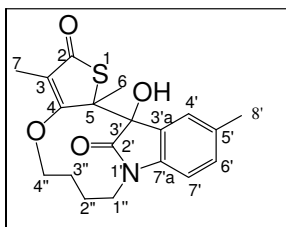
Light yellow solid (188 mg, 15%); m.p. 205-206°C;  $R_f$  (EtOAc:Hex 1:1) 0.29;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3439 (OH), 1715 ( $\text{C}_2=\text{O}$ ), 1631 ( $\text{C}_2=\text{O}$ ), 1480 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 8.09 (1H, d,  $J$  2.0, H-4'), 7.62 (1H, dd,  $J$  2.0 and 8.4, H-6'), 6.58 (1H, d,  $J$  8.4, H-7'), 4.62 (1H, m, H-4''a/b), 4.27 (1H, m, H-1''a/b), 3.55 (1H, m, H-4''a/b), 3.48 (1H, br. s, OH), 3.36 (1H, dt,  $J$  3.2 and 14.8, H-1''a/b), 2.07-1.84 (4H, m, H-2'' and H-3''), 1.97 (3H, s, H-7), 1.66 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.4, 175.8, 175.1, 141.4, 139.1, 134.6, 129.8, 111.1, 110.3, 84.8, 78.7, 74.5, 65.9, 39.5, 28.3, 26.7, 19.8, 9.5; HRMS(ESI) found  $m/z$  472.00759  $[\text{M}+\text{H}]^+$  for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSI}$  requires 471.00013; Anal Calc. for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSI}$ : C, 45.87%; H, 3.85%; N, 2.97%; S, 6.80%. Found: C, 45.62%; H, 3.34%; N, 2.06%; S, 6.66%.

**Compound 3.8f** (RFn4Y)

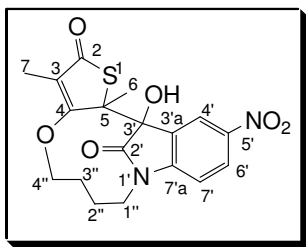
Light yellow solid (95.2 mg, 10%); m.p. 157°C;  $R_f$  (EtOAc:Hex 1:1) 0.26;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3306 (OH), 1701 ( $\text{C}_2=\text{O}$ ), 1625 ( $\text{C}_2=\text{O}$ ), 1489 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.59 (1H, dd,  $J$  2.8 and 8.0, H-4'), 7.01 (1H, ddd,  $J$  2.4, 8.6 and 8.8, H-6'), 6.73 (1H, dd,  $J$  4.0 and 8.4, H-7'), 4.63 (1H, m, H-4''a/b), 4.31 (1H, m, H-1''a/b), 3.57 (1H, m, H-4''a/b), 3.45 (1H, br. s, OH), 3.39 (1H, dt,  $J$  3.2 and 14.4, H-1''a/b), 2.09-1.83 (4H, m, H-2'' and H-3''), 1.99 (3H, s, H-7), 1.66 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.4, 177.2, 175.1, 159.8, 147.1, 137.6, 116.7, 114.6, 111.0, 108.9, 78.8, 74.5, 66.0, 39.6, 28.4, 26.7, 19.7, 9.4; HRMS(ESI) found  $m/z$  364.10153  $[\text{M}+\text{H}]^+$  for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSF}$  requires 363.09406; Anal Calc. for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSF}$ : C, 59.49%; H, 4.99%; N, 3.85%; S, 8.82%. Found: C, 59.37%; H, 4.88%; N, 3.21%; S, 8.72%.

**Compound 3.8g (RCl<sub>n</sub>4Y)**

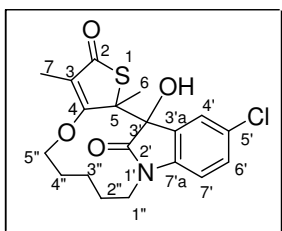
Light yellow solid (151 mg, 14%); m.p. 208-210°C;  $R_f$  (EtOAc:Hex 1:1) 0.24;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3415 (OH), 1707 ( $\text{C}_2=\text{O}$ ), 1618 ( $\text{C}_2=\text{O}$ ), 1484 (Ar C=C);  $\delta_{\text{H}}$  (400MHz, DMSO- $d_6$ ) 7.54 (1H, d,  $J$  2.4, H-4'), 7.37 (1H, dd,  $J$  2.4 and 8.8, H-6'), 7.04 (1H, d,  $J$  8.4, H-7'), 6.86 (1H, br. s, OH), 4.65 (1H, br. dd,  $J$  4.0 and 10.0, H-4''a/b), 4.07 (1H, m, H-1''a/b), 3.53 (1H, m, H-4''a/b), 3.46 (1H, m, H-1''a/b), 1.98-1.59 (4H, m, H-2'' and H-3''), 1.89 (3H, s, H-7), 1.56 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz, DMSO- $d_6$ ) 193.8, 175.8, 175.1, 140.6, 130.3, 129.6, 125.1, 124.8, 110.2, 109.8, 77.7, 74.7, 65.6, 48.5, 27.8, 25.9, 19.8, 8.8; HRMS(ESI) found  $m/z$  380.07214  $[\text{M}+\text{H}]^+$  for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSCl}$  requires 379.06451; Anal Calc. for  $\text{C}_{18}\text{H}_{18}\text{O}_4\text{NSCl}$ : C, 56.91%; H, 4.78%; N, 3.69%; S, 8.44%. Found: C, 55.91%; H, 4.68%; N, 3.49%; S, 8.37%.

**Compound 3.8h (RCH<sub>3n</sub>4Y)**

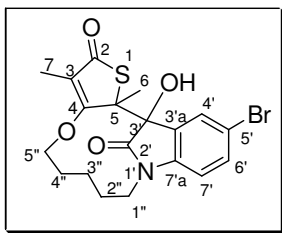
Light yellow solid (55.5 mg, 6%); m.p. 170-173°C;  $R_f$  (EtOAc:Hex 1:1) 0.31;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3377 (OH), 1700 ( $\text{C}_2=\text{O}$ ), 1613 ( $\text{C}_2=\text{O}$ ), 1494 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.63 (1H, d,  $J$  1.6, H-4'), 7.09 (1H, dd,  $J$  1.6 and 7.6, H-6'), 6.68 (1H, d,  $J$  8, H-7'), 4.61 (1H, m, H-4''a/b), 4.28 (1H, m, H-1''a/b), 3.56 (1H, m, H-4''a/b), 3.38 (1H, dt,  $J$  3.6 and 14.4, H-1''a/b), 3.34 (1H, br. s, OH), 2.28 (3H, s, H-8'), 2.08-1.85 (4H, m, H-2'' and H-3''), 2.00 (3H, s, H-7), 1.63 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 193.4, 176.3, 175.5, 139.2, 132.1, 130.5, 127.5, 126.8, 111.0, 108.1, 78.9, 74.5, 66.1, 39.4, 28.4, 26.8, 21.2, 19.6, 9.4; HRMS(ESI) found  $m/z$  360.12638  $[\text{M}+\text{H}]^+$  for  $\text{C}_{19}\text{H}_{21}\text{O}_4\text{NS}$  requires 359.11913; Anal Calc. for  $\text{C}_{19}\text{H}_{21}\text{O}_4\text{NS}$ : C, 63.49%; H, 5.89%; N, 3.90%; S, 8.92%. Found: C, 63.45%; H, 5.81%; N, 3.70%; S, 9.17%.

**Compound 3.8i** (RNO<sub>2</sub>n4Y)

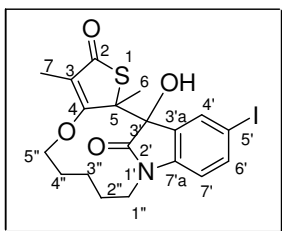
Light yellow crystalline solid (83.5 mg, 8%); m.p. 215-217°C;  $R_f$  (EtOAc:Hex 1:1) 0.14;  $IR_{\text{vmax}}(\text{KBr})/\text{cm}^{-1}$  3293 (OH), 1739 ( $\text{C}_2=\text{O}$ ), 1605 ( $\text{C}_2=\text{O}$ ), 1523 (Ar C=C), 1335/1523 (Asym./sym.  $\text{NO}_2$  str.);  $\delta_{\text{H}}$  (400MHz, DMSO- $d_6$ ) 7.54 (1H, d,  $J$  2.4, H-4'), 7.37 (1H, dd,  $J$  2.4 and 8.8, H-6'), 7.04 (1H, d,  $J$  8.4, H-7'), 6.86 (1H, br. s, OH), 4.65 (1H, m, H-4''a/b), 4.07 (1H, m, H-1''a/b), 3.53 (1H, m, H-4''a/b), 3.46 (1H, m, H-1''a/b), 2.01-1.61 (4H, m, H-2'' and H-3''), 1.89 (3H, s, H-7), 1.56 (3H, s, H-6);  $\delta_{\text{C}}$  (100MHz, DMSO- $d_6$ ) 193.4, 175.9, 175.3, 148.0, 141.3, 129.1, 127.0, 119.6, 109.9, 109.1, 77.2, 74.8, 65.5, 39.3, 27.8, 26.0, 19.8, 8.8; HRMS(ESI) found  $m/z$  391.09609  $[\text{M}+\text{H}]^+$  for  $\text{C}_{18}\text{H}_{18}\text{O}_6\text{N}_2\text{S}$  requires 390.08856; Anal Calc. for  $\text{C}_{18}\text{H}_{18}\text{O}_6\text{N}_2\text{S}$ : C, 55.38%; H, 4.86%; N, 7.18%; S, 8.21%. Found: C, 55.74%; H, 4.86%; N, 6.29%; S, 8.45%.

**Compound 3.8j** (RHH145A)

Light yellow solid (57.8 mg, 5%); m.p. 200-201°C;  $R_f$  (EtOAc:Hex 2:3) 0.32;  $IR_{\text{vmax}}(\text{KBr})/\text{cm}^{-1}$  3287 (OH), 1701 ( $\text{C}_2=\text{O}$ ), 1607 ( $\text{C}_2=\text{O}$ ), 1482 (Ar C=C);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.85 (1H, d,  $J$  2.1, H-4'), 7.29 (1H, dd,  $J$  2.4 and 8.4, H-6'), 6.74 (1H, d,  $J$  8.1, H-7'), 4.51 (1H, m, H-5''a/b), 4.17 (1H, m, H-1''a/b), 3.50 (1H, m, H-5''a/b) 3.33 (1H, br. s, OH), 3.26 (1H, dt,  $J$  3.0 and 14.1, H-1''a/b), 1.98 (3H, s, H-7), 1.96-1.71 (4H, m, H-2'' and H-4''), 1.68 (3H, s, H-6), 1.45 (2H, m, H-3'');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 194.9, 175.7, 174.8, 140.9, 130.4, 129.4, 128.4, 126.0, 112.1, 108.7, 78.2, 67.8, 65.8, 41.0, 28.4, 23.1, 19.8, 19.5, 9.8; HRMS(ESI) found  $m/z$  394.08725  $[\text{M}+\text{H}]^+$  for  $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{SCl}$  requires 393.08016; Anal Calc. for  $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{SCl}$ : C, 57.94%; H, 5.12%; N, 3.56%; S, 8.14%. Found: C, 57.45%; H, 5.10%; N, 3.39%; S, 8.04%.

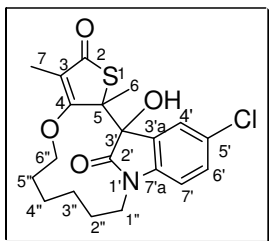
**Compound 3.8k** (RHH145D)

Light yellow solid (88.6 mg, 7%); m.p. 203-205°C;  $R_f$  (EtOAc:Hex 2:3) 0.34;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3321 (OH), 1706 ( $\text{C}_2=\text{O}$ ), 1604 ( $\text{C}_2=\text{O}$ ), 1476 (Ar C=C);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.98 (1H, d,  $J$  2.4, H-4'), 7.44 (1H, dd,  $J$  1.8 and 8.1, H-6'), 6.69 (1H, d,  $J$  8.1, H-7'), 4.50 (1H, m, H-5''a/b), 4.15 (1H, m, H-1''a/b), 3.46-3.54 (2H, m, H-5''a/b and OH), 3.25 (1H, dt,  $J$  3.0 and 14.0, H-1''a/b), 1.97 (3H, s, H-7), 1.95-1.71 (4H, m, H-2'' and H-4''), 1.68 (3H, s, H-6), 1.46 (2H, m, H-3'');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 194.8, 175.9, 174.8, 141.4, 133.3, 129.7, 128.6, 115.5, 112.1, 109.2, 78.2, 67.8, 65.8, 40.9, 28.4, 23.1, 19.8, 19.5, 9.8; HRMS(ESI) found  $m/z$  438.03618  $[\text{M}+\text{H}]^+$  for  $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{SBr}$  requires 437.02964; Anal Calc. for  $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{SBr}$ : C, 52.06%; H, 4.60%; N, 3.20%; S, 7.32%. Found: C, 51.88%; H, 4.11%; N, 3.01%; S, 7.22%.

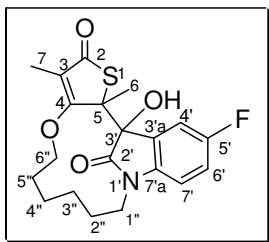
**Compound 3.8l** (RHH145E)

Light yellow solid (47.8 mg, 4%); m.p. 189-192°C;  $R_f$  (EtOAc:Hex 2:3) 0.35;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3378 (OH), 1709 ( $\text{C}_2=\text{O}$ ), 1601 ( $\text{C}_2=\text{O}$ ), 1482 (Ar C=C);  $\delta_{\text{H}}$  (300MHz,  $\text{DMSO-d}_6$ ) 7.90 (1H, d,  $J$  1.8, H-4'), 7.41 (1H, dd,  $J$  1.8 and 8.1, H-6'), 6.41 (1H, d,  $J$  8.4, H-7'), 6.17 (1H, br. s, OH), 4.31 (1H, m, H-5''a/b), 3.96 (1H, m, H-1''a/b), 3.30 (1H, m, H-5''a/b), 3.04 (1H, dt,  $J$  2.7 and 14.4, H-1''a/b), 1.82 (3H, s, H-7), 1.80-1.55 (4H, m, H-2'' and H-4''), 1.48 (3H, s, H-6), 1.25 (2H, m, H-3'');  $\delta_{\text{C}}$  (75MHz,  $\text{DMSO-d}_6$ ) 195.0, 175.2, 174.8, 141.7, 138.2, 133.5, 130.6, 111.3, 109.2, 83.9, 78.6, 67.3, 65.7, 40.1, 27.9, 22.6, 19.6, 19.0, 9.3; HRMS(ESI) found  $m/z$  486.02280  $[\text{M}+\text{H}]^+$  for  $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{SI}$  requires 485.01577; Anal Calc. for  $\text{C}_{19}\text{H}_{20}\text{NO}_4\text{SI}$ : C, 47.02%; H, 4.15%; N, 2.89%; S, 6.61%. Found: C, 46.98%; H, 3.97%; N, 2.57%; S, 6.22%.

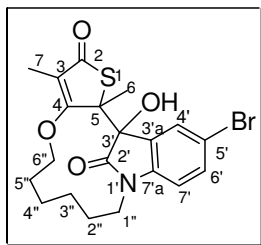


**Compound 3.8m** (RHH146A)

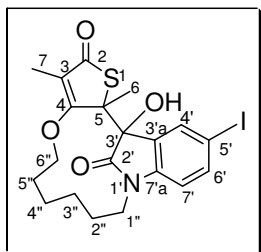
Yellow crystalline solid (65.5 mg, 6%); m.p. 214-215°C;  $R_f$  (EtOAc:Hex 1:1) 0.48;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3367 (OH), 1699 ( $\text{C}_2=\text{O}$ ), 1611 ( $\text{C}_2=\text{O}$ ), 1478 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.87 (1H, d,  $J$  2.4, H-4'), 7.28 (1H, dd,  $J$  2.4 and 8.4, H-6'), 6.78 (1H, d,  $J$  8.4, H-7'), 4.29-4.14 (3H, m, H-1''a/b and H-6''), 3.30 (1H, br. s, OH), 3.26 (1H, m, H-1''a/b), 2.05 (3H, s, H-7), 1.98-1.84 (3H, m, H-2''a/b and H-5''), 1.74 (3H, s, H-6), 1.64-1.30 (5H, m, H-2''a/b, H-3'' and H-4'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.8, 175.9, 175.0, 142.6, 130.5, 128.9, 125.7, 125.0, 112.0, 109.3, 77.4, 72.6, 65.2, 39.5, 26.0, 24.1, 23.7, 22.6, 21.5, 9.8; HRMS(EI) found  $m/z$  407.09528 for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{ClNS}$  requires 407.09581; Anal Calc. for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{ClNS}$ : C, 58.89%; H, 5.44%; N, 3.43%; S, 7.86%. Found: C, 58.85%; H, 5.42%; N, 3.38%; S, 7.89%

**Compound 3.8n** (RHH146C)

Light yellow crystalline solid (74.5 mg, 7%); m. p. 212-213°C;  $R_f$  (EtOAc:Hex 1:1) 0.45;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3358 (OH), 1699 ( $\text{C}_2=\text{O}$ ), 1614 ( $\text{C}_2=\text{O}$ ), 1491 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.40 (1H, dd,  $J$  2.8 and 8.8, H-4'), 6.78 (1H, ddd,  $J$  2.8, 8.4 and 8.8, H-6'), 6.57 (1H, dd,  $J$  4.0 and 8.4, H-7'), 4.08-3.97 (3H, m, H-1''a/b and H-6''), 3.06 (1H, m, H-1''a/b), 2.52 (1H, br. s, OH), 1.89 (3H, s, H-7), 1.78-1.66 (3H, m, H-2''a/b and H-5''), 1.54 (3H, s, H-6), 1.43-1.24 (5H, m, H-2''a/b, H-3'' and H-4'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.5, 176.3, 175.0, 159.4, 157.0, 139.9, 129.8, 116.0, 113.2, 111.2, 108.4, 76.5, 72.2, 65.4, 39.0, 25.8, 23.7, 23.5, 22.4, 21.6, 9.7; HRMS(EI) found  $m/z$  391.12484 for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{FNS}$  requires 391.12536; Anal Calc. for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{FNS}$ : C, 61.36%; H, 5.66%; N, 3.58%; S, 8.19%. Found: C, 60.92%; H, 5.74%; N, 3.24%; S, 7.98%.

**Compound 3.8o** (RHH146D)

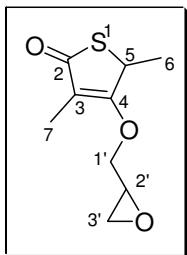
Yellow crystalline solid (68.9 mg, 6%); m.p. 211°C;  $R_f$  (EtOAc:Hex 1:1) 0.55;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3370 (OH), 1698 ( $\text{C}_2=\text{O}$ ), 1609 ( $\text{C}_2=\text{O}$ ), 1478 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 8.00 (1H, d,  $J$  2.0, H-4'), 7.44 (1H, dd,  $J$  2.0 and 8.4, H-6'), 6.73 (1H, br. d,  $J$  8.4, H-7'), 4.29-4.15 (3H, m, H-1''a/b and H-6''), 3.27 (2H, m, H-1''a/b and OH), 2.05 (3H, s, H-7), 1.96-1.85 (3H, m, H-2''a/b and H-5''), 1.75 (3H, s, H-6), 1.63-1.31 (5H, m, H-2''a/b, H-3'' and H-4'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.7, 175.8, 174.8, 143.1, 133.4, 129.2, 128.4, 115.3, 112.0, 109.8, 76.7, 72.6, 65.2, 39.4, 26.0, 24.1, 23.7, 22.6, 21.5, 9.9; HRMS(EI) found  $m/z$  451.04475 for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{BrNS}$  requires 451.04529; Anal Calc. for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{BrNS}$ : C, 53.10%; H, 4.90%; N, 3.10%; S, 7.09%. Found: C, 53.19%; H, 4.89%; N, 2.94%; S, 6.97%.

**Compound 3.8p** (RHH146E)

Yellow crystalline solid (90.8 mg, 7%); m.p. 214-215°C;  $R_f$  (EtOAc:Hex 1:1) 0.45;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3365 (OH), 1697 ( $\text{C}_2=\text{O}$ ), 1604 ( $\text{C}_2=\text{O}$ ), 1481 (Ar  $\text{C}=\text{C}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.99 (1H, d,  $J$  1.6, H-4'), 7.46 (1H, dd,  $J$  2.0 and 8.0, H-6'), 6.49 (1H, d,  $J$  8.0, H-7'), 4.15-4.02 (3H, m, H-1''a/b and H-6''), 3.10 (1H, m, H-1''a/b), 2.40 (1H, br. s, OH), 1.94 (3H, s, H-7), 1.84-1.70 (3H, m, H-2''a/b and H-5''), 1.61 (3H, s, H-6), 1.51-1.15 (5H, m, H-2''a/b, H-3'' and H-4'');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.1, 176.0, 174.4, 143.5, 138.5, 133.5, 130.2, 111.2, 109.8, 83.9, 76.2, 72.1, 65.2, 38.8, 25.6, 23.7, 23.3, 22.2, 21.3, 9.5; HRMS(EI) found  $m/z$  499.03088 for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{INS}$  requires 499.03142; Anal Calc. for  $\text{C}_{20}\text{H}_{22}\text{O}_4\text{INS}$ : C, 48.10%; H, 4.44%; N, 2.80%; S, 6.42%. Found: C, 48.14%; H, 4.50%; N, 2.69%; S, 6.23%.

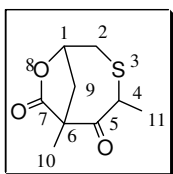
## 7.3. Experimental details pertaining to Chapters 4 and 5

## 3,5-Dimethyl-4-oxiranylmethoxy-5H-thiophen-2-one, 4.7a



The potassium salt of thiolactone **3.5** (1.00 g, 5.48 mmol, 1.0 eq) was added to 5 mL of ( $\pm$ )-epichlorohydrin. The reaction mixture was refluxed under nitrogen at 130°C for 7 hours. The product mixture was then cooled to room temperature and filtered over a celite pad. The filtrate was concentrated and the residue purified using column chromatography (eluent EtOAc:Hex 2:3) to afford **4.7a** as a colourless oil (823.0 mg, 75%);  $R_f$  (EtOAc:Hex 2:3) 0.58;  $IR_{\text{max}}(\text{film})/\text{cm}^{-1}$  1677 (C=O), 1633 (C=C-O vinyl ether);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) (50:50 diastereomeric mixture) 4.56/4.47 (1H, dd,  $J$  2.8 and 11.2, H-1'a/b), 4.21/4.19 (1H, m, H-5), 4.17/4.06 (1H, dd,  $J$  6.4 and 11.2, H-1'a/b), 3.27 (1H, m, H-2'), 2.89/2.88 (1H, dd,  $J$  1.6 and 4.0, H-3'a/b), 2.71/2.70, (1H, dd,  $J$  2.4 and 4.4, H-3'a/b), 1.83 (3H, d,  $J$  1.2, H-7), 1.58/1.57 (3H, d,  $J$  6.8, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.7, 177.4 (177.5), 115.3 (115.7), 71.3 (71.7), 50.0 (50.1), 44.1, 41.8 (41.9), 19.6, 8.9. Chemical shift values given in brackets represent those of the other diastereomer; HRMS (ESI) found  $m/z$  201.05809  $[\text{M}+\text{H}]^+$  for  $\text{C}_9\text{H}_{12}\text{O}_3\text{S}$  requires 200.05071; Anal. Calc. for  $\text{C}_9\text{H}_{12}\text{O}_3\text{S}$ : C, 53.98%; H, 6.04%; S, 16.01%. Found: C, 53.88%; H, 7.22%; S, 15.02%.

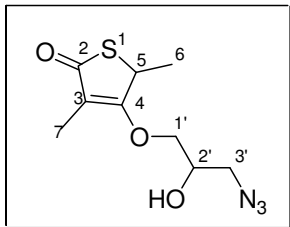
## 4,6-Dimethyl-8-oxa-3-thiabicyclo[4.2.1]nonane-5,7-dione, 4.7b



The potassium salt of thiolactone **3.5** (1.00g, 5.48 mmol, 1.0 eq) was added to 5 mL of ( $\pm$ )-epichlorohydrin. The reaction mixture was refluxed under nitrogen at 130°C for 7 hours. The product mixture was then cooled to room temperature and filtered over a celite pad. The filtrate was concentrated and the residue purified using column chromatography (eluent EtOAc:Hex 3:2) to afford the by-product **4.7b** as a colourless crystalline solid (109.7 mg, 10%); m.p. 164°C;  $R_f$  (EtOAc:Hex 2:3) 0.14;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  1762 (C<sub>7</sub>=O), 1699 (C<sub>5</sub>=O), 1350 (C<sub>1</sub>-O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 5.14 (1H, dd,  $J$  4.8 and 8.8, H-1), 3.92 (1H, q,  $J$  6.8, H-4), 3.23 (1H, dd,  $J$  1.2 and 14.4, H-2a/b), 3.03 (1H, ddd,  $J$  1.2, 4.8 and 14.8, H-2a/b), 2.62 (1H, br. d,  $J$  14.4, H-9a/b), 2.40 (1H, ddd,  $J$  1.6, 8.8 and 14.4, H-9a/b), 1.49 (3H, s, H-10), 1.34 (3H, d,  $J$  6.8, H-11);  $\delta_{\text{C}}$ (100MHz,  $\text{CDCl}_3$ ) 199.1, 175.0, 74.4, 52.9, 45.0, 39.1, 39.0, 23.5, 15.3; HRMS(ESI) found  $m/z$  201.05803

$[M+H]^+$  for  $C_9H_{12}O_3S$  requires 200.05071; Anal. Calc. for  $C_9H_{12}O_3S$ : C, 53.98%; H, 6.04%; S, 16.01%. Found: C, 53.99%; H, 6.03%; S, 16.10%.

#### 4-(3-Azido-2-hydroxy-propoxy)-3,5-dimethyl-5H-thiophen-2-one, 4.8

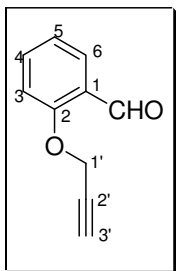


Sodium azide (34.1 mmol, 5.0 eq) was added to a stirred solution of the thiolactone epoxide **4.7a** (6.81 mmol, 1.0 eq) and  $NH_4Cl$  (20.4 mmol, 3.0 eq) in 10 mL of MeOH:H<sub>2</sub>O (8:1). The resulting mixture was stirred at 25°C for 48 hours. The reaction mixture

was then diluted with water, extracted with Et<sub>2</sub>O and the combined organic layer washed with water, brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Evaporation of the solvent under reduced pressure followed by purification with column chromatography (eluent EtOAc:Hex 3:2) afforded a colourless oil (1.38 g, 83%);  $R_f$  (MeOH:DCM 1:39) 0.28; IR $_{\max}$ (film)/cm<sup>-1</sup> 3428 (OH), 2103 (N<sub>3</sub>), 1626 (C<sub>2</sub>=O);  $\delta_H$  (300MHz, CDCl<sub>3</sub>) 4.33-4.16 (3H, m, H-1' and H-5), 4.08 (1H, m, H-2'), 3.48 (2H, m, H-3'), 3.22 (1H, br. s, OH), 1.79 (3H, d,  $J$  1.8, H-7), 1.57 (3H, d,  $J$  6.9, H-6);  $\delta_C$  (75MHz, CDCl<sub>3</sub>) 196.3, 177.5, 115.3, 71.7, 69.3, 53.0, 41.8, 19.6, 8.8; LRMS(EI) found  $m/z$  242.9 for  $C_9H_{13}N_3O_3S$  requires 243.0; Anal. Calc. for  $C_9H_{13}N_3O_3S$ : C, 44.43%; H, 5.39%; N, 17.27%; S, 13.18%. Found C, 44.37%; H, 5.28%; N, 16.99%; S, 13.00%.

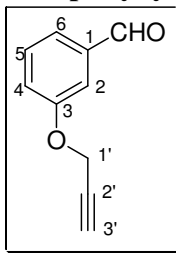
#### C. General procedure for preparing compounds 4.9a-c

Anhydrous K<sub>2</sub>CO<sub>3</sub> (1.70 g, 12.3 mmol, 1.5 eq) was added to hydroxybenzaldehyde (1.0 g, 8.19 mmol, 1.0 eq) dissolved in 5 mL anhydrous DMF. Propargyl bromide, 80% in toluene, (1.17 g, 9.83 mmol, 1.2 eq) was added to the mixture. The resulting mixture was stirred for 16 hours at 25°C. After consumption of the starting material, as indicated by tlc, ice-cold water was added to the reaction mixture. The precipitate so obtained was filtered, washed with water and recrystallized from MeOH to afford the pure product in excellent yield. If no precipitate formed upon addition of ice-cold water, the product mixture was extracted with EtOAc and the combined organic layer washed with water to remove DMF. This was followed with the drying of the resulting organic layer over anhydrous Na<sub>2</sub>SO<sub>4</sub> and the subsequent concentration thereof under reduced pressure. The crude was then subjected to column chromatography with EtOAc/Hexane 3:7, as eluent.

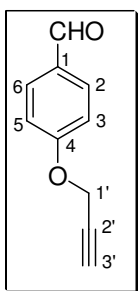
**2-Prop-2-ynyloxy-benzaldehyde, 4.9a**

77.6, 76.4, 56.4

Off-white crystalline solid (1.38 g, quant); m.p. 68°C;  $R_f$  (EtOAc:Hex 3:7) 0.72;  $IR_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C≡C), 1688 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 10.5 (1H, s, CHO), 7.85 (1H, dd,  $J$  2.0 and 7.6, H-6), 7.55 (1H, m, H-4), 7.12-7.05 (2H, m, H-3, H-5), 4.82 (2H, d,  $J$  2.4, H-1'), 2.56 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 189.4, 159.7, 135.6, 128.5, 125.5, 121.7, 113.2,

**3-Prop-2-ynyloxy-benzaldehyde, 4.9b**

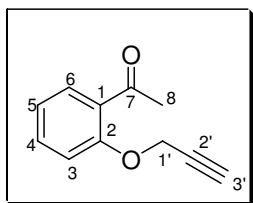
Yellow oil (1.37 g, quant);  $R_f$  (EtOAc:Hex 3:7) 0.66;  $IR_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C≡C), 1701 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 9.95 (1H, s, CHO), 7.49-7.42 (3H, m, H-2, H-5, H-6), 7.22 (1H, ddd,  $J$  2.4, 2.8 and 8.0, H-4), 4.73 (2H, d,  $J$  2.4, H-1'), 2.54 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 190.8, 158.1, 137.8, 130.2, 124.0, 122.0, 113.7, 78.0, 76.2, 56.0

**4-Prop-2-ynyloxy-benzaldehyde, 4.9c**

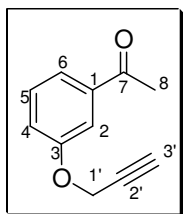
Off-white crystalline solid (1.20 g, 91%) m.p. 79-80°C;  $R_f$  (EtOAc:Hex 3:7) 0.59;  $IR_{\text{max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C≡C), 1691 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 9.90 (1H, s, CHO), 7.85 (2H, d,  $J$  8.7, H-2, H-6), 7.08 (2H, d,  $J$  8.7, H-3, H-5), 4.77 (2H, d,  $J$  2.4, H-1'), 2.56 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 190.7, 162.4, 131.8 (x2), 130.6, 115.2 (x2), 77.5, 76.3, 55.9

**D. General procedure for preparing compounds 4.12a-c**

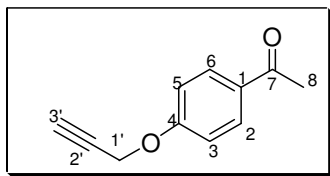
Anhydrous  $\text{K}_2\text{CO}_3$  (3.04 g, 22.0 mmol, 1.5 eq) was added to hydroxyacetophenone (2.0 g, 14.7 mmol, 1.0 eq) dissolved in 5 mL anhydrous DMF. Propargyl bromide, 80% in toluene, (2.62 g, 17.6 mmol, 1.2 eq) was added to the mixture. The resulting mixture was stirred for 24 hours at 25°C. Upon completion ice-cold water was added to the reaction mixture. The precipitate so obtained was filtered and washed with water. Recrystallization from MeOH afforded the pure product in excellent yield.

**1-(2-(Prop-2-ynoxy)phenyl)ethanone, 4.12a**

Off-white crystalline solid (2.45 g, quant); m.p. 38°C;  $R_f$  (EtOAc:Hex 3:7) 0.64;  $\delta_H$  (400MHz,  $CDCl_3$ ) 7.75 (1H, dd,  $J$  1.6 and 7.6, H-6), 7.48 (1H, m, H-4), 7.09-7.04 (2H, m, H-3, H-5), 4.81 (2H, d,  $J$  2.4, H-1'), 2.65 (3H, s, H-8), 2.56 (1H, t,  $J$  2.4, H-3');  $\delta_C$  (100MHz,  $CDCl_3$ ) 31.9, 56.3, 76.1, 77.9, 113.1, 121.6, 129.2, 130.5, 133.4, 156.8, 199.6

**1-(3-(Prop-2-ynoxy)phenyl)ethanone, 4.12b**

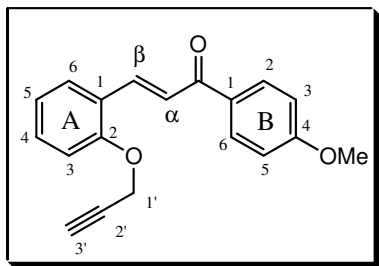
Off-white crystalline solid (2.41 g, quant); m.p. 29°C;  $R_f$  (EtOAc:Hex 3:7) 0.65;  $\delta_H$  (400MHz,  $CDCl_3$ ) 7.60-7.56 (2H, m, H-6 and H-2), 7.39 (1H, t,  $J$  8.4, H-5), 7.18 (1H, ddd,  $J$  2.4, 2.8 and 8.4, H-4), 4.74 (2H, d,  $J$  2.4, H-1'), 2.59 (3H, s, H-8), 2.55 (1H, t,  $J$  2.4, H-3');  $\delta_C$  (100MHz,  $CDCl_3$ ) 197.6, 157.8, 138.6, 129.7, 121.9, 120.3, 113.8, 78.1, 75.9, 56.0, 26.7

**1-(4-(Prop-2-ynoxy)phenyl)ethanone, 4.12c**

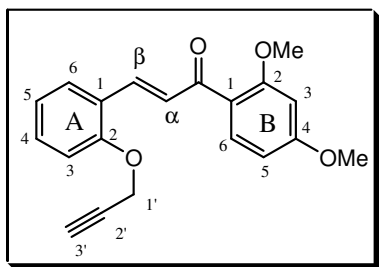
White crystalline solid (2.76 g, quant); m.p. 73°C;  $R_f$  (EtOAc:Hex 3:7) 0.58;  $\delta_H$  (400MHz,  $CDCl_3$ ) 7.95 (2H, d,  $J$  8.8, H-2, H-6), 7.02 (2H, d,  $J$  8.8, H-3, H-5), 4.76 (2H, d,  $J$  2.4, H-1'), 2.56 (4H, m, H-3', H-8);  $\delta_C$  (100MHz,  $CDCl_3$ ) 196.6, 161.3, 131.1, 130.5 (2xC), 114.6 (2xC), 77.8, 76.1, 55.9, 26.3

**E. General procedure for preparation of compounds 4.10 a-i**

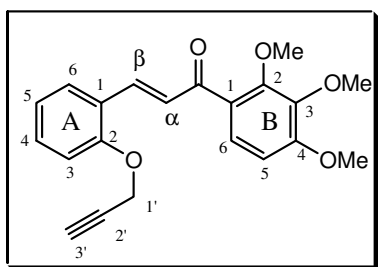
To a solution of the acetylenic aldehyde **4.9a-c** (6.25 mmol, 1.0 eq) in MeOH was added 5 mL of methanolic NaOH (3% w/v). The resulting mixture was stirred at 25°C for 30 minutes. A methanolic solution of the commercially available, substituted acetophenone (6.25 mmol, 1.0 eq) was added drop-wise and the mixture stirred overnight at the same temperature. A precipitate was formed which was filtered and washed with cold MeOH. Recrystallization from MeOH afforded the pure product. For the reactions in which no precipitation occurred, the product mixture was diluted with  $H_2O$ , neutralized with 10% HCl and extracted with EtOAc. The combined organic layer was dried and concentrated. The crude product was then recrystallized from MeOH.

**1-(4-Methoxy-phenyl)-3-(2-prop-2-ynyloxy-phenyl)-propenone, 4.10a**

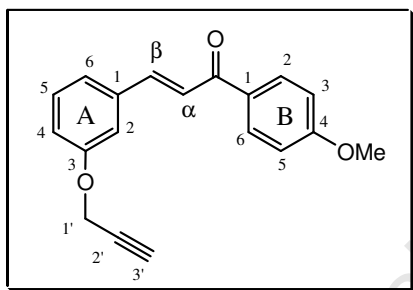
Light yellow amorphous solid (1.67 g, 91%); m. p. 78-79°C;  $R_f$  (EtOAc:Hex 1:4) 0.24;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 ( $\text{C}\equiv\text{C}$ ), 1657 ( $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 8.07 (1H, d,  $J$  16.2, H $\beta$ ), 8.04 (2H, d,  $J$  8.7, H-2B, H-6B), 7.67 (1H, d,  $J$  15.6, H $\alpha$ ), 7.64 (1H, dd,  $J$  1.5 and 7.2, H-6A), 7.36 (1H, m, H-4A), 7.05, (2H, m, H-3A, H-5A), 6.97 (2H, d,  $J$  8.7, H-3B, H-5B), 4.80 (2H, d,  $J$  2.4, H-1'), 3.88 (3H, s, OMe), 2.55 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 189.2, 163.3, 156.7, 139.1, 131.3, 131.2, 130.2 (x2), 129.5, 124.8, 123.3, 121.7, 113.7 (x2), 112.8, 78.2, 75.9, 55.2, 55.4; LRMS(ESI) found  $m/z$  293.1 (100%;  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{19}\text{H}_{16}\text{O}_3$  requires 292.1; Anal Calc. for  $\text{C}_{19}\text{H}_{16}\text{O}_3$ : C, 78.06%; H, 5.52%. Found: C, 77.66%; H, 5.52%

**1-(2,4-Dimethoxy-phenyl)-3-(2-prop-2-ynyloxy-phenyl)-propene, 4.10b**

Light yellow amorphous solid (1.76 g, 89%); m. p. 76°C;  $R_f$  (EtOAc:Hex 1:4) 0.16;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 ( $\text{C}\equiv\text{C}$ ), 1652 ( $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.99 (1H, d,  $J$  16, H $\beta$ ), 7.75 (1H, d,  $J$  8.4, H-6B), 7.62 (1H, dd,  $J$  1.6 and 7.6, H-6A), 7.55 (1H, d,  $J$  16, H $\alpha$ ), 7.34 (1H, m, H-4A), 7.06-7.00 (2H, m, H-3A, H-5A), 6.56 (1H, dd,  $J$  2.4 and 8.4, H-5B), 6.49 (1H, d,  $J$  2.4, H-3B), 4.77 (2H, d,  $J$  2.4, H-1'), 3.89 (3H, s, OMe), 3.86 (3H, s, OMe), 2.51 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 190.9, 164.0, 160.4, 156.5, 137.1, 132.8, 130.9, 128.7, 128.1, 125.2, 122.5, 121.6, 112.8, 105.1, 98.7, 78.3, 75.8, 56.1, 55.7, 55.5; LRMS(ESI) found  $m/z$  322.8 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{20}\text{H}_{18}\text{O}_4$  requires 322.1; Anal Calc. for  $\text{C}_{20}\text{H}_{18}\text{O}_4$ : C, 74.52%; H, 5.63%. Found: C, 74.36%; H, 5.62%

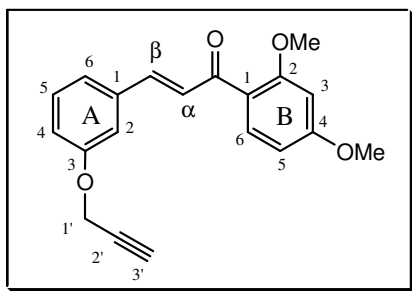
**3-(2-Prop-2-ynyloxy-phenyl)-1-(2,3,4-trimethoxy-phenyl)-propene, 4.10c**

Yellow oil (1.25 g, 94%);  $R_f$  (EtOAc:Hex 1:4) 0.22;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C $\equiv$ C), 1651 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 8.00 (1H, d,  $J$  15.9, H $\beta$ ), 7.65 (1H, dd,  $J$  2.1 and 7.8, H-6A), 7.52 (1H, d,  $J$  15.9, H $\alpha$ ), 7.47 (1H, d,  $J$  8.7, H-6B), 7.36, (1H, m, H-4A), 7.04 (2H, m, H-3A, H-5A), 6.74 (1H, d,  $J$  9.0, H-5B), 4.78 (2H, d,  $J$  2.4, H-1'), 3.89 (3H, s, OMe), 3.91 (3H, s, OMe), 3.92 (3H, s, OMe), 2.51 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 191.4, 156.9, 156.5, 153.7, 142.2, 138.1, 131.2, 128.8, 127.5, 127.1, 125.7, 124.9, 121.7, 112.9, 107.3, 78.3, 75.9, 62.1, 61.0, 56.2, 56.1; LRMS(ESI) found  $m/z$  353.0 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{21}\text{H}_{20}\text{O}_5$  requires 352.1; Anal Calc. for  $\text{C}_{21}\text{H}_{20}\text{O}_5$ : C, 71.58%; H, 5.72%. Found: C, 71.19%; H, 5.74%

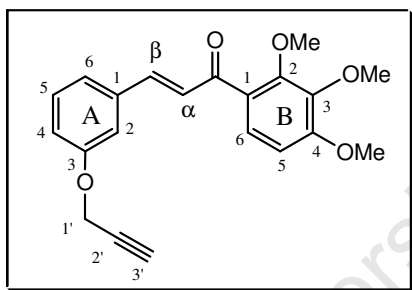
**1-(4-Methoxy-phenyl)-3-(3-prop-2-ynyloxy-phenyl)-propenone, 4.10d**

Light yellow amorphous solid (1.42 g, 78%); m.p. 68-69°C;  $R_f$  (EtOAc:Hex 1:4) 0.28;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C $\equiv$ C), 1660 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 8.03 (2H, d,  $J$  8.8, H-2B, H-6B), 7.72 (1H, d,  $J$  15.6, H $\beta$ ), 7.51 (1H, d,  $J$  15.6, H $\alpha$ ), 7.34 (1H, t,  $J$  8.0, H-5A), 7.28-7.23 (2H, m, H-2A, H-6A), 7.02 (1H, ddd,  $J$  2.4, 2.6 and 8.4, H-4A), 6.97 (2H, d,  $J$  8.8, H-3B, H-5B), 4.74 (2H, d,  $J$  2.4, H-1'), 3.88 (3H, s, OMe), 2.55 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 188.6, 163.5, 158.0, 143.6, 136.6, 131.1, 130.8 (x2), 129.9, 122.5, 121.8, 116.9, 114.6, 113.9 (x2), 78.3, 75.9, 56.0, 55.5; LRMS(ESI) found  $m/z$  293.1 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{19}\text{H}_{16}\text{O}_3$  requires 292.1; Anal Calc. for  $\text{C}_{19}\text{H}_{16}\text{O}_3$ : C, 78.06%; H, 5.52%. Found: C, 77.81%; H, 5.39%

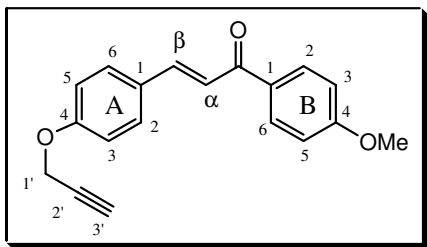


**1-(2,4-Dimethoxy-phenyl)-3-(3-prop-2-ynyloxy-phenyl)-propene, 4.10e**

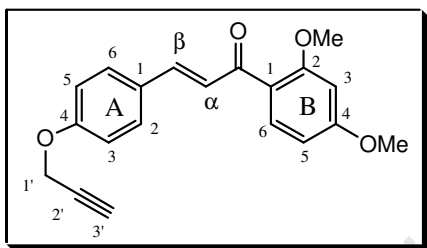
Light yellow amorphous solid (1.50 g, 76%); m.p. 72-73°C;  $R_f$  (EtOAc:Hex, 1:4) 0.18;  $IR_{\max}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C≡C), 1652 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.76, (1H, d,  $J$  8.8, H-6B), 7.63 (1H, d,  $J$  15.6, H $\beta$ ), 7.50 (1H, d,  $J$  15.6, H $\alpha$ ), 7.32 (1H, t,  $J$  8.0, H-5A), 7.22 (2H, m, H-2A, H-6A), 7.00 (1H, ddd,  $J$  2.4, 2.7 and 8.0, H-4A), 6.57 (1H, dd,  $J$  2.0 and 8.4, H-5B), 6.50 (1H, d,  $J$  2.4, H-3B), 4.73 (2H, d,  $J$  2.4, H-1'), 3.90 (3H, s, OMe), 3.87 (3H, s, OMe), 2.54 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 190.3, 164.3, 160.5, 157.9, 141.6, 137.1, 132.9, 129.8, 127.8, 122.2, 121.9, 116.6, 114.4, 105.3, 98.7, 78.4, 75.8, 55.9, 55.8, 55.6; LRMS(ESI) found  $m/z$  323.4 (59%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{20}\text{H}_{18}\text{O}_4$  requires 322.1; Anal Calc. for  $\text{C}_{20}\text{H}_{18}\text{O}_4$ : C, 74.52%; H, 5.63%. Found: C, 74.32%; H 5.56%

**3-(3-Prop-2-ynyloxy-phenyl)-1-(2,3,4-trimethoxy-phenyl)-propene, 4.10f**

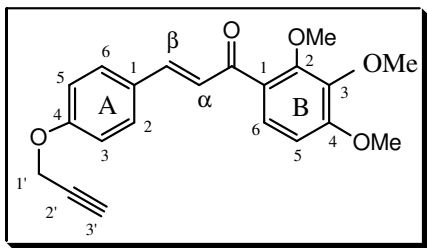
Light yellow amorphous solid, (998 mg, 76%); m.p. 61-62°C;  $R_f$  0.19 (EtOAc:Hex 1:4);  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  2106 (C≡C), 1654 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.63 (1H, d,  $J$  15.6, H $\beta$ ), 7.48 (1H, d,  $J$  9.0, H-6B), 7.47 (1H, d,  $J$  15.6, H $\alpha$ ), 7.32, (1H, t,  $J$  7.6, H-5A), 7.24-7.21 (2H, m, H-2A, H-6A), 7.00 (1H, ddd,  $J$  2.4, 2.8 and 8.0, H-4A), 6.75 (1H, d,  $J$  9.2, H-5B), 4.72 (2H, d,  $J$  2.0, H-1'), 3.91 (3H, s, OMe), 3.91 (3H, s, OMe), 3.89 (3H, s, OMe), 2.53 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 190.7, 157.9, 157.1, 153.8, 142.5, 142.2, 136.8, 129.9, 127.1, 126.7, 125.8, 121.9, 116.8, 114.4, 107.4, 78.3, 75.8, 62.1, 61.0, 56.1, 55.9; LRMS(ESI) found  $m/z$  353.0 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{20}\text{H}_{18}\text{O}_4$  requires 352.1; Anal Calc. for  $\text{C}_{21}\text{H}_{20}\text{O}_5$ : C, 71.58%; H, 5.72%. Found: C, 71.32%; H, 5.73%

**1-(4-Methoxy-phenyl)-3-(4-prop-2-ynyloxy-phenyl)-propenone, 4.10g**

Light yellow amorphous solid (1.46 g, 80%); m.p. 135-136°C;  $R_f$  (EtOAc:Hex 1:4) 0.34;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2126 (C≡C), 1657 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 8.02 (2H, d,  $J$  9.3, H-2B, H-6B), 7.76 (1H, d,  $J$  15.6, H $\beta$ ), 7.60 (2H, d,  $J$  8.1, H-2A, H-6A), 7.43 (1H, d,  $J$  15.6, H $\alpha$ ), 7.00-6.97 (4H, m, H-3B, H-5B, H-3A, H-5A), 4.73 (2H, d,  $J$  3.2, H-1'), 3.88 (3H, s, OMe), 2.55 (1H, t,  $J$  3.2, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 188.7, 163.3, 159.3, 143.5, 131.3(x2), 130.7(x2), 130.0, 128.7, 120.1, 115.3 (x2), 113.8 (x2), 78.0, 75.9, 55.8, 55.4; LRMS(ESI) found  $m/z$  293.0 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{19}\text{H}_{16}\text{O}_3$  requires 292.1; Anal Calc. for  $\text{C}_{19}\text{H}_{16}\text{O}_3$ : C, 78.06%; H, 5.52%. Found: C, 77.86%; H, 5.44%

**1-(2,4-Dimethoxy-phenyl)-3-(4-prop-2-ynyloxy-phenyl)-propene, 4.10h**

Light yellow amorphous solid (1.75 g, 89%); m.p. 113-114°C;  $R_f$  (EtOAc:Hex 1:4) 0.17;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2126 (C≡C), 1653 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.74 (1H, d,  $J$  9.0, H-6B), 7.64 (1H, d,  $J$  16.2, H $\beta$ ), 7.55 (2H, d,  $J$  8.7, H-2A, H-6A), 7.39 (1H, d,  $J$  15.6, H $\alpha$ ), 6.98 (2H, d,  $J$  8.7, H-3A, H-5A), 6.55 (1H, dd,  $J$  2.4 and 9.0, H-5B), 6.49 (1H, d,  $J$  2.4, H-3B), 4.72 (2H, d,  $J$  3.2, H-1'), 3.89 (3H, s, OMe), 3.86 (3H, s, OMe), 2.54 (1H, t,  $J$  2.7, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 190.5, 164.0, 160.3, 159.0, 141.7, 132.7, 129.8, 129.0 (x2), 125.5, 122.4, 115.2 (x2), 105.2, 98.7, 78.1, 75.8, 55.8, 55.7, 55.5; LRMS(ESI) found  $m/z$  323.4 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{20}\text{H}_{18}\text{O}_4$  requires 322.1; Anal Calc. for  $\text{C}_{20}\text{H}_{18}\text{O}_4$ : C, 74.52%; H, 5.63%. Found: C, 74.59%; H, 5.58%

**3-(4-Prop-2-ynyloxy-phenyl)-1-(2,3,4-trimethoxy-phenyl)-propene, 4.10i**

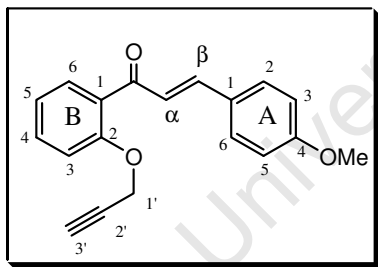
Light yellow amorphous solid (1.10 g, 83%);  $R_f$  (EtOAc:Hex 1:4) 0.17; m.p. 113-114°C;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  2125 (C≡C), 1653 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.64 (1H, d,  $J$  16, H $\beta$ ), 7.57 (2H, d,  $J$  8.6, H-2A, H-6A), 7.46 (1H, d,  $J$  8.8, H-6B), 7.37 (1H, d,  $J$  16, H $\alpha$ ), 6.99 (2H, d,  $J$  8.8, H-

3A, H-5A), 6.74 (1H, d,  $J$  9.2, H-5B), 4.72 (2H, d,  $J$  2.0, H-1'), 3.91 (3H, s, OMe), 3.91 (3H, s, OMe), 3.90 (3H, s, OMe), 2.54 (1H, t,  $J$  2.4, H-3');  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 190.9, 159.3, 156.9, 153.7, 142.7, 142.2, 130.0(x2), 128.8, 127.0, 125.7, 124.9, 115.3(x2), 107.4, 78.1, 75.9, 62.1, 61.0, 56.1, 55.9; LRMS(ESI) found  $m/z$  353.0 (100%, [M+H]<sup>+</sup>) for C<sub>20</sub>H<sub>18</sub>O<sub>4</sub> requires 352.1; Anal Calc. for C<sub>21</sub>H<sub>20</sub>O<sub>5</sub>: C, 71.58%; H, 5.72%. Found: C, 71.51%; H, 5.63%

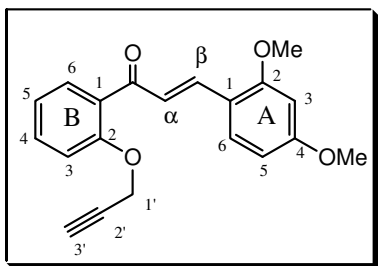
#### F. General procedure for preparation of compounds 4.13 a-i

To a solution of the acetylenic acetophenone **4.12a-c** (6.25 mmol, 1.0 eq) in MeOH was added 5 mL of methanolic NaOH (3% w/v). The resulting mixture was stirred at 25°C for 30 minutes. A methanolic solution of the commercially available, substituted aldehyde (6.25 mmol, 1.0 eq) was added drop-wise and the mixture stirred for a period of 16 hours at the same temperature. A precipitate was formed which was filtered and washed with cold MeOH. Recrystallization from MeOH afforded the pure product. For the reactions in which no precipitation occurred, the product mixture was diluted with H<sub>2</sub>O, neutralized with 10% HCl and extracted with EtOAc. The combined organic layer was dried over anhydrous NaSO<sub>4</sub> and concentrated under reduced pressure. The crude so obtained was subjected to recrystallization from MeOH to afford the acetylenic chalcones in good to excellent yields.

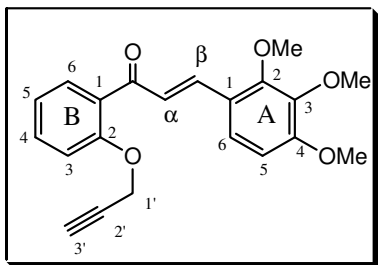
#### 3-(4-Methoxy-phenyl)-1-(2-prop-2-ynyloxy-phenyl)-propenone, 4.13a



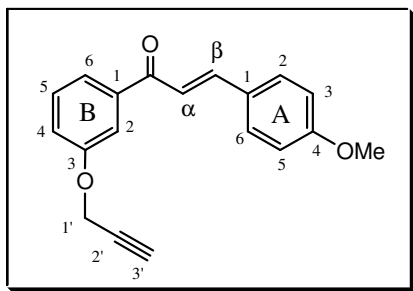
Yellow oil (1.44 g, 85%);  $R_f$  (30% EtOAc:Hex 3:7) 0.60; IR $v_{max}$ (film)/cm<sup>-1</sup> 2121 (C≡C), 1655 (C=O);  $\delta_H$  (400MHz, CDCl<sub>3</sub>) 7.61 (1H, dd,  $J$  2.0 and 8.0, H-6B), 7.56 (1H, d,  $J$  15.6, H $\beta$ ), 7.53 (2H, d,  $J$  8.8, H-2A, H-6A), 7.45 (1H, m, H-4B), 7.26 (1H, d,  $J$  16, H $\alpha$ ), 7.10-7.05 (2H, m, H-3B, H-5B), 6.89 (2H, d,  $J$  8.8, H-3A, H-5A), 4.76 (2H, d,  $J$  2.4, H-1'), 3.82 (3H, s, OMe), 2.50 (1H, t,  $J$  2.4, H-3');  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 192.7, 161.5, 155.9, 143.6, 132.4, 132.0, 130.4, 130.2(x2), 127.9, 125.0, 121.8, 114.4(x2), 113.5, 78.2, 76.0, 56.5, 55.4; LRMS(ESI) found  $m/z$  293.3 (100%, [M+H]<sup>+</sup>) for C<sub>19</sub>H<sub>16</sub>O<sub>3</sub> requires 292.1; Anal Calc. for C<sub>19</sub>H<sub>16</sub>O<sub>3</sub>: C, 78.06%; H, 5.52%. Found: C, 77.86%; H, 5.54%

**3-(2,4-Dimethoxy-phenyl)-1-(2-prop-2-ynyloxy-phenyl)-propene, 4.13b**

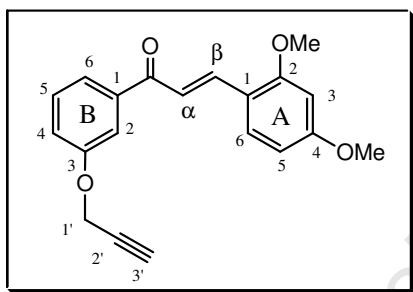
Yellow oil (1.86 g, quant);  $R_f$  (EtOAc:Hex 3:7) 0.47;  $IR_{\nu_{\max}}(\text{film})/\text{cm}^{-1}$  2121 (C $\equiv$ C), 1653 (C=O);  $\delta_H$  (400MHz, CDCl<sub>3</sub>) 7.86 (1H, d,  $J$  16, H $\beta$ ), 7.59 (1H, dd,  $J$  2.0 and 7.6, H-6B), 7.52 (1H, d,  $J$  8.8, H-6A), 7.43 (1H, m, H-4B), 7.35 (1H, d,  $J$  16, H $\alpha$ ), 7.11-7.04 (2H, m, H-3B, H-5B), 6.49 (1H, dd,  $J$  2.4 and 8.4, H-5A), 6.43 (1H, d,  $J$  2.4, H-3A), 4.75 (2H, d,  $J$  2.4, H-1'), 3.84 (3H, s, OMe), 3.82 (3H, s, OMe), 2.49 (1H, t,  $J$  2.4, H-3');  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 193.2, 162.9, 160.3, 155.8, 139.3, 132.0, 130.8, 130.6, 130.4, 125.3, 121.7, 117.3, 113.6, 105.4, 98.4, 78.3, 75.9, 55.56, 55.5, 55.5; LRMS(ESI) found  $m/z$  323.0 (100%, [M+H]<sup>+</sup>) for C<sub>20</sub>H<sub>18</sub>O<sub>4</sub> requires 322.1; Anal Calc. for C<sub>20</sub>H<sub>18</sub>O<sub>4</sub>: C, 74.52%; H, 5.63%. Found: C, 74.58%; H, 5.64%

**1-(2-Prop-2-ynyloxy-phenyl)-3-(2,3,4-trimethoxy-phenyl)-propene, 4.13c**

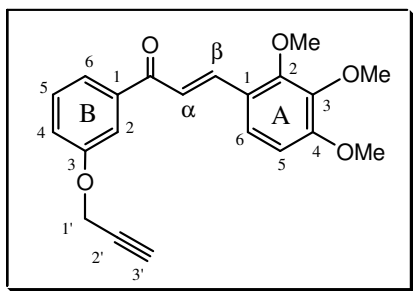
Yellow oil (2.05 g, quant.);  $R_f$  (EtOAc:Hex 1:4) 0.44;  $IR_{\nu_{\max}}(\text{film})/\text{cm}^{-1}$  2121 (C $\equiv$ C), 1654 (C=O);  $\delta_H$  (300MHz, CDCl<sub>3</sub>) 7.81 (1H, d,  $J$  16.2, H $\beta$ ), 7.60 (1H, dd,  $J$  1.8 and 7.5, H-6B), 7.45 (1H, m, H-4B), 7.35 (1H, d,  $J$  8.7, H-6A), 7.35 (1H, d,  $J$  15.9, H $\alpha$ ), 7.08 (2H, m, H-3B, H-5B), 6.68 (1H, d,  $J$  8.7, H-5A), 4.76 (2H, d,  $J$  2.4, H-1'), 3.90 (3H, s, OMe), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 2.51 (1H, t,  $J$  2.4, H-3');  $\delta_C$  (75MHz, CDCl<sub>3</sub>) 192.9, 155.7, 155.6, 153.7, 142.4, 138.8, 132.2, 130.5, 130.3, 126.1, 123.5, 122.1, 121.7, 113.4, 107.6, 78.2, 76.0, 60.8, 61.4, 56.5, 56.0; LRMS(ESI) found  $m/z$  353.3 (100%, [M+H]<sup>+</sup>) for C<sub>21</sub>H<sub>20</sub>O<sub>5</sub> requires 352.1; Anal Calc. for C<sub>21</sub>H<sub>20</sub>O<sub>5</sub>: C, 71.58%; H, 5.72%. Found: C, 71.85%; H, 5.78%

**3-(4-Methoxy-phenyl)-1-(3-prop-2-ynyloxy-phenyl)-propenone, 4.13d**

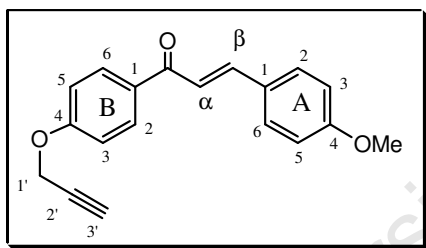
Light yellow powder (1.13 g, 67%); m.p. 83°C;  $R_f$  (EtOAc:Hex, 3:7) 0.61;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  2124 (C≡C), 1650 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.78 (1H, d,  $J$  15.6,  $H\beta$ ), 7.59-7.64 (4H, m, H-2A, H-6A, H-2B, H-6B), 7.42 (1H, t,  $J$  8.4, H-5B), 7.37 (1H, d,  $J$  15.6,  $H\alpha$ ), 7.19 (1H, ddd,  $J$  2.4, 2.7 and 8.1, H-4B), 6.94 (2H, d,  $J$  8.7, H-3A, H-5A), 4.77 (2H, d,  $J$  2.1, H-1'), 3.86 (3H, s, OMe), 2.54 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 190.1, 161.7, 157.8, 144.8, 140.0, 130.2(x2), 129.6, 127.6, 121.8, 119.8, 119.7, 114.4(x2), 114.2, 78.1, 75.9, 56.0, 55.4; LRMS(ESI) found  $m/z$  293.1 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{19}\text{H}_{16}\text{O}_3$  requires 292.1; Anal Calc. for  $\text{C}_{19}\text{H}_{16}\text{O}_3$ : C, 78.06%; H, 5.52%. Found: C, 77.64%; H, 5.55%

**3-(2,4-Dimethoxy-phenyl)-1-(3-prop-2-ynyloxy-phenyl)-propene, 4.13e**

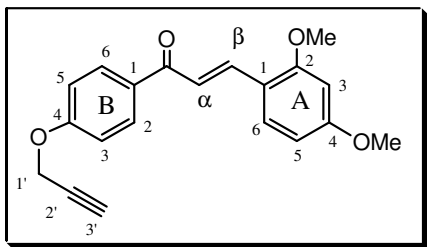
Light yellow amorphous solid (1.60 g, 88%); m.p. 132.°C;  $R_f$  (EtOAc:Hex 1:4) 0.53;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  2123 (C≡C), 1653 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 8.05 (1H, d,  $J$  15.6,  $H\beta$ ), 7.63 (2H, m, H-2B, H-6B), 7.56 (1H, d,  $J$  8.4, H-6A), 7.51 (1H, d,  $J$  15.6,  $H\alpha$ ), 7.41 (1H, t,  $J$  8.1, H-5B), 7.17 (1H, ddd,  $J$  2.4, 2.7 and 8.1, H-4B), 6.53 (1H, dd,  $J$  2.7 and 8.4, H-5A), 6.47 (1H, d,  $J$  2.7, H-3A), 4.76 (2H, d,  $J$  2.4, H-1'), 3.90 (3H, s, OMe), 3.85 (3H, s, OMe), 2.54, (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 190.7, 163.1, 160.5, 157.7, 140.7, 140.4, 131.0, 129.5, 121.8, 120.4, 119.5, 117.2, 114.2, 105.5, 98.5, 78.3, 75.8, 56.0, 55.6, 55.5; LRMS(ESI) found  $m/z$  323.3 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{20}\text{H}_{18}\text{O}_4$  requires 322.1; Anal Calc. for  $\text{C}_{20}\text{H}_{18}\text{O}_4$ : C, 74.52%; H, 5.63%. Found: C, 74.20%; H, 5.61%

**1-(3-Prop-2-ynyloxy-phenyl)-3-(2,3,4-trimethoxy-phenyl)-propene, 4.13f**

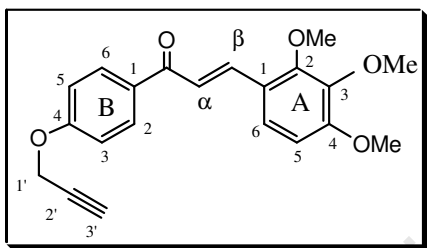
Light yellow amorphous solid (1.49 g, 74%); m.p. 74°C;  $R_f$  (EtOAc:Hex 1:4) 0.49;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  2113 (C≡C), 1653 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.99 (1H, d,  $J$  15.6, H $\beta$ ), 7.63 (2H, m, H-2B, H-6B), 7.52 (1H, d,  $J$  15.6, H $\alpha$ ), 7.43 (1H, d,  $J$  8.4, H-6A), 7.38 (1H, t,  $J$  8.7, H-5B), 7.18 (1H, ddd,  $J$  2.4, 2.7 and 8.1, H-4B), 6.71 (1H, d,  $J$  9.0, H-5A), 4.76 (2H, d,  $J$  2.4, H-1'), 3.94 (3H, s, OMe), 3.90 (3H, s, OMe), 3.88 (3H, s, OMe), 2.54 (1H, t,  $J$  2.7, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 190.3, 157.7, 155.8, 153.8, 142.5, 140.3, 140.0, 129.5, 123.9, 122.0, 121.8, 121.3, 119.6, 114.2, 107.6, 78.2, 75.8, 61.4, 60.9, 56.1, 56.0; LRMS(ESI) found  $m/z$  353.2 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{21}\text{H}_{20}\text{O}_5$  requires 352.1; Anal Calc. for  $\text{C}_{21}\text{H}_{20}\text{O}_5$ : C, 71.58%; H, 5.72%. Found: C, 71.20%; H, 5.78%

**3-(4-Methoxy-phenyl)-1-(4-prop-2-ynyloxy-phenyl)-propenone, 4.13g**

Light yellow amorphous solid (1.55 g, 87%); m.p. 112°C;  $R_f$  (EtOAc:Hex 3:7) 0.60;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  2111 (C≡C), 1651 (C=O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 8.02 (2H, d,  $J$  8.8, H-2B, H-6B), 7.76 (1H, d,  $J$  15.6, H $\beta$ ), 7.58 (2H, d,  $J$  8.8, H-2A, H-6A), 7.40 (1H, d,  $J$  15.6, H $\alpha$ ), 7.04 (2H, d,  $J$  8.8, H-3A, H-5A), 6.92 (2H, d,  $J$  8.8, H-3B, H-5B), 4.67 (2H, d,  $J$  2.4, H-1'), 3.84 (3H, s, OMe), 2.54 (1H, t,  $J$  2.0, H-3');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 188.8, 161.5, 161.0, 144.0, 132.2, 130.6(x2), 130.1(x2), 121.8, 119.5, 114.7 (x2), 114.4 (x2), 77.8, 76.0, 55.9, 55.4; LRMS(ESI) found  $m/z$  293.3 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{19}\text{H}_{16}\text{O}_3$  requires 292.1; Anal Calc. for  $\text{C}_{19}\text{H}_{16}\text{O}_3$ : C, 78.06%; H, 5.52%. Found: C, 77.89%; H, 5.56%

**3-(2,4-Dimethoxy-phenyl)-1-(4-prop-2-ynyloxy-phenyl)-propene, 4.13h**

Light yellow amorphous solid (1.42 g, 78%); m.p. 76-77°C;  $R_f$  (EtOAc:Hex 1:4) 0.48;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  2123 (C≡C), 1652 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 8.01-8.06 (3H, m, H-2B, H-6B, H $\beta$ ), 7.56 (1H, d,  $J$  8.4, H-6A), 7.54 (1H, d,  $J$  15.6, H $\alpha$ ), 7.05 (2H, d,  $J$  9.0, H-3B, H-5B), 6.53 (1H, dd,  $J$  2.4 and 8.4, H-5A), 6.48 (1H, d,  $J$  2.4, H-3A), 4.77 (2H, d,  $J$  3.2, H-1'), 3.90 (3H, s, OMe), 3.85 (3H, s, OMe), 2.55 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 189.4, 162.9, 160.9, 160.4, 139.9, 132.5, 130.9, 130.6(x2), 120.3, 117.3, 114.6(x2), 105.4, 98.5, 78.0, 76.0, 55.9, 55.6, 55.5; LRMS(ESI) found  $m/z$  323.1 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{20}\text{H}_{18}\text{O}_4$  requires 322.1; Anal Calc. for  $\text{C}_{20}\text{H}_{18}\text{O}_4$ : C, 74.52%; H, 5.63%. Found: C, 74.27%; H, 5.66%

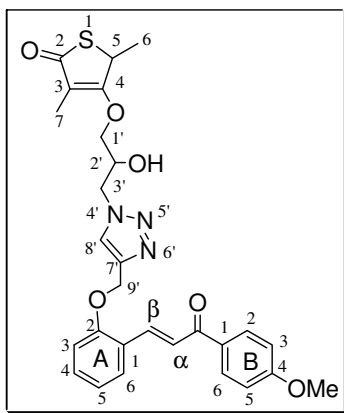
**1-(4-Prop-2-ynyloxy-phenyl)-3-(2,3,4-trimethoxy-phenyl)-propene, 4.13i**

Light yellow amorphous solid (1.43 g, 71%); m.p. 97-98°C;  $R_f$  (EtOAc:Hex 1:4) 0.43;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  2126 (C≡C), 1655 (C=O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 8.03 (2H, d,  $J$  9.0, H-2B, H-6B), 7.97 (1H, d,  $J$  15.6, H $\beta$ ), 7.55 (1H, d,  $J$  16.2, H $\alpha$ ), 7.36 (1H, d,  $J$  8.7, H-6A), 7.05 (2H, d,  $J$  8.7, H-3B, H-5B), 6.71 (1H, d,  $J$  8.7, H-5A), 4.76 (2H, d,  $J$  2.4, H-1'), 3.94 (3H s, OMe), 3.90 (3H, s, OMe), 3.88 (3H, s, OMe), 2.55 (1H, t,  $J$  2.4, H-3');  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 189.1, 161.0, 155.6, 153.7, 142.5, 139.5, 132.2, 130.6(x2), 123.8, 122.2, 121.2, 114.6 (x2), 107.6, 77.8, 76.1, 61.3, 60.9, 56.1, 55.8; LRMS(ESI) found  $m/z$  353.1 (100%,  $[\text{M}+\text{H}]^+$ ) for  $\text{C}_{21}\text{H}_{20}\text{O}_5$  requires 352.1; Anal Calc. for  $\text{C}_{21}\text{H}_{20}\text{O}_5$ : C, 71.58%; H, 5.72%. Found: C, 71.43%; H, 5.76%

**G. General procedure for preparation of compounds 4.14a-r**

The thiolactone azide **4.8** (0.822 mmol, 1.0 eq) was added to a stirred solution of the appropriate acetylene **4.10a-i**, **4.13a-i** (0.905 mmol, 1.1 eq), CuSO<sub>4</sub>·5H<sub>2</sub>O (0.0411 mmol, 0.05 eq) and sodium ascorbate (0.123 mmol, 0.15 eq) in 3 mL CH<sub>2</sub>Cl<sub>2</sub>/H<sub>2</sub>O (1:1). The resulting mixture was vigorously stirred for 16 hours at 25°C. The precipitated product was extracted with EtOAc and the combined organic layer washed with water, brine and dried over anhydrous NaSO<sub>4</sub>. Evaporation of the solvent under reduced pressure afforded a residue which was purified by column chromatography (MeOH:DCM 1:4).

**4-[2-Hydroxy-3-(4-{2-[3-(4-methoxy-phenyl)-3-oxo-propenyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.11a** (RHH138E)

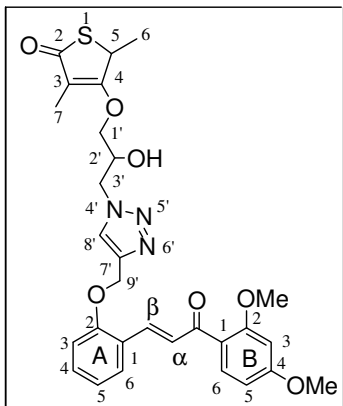


Yellow gum (84.9 mg, 19%);  $R_f$  (MeOH:DCM 1:39) 0.18;  $IR_{\text{vmax}}(\text{KBr})/\text{cm}^{-1}$  3400 (OH), 1625 (C<sub>2</sub>=O), 1600 ( $\alpha\beta$ -unsat C=O);  $\delta_{\text{H}}$  (400MHz, CDCl<sub>3</sub>) 7.98 (1H, d,  $J$  15.6,  $\beta$ H), 7.93 (2H, d,  $J$  8.0, H-2B, H-6B), 7.87 (1H, s, H-8'), 7.60 (1H, dd,  $J$  1.6 and 7.6, H-6A), 7.55 (1H, d,  $J$  16,  $\alpha$ H), 7.34 (1H, m, H-4A), 6.98-7.06 (2H, m, H-3A, H-5A), 6.92 (2H, d,  $J$  8.8, H-3B, H-5B), 5.21 (2H, s, H-9'), 4.63 (1H, dd,  $J$  3.2 and 14.0, H-1'a/b), 4.47 (1H, m, H-1'a/b), 4.36 (1H, m, H-2'), 4.26 (1H, m, H-3'a/b), 4.17 (2H, m, H-5, H-3'a/b), 3.84 (3H, s, OMe), 1.75 (3H, dd<sup>‡</sup>,  $J$  1.2 and 4.8, H-7), 1.51 (3H, d,  $J$  6.8, H-6);  $\delta_{\text{C}}$  (100MHz, CDCl<sub>3</sub>) 195.8, 189.6, 177.4, 163.6, 157.4, 143.7, 139.4, 134.6, 131.8, 131.4, 130.9(x2), 128.9, 124.8, 122.7, 121.7, 113.9(x2), 113.6, 113.3, 71.8, 69.0(x2), 62.9, 52.8, 41.8, 19.6, 8.9; HRMS(ESI) found  $m/z$  536.18485 [M+H]<sup>+</sup> for C<sub>28</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>S requires 535.17771; Anal Calc. for C<sub>28</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>S C, 62.79%; H, 5.46%; N, 7.85%; S, 5.99%. Found: C, 62.39%; H, 5.55%; N, 7.51%; S, 5.78%.

<sup>‡</sup> The expected multiplicity of H-7 is a doublet, however, a doublet of doublets and in some cases (**4.11f**) a broad doublet was observed in the <sup>1</sup>H-NMR spectra of compounds in this series (fig. 4.13).

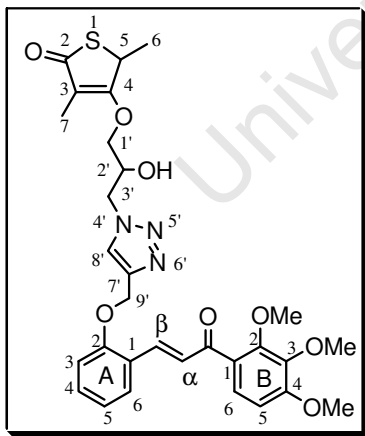


**4-[3-(4-{2-[3-(2,4-Dimethoxy-phenyl)-3-oxo-propenyl]-phenoxy-methyl}-[1,2,3]-triazol-1-yl)-2-hydroxy-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.11b (RHH138M)**



Yellow gum (179.0 mg, 38%);  $R_f$  (MeOH:DCM 1:39) 0.31;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3413 (OH), 1629 ( $\text{C}_2=\text{O}$ ), 1603 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.92 (1H, d,  $J$  15.6,  $\beta\text{H}$ ), 7.89 (1H, s, H-8'), 7.66 (1H, d,  $J$  8.4, H-6B), 7.59 (1H, br.d,  $J$  8.1, H-6A), 7.48 (1H, d,  $J$  16.2,  $\alpha\text{H}$ ), 7.34 (1H, m, H-4A), 7.08-6.98 (2H, m, H-3A, H-5A), 6.53 (1H, dd,  $J$  2.7 and 8.8, H-5B), 6.47 (1H, d,  $J$  2.4, H-3B), 5.24 (2H, s, H-9'), 4.49 (1H, dd,  $J$  3.0 and 13.8, H-1'a/b), 4.47 (1H, m, H-1'a/b), 4.35 (1H, m, H-2'), 4.28 (1H, m, H-3'a/b), 4.18 (2H, m, H-5, H-3'a/b), 3.80 (3H, s, OMe), 3.77 (3H, s, OMe), 1.79 (3H, dd,  $J$  0.9 and 3.3, H-7), 1.54 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.8, 191.4, 177.4, 164.4, 160.5, 157.4, 137.5, 133.4, 132.8, 131.6, 128.3, 127.6, 124.7, 121.7, 121.0, 113.5, 112.6, 105.4, 98.8, 98.4, 71.8, 69.0, 63.1, 55.8, 55.6, 52.7, 41.8, 19.7, 8.9; HRMS(ESI) found  $m/z$  566.19528  $[\text{M}+\text{H}]^+$  for  $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_7\text{S}$  requires 565.18827; Anal Calc. for  $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_7\text{S}$ : C, 61.58%; H, 5.52%; N, 7.43%; S, 5.67%. Found: C, 61.09%; H, 5.34%; N, 6.98%; S, 5.27%.

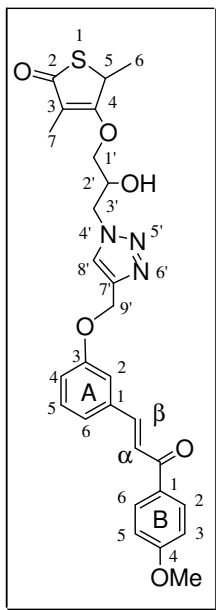
**2-[2-Hydroxy-3-(4-{4-[3-(2,3,4-trimethoxy-phenyl)-propenyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.11c (RHH138SA)**



Yellow foam (156.2 mg, 32%);  $R_f$  (MeOH:DCM 1:39) 0.14;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3401 (OH), 1630 ( $\text{C}_2=\text{O}$ ), 1601 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.91 (1H, s, H-8'), 7.89 (1H, d,  $J$  16.2,  $\beta\text{H}$ ), 7.60 (1H, dd,  $J$  2.1 and 7.8, H-6A), 7.44-7.50 (2H, m,  $\alpha\text{H}$ , H-6B), 7.37 (1H, m, H-4A), 7.08-6.99 (2H, m, H-3A, H-5A), 6.74 (1H, d,  $J$  8.7, H-5B), 5.26 (2H, s, H-9'), 4.67 (1H, dd,  $J$  3.3 and 14.1, H-1'a/b), 4.17-4.54 (5H, m, H-1'a/b, H-2', H-3', H-5), 3.91 (3H, s, OMe), 3.89 (3H, s, OMe), 3.86 (3H, s, OMe), 1.81 (3H, dd,  $J$  1.5 and 3.6, H-7), 1.56 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.6, 192.1, 177.2, 157.4, 156.8, 153.4, 139.2, 131.8, 131.6, 129.3, 128.4, 127.2, 126.8, 125.8, 124.5, 124.3, 121.7, 121.6, 113.1, 107.5, 71.8, 69.1, 63.1, 62.2, 61.1, 56.1, 52.7, 41.7, 19.6, 8.9; HRMS(ESI) found  $m/z$  596.20614  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{33}\text{N}_3\text{O}_8\text{S}$

requires 595.19884; Anal Calc. for  $C_{30}H_{33}N_3O_8S$ : C, 60.49%; H, 5.58%; N, 7.05%; S, 5.38%. Found: C, 60.12%; H, 5.24%; N, 7.04%; S, 5.30%.

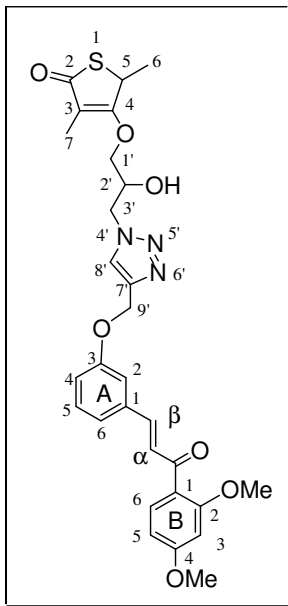
**4-[2-Hydroxy-3-(4-{3-[3-(4-methoxy-phenyl)-3-oxo-propenyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.11d (RHH138F)**



Yellow gum (90.1 mg, 20%);  $R_f$  (MeOH:DCM 1:39) 0.19;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3338 (OH), 1629 ( $\text{C}_2=\text{O}$ ), 1610 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.98 (2H, d,  $J$  9.2, H-2B, H-6B), 7.79 (1H, s, H-8'), 7.65 (1H, d,  $J$  15.6,  $\beta\text{H}$ ), 7.46 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 7.27 (1H, t,  $J$  8.0, H-5A), 7.21-7.17 (2H, m, H-2A, H-6A), 6.97-6.92 (3H, m, H-4A, H-3B, H-5B), 5.15 (2H, s, H-9'), 4.62 (1H, dd,  $J$  3.6 and 14.0, H-1'a/b), 4.48 (1H, m, H-1'a/b), 4.38 (1H, m, H-2'), 4.27 (1H, m, H-3'a/b), 4.18 (2H, m, H-5, H-3'a/b), 3.84 (3H, s, OMe), 1.75 (3H, dd,  $J$  1.2 and 6.8, H-7), 1.52 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.9, 188.9, 177.5, 163.6, 158.5, 143.8, 143.6, 136.6, 131.4, 130.9(x2), 130.1, 124.7, 122.5, 121.6, 116.8, 114.5, 114.0(x3), 71.8, 68.9, 61.9, 55.5, 52.8, 41.8, 19.6,

8.9; HRMS(ESI) found  $m/z$  536.18555 for  $C_{28}H_{29}N_3O_6S$  requires 535.17771; Anal Calc. for  $C_{28}H_{29}N_3O_6S$ : C, 62.79%; H, 5.46%; N, 7.85%; S, 5.99%. Found: C, 62.43%; H, 5.42%; N, 7.62%; S, 5.60%.

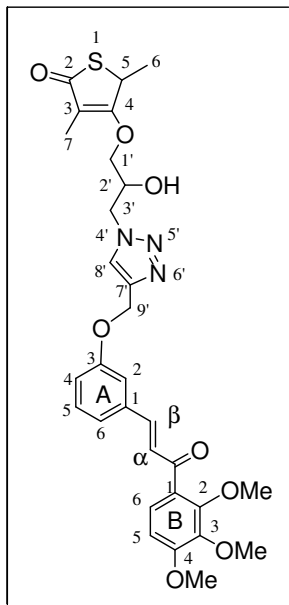
**4-[3-(4-{3-[3-(2,4-Dimethoxy-phenyl)-3-oxo-propenyl]-phenoxy-methyl}-[1,2,3] triazol-1-yl)-2-hydroxy-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.11e (RHH138U)**



Yellow gum (120.7 mg, 26%);  $R_f$  (MeOH:DCM 1:39) 0.17; IR $v_{\max}$ ((KBr)/cm $^{-1}$ ) 3400 (OH), 1630 (C $_2$ =O), 1601 ( $\alpha\beta$ -unsat C=O);  $\delta_H$  (300MHz, CDCl $_3$ ) 7.79 (1H, s, H-8'), 7.71 (1H, d,  $J$  8.4, H-6B), 7.57 (1H, d,  $J$  15.6,  $\beta$ H), 7.45 (1H, d,  $J$  15.6,  $\alpha$ H), 7.28 (1H, t,  $J$  8.1, H-5A), 7.20-7.14 (2H, m, H-2A, H-6A), 6.97 (1H, m, H-4A), 6.54 (1H, dd,  $J$  2.4 and 9.0, H-5B), 6.48 (1H, d,  $J$  2.4, H-3B), 5.18 (2H, s, H-9'), 4.62 (1H, dd,  $J$  3.6 and 13.8, H-1'a/b), 4.48 (1H, m, H-1'a/b), 4.37 (1H, m, H-2'), 4.26 (1H, m, H-3'a/b), 4.18 (2H, m, H-5, H-3'a/b), 3.88 (3H, s, OMe), 3.85 (3H, s, OMe), 1.77 (3H, dd,  $J$  1.2 and 4.2, H-7), 1.53 (3H, d,  $J$  6.9, H-6);  $\delta_C$  (75MHz, CDCl $_3$ ) 195.8, 190.5, 177.4, 165.0, 164.4, 160.5, 158.4,

143.8, 141.7, 136.9, 132.8, 129.9, 127.6, 124.6, 121.4, 116.4, 114.4, 105.4, 98.6, 98.4, 71.7, 68.8, 61.8, 55.8, 55.5, 52.6, 41.7, 19.7, 8.9; HRMS(ESI) found  $m/z$  566.21003 [M+H] $^+$  for C $_{29}$ H $_{31}$ N $_3$ O $_7$ S requires 565.18827; Anal Calc. for C $_{29}$ H $_{31}$ N $_3$ O $_7$ S: C, 61.58%; H, 5.52%; N, 7.43%; S, 5.67%. Found: C, 61.96%; H, 5.41%; N, 7.23%; S, 5.58%.

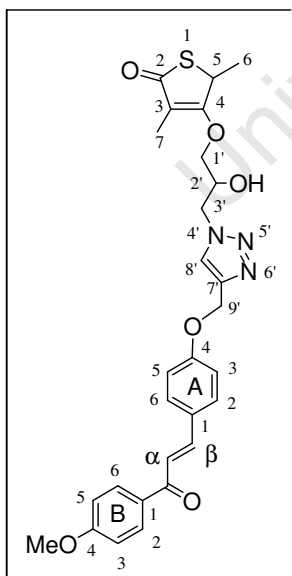
**3-[2-Hydroxy-3-(4-{4-[3-(2,3,4-trimethoxy-phenyl)-propenyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5*H*-thiophen-2-one, 4.11f (RHH138N)**



Yellow foam (108.8 mg, 22%);  $R_f$  (MeOH:DCM 1:39) 0.15;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3401 (OH), 1629 ( $\text{C}_2=\text{O}$ ), 1603 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.80 (1H, s, H-8'), 7.57 (1H, d,  $J$  16.2,  $\beta\text{H}$ ), 7.46-7.40 (2H, m, H-6B,  $\alpha\text{H}$ ), 7.29-7.16 (3H, m, H-2A, H-5A, H-6A), 6.98 (1H, m, H-4A), 6.74 (1H, d,  $J$  8.7, H-5B), 5.18 (2H, s, H-9'), 4.63 (1H, dd,  $J$  3.3 and 14.1, H-1'a/b), 4.51-4.14 (5H, m, H-1'a/b, H-2', H-3', H-5), 3.88 (3H, s, OMe), 3.89 (3H, s, OMe), 3.91 (3H, s, OMe), 1.78 (3H, br. d,  $J$  4.5, H-7), 1.54 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.7, 190.9, 177.3, 158.4, 157.2, 153.7, 143.7, 142.6, 136.6, 130.3, 130.0, 129.3, 127.0, 125.8, 124.6, 121.5, 116.6, 115.9, 114.4, 107.4, 71.7, 68.9, 62.1, 61.8, 61.0, 56.1,

52.6, 41.7, 19.6, 8.9; HRMS(ESI) found  $m/z$  596.20647  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{33}\text{N}_3\text{O}_8\text{S}$  requires 595.19884; Anal Calc. for  $\text{C}_{30}\text{H}_{33}\text{N}_3\text{O}_8\text{S}$ : C, 60.49%; H, 5.58%; N, 7.05%; S, 5.38%. Found: C, 60.76%; H, 5.41%; N, 7.23%; S, 5.58%

**4-[2-Hydroxy-3-(4-{4-[3-(4-methoxy-phenyl)-3-oxo-propenyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)propoxy]-3,5-dimethyl-5*H*-thiophen-2-one, 4.11g (RHH138O)**

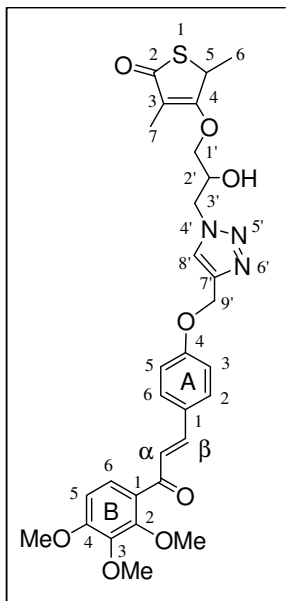


Yellow gum (101.1 mg, 23%);  $R_f$  (MeOH:DCM 1:39) 0.31;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3393 (OH), 1628 ( $\text{C}_2=\text{O}$ ), 1600 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 8.00 (2H, d,  $J$  8.7, H-2B, H-6B), 7.79 (1H, s, H-8'), 7.71 (1H, d,  $J$  15.6,  $\beta\text{H}$ ), 7.56 (2H, d,  $J$  8.7, H-2A, H-6A), 7.40 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 6.99-6.94 (4H, m, H-3A, H-5A, 3B, H-5B), 5.19 (2H, s, H-9'), 4.64 (1H, dd,  $J$  3.3 and 14.1, H-1'a/b), 4.50 (1H, m, H-1'a/b), 4.47 (1H, m, H-2'), 4.28 (1H, m, H-3'a/b), 4.20 (2H, m, H-5, H-3'a/b), 3.87 (3H, s, OMe), 1.79 (3H, dd,  $J$  0.9 and 4.8, H-7), 1.55 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.8, 188.9, 177.3, 163.5, 160.0, 143.6, 131.2,

130.8(x2), 130.2(x2), 128.4, 124.6, 120.0, 115.2(x2), 114.6, 113.9(x2), 73.1, 71.7, 68.9, 61.9, 55.5, 52.7, 41.8, 19.6, 8.9; HRMS(ESI) found  $m/z$  536.18482  $[\text{M}+\text{H}]^+$  for  $\text{C}_{28}\text{H}_{29}\text{N}_3\text{O}_6\text{S}$

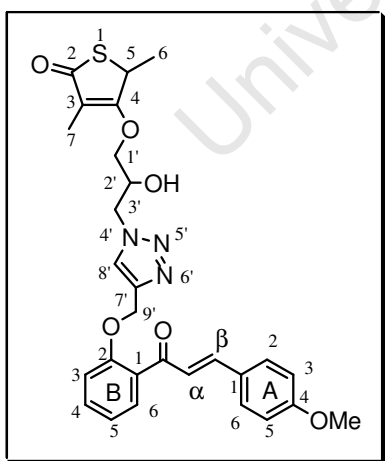


**4-[2-Hydroxy-3-(4-{4-[3-(2,3,4-trimethoxy-phenyl)-propenyl]-phenoxy-methyl]-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.11i (RHH96A)**



Yellow gum (208.9 mg, 43%);  $R_f$  (MeOH:DCM 1:39) 0.32;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3420 (OH), 1630 ( $\text{C}_2=\text{O}$ ), 1600 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.78 (1H, s, H-8'), 7.59 (1H, d,  $J$  16,  $\beta\text{H}$ ), 7.53 (2H, d,  $J$  8.8, H-2A, H-6A), 7.43 (1H, d,  $J$  8.8, H-6B), 7.34 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 6.97 (2H, d,  $J$  8.8, H-3A, H-5A), 6.73 (1H, d,  $J$  8.8, H-5B), 5.19 (2H, s, H-9'), 4.64 (1H, dd,  $J$  3.6 and 14.0, H-1'a/b), 4.50 (1H, m, H-1'a/b), 4.40 (1H, m, H-2'), 4.29 (1H, m, H-3'a/b), 4.19 (2H, m, H-5, H-3'a/b), 3.91 (3H, s, OMe), 3.90 (3H, s, OMe), 3.89 (3H, s, OMe), 1.78 (3H, dd,  $J$  0.8 and 6.0, H-7), 1.55 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 195.7, 191.1, 177.2, 159.9, 157.0, 153.6, 143.7, 142.8, 142.2, 130.1(x2), 128.5, 126.9, 125.6, 124.8, 124.6, 115.2(x2), 113.2, 107.4, 71.7, 69.0, 62.1, 61.9, 61.0, 56.1, 52.7, 41.8, 19.6, 8.9; HRMS(EI) found  $m/z$  595.1993 for  $\text{C}_{30}\text{H}_{33}\text{N}_3\text{O}_8\text{S}$  requires 595.19884; Anal Calc. for  $\text{C}_{30}\text{H}_{33}\text{N}_3\text{O}_8\text{S}$ : C, 60.49%; H, 5.58%; N, 7.05%; S, 5.38%. Found: C, 60.42%; H, 5.86%; N, 6.92%; S, 5.20%

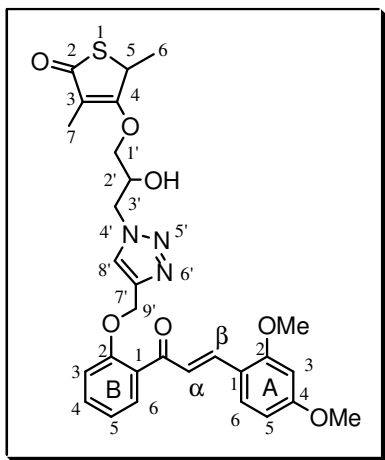
**4-[2-Hydroxy-3-(4-{2-[3-(4-methoxy-phenyl)-acryloyl]-phenoxy-methyl]-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14a (RHH138Q)**



Yellow gum (138.7 mg, 32%);  $R_f$  (MeOH:DCM 1:39) 0.15;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3400 (OH), 1627 ( $\text{C}_2=\text{O}$ ), 1602 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.59 (1H, s, H-8'), 7.51 (1H, dd,  $J$  1.8 and 7.5, H-6B), 7.46 (1H, d,  $J$  15.9, H $\beta$ ), 7.45-7.42 (3H, m, H-4B, H-2A, H-6A), 7.15 (1H, d,  $J$  15.9,  $\alpha\text{H}$ ), 7.10-7.02 (2H, m, H-3B, H-5B), 6.97 (2H, d,  $J$  8.7, H-3A, H-5A), 5.21 (2H, s, H-9'), 4.43 (1H, dd,  $J$  3.3 and 14.1, H-1'a/b), 4.29-4.06 (5H, m, H-1'a/b, H-2', H-3', H-5), 3.80 (3H, s, OMe), 1.74 (3H, dd,  $J$  0.9 and 2.4, H-7), 1.51 (3H, d,  $J$  7.2, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.9, 193.4, 177.5, 161.7, 156.4, 143.7, 143.5, 132.9, 131.9, 130.3(x2), 130.1, 127.5, 125.0, 124.7, 121.9, 114.5(x2), 114.1, 113.5, 71.7, 68.9, 63.3, 55.4, 52.6, 41.8,

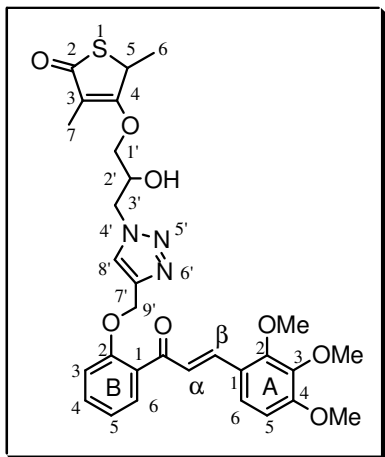
19.6, 8.9; HRMS(ESI) found  $m/z$  536.18492  $[M+H]^+$  for  $C_{28}H_{29}N_3O_6S$  requires 535.17771; Anal Calc. for  $C_{28}H_{29}N_3O_6S$ : C, 62.79%; H, 5.46%; N, 7.85%; S, 5.99%. Found: C, 62.49%; H, 5.43%; N, 7.49%; S, 5.86%.

**4-[3-(4-{2-[3-(2,4-Dimethoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-2-hydroxy-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14b (RHH138A)**



Yellow foam (168.9 mg, 36%);  $R_f$  (MeOH:DCM 1:39) 0.42;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3412 (OH), 1633 ( $C_2=O$ ), 1600 ( $\alpha\beta$ -unsat  $C=O$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.79 (1H, d,  $J$  16.2,  $\beta$ H), 7.57 (1H, s, H-8'), 7.48 (2H, m, H-4B, H-6B), 7.44 (1H, d,  $J$  8.4, H-6A), 7.23 (1H, d,  $J$  16.6,  $\alpha$ H), 7.14-7.04 (2H, m, H-3B, H-5B), 6.49 (1H, dd,  $J$  2.4 and 8.4, H-5A), 6.42 (1H, d,  $J$  2.4, H-3A), 5.24 (2H, s, H-9'), 4.62 (1H, dd,  $J$  3.2 and 14.1, H-1'a/b), 4.29-4.13 (5H, m, H-5, H-3', H-2', H-1'a/b), 3.82 (3H, s, OMe), 3.80 (3H, s, OMe), 1.77 (3H, br. d,  $J$  1.2, H-7), 1.54 (3H, d,  $J$  7.5, H-6);  $\delta_C$  (75MHz,  $CDCl_3$ ) 194.1, 191.5, 177.2, 163.2, 160.3, 156.2, 143.7, 139.6, 132.6, 130.5, 130.0, 125.3, 124.6, 122.0, 116.9, 114.6, 105.6, 104.3, 98.4, 97.9, 71.6, 69.0, 63.8, 55.6, 55.5, 52.6, 41.8, 19.6, 8.9; HRMS(ESI) found  $m/z$  566.19536  $[M+H]^+$  for  $C_{29}H_{31}N_3O_7S$  requires 565.18827; Anal Calc. for  $C_{29}H_{31}N_3O_7S$ : C 61.58%, H 5.52%, N 7.43%, S 5.67%. Found: C, 61.17%; H, 5.63%; N, 7.11%; S, 5.26%.

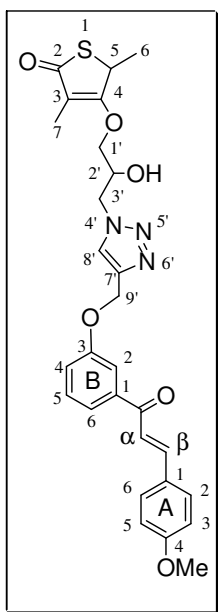
**4-[2-Hydroxy-3-(4-{2-[3-(2,3,4-trimethoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14c (RHH138H)**



Yellow foam (160.7 mg, 33%);  $R_f$  (MeOH:DCM 1:39) 0.33;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3415 (OH), 1631 ( $C_2=O$ ), 1600 ( $\alpha\beta$ -unsat  $C=O$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.71 (1H, d,  $J$  15.9,  $\beta$ H), 7.59 (1H, s, H-8'), 7.52 (1H, dd,  $J$  2.1 and 7.8, H-6B), 7.47 (1H, m, H-4B), 7.26 (1H, d,  $J$  8.7 H-6A), 7.24 (1H, d,  $J$  15.9,  $\alpha$ H), 7.14 (1H, d,  $J$  7.8, H-3B), 7.08 (1H, ddd,  $J$  1.2, 7.2 and 7.5, H-5B), 6.69 (1H, d,  $J$  9.3, H-5A), 5.25 (2H, s, H-9'), 4.67 (1H, dd,  $J$  3.3 and 14.7, H-1'a/b),

4.31-4.10 (5H, m, H-1'a/b, H-2', H-3', H-5), 3.88 (3H, s, OMe), 3.87 (3H, s, OMe), 3.86, (3H, s, OMe), 1.79 (3H, dd,  $J$  1.8 and 2.4, H-7), 1.55, (3H, d,  $J$  6.9, H-6);  $\delta_C$  (75MHz, CDCl<sub>3</sub>) 195.9, 194.0, 177.4, 156.3, 155.8, 153.5, 143.6, 142.3, 139.2, 132.9, 130.9 130.1, 126.4, 124.7, 123.8, 122.0, 121.8, 115.5, 114.6, 107.8, 71.6, 69.0, 63.7, 61.7, 61.1, 56.1, 52.7, 41.8, 19.7, 9.0; HRMS(ESI) found  $m/z$  596.20609 [M+H]<sup>+</sup> for C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>O<sub>8</sub>S requires 595.19884; Anal Calc. for C<sub>30</sub>H<sub>33</sub>N<sub>3</sub>O<sub>8</sub>S: C, 60.49%; H, 5.58%; N, 7.05%; S, 5.38%. Found: C, 60.28%; H, 5.90%; N, 6.68%; S, 5.21%.

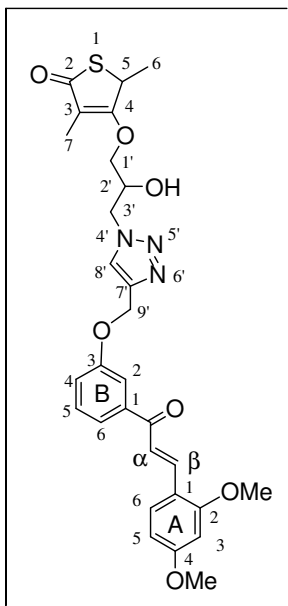
**4-[2-Hydroxy-3-(4-{3-[3-(4-methoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14d (RHH138KA)**



Yellow gum (99.8 mg, 23%);  $R_f$  (MeOH:DCM 1:39) 0.30; IR<sub>vmax</sub>(KBr)/cm<sup>-1</sup> 3401 (OH), 1629 (C<sub>2</sub>=O), 1601 ( $\alpha\beta$ -unsat C=O);  $\delta_H$  (300MHz, CDCl<sub>3</sub>) 7.82 (1H, s, H-8'), 7.73 (1H, d,  $J$  15.6,  $\beta$ H), 7.54 – 7.58 (4H, m, H-2A, H-6A, H-6B, H-2B), 7.36 (1H, t,  $J$  8.7, H-5B), 7.34 (1H, d,  $J$  15.6,  $\alpha$ H), 7.14 (1H, ddd,  $J$  2.4, 2.8 and 8.4, H-4B), 6.91 (2H, d,  $J$  9.0, H-3A, H-5A), 5.19 (2H, s, H-9'), 4.64 (1H, dd,  $J$  3.0 and 13.6, H-1'a/b), 4.49 (1H, m, H-1'a/b), 4.41 (1H, m, H-2'), 4.28 (1H, m, H-3'a/b), 4.20 (2H, m, H-5, H-3'a/b), 3.83 (3H, s, OMe), 1.77 (3H, dd,  $J$  1.2 and 6.8, H-7), 1.54 (3H, d,  $J$  6.9, H-6);  $\delta_C$  (75MHz, CDCl<sub>3</sub>) 195.9, 190.4, 177.5, 161.9, 158.4, 145.2, 143.6, 139.9, 131.8, 130.4(x2), 129.8, 127.4, 121.6, 119.7, 119.6, 114.5(x2), 114.1, 113.7, 71.8, 68.9, 62.0, 55.4, 52.8, 41.8, 19.6, 8.9; HRMS(ESI) found  $m/z$  536.18510 [M+H]<sup>+</sup> for C<sub>28</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>S requires 535.17771; Anal Calc. for C<sub>28</sub>H<sub>29</sub>N<sub>3</sub>O<sub>6</sub>S: C, 62.79%; H, 5.46%; N, 7.85%; S, 5.99%. Found: C, 62.61%; H, 5.51%; N, 7.25%; S, 5.80%.

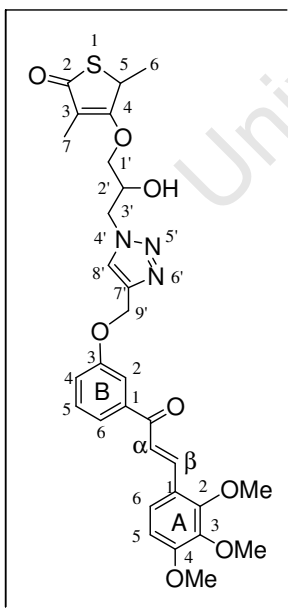


**4-[3-(4-{3-[3-(2,4-Dimethoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-2-hydroxy-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14e (RHH138J)**



Yellow foam (168.9 mg, 36%);  $R_f$  (MeOH:DCM 1:39), 0.15;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3401 (OH), 1629 ( $\text{C}_2=\text{O}$ ), 1604 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 8.03 (1H, d,  $J$  16.2,  $\beta\text{H}$ ), 7.83 (1H, s, H-8'), 7.57-7.55 (3H, m, H-2B, H-6B, H-6A), 7.48 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 7.38 (1H, t,  $J$  8.0, H-5B), 7.16 (1H, ddd,  $J$  2.4, 2.7 and 8.4, H-4B), 6.52 (1H, dd,  $J$  2.0 and 8.8, H-5A), 6.47 (1H, d,  $J$  2.0, H-3A), 5.22 (2H, s, H-9'), 4.64 (1H, m, H-1'a/b), 4.29-4.13 (5H, m, H-5, H-3', H-2', H-1'a/b), 3.89 (3H, s, OMe), 3.85 (3H, s, OMe), 1.79 (3H, dd,  $J$  1.7 and 4.0, H-7), 1.55 (3H, d,  $J$  6.8, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 191 (x2), 177.5, 163.3, 160.5, 158.4, 143.7, 141.1, 140.2, 131.9, 131.0, 129.7, 124.8, 121.6, 120.1, 119.6, 116.9, 114.1, 105.6, 98.5, 71.8, 68.9, 62.0, 55.6, 55.5, 52.7, 41.8, 19.7, 8.9; HRMS(ESI) found  $m/z$  566.19536  $[\text{M}+\text{H}]^+$  for  $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_7\text{S}$  requires 565.18827; Anal Calc. for  $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_7\text{S}$ : C 61.58%, H 5.52%, N 7.43%, S 5.67%. Found: C, 61.11%; H, 5.63%; N, 7.11%; S, 5.26%.

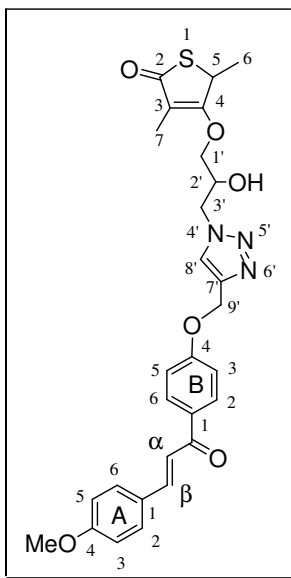
**4-[2-Hydroxy-3-(4-{3-[3-(2,3,4-trimethoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14f (RHH138D)**



Yellow gum (89.4 mg, 18%);  $R_f$  (MeOH:DCM 1:39) 0.33;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3400 (OH), 1628 ( $\text{C}_2=\text{O}$ ), 1600 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.96 (1H, d,  $J$  16,  $\beta\text{H}$ ), 7.82 (1H, s, H-8'), 7.55 (2H, m, H-2B, H-6B), 7.48 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 7.39-7.34 (2H, m, H-6A, H-5B), 7.14 (1H, ddd,  $J$  2.4, 2.8 and 8.7, H-4B), 6.70 (1H, d,  $J$  9.0, H-5A), 5.19 (2H, s, H-9'), 4.64 (1H, dd,  $J$  2.7 and 14.1, H-1'a/b), 4.49 (1H, m, H-1'a/b), 4.38 (1H, m, H-2'), 4.29 (1H, m, H-3'a/b), 4.21 (2H, m, H-5, H-3'a/b), 3.92 (3H, s, OMe), 3.88 (3H, s, OMe), 3.86 (3H, s, OMe), 1.76 (3H, dd,  $J$  1.2 and 3.9, H-7), 1.53 (3H, d,  $J$  6.9, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.8, 190.6, 177.4, 158.3, 156.0, 153.8, 143.5, 142.4, 140.6, 139.9, 129.7, 124.7, 123.9, 121.7, 121.6, 121.0, 119.6, 115.3, 114.0, 107.7, 71.7, 68.8, 61.9, 61.4,

60.8, 56.0, 52.6, 41.7, 19.6, 8.8; HRMS(ESI) found  $m/z$  596.20684  $[M+H]^+$  for  $C_{30}H_{33}N_3O_8S$  requires 595.19884; Anal Calc. for  $C_{30}H_{33}N_3O_8S$ : C, 60.49%; H, 5.58%; N, 7.05%; S, 5.38%. Found: C, 60.19%; H, 5.56%; N, 6.58%; S, 5.08%

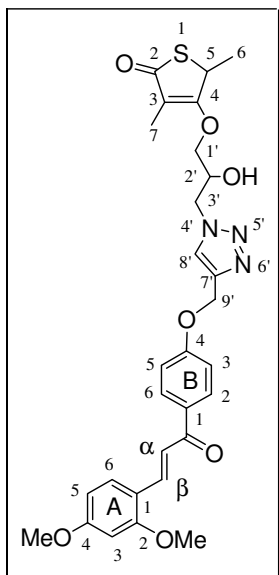
**4-[2-Hydroxy-3-(4-{3-[3-(4-methoxy-phenyl)-acryloyl]-phenoxy)methyl]-[1,2,3]triazol-1-yl)-prooxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14g (RHH138G)**



Yellow gum (86 mg, 20%);  $R_f$  (MeOH:DCM 1:39) 0.12;  $IR_{\nu_{max}}$  (KBr)/ $cm^{-1}$  3400 (OH), 1628 ( $C_2=O$ ), 1600 ( $\alpha\beta$ -unsat  $C=O$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.96 (2H, d,  $J$  8.7, H-2B and H-6B), 7.81 (1H, s, H-8'), 7.72 (1H, d,  $J$  15.6,  $\beta$ H), 7.57 (2H, d,  $J$  9.0, H-2A, H-6A), 7.37 (1H, d,  $J$  15.6,  $\alpha$ H), 7.01 (2H, d,  $J$  8.7, H-3A, H-5A), 6.91 (2H, d,  $J$  8.7, H-3B, H-5B), 5.21 (2H, s, H-9'), 4.64 (1H, dd,  $J$  3.0 and 14.1, H-1'a/b), 4.50 (1H, m, H-1'a/b), 4.39 (1H, m, H-2'), 4.28 (1H, m, H-3'a/b), 4.19 (2H, m, H-5, H-3'a/b), 3.83 (3H, s, OMe), 1.77 (3H, dd,  $J$  1.5 and 5.7, H-7), 1.54 (3H, d,  $J$  6.9, H-6);  $\delta_C$  (75MHz,  $CDCl_3$ ) 195.9, 189.0, 177.4, 161.8, 161.7, 144.3,

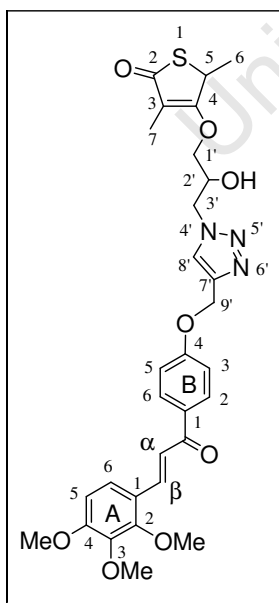
143.4, 131.5, 130.7(x2), 130.2(x2), 127.6, 124.7, 119.4, 114.6(x2), 114.5(x2), 113.7, 71.8, 68.9, 61.9, 55.4, 52.8, 41.8, 19.6, 8.9; HRMS(ESI) found  $m/z$  536.18526  $[M+H]^+$  for  $C_{28}H_{29}N_3O_6S$  requires 535.17771; Anal Calc. for  $C_{28}H_{29}N_3O_6S$ : C, 62.79%; H, 5.46%; N, 7.85%; S, 5.99%. Found C, 62.71%; H, 5.40%; N, 7.82%; S, 5.84%.

**4-[3-(4-{4-[3-(2,4-Dimethoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-2-hydroxy-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14h (RHH138B)**



Yellow foam (91.4 mg, 20%);  $R_f$  (MeOH:DCM 1:39) 0.34;  $IR_{\nu_{\max}}(\text{CHCl}_3)/\text{cm}^{-1}$  3413 (OH), 1630 ( $\text{C}_2=\text{O}$ ), 1600 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.99 (1H, d,  $J$  15.6,  $\beta\text{H}$ ), 7.95 (2H, d,  $J$  8.8, H-2B and H-6B), 7.78 (1H, s, H-8'), 7.53 (1H, d,  $J$  8.8, H-6A), 7.50 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 7.00 (2H, d,  $J$  8.8, H-3B, H-5B), 6.51 (1H, dd,  $J$  2.4 and 8.4, H-5A), 6.45 (1H, d,  $J$  2.4, H-3A), 5.22 (2H, s, H-9'), 4.62 (1H, dd,  $J$  3.2 and 14.1, H-1'a/b), 4.48 (1H, m, H-1'a/b), 4.38 (1H, m, H-2'), 4.27 (1H, m, H-3'a/b), 4.17 (2H, m, H-5, H-3'a/b), 3.87 (3H, s, OMe), 3.83 (3H, s, OMe), 1.77 (3H, dd,  $J$  1.2 and 6.4, H-7), 1.54 (3H, d,  $J$  6.8, H-6);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 194.6, 189.6, 177.2, 163.1, 161.6, 160.4, 143.6, 140.2, 132.2, 131.2, 130.9, 130.7(x2), 124.6, 120.0, 117.1, 114.5(x2), 105.5, 98.5, 71.7, 69.0, 61.9, 55.6, 55.5, 52.6, 41.7, 19.7, 8.9; HRMS(ESI) found  $m/z$  566.19509  $[\text{M}+\text{H}]^+$  for  $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_7\text{S}$  requires 565.18827; Anal Calc. for  $\text{C}_{29}\text{H}_{31}\text{N}_3\text{O}_7\text{S}$ : C, 61.58%; H, 5.52%; N, 7.43%; S, 5.67%. Found: C, 61.17%; H, 5.58%; N, 7.33%; S, 5.78%.

**4-[2-Hydroxy-3-(4-{4-[3-(2,3,4-trimethoxy-phenyl)-acryloyl]-phenoxy-methyl}-[1,2,3]triazol-1-yl)-propoxy]-3,5-dimethyl-5H-thiophen-2-one, 4.14i (RHH138C)**



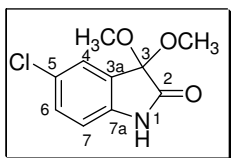
Yellow gum (84.2 mg, 17%);  $R_f$  (MeOH:DCM 1:39) 0.30;  $IR_{\nu_{\max}}(\text{KBr})/\text{cm}^{-1}$  3418 (OH), 1630 ( $\text{C}_2=\text{O}$ ), 1601 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.95 (1H, d,  $J$  16, H $\beta$ ), 7.93 (2H, d,  $J$  8.7, H-2B, H-6B), 7.78 (1H, s, H-8'), 7.50 (1H, d,  $J$  15.6,  $\alpha\text{H}$ ), 7.35 (1H, d,  $J$  8.7, H-6A), 7.00 (2H, d,  $J$  8.7, H-3B, H-5B), 6.69 (1H, d,  $J$  8.7, H-5A), 5.19 (2H, s, H-9'), 4.64 (1H, dd,  $J$  3.0 and 14.1, H-1'a/b), 4.50 (1H, m, H-1'a/b), 4.38 (1H, m, H-2'), 4.29 (1H, m, H-3'a/b), 4.20 (2H, m, H-5, H-3'a/b), 3.91 (3H, s, OMe), 3.87 (3H, s, OMe), 3.85 (3H, s, OMe), 1.75 (3H, dd,  $J$  0.9 and 4.5, H-7), 1.52 (3H, d,  $J$  6.9, H-6);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 195.8, 189.3, 177.4, 161.7, 155.8, 153.6, 143.2, 139.6, 131.8, 131.1, 130.7(x2), 124.7,

123.8, 121.8, 120.8, 114.5(x2), 114.3, 107.6, 71.7, 68.8, 61.8, 61.4, 60.8, 56.0, 52.6, 41.8, 19.6, 8.8; HRMS(ESI) found  $m/z$  596.20590  $[M+H]^+$  for  $C_{30}H_{33}N_3O_8S$  requires 595.19884; Anal Calc. for  $C_{30}H_{33}N_3O_8S$ : C, 60.49%; H, 5.58%; N, 7.05%; S, 5.38%. Found: C, 60.29%; H, 5.54%; N, 7.07%; S, 4.98%.

### I. General procedure for preparation of compounds 4.19a-f

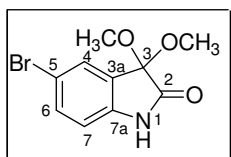
A mixture of the isatin/5-substituted isatin (11.0 mmol, 1.0 eq), a catalytic amount of PTSA and trimethyl orthoformate (330 mmol, 30 eq) in 30 mL of anhydrous MeOH was refluxed for 48 hours under an inert atmosphere. Upon completion the product mixture was neutralized with 5% aqueous sodium bicarbonate and the resulting mixture extracted with EtOAc. The combined organic layer was washed with  $H_2O$ , brine, dried over anhydrous  $Na_2SO_4$  and concentrated under reduced pressure to afford a crude residue. The latter was purified with column chromatography (eluent EtOAc:Hex, 2:3) to yield the pure product.

#### 5-Chloro-3,3-dimethoxyindolin-2-one, 4.19a



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure I, which gave **4.19a** as a light yellow-orange crystalline solid (1.63 g, 65%). m. p. 152°C;  $R_f$  (EtOAc:Hex 2:3) 0.47;  $IR_{\nu_{max}}$ (KBr)/ $cm^{-1}$  3327 (N-H), 1625 (C=O), 1480 (Ar C=C);  $\delta_H$  (400MHz,  $CDCl_3$ ) 8.66 (1H, s, NH), 7.39 (1H, d,  $J$  2.4, H-4), 7.31 (1H, dd,  $J$  2.0 and 8.0, H-6), 6.85, (1H, d,  $J$  8.4, H-7), 3.58 (6H, s, OMe);  $\delta_C$  (100MHz,  $CDCl_3$ ) 172.7, 139.0, 130.6, 128.3, 126.9, 125.6, 111.9, 97.2, 50.9(x2); LRMS(EI) found  $m/z$  226.8 (49%,  $M^+$ ) for  $C_{10}H_{10}ClNO_3$  requires 227.0

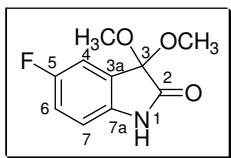
#### 5-Bromo-3,3-dimethoxyindolin-2-one, 4.19b



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure I. However, 8.85 mmol of 5-bromoisatin and 265 mmol of trimethyl orthoformate were used, which gave **4.19b** as a light yellow crystalline solid (1.08 g, 45%). m. p. 156-157°C;  $R_f$  (EtOAc:Hex 2:3) 0.55;  $IR_{\nu_{max}}$ (KBr)/ $cm^{-1}$  3332 (N-H), 1621 (C=O), 1476 (Ar C=C);  $\delta_H$  (400MHz,  $CDCl_3$ ) 8.65 (1H, s, NH), 7.53 (1H, d,  $J$  2.0, H-4), 7.45

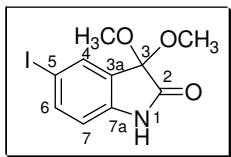
(1H, dd, *J* 2.0 and 8.4, H-6), 6.81, (1H, d, *J* 8.4, H-7), 3.58 (6H, s, OMe);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 172.5, 139.4, 133.5, 128.3, 127.2, 115.5, 112.4, 96.6, 50.9(x2); LRMS(EI) found *m/z* 270.5 for C<sub>10</sub>H<sub>10</sub>BrNO<sub>3</sub> requires 271.0

#### 5-Fluoro-3,3-dimethoxyindolin-2-one, 4.19c

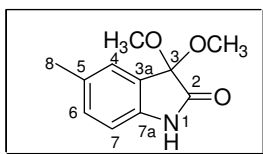


The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure I. However, 12.1 mmol of the 5-fluoroisatin and 363 mmol of trimethyl orthoformate were used, which gave **4.19c** as an orange crystalline solid (1.22 g, 48%). m. p. 101-103°C; *R<sub>f</sub>* (EtOAc:Hex 2:3) 0.51; IR<sub>v<sub>max</sub></sub>(KBr)/cm<sup>-1</sup> 3188 (N-H), 1633 (C=O), 1485 (Ar C=C);  $\delta_H$  (400MHz, CDCl<sub>3</sub>) 8.86 (1H, s, NH), 7.15 (1H, dd, *J* 2.8 and 7.6, H-4), 7.03 (1H, ddd, *J* 2.8, 8.0 and 8.8, H-6), 6.86 (1H, dd, *J* 4.0 and 8.8, H-7), 3.55 (6H, s, OMe);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 173.2, 160.2, 136.4, 126.7, 117.2, 113.2, 111.7, 97.4, 50.9(x2); LRMS(EI) found *m/z* 211.4 for C<sub>10</sub>H<sub>10</sub>FNO<sub>3</sub> requires 211.1

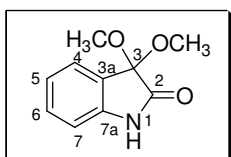
#### 5-Iodo-3,3-dimethoxyindolin-2-one, 4.19d



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure I. However, 7.32 mmol of the 5-iodoisatin and 220 mmol of trimethyl orthoformate were used, which gave **4.19d** as a yellow-orange crystalline solid (1.18 g, 51%). m. p. 155°C; *R<sub>f</sub>* (EtOAc:Hex 2:3) 0.57; IR<sub>v<sub>max</sub></sub>(KBr)/cm<sup>-1</sup> 3321 (N-H), 1615 (C=O), 1475 (Ar C=C);  $\delta_H$  (300MHz, CDCl<sub>3</sub>) 8.42 (1H, s, NH), 7.68 (1H, d, *J* 1.8, H-4), 7.63 (1H, dd, *J* 2.0 and 8.4, H-6), 6.68 (1H, d, *J* 8.4, H-7), 3.56 (6H, s, OMe);  $\delta_C$  (75MHz, CDCl<sub>3</sub>) 170.1, 140.0, 139.4, 133.9, 127.5, 112.8, 96.5, 85.2, 50.9(x2); LRMS(EI) found *m/z* 318.8 for C<sub>10</sub>H<sub>10</sub>INO<sub>3</sub> requires 319.0

**3,3-Dimethoxy-5-methylindolin-2-one, 4.19e**

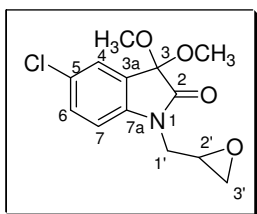
The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure I. However, 12.4 mmol of the 5-methylisatin and 372 mmol of trimethyl orthoformate were used, which gave **4.19e** as off-white needles (1.41 g, 55%). m. p. 106°C;  $R_f$  (EtOAc:Hex 2:3) 0.52;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3310 (N-H), 1626 (C=O), 1495 (Ar C=C);  $\delta_H$  (400MHz,  $\text{CDCl}_3$ ) 8.53 (1H, s, NH), 7.23 (1H, d,  $J$  1.2, H-4), 7.11 (1H, dd,  $J$  1.2 and 8.0, H-6), 6.80, (1H, d,  $J$  8.0, H-7), 3.58 (6H, s, OMe), 2.35 (3H, s, H-8);  $\delta_C$  (100MHz,  $\text{CDCl}_3$ ) 173.1, 139.1, 132.4, 131.0, 125.8, 112.3, 110.6, 97.5, 50.9(x2), 21.1; LRMS(EI) found  $m/z$  207.5 for  $\text{C}_{11}\text{H}_{13}\text{NO}_3$  requires 207.1

**3,3-Dimethoxyindolin-2-one, 4.19f**

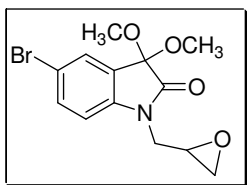
The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure I. However, 13.6 mmol of isatin and 408 mmol of trimethyl orthoformate were used, which gave **4.19f** as colourless prisms (1.42 g, 54%); m. p. 90°C;  $R_f$  (EtOAc:Hex 2:3) 0.43;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3165 (N-H), 1623 (C=O), 1474 (Ar C=C);  $\delta_H$  (400MHz,  $\text{CDCl}_3$ ) 9.00 (1H, s, NH), 7.40 (1H, dd,  $J$  1.2 and 8.0, H-4), 7.31 (1H, td,  $J$  1.2 and 7.6, H-6), 7.07, (1H, td,  $J$  0.8 and 8.4, H-5), 6.92 (1H, d,  $J$  7.6, H-7), 3.58 (6H, s, OMe);  $\delta_C$  (100MHz,  $\text{CDCl}_3$ ) 173.3, 140.6, 130.7, 125.1, 122.7, 112.6, 111.0, 97.4, 50.9 (x2); LRMS(EI) found  $m/z$  193.4 for  $\text{C}_{10}\text{H}_{11}\text{NO}_3$  requires 193.1

**J. General procedure for preparation of compounds 4.20a-f**

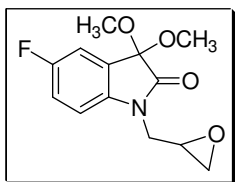
The ketal **4.19** (4.39 mmol, 1.0 eq), and  $\text{KF-Al}_2\text{O}_3$  (22.0 mol, 5.0 eq) was suspended in 10 ml anhydrous  $\text{CH}_2\text{Cl}_2$ . ( $\pm$ )-Epichlorohydrin (22.0 mmol, 5.0 eq) was added and the reaction mixture stirred for 20 hours at 25°C. When the reaction was completed, as indicated by tlc, the product mixture was filtered, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated to afforded the crude epoxide which was purified using flash column chromatography (10-40% EtOAc:Hex).

**5-Chloro-3,3-dimethoxy-1-(oxiran-2-ylmethyl)indolin-2-one, 4.20a**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure J; the ketal **4.19a** gave **4.20a** as a colourless oil (1.05 g, 85%).  $R_f$  (MeOH:DCM 1:99) 0.42;  $IR_{\nu_{\max}}(\text{film})/\text{cm}^{-1}$  1612 (C=O), 1481 (Ar C=C), 1069 (C-O);  $\delta_H$  (400MHz,  $\text{CDCl}_3$ ) 7.38 (1H, d,  $J$  2.0, H-4), 7.33 (1H, dd,  $J$  2.0 and 8.4, H-6), 6.99 (1H, d,  $J$  8.4, H-7), 4.23 (1H, dd,  $J$  2.8 and 15.2, H-1'a/b), 3.56 (3H, s, OMe), 3.55 (3H, s, OMe), 3.48 (1H, dd,  $J$  5.6 and 15.2, H-1'a/b), 3.15 (1H, m, H-2'), 2.81 (1H, dd,  $J$  4.0 and 4.4, H-3'a/b), 2.62 (1H, dd,  $J$  2.4 and 4.8, H-3'a/b);  $\delta_C$  (100MHz,  $\text{CDCl}_3$ ) 170.4, 141.2, 130.6, 128.4, 126.3, 125.2, 111.1, 96.7, 50.9(x2), 49.8, 44.8, 41.8; LRMS(EI) found  $m/z$  283.5 for  $\text{C}_{13}\text{H}_{14}\text{ClNO}_4$  requires 283.1

**5-Bromo-3,3-dimethoxy-1-(oxiran-2-ylmethyl)indolin-2-one, 4.20b**

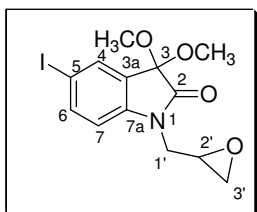
The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure J. However, 3.40 mmol of the ketal **4.19b** and 17.0 mmol each of  $\text{KF-Al}_2\text{O}_3$  and ( $\pm$ )-epichlorohydrin were used, which gave **4.20b** as a colourless oil (1.28 g, 89%).  $R_f$  (DCM) 0.29;  $IR_{\nu_{\max}}(\text{film})/\text{cm}^{-1}$  1610 (C=O), 1484 (Ar C=C), 1070 (C-O);  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.50 (1H, d,  $J$  1.8, H-4), 7.47 (1H, dd,  $J$  2.1 and 8.4, H-6), 6.93 (1H, d,  $J$  8.4, H-7), 4.21 (1H, dd,  $J$  3.0 and 15.0, H-1'a/b), 3.55 (3H, s, OMe), 3.54 (3H, s, OMe), 3.46 (1H, dd,  $J$  6.0 and 15.0, H-1'a/b), 3.13 (1H, m, H-2'), 2.80 (1H, dd,  $J$  4.1 and 4.5, H-3'a/b), 2.60 (1H, dd,  $J$  2.4 and 4.8, H-3'a/b);  $\delta_C$  (75MHz,  $\text{CDCl}_3$ ) 170.2, 141.7, 133.4, 127.8, 126.6, 115.6, 111.5, 96.6, 50.8(x2), 49.7, 44.7, 41.7; LRMS(EI) found  $m/z$  327.2 for  $\text{C}_{13}\text{H}_{14}\text{BrNO}_4$  requires 327.0

**5-Fluoro-3,3-dimethoxy-1-(oxiran-2-ylmethyl)indolin-2-one, 4.20c**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure J. However, 4.99 mmol of the ketal **4.19c** and 24.9 mmol each of  $\text{KF-Al}_2\text{O}_3$  and ( $\pm$ )-epichlorohydrin were used, which gave **4.20c** as a colourless oil (1.02 g, 87%).  $R_f$  (DCM) 0.29;  $IR_{\nu_{\max}}(\text{film})/\text{cm}^{-1}$  1621 (C=O), 1495 (Ar C=C), 1068 (C-O);

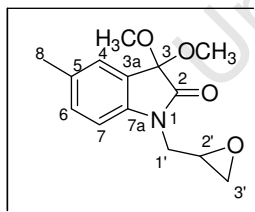
$\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.12 (1H, dd,  $J$  2.4 and 7.2, H-4), 7.04 (1H, ddd,  $J$  2.4, 8.7 and 9.0, H-6), 6.95 (1H, dd,  $J$  4.2 and 8.7, H-7), 4.22 (1H, dd,  $J$  2.4 and 15.0, H-1'a/b), 3.54 (3H, s, OMe), 3.53 (3H, s, OMe), 3.45 (1H, dd,  $J$  6.0 and 15.3, H-1'a/b), 3.13 (1H, m, H-2'), 2.79 (1H, dd,  $J$  4.1 and 4.5, H-3'a/b), 2.61 (1H, dd,  $J$  2.4 and 4.8, H-3'a/b);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 170.7, 160.3, 138.6, 126.2, 117.0, 112.8, 110.8, 96.8, 50.8(x2), 49.9, 44.8, 41.9; LRMS(EI) found  $m/z$  267.9 for  $\text{C}_{13}\text{H}_{14}\text{FNO}_4$  requires 267.1

### 5-Iodo-3,3-dimethoxy-1-(oxiran-2-ylmethyl)indolin-2-one, 4.20d



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure J. However, 3.37 mmol of the ketal **4.19d** and 16.8 mmol each of  $\text{KF-Al}_2\text{O}_3$  and ( $\pm$ )-epichlorohydrin were used, which gave **4.20d** as a colourless oil (1.33 g, 81%).  $R_f$  (DCM) 0.36;  $\text{IR}_{\text{max}}(\text{film})/\text{cm}^{-1}$  1607 (C=O), 1481 (Ar C=C), 1070 (C-O);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.69 (1H, d,  $J$  2.0, H-4), 7.68 (1H, dd,  $J$  2.0 and 8.4, H-6), 6.85 (1H, d,  $J$  8.8, H-7), 4.22 (1H, dd,  $J$  2.8 and 15.2, H-1'a/b), 3.57 (3H, s, OMe), 3.56 (3H, s, OMe), 3.48 (1H, dd,  $J$  6.0 and 15.2, H-1'a/b), 3.16 (1H, m, H-2'), 2.82 (1H, dd,  $J$  4.0 and 4.4, H-3'a/b), 2.62 (1H, dd,  $J$  2.8 and 4.8, H-3'a/b);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 170.2, 142.4, 139.5, 133.4, 127.0, 112.1, 96.5, 85.4, 50.9(x2), 49.8, 44.8, 41.8; LRMS(EI) found  $m/z$  375.1 for  $\text{C}_{13}\text{H}_{14}\text{INO}_4$  requires 375.0

### 3,3-Dimethoxy-5-methyl-1-(oxiran-2-ylmethyl)indolin-2-one, 4.20e

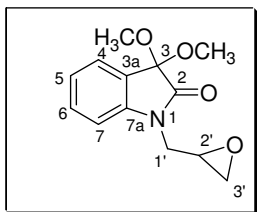


The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure J. However 6.29 mmol of the ketal **4.19e** and 31.5 mmol each of  $\text{KF-Al}_2\text{O}_3$  and ( $\pm$ )-epichlorohydrin were used, which gave **4.20e** as a colourless oil (1.10 g, 95%).  $R_f$  (DCM) 0.28;  $\text{IR}_{\text{max}}(\text{film})/\text{cm}^{-1}$  1623 (C=O), 1499 (Ar C=C), 1069 (C-O);  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.21 (1H, d,  $J$  1.2, H-4), 7.14 (1H, dd,  $J$  1.2 and 8.4, H-6), 6.91 (1H, d,  $J$  8.8, H-7), 4.14 (1H, dd,  $J$  3.0 and 15.0, H-1'a/b), 3.55 (3H, s, OMe), 3.54 (3H, s, OMe), 3.52 (1H, dd,  $J$  6.0 and 15.0, H-1'a/b), 3.14 (1H, m, H-2'), 2.78 (1H, dd,  $J$  4.0 and 4.4, H-3'a/b), 2.62 (1H, dd,  $J$  2.8 and 4.8, H-3'a/b), 2.33 (3H, s, H-8);  $\delta_{\text{C}}$  (75MHz,



CDCl<sub>3</sub>) 170.9, 140.3, 132.6, 131.0, 125.5, 124.7, 109.7, 97.1, 50.9(x2), 49.8, 45.0, 41.7, 21.0  
LRMS(EI) found m/z 263.6 for C<sub>14</sub>H<sub>17</sub>NO<sub>4</sub> requires 263.1

### 3,3-Dimethoxy-1-(oxiran-2-ylmethyl)indolin-2-one, **4.20f**

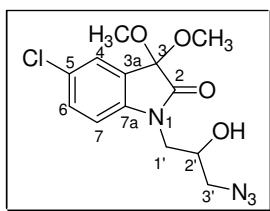


The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure J. However, 6.59 mmol of the ketal **4.19f** and 32.9 mmol each of KF-Al<sub>2</sub>O<sub>3</sub> and (±)-epichlorohydrin were used, which gave **4.20f** as a colourless oil (1.02 g, 93%). R<sub>f</sub> (DCM) 0.28; IR<sub>v</sub><sub>max</sub>(film)/cm<sup>-1</sup> 1615 (C=O), 1489 (Ar C=C), 1068 (C-O); δ<sub>H</sub> (300MHz, CDCl<sub>3</sub>) 7.31-7.39 (2H, m, H-4, H-6), 7.06 (1H, td, *J* 0.8 and 7.8, H-5), 7.00 (1H, d, *J* 7.8, H-7), 4.16 (1H, dd, *J* 3.0 and 15.0, H-1'a/b), 3.54 (3, s, OMe), 3.53 (3H, s, OMe), 3.52 (1H, dd, *J* 6.0 and 15.0, H-1'a/b), 3.13 (1H, m, H-2'), 2.78 (1H, dd, *J* 3.0 and 3.3, H-3'a/b), 2.62 (1H, dd, *J* 3.0 and 6.0, H-3'a/b); δ<sub>C</sub> (75MHz, CDCl<sub>3</sub>) 170.8, 142.6, 130.6, 124.6, 124.5, 122.7, 109.8, 96.8, 50.7(x2), 49.6, 44.9, 41.5; LRMS(EI) found m/z 249.4 for C<sub>13</sub>H<sub>15</sub>NO<sub>4</sub> requires 249.1

### K. General procedure for preparation of compounds **4.21a-f**

Sodium azide (34.0 mmol, 5.0 eq) was added to a mixture of the epoxide **4.20** (6.79 mmol, 1.0 eq) and NH<sub>4</sub>Cl (20.4 mmol, 3.0 eq) in 6 mL MeOH-H<sub>2</sub>O (8:1). The resulting mixture was stirred at 25°C for 16 hours. The product mixture was diluted with deionized water and extracted with EtOAc. The combined organic extracted was washed with 10% aqueous NaHCO<sub>3</sub>, H<sub>2</sub>O and brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Concentration of the organic layer under reduced pressure afforded the azide.

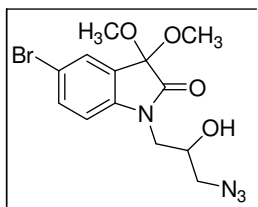
### 1-(3-Azido-2-hydroxypropyl)-5-chloro-3,3-dimethoxyindolin-2-one, **4.21a**



The conditions employed for the preparation of this compound is the same as the conditions described in General Procedure K; the epoxide **4.20a** gave **4.21a** as a colourless oil (2.06 g, 93%). R<sub>f</sub> (MeOH:DCM 1:99) 0.12; IR<sub>v</sub><sub>max</sub>(film)/cm<sup>-1</sup> 3457 (OH), 2105 (N<sub>3</sub>), 1613 (C=O); δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 7.38 (1H, d, *J* 2.0, H-4), 7.34 (1H, dd, *J* 2.4 and 8.0, H-6), 6.95 (1H, d, *J* 8.4, H-7), 4.08 (1H, m, H-2'), 3.75 (2H, m, H-3'), 3.55 (3H, s, OMe), 3.54

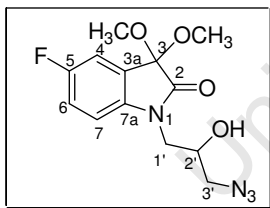
(3H, s, OMe), 3.45 (1H, dd, *J* 4.4 and 12.4, H-1'a/b), 3.36 (1H, dd, *J* 6.4 and 12.8, H-1'a/b), 2.84 (1H, br. s, OH);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 171.5, 141.3, 130.6, 128.6, 126.4, 125.3, 110.8, 96.5, 70.0, 54.4, 50.9 (x2), 43.7; LRMS(EI) found *m/z* 326.5 (42%, M<sup>+</sup>) for C<sub>13</sub>H<sub>15</sub>ClN<sub>4</sub>O<sub>4</sub> requires 326.1

### 1-(3-Azido-2-hydroxypropyl)-5-bromo-3,3-dimethoxyindolin-2-one, 4.21b

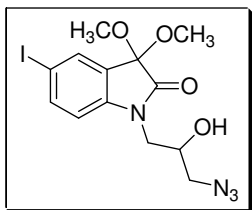


The conditions employed for the preparation of this compound is the same as the conditions described in General Procedure K. However, 5.99 mmol of the epoxide **4.20b**, 29.9 mmol of NaN<sub>3</sub> and 18.0 mmol of NH<sub>4</sub>Cl were used, which gave **4.21b** as a colourless oil (1.02g, 50%). *R<sub>f</sub>* (MeOH:DCM 1:99) 0.18; IR<sub>v<sub>max</sub></sub>(thin film)/cm<sup>-1</sup> 3457 (OH), 2105 (N<sub>3</sub>), 1610 (C=O);  $\delta_H$  (400MHz, CDCl<sub>3</sub>) 7.53 (1H, d, *J* 2.0, H-4), 7.50 (1H, dd, *J* 2.0 and 8.4, H-6), 6.91 (1H, d, *J* 8.4, H-7), 4.08 (1H, m, H-2'), 3.76 (2H, m, H-3'), 3.56 (3H, s, OMe), 3.55 (3H, s, OMe), 3.46 (1H, dd, *J* 4.4 and 12.8, H-1'a/b), 3.37 (1H, dd, *J* 6.4 and 12.8, H-1'a/b), 2.79 (1H, br. s, OH);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 166.1, 136.5, 128.2, 122.7, 121.5, 110.5, 106.0, 91.2, 63.7, 49.1, 45.6, 45.5, 38.3; LRMS(EI) found *m/z* 370.5 (10%, M<sup>+</sup>) for C<sub>13</sub>H<sub>15</sub>BrN<sub>4</sub>O<sub>4</sub> requires 370.0

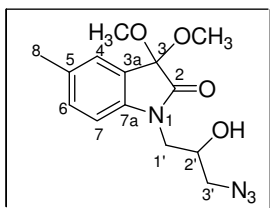
### 1-(3-Azido-2-hydroxypropyl)-5-fluoro-3,3-dimethoxyindolin-2-one, 4.21c



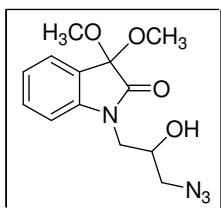
The conditions employed for the preparation of this compound is the same as the conditions described in General Procedure K. However, 5.26 mmol of the epoxide **4.20c**, 26.3 mmol of NaN<sub>3</sub> and 15.8 mmol of NH<sub>4</sub>Cl were used, which gave **4.21c** as a colourless oil (1.02 g, 50%). *R<sub>f</sub>* (MeOH:DCM 1:99) 0.24; IR<sub>v<sub>max</sub></sub>(thin film)/cm<sup>-1</sup> 3456 (OH), 2106 (N<sub>3</sub>), 1622 (C=O);  $\delta_H$  (400MHz, CDCl<sub>3</sub>) 7.16 (1H, dd, *J* 2.0 and 7.6, H-4), 7.08 (1H, ddd, *J* 2.4, 8.4 and 8.8, H-6), 6.96 (1H, dd, *J* 4.0 and 8.8, H-7), 4.09 (1H, m, H-2'), 3.77 (2H, m, H-3'), 3.56 (3H, s, OMe), 3.55 (3H, s, OMe), 3.46 (1H, dd, *J* 4.4 and 12.8, H-1'a/b), 3.37 (1H, dd, *J* 6.0 and 12.4, H-1'a/b), 2.82 (1H, br. s, OH);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 166.5, 155.0, 133.3, 121.0, 111.6, 107.7, 105.2, 91.3, 63.7, 49.1, 45.5(x2), 38.4; LRMS(EI) found *m/z* 310.6 (19%, M<sup>+</sup>) for C<sub>13</sub>H<sub>15</sub>FN<sub>4</sub>O<sub>4</sub> requires 310.1

**1-(3-Azido-2-hydroxypropyl)-5-iodo-3,3-dimethoxyindolin-2-one, 4.21d**

The conditions employed for the preparation of this compound is the same as the conditions described in General Procedure K. However, 3.67 mmol of the epoxide **4.20d**, 18.3 mmol of NaN<sub>3</sub> and 11.0 mmol of NH<sub>4</sub>Cl were used, which gave **4.21d** as a colourless oil (1.17 g, 88%). R<sub>f</sub> (MeOH:DCM 1:99) 0.19; IR<sub>v</sub>max(film)/cm<sup>-1</sup> 3454 (OH), 2104 (N<sub>3</sub>), 1607 (C=O); δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 7.70 (1H, d, *J* 2.0, H-4), 7.69 (1H, dd, *J* 1.8 and 8.0, H-6), 6.81 (1H, d, *J* 8.4, H-7), 4.07 (1H, m, H-2'), 3.74 (2H, m, H-3'), 3.55 (3H, s, OMe), 3.54 (3H, s, OMe), 3.46 (1H, dd, *J* 4.8 and 12.8, H-1'a/b), 3.36 (1H, dd, *J* 6.4 and 12.8, H-1'a/b), 2.65 (1H, br. s, OH); δ<sub>C</sub> (100MHz, CDCl<sub>3</sub>) 171.2, 142.5, 139.5, 133.6, 127.1, 111.8, 96.4, 85.6, 69.0, 54.4, 50.8, 50.9, 43.6; LRMS(EI) found *m/z* 418.4 (9%, M<sup>+</sup>) for C<sub>13</sub>H<sub>15</sub>IN<sub>4</sub>O<sub>4</sub> requires 418.0

**1-(3-Azido-2-hydroxypropyl)-3,3-dimethoxy-5-methylindolin-2-one, 4.21e**

The conditions employed for the preparation of this compound is the same as the conditions described in General Procedure K. However, 7.46 mmol of the epoxide **4.20e**, 37.3 mmol of NaN<sub>3</sub> and 22.4 mmol of NH<sub>4</sub>Cl were used, which gave **4.21e** as a colourless oil (1.90 g, 90%). R<sub>f</sub> (MeOH:DCM 1:99) 0.15; IR<sub>v</sub>max(film)/cm<sup>-1</sup> 3454 (OH), 2104 (N<sub>3</sub>), 1622 (C=O); δ<sub>H</sub> (400 MHz, CDCl<sub>3</sub>) 7.24 (1H, d, *J* 2.0, H-4), 7.16 (1H, dd, *J* 2.0 and 8.0, H-6), 6.88 (1H, d, *J* 8.0, H-7), 4.09 (1H, m, H-2'), 3.76 (2H, m, H-3'), 3.56 (3H, s, OMe), 3.55 (3H, s, OMe), 3.45 (1H, dd, *J* 4.4 and 12.8, H-1'a/b), 3.41 (1H, dd, *J* 5.6 and 12.8, H-1'a/b), 2.81 (1H, br. s OH), 2.36 (3H, s, H-8); δ<sub>C</sub> (100 MHz, CDCl<sub>3</sub>) 166.8, 134.9, 127.5, 125.7, 120.4, 119.6, 104.0, 91.7, 63.8, 49.1, 45.6, 45.5, 38.4, 15.7; LRMS(EI) found *m/z* 305.9 (55%, M<sup>+</sup>) for C<sub>14</sub>H<sub>18</sub>N<sub>4</sub>O<sub>4</sub> requires 306.1

**1-(3-Azido-2-hydroxypropyl)-3,3-dimethoxyindolin-2-one, 4.21f**

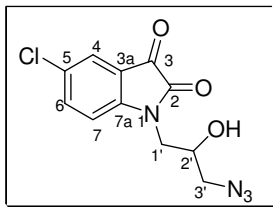
The conditions employed for the preparation of this compound is the same as the conditions described in General Procedure K. However, 7.46 mmol of the epoxide **4.20f**, 37.3 mmol of NaN<sub>3</sub> and 22.4 mmol of NH<sub>4</sub>Cl were used, which gave **4.21f** as a colourless oil (1.84 g, 92%). R<sub>f</sub> (MeOH:DCM 1:99) 0.14; IR<sub>v</sub>max(thin film)/cm<sup>-1</sup> 3454 (OH), 2104 (N<sub>3</sub>), 1615 (C=O); δ<sub>H</sub>

(400MHz, CDCl<sub>3</sub>) 7.42 (1H, d, *J* 0.8 and 7.6, H-4), 7.38 (1H, td, *J* 1.2 and 8.0, H-6), 7.12 (1H, td, *J* 0.8 and 7.6, H-5), 7.00 (1H, d, *J* 8.0, H-7), 4.10 (1H, m, H-2'), 3.79 (2H, m, H-3'), 3.57 (3H, s, OMe), 3.56 (3H, s, OMe), 3.45 (1H, dd, *J* 4.4 and 12.0, H-1'a/b), 3.39 (1H, dd, *J* 6.4 and 12.8, H-1'a/b); 2.78 (1H, br. s, OH);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 166.8, 137.3, 125.5, 119.7, 119.6, 117.7, 104.2, 91.5, 63.8, 49.1, 45.5(x2), 38.3; LRMS(EI) found *m/z* 292.6 (19%, M<sup>+</sup>) for C<sub>13</sub>H<sub>16</sub>N<sub>4</sub>O<sub>4</sub> requires 292.1

#### L. General procedure for preparation of compounds 4.22a-f

A solution of the azide **4.21** (500 mg, 1.53 mmol) in 9 ml acetone was treated with 1 mL of concentrated HCl. The resulting solution was stirred for 3 hours at 25°C. The product mixture was then neutralized with 2M Na<sub>2</sub>CO<sub>3</sub>. Extraction with EtOAc afforded the organic layer which was washed with water, brine and dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Removal of the solvent under reduced pressure afforded a crude residue which was recrystallized from methanol.

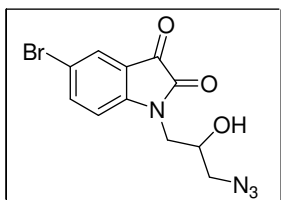
#### 1-(3-Azido-2-hydroxypropyl)-5-chloroindoline-2,3-dione, **4.22a**



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure L; the azide **4.21a** gave **4.22a** as a red crystalline solid (429 mg, 97%). m. p. 86°C; *R<sub>f</sub>* (MeOH:DCM 1:39) 0.44;

IR<sub>v<sub>max</sub></sub>(KBr)/cm<sup>-1</sup> 3470 (OH), 2100 (N<sub>3</sub>), 1744 (C<sub>3</sub>=O), 1608 (C<sub>2</sub>=O);  $\delta_H$  (400MHz, CDCl<sub>3</sub>) 7.55 (1H, dd, *J* 2.4 and 8.4, H-6), 7.51 (1H, d, *J* 2.4, H-4), 7.09 (1H, d, *J* 8.4, H-7), 4.14 (1H, m, H-2'), 3.80 (2H, m, H-1'), 3.53 (1H, *J* 4.4 and 12.8, H-3'a/b), 3.41 (1H, dd, *J* 6.0 and 12.4, H-3'a/b), 2.80 (1H, br. s, OH);  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 182.0, 158.6, 149.5, 137.8, 129.8, 125.7, 118.3, 112.6, 68.8, 54.4, 44.0; LRMS(EI) found *m/z* 279.8 (41%, M<sup>+</sup>) for C<sub>11</sub>H<sub>9</sub>ClN<sub>4</sub>O<sub>3</sub> requires 280.0

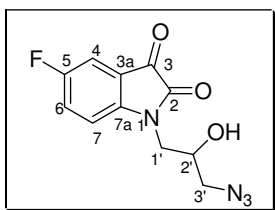
#### 1-(3-Azido-2-hydroxypropyl)-5-bromoindoline-2,3-dione, **4.22b**



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure L. However, 2.76 mmol (1.02 g) of the azide **4.21b** was

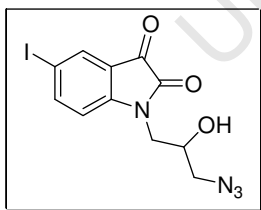
used, which gave **4.22b** as a red crystalline solid (773.0 mg, 86%). m. p. 85°C;  $R_f$  (EtOAc:Hex 2:3) 0.42;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3453 (OH), 2104 ( $\text{N}_3$ ), 1734 ( $\text{C}_3=\text{O}$ ), 1617 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.70 (1H, dd,  $J$  2.1 and 8.4, H-6), 7.66 (1H, d,  $J$  2.1, H-4), 7.02 (1H, d,  $J$  8.4, H-7), 4.13 (1H, m, H-2'), 3.79 (2H, m, H-3'), 3.53 (1H, dd,  $J$  4.2 and 12.6, H-1'a/b), 3.40 (1H, dd,  $J$  6.0 and 12.3, H-1'a/b), 2.98 (1H, br. s, OH);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 181.8, 158.4, 150.0, 140.7, 128.0, 118.7, 116.8, 113.0, 68.8, 54.4, 44.0; LRMS(EI) found  $m/z$  323.8 (31%,  $\text{M}^+$ ) for  $\text{C}_{11}\text{H}_9\text{BrN}_4\text{O}_3$  requires 324.0

### 1-(3-Azido-2-hydroxypropyl)-5-fluoroindoline-2,3-dione, **4.22c**

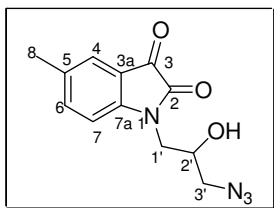


The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure L. However, 4.30 mmol (1.33 g) of the azide **4.21c** was used, which gave **4.22c** as an orange crystalline solid (1.11 g, 98%). m. p. 104°C,  $R_f$  (EtOAc:Hex 2:3) 0.30;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3444 (OH), 2090 ( $\text{N}_3$ ), 1730 ( $\text{C}_3=\text{O}$ ), 1612 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.28-7.35 (2H, m, H-4, H-6), 7.10 (1H, dd,  $J$  3.6 and 8.8, H-7), 4.15 (1H, m, H-2'), 3.82 (2H, m, H-3'), 3.54 (1H, dd,  $J$  4.4 and 12.8, H-1'a/b), 3.42 (1H, dd,  $J$  6.4 and 12.8, H-1'a/b), 3.07 (1H, br. s, OH);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 182.4, 160.7, 158.6, 147.4, 124.8, 118.2, 112.6, 112.3, 68.9, 54.5, 44.1; LRMS(EI) found  $m/z$  263.7 (45%,  $\text{M}^+$ ) for  $\text{C}_{11}\text{H}_9\text{FN}_4\text{O}_3$  requires 264.1

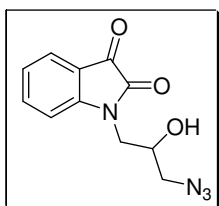
### 1-(3-Azido-2-hydroxypropyl)-5-iodoindoline-2,3-dione, **4.22d**



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure L. However, 2.81 mmol (1.17 g) of the azide **4.21d** was used, which gave **4.22d** as a red crystalline solid (974.5 mg, 93%). m. p. 92-93°C;  $R_f$  (EtOAc:Hex 2:3) 0.46;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3460 (OH), 2091 ( $\text{N}_3$ ), 1740 ( $\text{C}_3=\text{O}$ ), 1619 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.91 (1H, dd,  $J$  2.0 and 8.4, H-6), 7.88 (1H, d,  $J$  1.6, H-4), 6.93 (1H, d,  $J$  8.4, H-7), 4.14 (1H, m, H-2'), 3.82 (2H, m, H-3'), 3.56 (1H, dd,  $J$  4.4 and 12.4, H-1'a/b), 3.42 (1H, dd,  $J$  6.0 and 12.8, H-1'a/b), 2.64 (1H, br. s, OH);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 181.5, 158.1, 150.6, 146.5, 133.8, 119.1, 113.4, 86.3, 69.0, 54.5, 43.9; LRMS(EI) found  $m/z$  371.8 (45%,  $\text{M}^+$ ) for  $\text{C}_{11}\text{H}_9\text{IN}_4\text{O}_3$  requires 372.0

**1-(3-Azido-2-hydroxypropyl)-5-methylindoline-2,3-dione, 4.22e**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure L. However, 6.19 mmol (1.90 g) of the azide **4.21e** was used, which gave **4.22e** as a red crystalline solid (1.45 g, 90%). m. p. 97°C;  $R_f$  (EtOAc:Hex 2:3) 0.28;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3473 (OH), 2094 ( $\text{N}_3$ ), 1736 ( $\text{C}_3=\text{O}$ ), 1619 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.40-7.37 (2H, m, H-4, H-6), 6.96 (1H, d,  $J$  8.7, H-7), 4.14 (1H, m, H-2'), 3.78 (2H, m, H-3'), 3.51 (1H, dd,  $J$  4.2 and 12.9, H-1'a/b), 3.40 (1H, dd,  $J$  6.3 and 12.6, H-1'a/b), 3.05 (1H, br. s, OH), 2.31 (3H, s, H-8);  $\delta_{\text{C}}$  (75MHz,  $\text{CDCl}_3$ ) 183.1, 159.3, 149.0, 138.9, 134.0, 125.7, 117.7, 110.8, 68.9, 54.5, 44.0, 20.6; LRMS(EI) found  $m/z$  260.6 (11%,  $\text{M}^+$ ) for  $\text{C}_{12}\text{H}_{12}\text{N}_4\text{O}_3$  requires 260.1

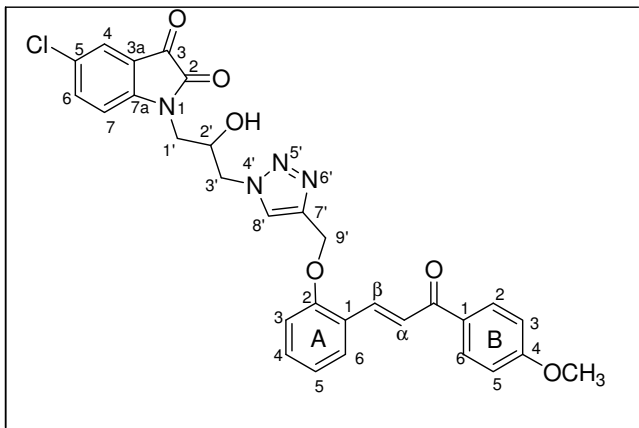
**1-(3-Azido-2-hydroxypropyl)indoline-2,3-dione, 4.22f**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure L. However, 6.29 mmol (1.84 g) of the azide **4.21f** was used, which gave **4.22f** as a red needles (1.72 g, quant). m. p. 96-97°C;  $R_f$  (EtOAc:Hex 2:3) 0.29;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3453 (OH), 2093 ( $\text{N}_3$ ), 1734 ( $\text{C}_3=\text{O}$ ), 1610 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 7.63-7.59 (2H, m, H-4, H-6), 7.14 (1H, td,  $J$  0.8 and 7.6, H-5), 7.09 (1H, d,  $J$  8.8, H-7), 4.16 (1H, m, H-2'), 3.83 (2H, m, H-3'), 3.54 (1H, dd,  $J$  4.4 and 12.4, H-1'a/b), 3.43 (1H, dd,  $J$  6.4 and 12.8, H-1'a/b), 3.15 (1H, br. s, OH);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 182.8, 159.2, 151.2, 138.5, 125.4, 124.1, 117.7, 111.0, 68.3, 54.5, 44.0; LRMS(EI) found  $m/z$  246.6 (3%,  $\text{M}^+$ ) for  $\text{C}_{11}\text{H}_{10}\text{N}_4\text{O}_3$  requires 246.1

**M. General procedure for preparation of compounds 4.23 a-i and 4.24 a-i**

The azide **4.22a** (0.356 mmol, 1.0 eq) and the appropriate acetylene **4.10a-i**, **4.13a-i** (0.392 mmol, 1.1 eq) was dissolved in 3 mL of  $\text{CH}_2\text{Cl}_2:\text{H}_2\text{O}$  (1:1). Copper(II) sulphate pentahydrate (0.0178 mmol, 5 mol%) and sodium ascorbate (0.0534 mmol, 15 mol%) was added and the resulting mixture stirred for 3 hours at 25°C. The precipitated product was filtered and washed with water and cold DCM.

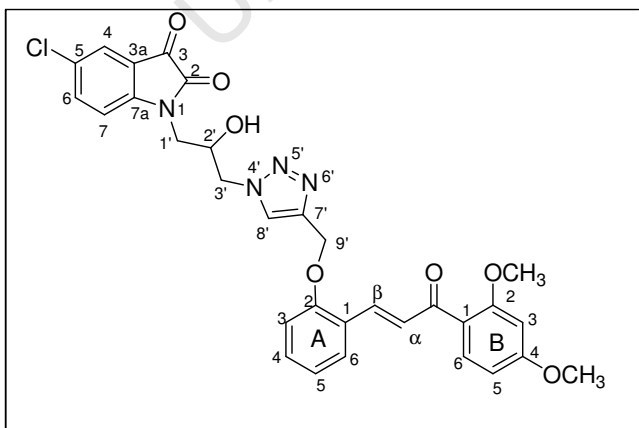
**(E)-5-chloro-1-(2-hydroxy-3-(4-((2-(3-(4-methoxyphenyl)-3-oxoprop-1-enyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.23a (EXP60A)**



Orange amorphous solid (118.4 mg, 58%); m. p. 178-180°C;  $R_f$  (MeOH:DCM 1:39) 0.13;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3470 (OH), 1753 ( $\text{C}_3=\text{O}$ ), 1662 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1607 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{DMSO-d}_6$ ) 8.24 (1H, s, H-8'), 8.02 (2H, d,  $J$  8.8, H-2B, H-6B), 7.90 (3H, m,  $H_{\alpha}$ ,  $H_{\beta}$ ,

H-6A), 7.68 (1H, dd,  $J$  2.0 and 8.4, H-6), 7.59 (1H, d,  $J$  2.0, H-4), 7.44 (1H, m, H-4A), 7.33 (1H, br. d,  $J$  8.0, H-3A), 7.23 (1H, d,  $J$  8.4, H-7), 7.06-7.02 (3H, m, H-5A, H-3B, H-5B), 5.51 (1H, d,  $J$  5.2, OH), 5.29 (2H, s, H-9'), 4.64 (1H, dd,  $J$  3.2 and 13.6, H-3'a/b), 4.45 (1H, dd,  $J$  8.8 and 13.6, H-3'a/b), 4.24 (1H, m, H-2'), 3.84 (3H, s, OMe), 3.76 (2H, m, H-1');  $\delta_{\text{C}}$  (100MHz,  $\text{DMSO-d}_6$ ) 188.1, 182.9, 163.8, 159.0, 158.0, 150.4, 142.8, 138.7, 137.5, 132.6, 131.4(x2), 131.2, 130.2, 128.0, 126.2, 124.4, 124.1, 122.9, 121.8, 119.7, 114.7(x2), 113.8, 113.6, 67.5, 62.3, 56.2, 53.7, 44.7; HRMS(ESI) found  $m/z$  573.1542  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$  requires 572.14626; Anal. Calc. for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$ : C, 62.88%; H, 4.40%; N, 9.78%. Found: C, 62.74%; H, 4.48%; N, 9.62%.

**(E)-5-chloro-1-(3-(4-((2-(3-(2,4-dimethoxyphenyl)-3-oxoprop-1-enyl)phenoxy) methyl)-1H-1,2,3-triazol-1-yl)-2-hydroxypropyl)indoline-2,3-dione, 4.23b (EXP60B)**

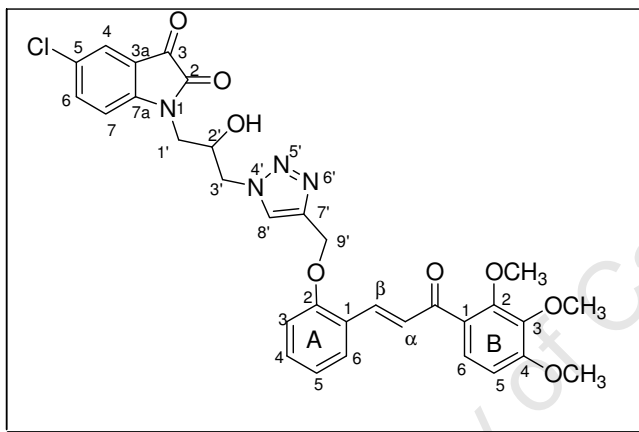


Orange amorphous solid (130.2 mg, 61%); m. p. 189°C;  $R_f$  (MeOH:DCM 1:39) 0.15;  $IR_{\nu_{\max}}$ (KBr)/ $\text{cm}^{-1}$  3429 (OH), 1748 ( $\text{C}_3=\text{O}$ ), 1658 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1607 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz,  $\text{DMSO-d}_6$ ) 8.18 (1H, s, H-8'), 7.76 (1H, d,  $J$  15.6,  $H_{\beta}$ ), 7.71-7.67 (2H, m, H-6, H-6A), 7.59 (1H, d,  $J$  2.0, H-

4), 7.54 (1H, d,  $J$  8.4, H-6B), 7.52 (1H, d,  $J$  15.6,  $H_{\alpha}$ ), 7.41 (1H, m, H-4A), 7.31 (1H, br. d,  $J$  8.0, H-3A), 7.23 (1H, d,  $J$  8.4, H-7), 7.02 (1H, br. t,  $J$  7.2, H-5A), 6.63 (1H, d,  $J$  2.0, H-3B),

6.59 (1H, dd,  $J$  2.0 and 8.8, H-5B), 5.51 (1H, d,  $J$  5.2, OH), 5.27 (2H, s, H-9'), 4.61 (1H, dd,  $J$  3.2 and 13.6, H-3'a/b), 4.42 (1H, dd,  $J$  8.4 and 13.6, H-3'a/b), 4.22 (1H, m, H-2'), 3.83 (6H, s, 2xOMe), 3.74 (2H, m, H-1');  $\delta_C$  (100MHz, DMSO- $d_6$ ) 190.1, 183.0, 164.6, 160.8, 159.0, 157.6, 150.4, 142.8, 137.5, 136.7, 132.7, 132.3, 129.1(x2), 128.0, 126.2, 124.4, 124.3, 122.2, 121.9, 119.7, 113.8, 113.6, 106.7, 99.3, 67.4, 62.3, 56.6, 56.3, 53.7, 44.7; HRMS(ESI) found  $m/z$  603.1639  $[M+H]^+$  for  $C_{31}H_{27}ClN_4O_7$  requires 602.15683; Anal Calc. for  $C_{31}H_{27}ClN_4O_7$ : C, 61.74%; H, 4.51%; N, 9.29%. Found: C, 61.63%; H, 4.85%; N, 8.99%.

**(*E*)-5-chloro-1-(2-hydroxy-3-(4-((2-(3-oxo-3-(2,3,4-trimethoxyphenyl)prop-1-enyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.23c (EXP60C)**

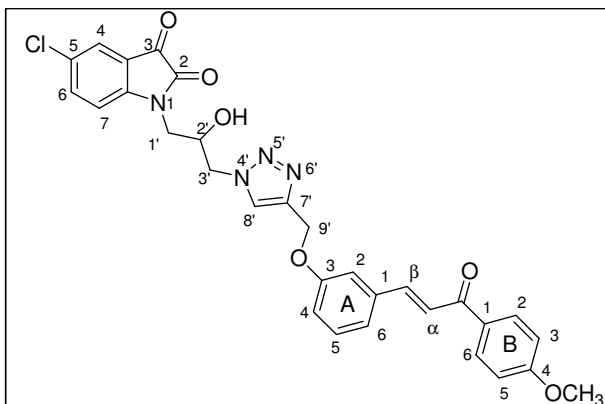


Orange amorphous solid (143.6 mg, 64%); m. p. 93°C;  $R_f$  (MeOH:DCM 1:39) 0.13;  $IR_{\max}$ (KBr)/ $cm^{-1}$  3446 (OH), 1744 ( $C_3=O$ ), 1649 ( $\alpha\beta$ -unsat  $C=O$ ), 1608 ( $C_2=O$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.93 (1H, s, H-8'), 7.84 (1H, d,  $J$  15.9, H $\beta$ ), 7.57 (1H, dd,  $J$  1.5

and 7.8, H-6), 7.45-7.39 (3H, m H $\alpha$ , H-4, H-6B), 7.34 (1H, m, H-4A), 7.28 (1H, d,  $J$  8.7, H-6A), 7.06-6.96 (3H, m, H-3A, H-5A, H-7), 6.70 (1H, d,  $J$  9.0, H-5B), 5.17 (2H, s, H-9'), 4.37 – 4.74 (3H, m, H-2', H-3'), 3.89 (3H, s, OMe), 3.85 (3H, s, OMe), 3.80 (3H, s, OMe), 3.79 (2H, m, H-1');  $\delta_C$  (75MHz,  $CDCl_3$ ) 191.7, 182.0, 158.6, 157.3, 156.9, 153.5, 149.6, 143.6, 141.8, 138.7, 137.7, 131.8, 130.6, 129.5, 128.9, 127.0, 126.5, 125.8, 124.8, 124.3, 121.6, 118.5, 113.0, 112.9, 107.4, 68.6, 62.8, 62.2, 61.1, 56.1, 53.8, 44.4; HRMS(EI) found  $m/z$  633.04956 for  $C_{32}H_{29}ClN_4O_8$  requires 633.04766; Anal Calc. for  $C_{32}H_{29}ClN_4O_8$ : C, 60.71%; H, 4.62%; N, 8.85%. Found: C, 60.31%; H, 4.53%; N, 8.64%.



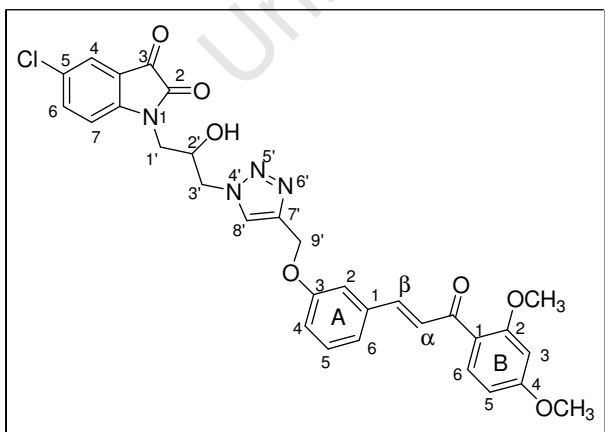
**(E)-5-chloro-1-(2-hydroxy-3-(4-((3-(3-(4-methoxyphenyl)-3-oxoprop-1-enyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.23d (EXP10/08)**



Orange amorphous solid (142.9 mg, 70%); m. p. 149-150°C;  $R_f$  (MeOH:DCM 1:39) 0.12;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3367 (OH), 1747 ( $\text{C}_3=\text{O}$ ), 1659 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1608 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (400MHz, DMSO- $d_6$ ) 8.12-8.15 (3H, m, H-2B, H-6B, H-8'), 7.87

(1H, d,  $J$  15.6, H $\beta$ ), 7.68-7.62 (2H, m, H $\alpha$ , H-6), 7.56 (2H, m, H-2A, H-4), 7.41-7.33 (2H, m, H-5A, H-6A), 7.22 (1H, d,  $J$  8.4, H-7), 7.10 (1H, m, H-4A), 7.06 (2H, d,  $J$  8.8, H-3B, H-5B), 5.44 (1H, br. s, OH), 5.23 (2H, s, H-9'), 4.59 (1H, dd,  $J$  3.6 and 13.6, H-3'a/b), 4.42 (1H, dd,  $J$  8.0 and 13.6, H-3'a/b), 4.24 (1H, m, H-2'), 3.86 (3H, s, OMe), 3.74 (2H, m, H-1');  $\delta_{\text{C}}$  (100MHz, DMSO- $d_6$ ) 187.3, 182.2, 163.2, 158.4, 158.2, 149.7, 142.9, 142.2, 136.7, 136.2, 130.9(x2), 130.4, 129.9, 127.2, 125.4, 123.6, 122.3, 121.9, 118.9, 117.1, 115.0, 114.0(x2), 112.9, 66.7, 61.2, 55.5, 45.9, 43.9; HRMS(EI) found  $m/z$  572.14574 for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$  requires 572.14626; Anal Calc. for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$ : C, 62.88%; H, 4.40%; N, 9.78%. Found: C, 62.84%; H, 4.38%; N, 9.70%.

**(E)-5-chloro-1-(3-(4-((3-(3-(2,4-dimethoxyphenyl)-3-oxoprop-1-enyl)phenoxy) methyl)-1H-1,2,3-triazol-1-yl)-2-hydroxypropyl)indoline-2,3-dione, 4.23e (EXP9/08)**

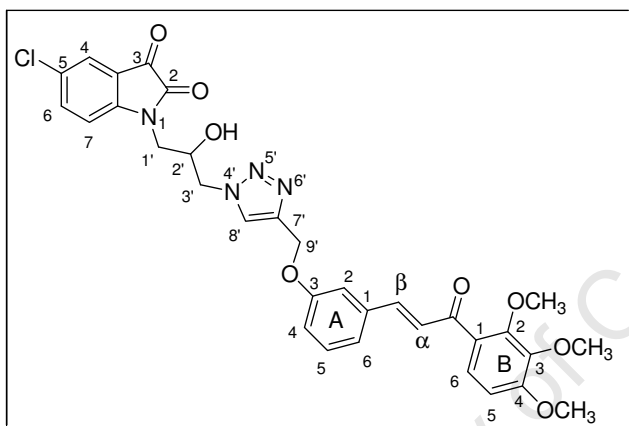


Orange amorphous solid (115.9 mg, 54%); m.p. 76°C;  $R_f$  (MeOH:DCM 1:39) 0.22;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3400 (OH), 1744 ( $\text{C}_3=\text{O}$ ), 1651 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1608 (amide  $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$ (400MHz, DMSO- $d_6$ ) 8.15 (1H, s, H-8'), 7.68 (1H, dd,  $J$  2.1 and 8.4, H-6), 7.61-7.48 (3H, m, H $\beta$ , H-6B, H-4), 7.26-7.38 (4H, m, H $\alpha$ , H-2A, H-5A,

H-6A), 7.23 (1H, d,  $J$  8.4, H-7), 7.08 (1H, ddd,  $J$  2.4, 2.7 and 7.8, H-4A), 6.72 (1H, d,  $J$  2.7, H-3B), 6.64 (1H, dd,  $J$  2.4 and 8.4, H-5B), 5.45 (1H, d,  $J$  5.4, OH), 5.21 (2H, s, H-9'), 4.59

(1H, dd, *J* 3.6 and 13.8, H-3'a/b), 4.13 (1H, dd, *J* 8.4 and 13.8, H-3'a/b), 4.24 (1H, m, H-2'), 3.90 (3H, s, OMe), 3.85 (3H, s, OMe), 3.75 (2H, m, H-1');  $\delta_C$  (100MHz, DMSO- $d_6$ ) 189.2, 182.0, 163.8, 160.1, 158.3, 158.0, 149.6, 142.2, 140.8, 136.6, 136.2, 131.7, 129.8, 127.4, 127.2, 125.1, 123.4, 121.3, 120.9, 118.7, 116.7, 113.9, 112.8, 105.9, 98.6, 66.6, 61.1, 55.8, 55.4, 52.8, 43.9; HRMS(EI) found *m/z* 602.15624 for  $C_{31}H_{27}ClN_4O_7$  requires 602.15683; Anal Calc. for  $C_{31}H_{27}ClN_4O_7$ : C, 61.74%; H, 4.51%; N, 9.29%. Found: C, 61.72%; H, 4.55%; N, 8.96%.

**(*E*)-5-chloro-1-(2-hydroxy-3-(4-((3-(3-oxo-3-(2,3,4-trimethoxyphenyl)prop-1-enyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.23f (EXP7/08)**

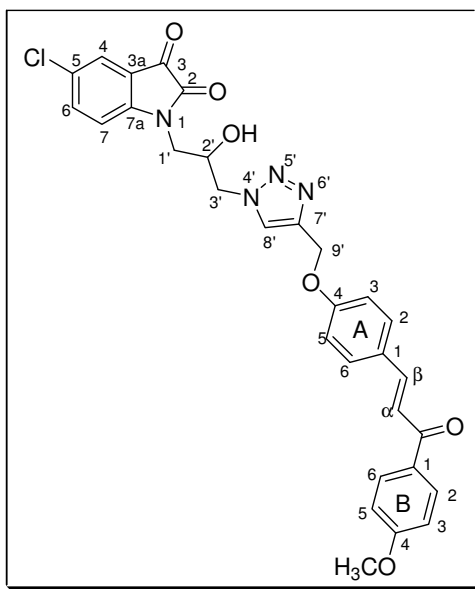


Orange amorphous solid (117.2 mg, 52%); m.p. 127-128°C;  $R_f$  (MeOH:DCM 1:39) 0.17;  $IR_{\max}(KBr)/cm^{-1}$  3487 (OH), 1749 ( $C_3=O$ ), 1654 ( $\alpha\beta$ -unsat  $C=O$ ), 1603 (amide  $C_2=O$ );  $\delta_H$  (300MHz,  $CDCl_3$ ) 7.85 (1H, s, H-8'), 7.54 (1H, d, *J* 15.6, H $\beta$ ), 7.48-7.36 (4H, m, H-6B,

H-4, H-6, H $\alpha$ ), 7.27 (1H, t, *J* 7.8, H-5A), 7.20-7.13 (2H, m, H-2A, H-6A), 7.05 (1H, d, *J* 8.7, H-7), 6.95 (1H, m, H-4A), 6.73 (1H, d, *J* 8.7, H-5B), 5.13 (2H, s, H-9'), 4.58 (1H, m, H-3'a/b), 4.50 (1H, m, H-2'), 4.40 (1H, dd, *J* 6.9, 13.2, H-3'a/b), 3.91 (3H, s, OMe), 3.89 (3H, s, OMe), 3.88 (3H, s, OMe), 3.75-3.94 (2H, m, H-1'), 2.75 (1H, br. s, OH);  $\delta_C$  (75MHz,  $CDCl_3$ ) 190.9, 181.9, 158.7, 158.4, 157.2, 153.8, 149.5, 142.6, 137.8, 136.5, 130.0, 129.7, 127.0, 126.5, 125.8, 124.9, 121.5, 118.4, 116.8, 114.5, 112.9, 107.4, 68.5, 62.1, 61.8, 61.0, 56.1, 53.9, 44.4; HRMS(EI) found *m/z* 632.16686 for  $C_{32}H_{29}ClN_4O_8$  requires 632.16739; Anal Calc. for  $C_{32}H_{29}ClN_4O_8$ : C, 60.71%; H, 4.62%; N, 8.85%. Found: C, 60.76%; H, 4.62%; N, 8.65%.

**(E)-5-chloro-1-(2-hydroxy-3-(4-((4-(3-(4-methoxyphenyl)-3-oxoprop-1-enyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.23g**

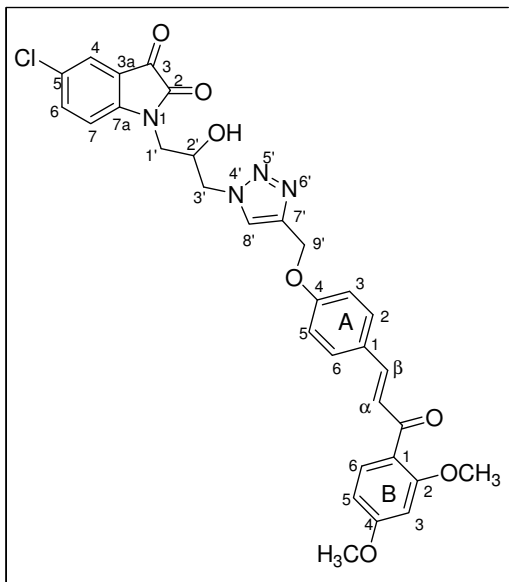
(EXP59A)



Orange amorphous solid (142.4 mg, 70%); m. p. 202-205°C;  $R_f$  (MeOH:DCM 1:39) 0.10;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3488 (OH), 1750 ( $\text{C}_3=\text{O}$ ), 1657 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1602 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz, DMSO- $d_6$ ) 8.17 (1H, s, H-8'), 8.12 (2H, d,  $J$  8.1, H-2B, H-6B), 7.83-7.58 (6H, m, H $\alpha$ , H $\beta$ , H-4, H-6, H-2A, H-6A), 7.24 (1H, d,  $J$  8.1, H-7), 7.12 (2H, d,  $J$  8.4, H-3B, H-5B), 7.07 (2H, d,  $J$  8.4, H-3A, H-5A), 5.44 (1H, br. s, OH), 5.24 (2H, s, H-9'), 4.60 (1H, br. d,  $J$  13.0, H-3'a/b), 4.44 (1H, dd,  $J$  7.8 and 13.8, H-3'a/b), 4.25 (1H, m, H-2'), 3.87 (3H, s,

OMe), 3.77 (2H, m, H-1');  $\delta_{\text{C}}$  (75MHz, DMSO- $d_6$ ) 187.2, 182.1, 162.9, 159.8, 158.1, 149.6, 142.7, 142.0, 136.7, 130.5(x2), 130.4(x2), 127.6, 127.2, 125.2, 123.5, 120.0, 119.7, 118.8, 115.0(x2), 113.8(x2), 112.8, 66.6, 61.2, 55.4, 52.9, 43.8; HRMS(ESI) found  $m/z$  573.1550  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$  requires 572.14626; Anal Calc. for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$ : C, 62.88%; H, 4.40%; N, 9.78%. Found: C, 62.64%; H, 4.38%; N, 9.48%.

**(E)-5-chloro-1-(3-(4-((4-(3-(2,4-dimethoxyphenyl)-3-oxoprop-1-enyl)phenoxy) methyl)-1H-1,2,3-triazol-1-yl)-2-hydroxypropyl)indoline-2,3-dione, 4.23h (EXP59B)**

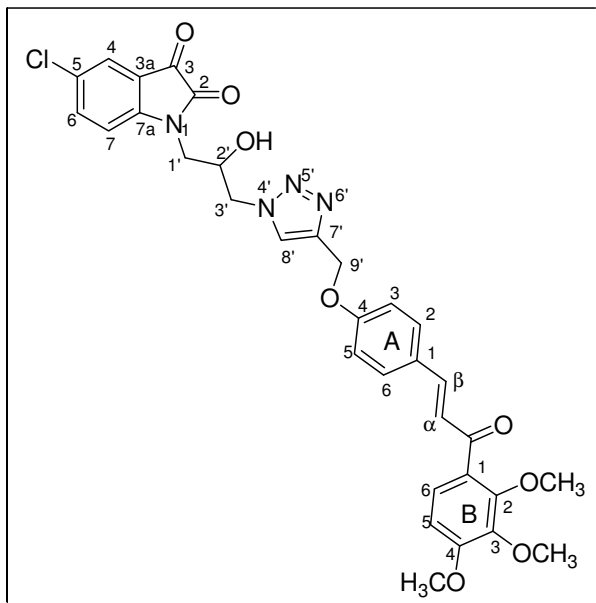


Orange amorphous solid (102.6 mg, 48%); m. p. 126-128°C;  $R_f$  (MeOH:DCM 1:39) 0.13;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3488 (OH), 1750 ( $\text{C}_3=\text{O}$ ), 1649 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1610 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{DMSO-d}_6$ ) 8.17 (1H, s, H-8'), 7.56-7.71 (5H, m, H-4, H-6, H-2A, H-6A, H-6B), 7.50 (1H, d,  $J$  15.6, H $\beta$ ), 7.38 (1H, d,  $J$  15.6, H $\alpha$ ), 7.24 (1H, d,  $J$  8.4, H-7), 7.10 (2H, d,  $J$  8.7, H-3A, H-5A), 6.67 (1H, d,  $J$  2.0, H-3B), 6.63 (1H, dd,  $J$  2.1 and 8.7, H-5B), 5.46 (1H, d,  $J$  4.8, OH), 5.22 (2H, s, H-9'), 4.60 (1H, dd,  $J$  3.3 and

13.5, H-3'a/b), 4.42 (1H, dd,  $J$  7.8 and 13.5, H-3'a/b), 4.24 (1H, m, H-2'), 3.89 (3H, s, OMe), 3.85 (3H, s, OMe), 3.76 (2H, m, H-1');  $\delta_{\text{C}}$  (75MHz,  $\text{DMSO-d}_6$ ) 189.2, 182.1, 163.6, 159.9, 159.7, 158.1, 149.6, 142.0, 141.0, 136.6, 131.6, 129.8(x2), 127.6, 127.2, 125.2, 124.9, 123.5, 121.6, 118.8, 115.1(x2), 112.8, 105.8, 98.6, 66.6, 61.2, 55.8, 55.4, 52.8, 43.9; HRMS(ESI) found  $m/z$  603.1675  $[\text{M}+\text{H}]^+$  for  $\text{C}_{31}\text{H}_{27}\text{ClN}_4\text{O}_7$  requires 602.15683; Anal Calc. for  $\text{C}_{31}\text{H}_{27}\text{ClN}_4\text{O}_7$ : C, 61.74%; H, 4.51%; N, 9.29%. Found: C, 61.36%; H, 4.32%; N, 9.07%.

**(E)-5-chloro-1-(2-hydroxy-3-(4-((4-(3-oxo-3-(2,3,4-trimethoxyphenyl)prop-1-enyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.23i**

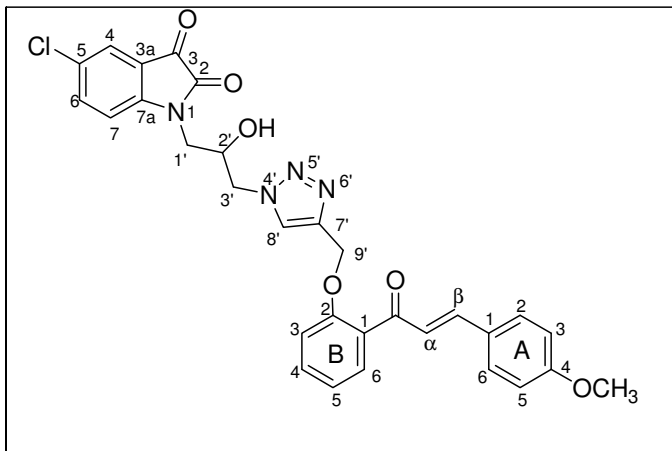
(EXP59C)



Orange amorphous solid (134.6 mg, 60%); m. p. 143-145°C;  $R_f$  (MeOH:DCM 1:39) 0.10;  $IR_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3487 (OH), 1749 ( $\text{C}_3=\text{O}$ ), 1655 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1608 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz, DMSO- $d_6$ ) 8.16 (1H, s, H-8'), 7.69-7.58 (4H, m, H-4, H-6, H-2A, H-6A), 7.50 (1H, d,  $J$  16.2, H $\beta$ ), 7.34-7.22 (3H, m, H-6B, H-7, H $\alpha$ ), 7.10 (2H, d,  $J$  8.4, H-3A, H-5A), 6.91 (1H, d,  $J$  8.7, H-5B), 5.47 (1H, d,  $J$  4.8,

OH), 5.22 (2H, s, H-9'), 4.59 (1H, br. d,  $J$  13.2, H-3'a/b), 4.40 (1H, dd,  $J$  8.4 and 13.2, H-3'a/b), 4.23 (1H, m, H-2'), 3.87 (3H, s, OMe), 3.83 (3H, s, OMe), 3.79 (3H, s, OMe), 3.76 (2H, m, H-1');  $\delta_{\text{C}}$  (75MHz, DMSO- $d_6$ ) 190.0 182.1, 159.9, 158.2., 156.3, 152.5, 149.6, 142.4, 142.3, 142.0, 136.7, 130.0(x2), 127.4, 127.2, 126.4, 125.3, 124.7, 124.4, 123.5, 118.8, 115.2(x2), 112.8, 107.8, 66.6, 61.5, 61.2, 60.3, 56.0, 53.0, 43.9; HRMS(ESI) found  $m/z$  633.1747  $[\text{M}+\text{H}]^+$  for  $\text{C}_{32}\text{H}_{29}\text{ClN}_4\text{O}_8$  requires 632.16739; Anal Calc. for  $\text{C}_{32}\text{H}_{29}\text{ClN}_4\text{O}_8$ : C, 60.71%; H, 4.62%; N, 8.85%. Found: C, 60.53%; H, 4.32%; N, 8.79%.

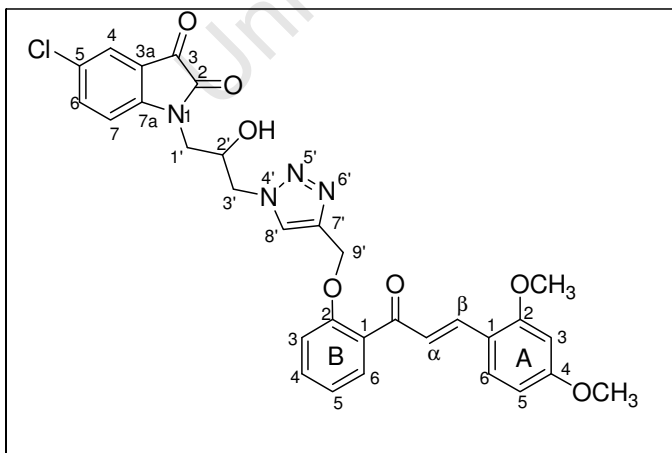
**(E)-5-chloro-1-(2-hydroxy-3-(4-((2-(3-(4-methoxyphenyl)acryloyl)phenoxy) methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.24a (EXP54A)**



Orange amorphous solid (171.0 mg, 84%); m. p. 93-94°C;  $R_f$  (MeOH:DCM 1:39) 0.24;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3435 (OH), 1744 ( $\text{C}_3=\text{O}$ ), 1650 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1598 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.62 (1H, s, H-8'), 7.48 (1H, dd,  $J$  1.5 and 7.2, H-6), 7.47-

7.35 (5H, m,  $\text{H}\beta$ , H-4, H-2A, H-6A, H-6B), 7.12-6.95 (5H, m,  $\text{H}\alpha$ , H-3B, H-4B, H-5B, H-7), 6.80 (2H, d,  $J$  8.7, H-3A, H-5A), 5.14 (2H, s, H-9'), 4.36 (1H, dd,  $J$  3.0 and 13.5, H-3'a/b), 4.27 (1H, m, H-2'), 4.16 (1H, dd,  $J$  7.2 and 13.2, H-3'a/b), 3.77 (3H, s, OMe), 3.54-3.72 (2H, m, H-1');  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 193.2, 182.0, 161.6, 158.6, 156.4, 149.6, 143.6, 143.4, 137.7, 132.9, 130.2(x3), 129.6, 127.4, 126.2, 124.9, 124.8, 122.8, 121.8, 118.5, 114.4(x2), 113.8, 112.9, 68.5, 63.1, 55.4, 53.8, 44.2; HRMS(ESI) found  $m/z$  573.1561  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$  requires 572.14626; Anal Calc. for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$ : C, 62.88%; H, 4.40%; N, 9.78%. Found: C, 62.55%; H, 4.12%; N, 9.66%.

**(E)-5-chloro-1-(3-(4-((2-(3-(2,4-dimethoxyphenyl)acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-hydroxypropyl)indoline-2,3-dione, 4.24b (EXP54B)**

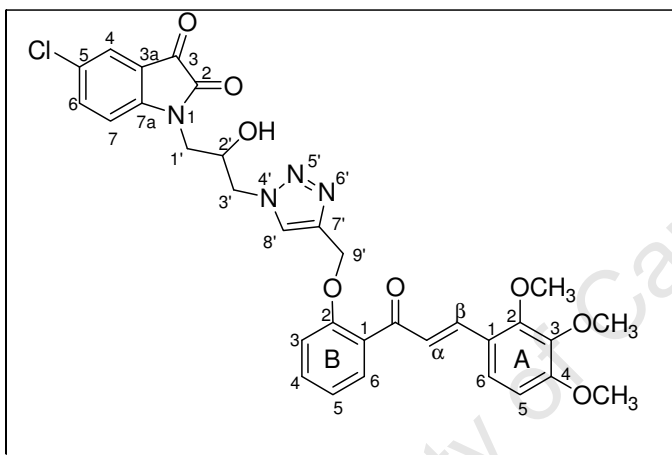


Orange amorphous solid (116.3 mg, 54%); m. p. 97-98°C;  $R_f$  (MeOH:DCM 1:39) 0.13;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3423 (OH), 1744 ( $\text{C}_3=\text{O}$ ), 1646 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1598 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz,  $\text{CDCl}_3$ ) 7.70 (1H, d,  $J$  15.6,  $\text{H}\beta$ ),

7.64 (1H, s, H-8'), 7.48-7.40 (4H, m, H-4, H-6, H-4B, H-6B), 7.32 (1H, d,  $J$  8.4, H-6A), 7.16 (1H, d,  $J$  15.6,  $\text{H}\alpha$ ), 7.06-6.93 (3H, m, H-7, H-3B, H-5B), 6.40 (1H, dd,  $J$  1.5 and 8.4, H-5A),

6.31 (1H, d,  $J$  1.8, H-3A), 5.12 (2H, s, H-9'), 4.37 (1H, dd,  $J$  2.4 and 13.2, H-3'a/b), 4.26 (1H, m, H-2'), 4.17 (1H, dd,  $J$  7.2 and 13.2, H-3'a/b), 3.75 (3H, s, OMe), 3.72 (3H, s, OMe), 3.56 (2H, m, H-1');  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 193.8, 182.0, 163.1, 160.2, 158.5, 156.3, 149.6, 143.5, 139.2, 137.7, 132.6, 130.5, 130.3, 130.2, 129.5, 125.0, 124.8, 124.7, 121.7, 118.4, 116.7, 113.7, 113.0, 105.6, 98.4, 68.5, 63.1, 55.5(x2), 53.8, 44.2; HRMS(ESI) found  $m/z$  603.1644 [M+H]<sup>+</sup> for C<sub>31</sub>H<sub>27</sub>ClN<sub>4</sub>O<sub>7</sub> requires 602.15683; Anal Calc. for C<sub>31</sub>H<sub>27</sub>ClN<sub>4</sub>O<sub>7</sub>: C, 61.74%; H, 4.51%; N, 9.29%. Found: C, 61.63%; H, 4.55%; N, 8.90%.

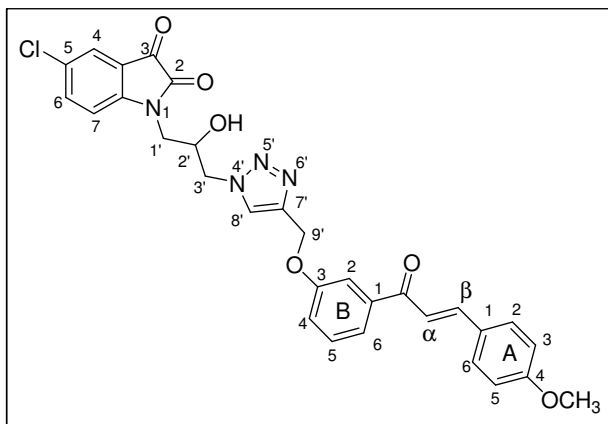
**(*E*)-5-chloro-1-(2-hydroxy-3-(4-((2-(3-(2,3,4-trimethoxyphenyl)acryloyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.24c (EXP54C)**



Orange amorphous solid (187.8 mg, 83%); m. p. 96°C;  $R_f$  (MeOH:DCM 1:39) 0.12; IR $\nu_{max}$ (KBr)/cm<sup>-1</sup> 3428 (OH), 1744 (C<sub>3</sub>=O), 1649 ( $\alpha\beta$ -unsat C=O), 1608 (C<sub>2</sub>=O);  $\delta_H$  (300MHz, CDCl<sub>3</sub>) 7.65 (1H, d,  $J$  15.9, H $\beta$ ), 7.64 (1H, s, H-8'), 7.49 (1H, dd,  $J$

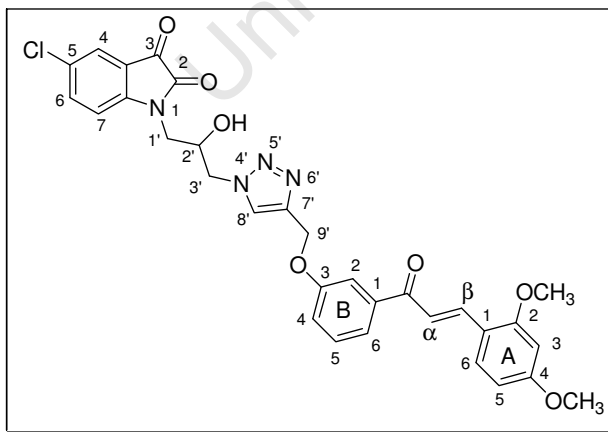
1.5 and 7.8, H-6), 7.46 (1H, d,  $J$  1.8, H-4), 7.44-7.40 (2H, m, H-4B, H-6B), 7.19 (1H, d,  $J$  9.3, H-6A), 7.18 (1H, d,  $J$  16.2, H $\alpha$ ), 6.97-7.09 (3H, m, H-7, H-3B, H-5B), 6.64 (1H, d,  $J$  8.7, H-5A), 5.14 (2H, s, H-9'), 4.41 (1H, dd,  $J$  3.0 and 13.5, H-3'a/b), 4.30 (1H, m, H-2'), 4.19 (1H, dd,  $J$  7.2 and 13.5, H-3'a/b), 3.85 (3H, s, OMe), 3.81 (3H, s, OMe), 3.79 (3H, s, OMe), 3.62-3.83 (2H, m, H-1');  $\delta_C$  (100MHz, CDCl<sub>3</sub>) 193.7, 182.0, 158.6, 156.4, 155.8, 153.4, 149.6, 143.4, 142.3, 139.1, 137.7, 132.8, 130.4, 130.2, 129.6, 126.2, 124.8(x2), 123.7, 121.8, 121.7, 118.5, 114.0, 112.9, 107.8, 68.6, 63.2, 61.6, 60.9, 56.1, 53.9, 44.3; HRMS(EI) found  $m/z$  633.04645 for C<sub>32</sub>H<sub>29</sub>ClN<sub>4</sub>O<sub>8</sub> requires 633.04766; Anal Calc. for C<sub>32</sub>H<sub>29</sub>ClN<sub>4</sub>O<sub>8</sub>: C, 60.71%; H, 4.62%; N, 8.85%. Found: C, 60.61%; H, 4.61%; N, 8.62%.

**(E)-5-chloro-1-(2-hydroxy-3-(4-((3-(3-(4-methoxyphenyl)acryloyl)phenoxy) methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.24d** (EXP13/08)



Orange amorphous solid (122.4 mg, 60%); m. p. 180°C;  $R_f$  (MeOH:DCM 1:39) 0.21;  $IR_{v_{max}}(KBr)/cm^{-1}$  3483 (OH), 1752 ( $C_3=O$ ), 1661 ( $\alpha\beta$ -unsat  $C=O$ ), 1611 (amide  $C_2=O$ );  $\delta_H$  (400MHz, DMSO- $d_6$ ) 8.18 (1H, s, H-8'), 7.84 (2H, d,  $J$  8.6, H-2A, H-6A), 7.76 (1H, d,  $J$  15.6, H $\beta$ ), 7.72-7.68 (4H, m, H $\alpha$ , H-2B, H-6B, H-6), 7.59 (1H, d,  $J$  2.0, H-4), 7.47 (1H, t,  $J$  8.0, H-5B), 7.31 (1H, m, H-4B), 7.24 (1H, d,  $J$  8.4, H-7), 7.00 (2H, d,  $J$  8.8, H-3A, H-5A), 5.49 (1H, d,  $J$  4.6, OH), 5.25 (2H, s, H-9'), 4.60 (1H, dd,  $J$  3.2 and 13.6, H-3'a/b), 4.42 (1H, dd,  $J$  8.0 and 13.6, H-3'a/b), 4.22 (1H, m, H-2'), 3.81 (3H, s, OMe), 3.74 (2H, m, H-1');  $\delta_C$  (100MHz, DMSO- $d_6$ ) 188.6, 182.2, 161.3, 158.2 (x2), 149.7, 144.0, 139.3, 136.7, 136.2, 130.8(x2), 129.8, 127.2, 125.4, 123.6, 121.0, 119.5, 119.4, 118.9, 114.3(x2), 114.1, 112.9, 110.3, 66.7, 61.3, 55.3, 52.9, 43.9; HRMS(EI) found  $m/z$  572.14569 for  $C_{30}H_{25}ClN_4O_6$  requires 572.14626; Anal Calc. for  $C_{30}H_{25}ClN_4O_6$ : C, 62.88%; H, 4.40%; N, 9.78%. Found: C, 62.79%; H, 4.38%; N, 9.50%.

**(E)-5-chloro-1-(3-(4-((3-(3-(2,4-dimethoxyphenyl)acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-hydroxypropyl)indoline-2,3-dione, 4.24e** (EXP12/08)

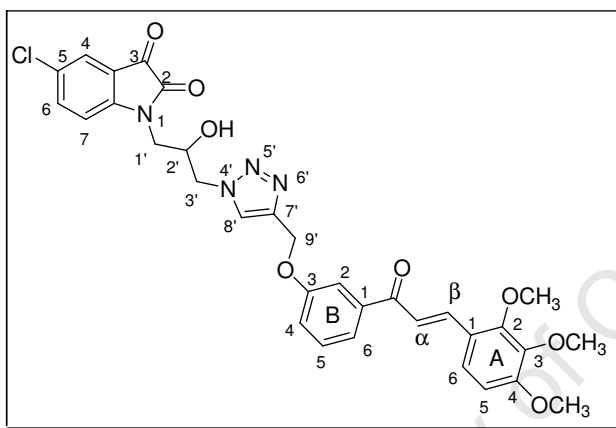


Orange amorphous solid (109.5 mg, 51%); m.p. 162°C;  $R_f$  (MeOH:DCM 1:39) 0.12;  $IR_{v_{max}}(KBr)/cm^{-1}$  3478 (OH), 1752 ( $C_3=O$ ), 1660 ( $\alpha\beta$ -unsat  $C=O$ ), 1611 (amide  $C_2=O$ );  $\delta_H$ (400MHz, DMSO- $d_6$ ) 8.18 (1H, s, H-8'), 7.97 (1H, d  $J$  15.6, H $\beta$ ), 7.91 (1H, d,  $J$  8.8, H-6A), 7.72-7.66 (4H, m, H $\alpha$ , H-2B, H-6B, H-6), 7.59 (1H, d,  $J$  2.0, H-4), 7.46 (1H, t,  $J$  8.0, H-5B), 7.30 (1H, m, H-4B), 7.23 (1H, d,  $J$  8.4, H-7), 6.59-6.62 (2H, m, H-3A, H-5A), 5.49 (1H, d,  $J$  4.8, OH), 5.25



(2H, s, H-9'), 4.60 (1H, dd,  $J$  2.8 and 13.6, H-3'a/b), 4.41 (1H, dd,  $J$  8.4 and 13.8, H-3'a/b), 4.21 (1H, m, H-2'), 3.83 (3H, s, OMe), 3.88 (3H, s, OMe), 3.74 (2H, m, H-1');  $\delta_C$  (100MHz,  $CDCl_3$ ) 188.7, 182.2, 163.1, 159.9, 158.3, 149.7, 142.2, 139.5, 138.8, 136.7, 130.0, 129.8, 127.2, 125.4, 123.6, 120.9, 119.3, 119.0, 118.9, 115.8, 113.9, 112.9, 106.3, 98.2, 94.9, 66.7, 61.3, 55.8, 55.5, 52.9, 43.9; HRMS(EI) found  $m/z$  602.15624 for  $C_{31}H_{27}ClN_4O_7$  requires 602.15683; Anal Calc. for  $C_{31}H_{27}ClN_4O_7$ : C, 61.74%; H, 4.51%; N, 9.29%. Found: C, 61.73%; H, 4.55%; N, 9.26%.

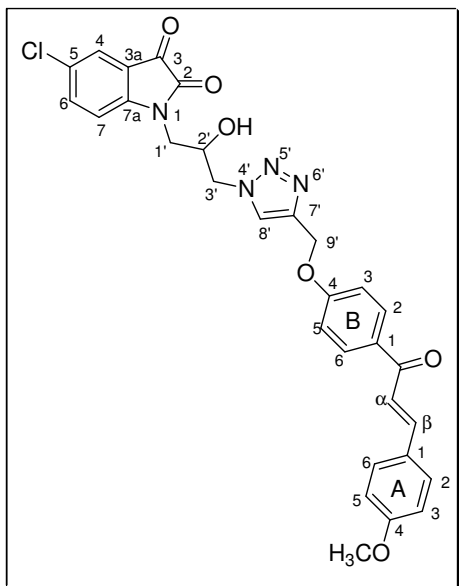
**(E)-5-chloro-1-(2-hydroxy-3-(4-((3-(3-(2,3,4-trimethoxyphenyl)acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.24f** (EXP11/08)



Orange amorphous solid (121.8 mg, 54%); m. p. 149-150°C;  $R_f$  (MeOH:DCM 1:39) 0.14,  $IR_{\max}(KBr)/cm^{-1}$  3478 (OH), 1751 ( $C_3=O$ ), 1660 ( $\alpha\beta$ -unsat  $C=O$ ), 1611 (amide  $C_2=O$ );  $\delta_H$  (400MHz,  $DMSO-d_6$ ) 8.18 (1H, s, H-8'), 7.90 (1H, d,  $J$  16.0, H $\beta$ ), 7.77 (1H, d,  $J$  9.2, H-6A),

7.76 (1H, d,  $J$  16.0, H $\alpha$ ), 7.70-7.68 (3H, m, H-6, H-2B, H-6B), 7.59 (1H, d,  $J$  2.0, H-4), 7.48 (1H, t,  $J$  8.0, H-5B), 7.32 (1H, ddd,  $J$  2.0, 2.4 and 8.0, H-4B), 7.24 (1H, d,  $J$  8.4, H-7), 6.90 (1H, d,  $J$  8.8, H-5A), 5.49 (1H, d,  $J$  4.8, OH), 5.25 (2H, s, H-9'), 4.60 (1H, dd,  $J$  3.2 and 13.6, H-3'a/b), 4.41 (1H, dd,  $J$  6.9, 13.2, H-3'a/b), 4.21 (1H, m, H-2'), 3.86 (3H, s, OMe), 3.85 (3H, s, OMe), 3.76 (3H, s, OMe), 3.75 (2H, m, H-1');  $\delta_C$  (100MHz,  $DMSO-d_6$ ) 188.8, 182.2, 158.3, 158.2, 155.8, 153.0, 149.7, 142.1, 141.80, 139.3, 138.5, 136.7, 129.8, 127.2, 125.4, 123.6, 123.4, 121.0, 120.9, 120.4, 119.4, 118.9, 114.0, 112.9, 108.4, 66.7, 61.4, 61.3, 60.4, 56.0, 52.9, 43.9; HRMS(EI) found  $m/z$  632.16686 for  $C_{32}H_{29}ClN_4O_8$  requires 632.16739; Anal Calc. for  $C_{32}H_{29}ClN_4O_8$ : C, 60.71%; H, 4.62%; N, 8.85%. Found: C, 60.64%; H, 4.63%; N, 8.25%.

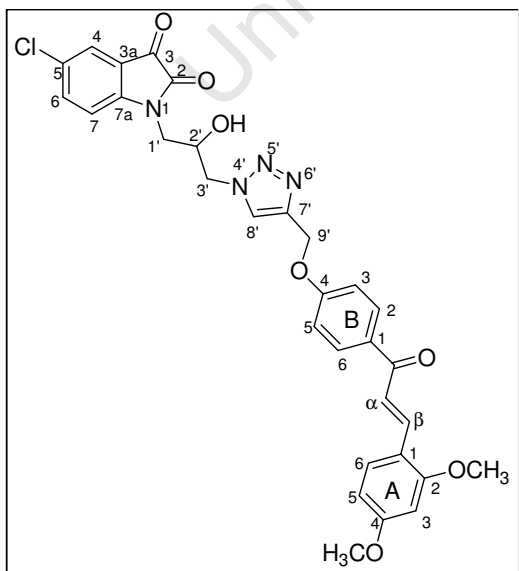
**(E)-5-chloro-1-(2-hydroxy-3-(4-((4-(3-(4-methoxyphenyl)acryloyl)phenoxy) methyl)-1H-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.24g (EXP53A)**



Orange amorphous solid (104.6 mg, 65%); m. p. 199-202°C;  $R_f$  (MeOH:DCM 1:39) 0.10;  $IR_{\max}$ (KBr)/ $\text{cm}^{-1}$  3489 (OH), 1750 ( $\text{C}_3=\text{O}$ ), 1658 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1601 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz, DMSO- $d_6$ ) 8.19 (1H, s, H-8'), 8.12 (2H, d,  $J$  9.0, H-2B, H-6B), 7.82-7.59 (6H, m,  $H_{\alpha}$ ,  $H_{\beta}$ , H-4, H-6, H-2A, H-6A), 7.24 (1H, d,  $J$  8.1, H-7), 7.28 (2H, d,  $J$  8.7, H-3A, H-5A), 7.01 (2H, d,  $J$  8.4, H-3B, H-5B), 5.48 (1H, br. s, OH), 5.29 (2H, s, H-9'), 4.61 (1H, dd,  $J$  2.1 and 13.2, H-3'a/b), 4.43 (1H, dd,  $J$  8.4 and 13.8, H-3'a/b), 4.24 (1H, m, H-2'), 3.82 (3H, s,

OMe), 3.76 (2H, m, H-1');  $\delta_{\text{C}}$  (100MHz, DMSO- $d_6$ ) 188.0, 183.0, 162.5, 161.9, 159.0, 150.4, 143.8, 142.6, 137.5, 131.6, 131.4(x2), 131.3(x2), 128.2, 128.0, 126.3, 124.4, 120.2, 119.7, 115.4(x2), 115.1(x2), 113.6, 67.4, 62.1, 56.1, 53.7, 44.7; HRMS(ESI) found  $m/z$  573.1558  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$  requires 572.14626; Anal Calc. for  $\text{C}_{30}\text{H}_{25}\text{ClN}_4\text{O}_6$ : C, 62.88%; H, 4.40%; N, 9.78%. Found: C, 62.28%; H, 4.36%; N, 9.59%.

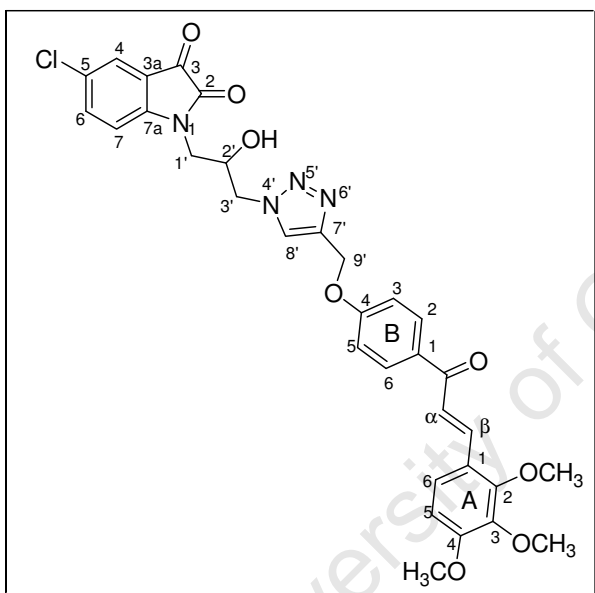
**(E)-5-chloro-1-(3-(4-((4-(3-(2,4-dimethoxyphenyl)acryloyl)phenoxy)methyl)-1H-1,2,3-triazol-1-yl)-2-hydroxypropyl)indoline-2,3-dione, 4.24h (EXP53B)**



Orange amorphous solid (171.9 mg, 80%); m. p. 185-187°C;  $R_f$  (MeOH:DCM 1:39) 0.11;  $IR_{\max}$ (KBr)/ $\text{cm}^{-1}$  3488 (OH), 1750 ( $\text{C}_3=\text{O}$ ), 1655 ( $\alpha\beta$ -unsat  $\text{C}=\text{O}$ ), 1611 ( $\text{C}_2=\text{O}$ );  $\delta_{\text{H}}$  (300MHz, DMSO- $d_6$ ) 8.18 (1H, s, H-8'), 8.07 (2H, d,  $J$  8.7, H-2B, H-6B), 7.94 (1H, d,  $J$  15.6,  $H_{\beta}$ ), 7.85 (1H, d,  $J$  8.4, H-6A), 7.70 (1H, d,  $J$  15.6,  $H_{\alpha}$ ), 7.68 (1H, dd,  $J$  1.8 and 8.7, H-6), 7.58 (1H, d,  $J$  2.1, H-4), 7.23 (1H, d,  $J$  8.7, H-7), 7.18 (2H, d,  $J$  8.7, H-3B, H-5B), 6.63-6.60

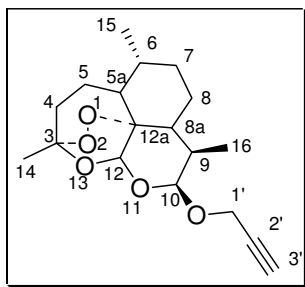
(2H, m, H-3A, H-5A), 5.49 (1H, br. s, OH), 5.28 (2H, s, H-9'), 4.60 (1H, dd,  $J$  3.3 and 14.1, H-3'a/b), 4.42 (1H, dd,  $J$  8.4 and 14.1, H-3'a/b), 4.24 (1H, m, H-2'), 3.90 (3H, s, OMe), 3.84 (3H, s, OMe), 3.75 (2H, m, H-1');  $\delta_C$  (75MHz, DMSO- $d_6$ ) 187.1, 181.8, 162.5, 161.2, 159.4, 157.8, 149.3, 141.5, 137.6, 137.4, 130.7, 130.0(x2), 129.6, 126.9, 125.0, 123.2, 118.8, 118.5, 115.7, 114.3(x2), 112.5, 105.9, 97.9, 66.3, 61.0, 55.4, 55.1, 52.6, 43.6; HRMS(ESI) found  $m/z$  603.1626  $[M+H]^+$  for  $C_{31}H_{27}ClN_4O_7$  requires 602.15683; Anal Calc. for  $C_{31}H_{27}ClN_4O_7$ : C, 61.74%; H, 4.51%; N, 9.29%. Found: C, 61.37%; H, 4.22%; N, 8.90%.

**(*E*)-5-chloro-1-(2-hydroxy-3-(4-((4-(3-(2,3,4-trimethoxyphenyl)acryloyl)phenoxy)methyl)-1*H*-1,2,3-triazol-1-yl)propyl)indoline-2,3-dione, 4.24i (EXP53C)**



Orange amorphous solid (203 mg, 90%); m. p. 155-157°C;  $R_f$  (MeOH:DCM 1:39) 0.10;  $IR_{\max}$ (KBr)/ $cm^{-1}$  3487 (OH), 1749 ( $C_3=O$ ), 1652 ( $\alpha\beta$ -unsat  $C=O$ ), 1603 ( $C_2=O$ );  $\delta_H$  (300MHz, DMSO- $d_6$ ) 8.18 (1H, s, H-8'), 8.09 (2H, d,  $J$  9.3, H-2B, H-6B), 7.86 (1H, d,  $J$  16.0, H $\beta$ ), 7.74 (1H, d,  $J$  15.6, H $\alpha$ ), 7.72-7.67 (2H, m, H-6, H-6A), 7.58 (1H, d,  $J$  2.7, H-4), 7.23 (1H, d,  $J$  8.7, H-7), 7.19 (2H, d,  $J$  8.7, H-3B, H-5B), 6.90 (1H, d,  $J$  8.7, H-5A),

5.48 (1H, d,  $J$  4.8, OH), 5.28 (2H, s, H-9'), 4.60 (1H, dd,  $J$  3.3 and 13.5, H-3'a/b), 4.42 (1H, dd,  $J$  8.4 and 13.5, H-3'a/b), 4.23 (1H, m, H-2'), 3.87 (3H, s, OMe), 3.86 (3H, s, OMe), 3.78 (3H, s, OMe), 3.75 (2H, m, H-1');  $\delta_C$  (75MHz, DMSO- $d_6$ ) 186.8, 181.5, 161.1, 157.5, 154.8, 152.2, 149.0, 141.2(x2), 137.0, 136.1, 130.3, 129.9(x2), 126.6, 124.8, 122.9, 122.6, 120.4, 119.9, 118.2, 114.0(x2), 112.2, 107.8, 66.0, 60.7(x2), 59.7, 55.4, 52.3, 43.3; HRMS(ESI) found  $m/z$  633.1751  $[M+H]^+$  for  $C_{32}H_{29}ClN_4O_8$  requires 632.16739; Anal Calc. for  $C_{32}H_{29}ClN_4O_8$ : C, 60.71%; H, 4.62%; N, 8.85%. Found: C, 60.59%; H, 4.36%; N, 8.79%.

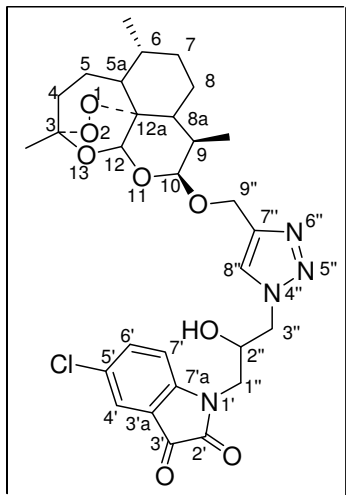
**Compound 5.4 (EXP39)**

Boron trifluoride diethyl etherate complex (5 drops) was added slowly to a solution of dihydroartemisinin **1.15** (1.00 g, 3.54 mmol, 1 eq) and propargyl alcohol (794 mg, 14.2 mmol, 4 eq) in 10 mL of dry DCM at 0°C under a nitrogen atmosphere. The resulting mixture was allowed to warm to 25°C and stirred at this temperature for 10 hours. The product mixture, after dilution with DCM, was washed successively with 5% aqueous NaHCO<sub>3</sub>, water and brine. The organic layer was dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated. Purification of the crude product using column chromatography (0-20% EtOAc:Hex) afforded the β-isomer as colourless needles (867.4 mg, 76%); m. p. 116°C; R<sub>f</sub> (EtOAc:Hex 1:4) 0.67; IR<sub>v</sub><sub>max</sub>(KBr)/cm<sup>-1</sup> 2113 (C≡C); δ<sub>H</sub> (300MHz, CDCl<sub>3</sub>) 5.39 (1H, s, H-12), 4.95 (1H, d, *J* 3.3, H-10), 4.29 (2H, d, *J* 2.4, H-1'), 2.65 (1H, m, H-9), 2.40-2.30 (2H, m, H-3' and a proton of the <sup>§</sup>DHA nucleus), 2.05-1.17 (10H, m, protons of the DHA nucleus), 1.42 (3H, s, H-14), 0.93 (3H, d, *J* 6.0, H-16), 0.91 (3H, d, *J* 7.2, H-15); δ<sub>C</sub> (75MHz, CDCl<sub>3</sub>) 104.1, 100.6, 88.0, 81.0, 79.7, 73.9, 54.9, 52.5, 44.3, 37.4, 36.4, 34.6, 30.6, 26.1, 24.7, 24.4, 20.3, 12.7; HRMS(ESI) found *m/z* 323.1207 [M+H]<sup>+</sup> for C<sub>18</sub>H<sub>26</sub>O<sub>5</sub> requires 322.17802; Anal Calc. for C<sub>18</sub>H<sub>26</sub>O<sub>5</sub>: C, 67.06%; H, 8.13%. Found: C, 66.70%; H, 8.10%.

**M. General procedure for preparation of compounds 5.5a-f**

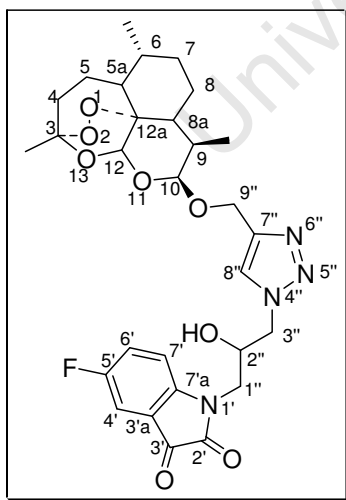
The azide **4.22** (0.356 mmol, 1.0 eq) and acetylene **5.4** (0.392 mmol, 1.1 eq) was dissolved in 3 mL of CH<sub>2</sub>Cl<sub>2</sub>:H<sub>2</sub>O (1:1). Copper (II) sulphate pentahydrate (0.0178 mmol, 5 mol%) and sodium ascorbate (0.0534 mmol, 15 mol%) was added and the resulting mixture stirred for 6 hours at 25°C. The product mixture was diluted with water and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous Na<sub>2</sub>SO<sub>4</sub> and concentrated. The crude product mixture was then subjected to column chromatography (0.5-10% MeOH/DCM).

<sup>§</sup> Apart from H-9, H-10, H-12 and the methyl protons H-14, H-15 and H-16, assignment of the remaining 11 protons of the DHA nucleus was not attempted. In the literature these protons are either not assigned or are omitted.

**Compound 5.5a (EXP57AA)**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure M; the azide **4.22a** was used, which gave **5.5a** as an orange foam (112 mg, 52%). m. p. 99-101°C;  $R_f$  (EtOAc:Hex 3:2) 0.13;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3463 (OH), 1747 ( $\text{C}_3=\text{O}$ ), 1611 ( $\text{C}_2=\text{O}$ ), 1475 (Ar C=C);  $\delta_{\text{H}}$  (400MHz, DMSO- $d_6$ ) 8.05 (1H, s, H-8''), 7.74 (1H, dd,  $J$  2.0 and 8.4, H-6'), 7.64 (1H, d,  $J$  2.0, H-4'), 7.29 (1H, d,  $J$  8.4, H-7'), 5.50 (1H, d,  $J$  4.4, OH), 5.44 (1H, d,  $J$  5.6, H-12), 4.85 (1H, br. s,

\*\*H-10), 4.79 (1H, d,  $J$  12.4, H-9''a/b), 4.61 (1H, dd,  $J$  3.2 and 14.0, H-3''a/b), 4.56 (1H, d,  $J$  12.8, H-9''a/b), 4.43 (1H, dd,  $J$  8.4 and 14.0, H-3''a/b), 4.25 (1H, m, H-2''), 3.77 (2H, m, H-1''), 2.45 (1H, m, H-9), 2.29-1.14 (11H, m, protons of the DHA nucleus), 1.34 (3H, s, H-14), 0.91 (3H, br. d,  $J$  6.0, H-16), 0.82 (3H, d,  $J$  7.2, H-15);  $\delta_{\text{C}}$  (100MHz, DMSO- $d_6$ ) 183.0, 159.0, 150.5, 144.3, 137.5, 128.0, 125.2, 124.4, 119.6, 113.7, 104.0, 100.6, 87.7, 81.2, 67.5, 61.1, 53.6, 52.8, 44.7, 44.5, 37.3, 36.7, 34.8, 31.0, 26.3, 24.9, 24.7, 20.8, 13.4; HRMS(ESI) found  $m/z$  603.2201  $[\text{M}+\text{H}]^+$  for  $\text{C}_{29}\text{H}_{35}\text{ClN}_4\text{O}_8$  requires 602.21434; Anal Calc. for  $\text{C}_{29}\text{H}_{35}\text{ClN}_4\text{O}_8$ : C, 57.76%; H, 5.85%; N, 9.29%. Found: C, 57.56%; H, 5.70%; N, 8.93%;

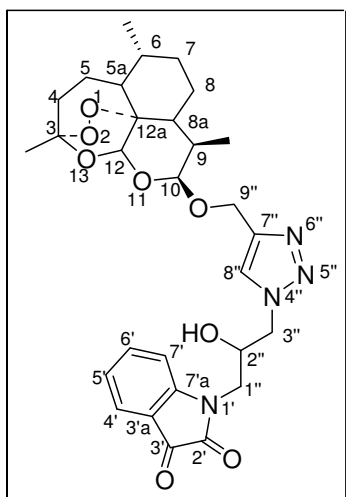
**Compound 5.5b (EXP57BA)**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure M. However, 0.380 mmol of the azide **4.22c** was used, which gave **5.5b** as an orange foam (69.1 mg, 31%). m. p. 101-103°C;  $R_f$  (EtOAc:Hex 3:2) 0.11;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3464 (OH), 1743 ( $\text{C}_3=\text{O}$ ), 1623 ( $\text{C}_2=\text{O}$ ), 1487 (Ar C=C);  $\delta_{\text{H}}$  (400MHz, DMSO- $d_6$ ) 8.06 (1H, s, H-8''), 7.57 (1H, ddd,  $J$  2.4, 8.8 and 9.2, H-6'), 7.49 (1H, dd,  $J$  2.4 and 6.8, H-4'), 7.28 (1H, dd,  $J$  4.0 and 8.4, H-7'), 5.51 (1H, d,  $J$  4.8, OH), 5.44 (1H, d,  $J$  6.0, H-12), 4.85 (1H, br. s, H-10), 4.79 (1H, d,  $J$  12.4, H-9''a/b),

\*\* H-10 for this series of compounds (**5.5**) consistently appeared as a broad singlet (fig. 5.10).

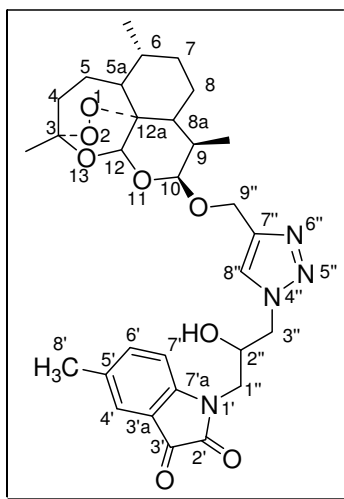
4.61 (1H, dd,  $J$  3.6 and 14.0, H-3''a/b), 4.56 (1H, d,  $J$  12.4, H-9''a/b), 4.43 (1H, dd,  $J$  8.0 and 14.0, H-3''a/b), 4.26 (1H, m, H-2''), 3.77 (2H, m, H-1''), 2.45 (1H, m, H-9), 2.25-1.14 (11H, m, protons of the DHA nucleus), 1.33 (3H, s, H-14), 0.91 (3H, dd,  $J$  2.0 and 6.0, H-16), 0.82 (3H, d,  $J$  7.2, H-15);  $\delta_C$  (100MHz, DMSO- $d_6$ ) 184.2, 160.3, 148.2, 144.3, 129.4, 125.3, 124.4, 113.4, 111.7, 104.1, 100.6, 87.7, 81.2, 67.4, 61.1, 55.6, 53.6, 52.8, 44.6, 44.5, 37.3, 36.7, 34.8, 31.0, 26.3, 24.9, 24.7, 20.8, 13.4; HRMS(ESI) found  $m/z$  587.2512  $[M+H]^+$  for  $C_{29}H_{35}FN_4O_8$  requires 586.24389; Anal Calc. for  $C_{29}H_{35}FN_4O_8$ : C, 59.38%; H, 6.01%; N, 9.55%. Found: C, 59.10%; H, 6.00%; N, 9.10%;

### Compound 5.5c (EXP57CC)



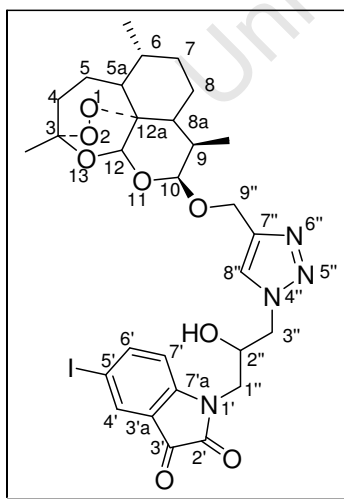
The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure M. However, 0.404 mmol of the azide **4.22f** was used, which gave **5.5c** as an orange foam (110.3 mg, 48%); m. p. 92°C;  $R_f$  (EtOAc:Hex 3:2) 0.10;  $IR_{\max}$ (KBr)/ $cm^{-1}$  3463 (OH), 1741 ( $C_3=O$ ), 1612 ( $C_2=O$ ), 1472 (Ar  $C=C$ );  $\delta_H$  (400MHz, DMSO- $d_6$ ) 8.02 (1H, s, H-8''), 7.65 (1H, br. t,  $J$  7.5, H-6'), 7.55 (1H, br. d,  $J$  6.9, H-4'), 7.19 (1H, d,  $J$  7.8, H-7'), 7.13 (1H, br. t,  $J$  7.2, H-5'), 5.39-5.45

(2H, m, H-12 and OH), 4.82 (1H, br. s, H-10), 4.76 (1H, d,  $J$  12.3, H-9''a/b), 4.52-4.60 (2H, m, H-3''a/b, H-9''a/b), 4.41 (1H, dd,  $J$  7.8 and 13.5, H-3''a/b), 4.25 (1H, m, H-2''), 3.73 (2H, m, H-1''), 2.43 (1H, m, H-9), 2.24-1.09 (11H, m, protons of the DHA nucleus), 1.30 (3H, s, H-14), 0.88 (3H, d,  $J$  4.8, H-16), 0.79 (3H, d,  $J$  7.2, H-15);  $\delta_C$  (100MHz, DMSO- $d_6$ ) 183.2, 158.3, 151.1, 143.5, 137.8, 124.3, 124.0, 122.9, 117.5, 111.0, 103.2, 99.9, 86.9, 80.3, 66.6, 60.3, 52.8, 52.0, 43.8, 43.7, 36.5, 35.9, 33.9, 30.2, 25.4, 24.1, 23.8, 19.9, 12.4; HRMS(ESI) found  $m/z$  569.2589  $[M+H]^+$  for  $C_{29}H_{36}N_4O_8$  requires 568.25331; Anal Calc. for  $C_{29}H_{36}N_4O_8$ : C, 61.26%; H, 6.38%; N, 9.85%. Found: C, 60.96%; H, 6.17%; N, 9.55%

**Compound 5.5d (EXP57D)**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure M. However, 0.387 mmol of the azide **4.22e** was used, which gave **5.5d** as an orange foam (101.4 mg, 45%). m. p. 103-105°C;  $R_f$  (EtOAc:Hex 3:2) 0.12;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3464 (OH), 1736 ( $\text{C}_3=\text{O}$ ), 1622 ( $\text{C}_2=\text{O}$ ), 1492 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{DMSO-d}_6$ ) 8.06 (1H, s, H-8''), 7.51 (1H, br. d,  $J$  8.4, H-6'), 7.41 (1H, br s, H-4'), 7.13 (1H, d,  $J$  8.0, H-7'), 5.51 (1H, d,  $J$  7.2, OH), 5.44 (1H, d,  $J$  3.6, H-

12), 4.85 (1H, br. s, H-10), 4.79 (1H, d,  $J$  12.4, H-9''a/b), 4.61 (dd,  $J$  3.6 and 14.0, H-3''a/b), 4.56 (1H, d,  $J$  12.8, H-9''a/b), 4.43 (1H, dd,  $J$  8.4 and 14.0, H-3''a/b), 4.25 (1H, m, H-2''), 3.74 (2H, d,  $J$  6.0, H-1''), 2.45 (1H, m, H-9), 2.32 (3H, s, H-8'), 2.25-1.16 (11H, m, protons of the DHA nucleus), 1.33 (3H, s, H-14), 0.91 (3H, dd,  $J$  2.8 and 6.0, H-16), 0.82 (3H, d,  $J$  7.2, H-15);  $\delta_{\text{C}}$  (100MHz,  $\text{DMSO-d}_6$ ) 184.3, 159.3, 149.8, 144.3, 138.9, 133.1, 125.3, 125.1, 118.3, 111.7, 104.1, 100.6, 87.7, 81.2, 67.4, 61.1, 53.7, 52.8, 44.6, 44.5, 37.3, 36.7, 34.8, 31.1, 26.3, 24.9, 24.7, 20.8, 20.7, 13.4; HRMS(ESI) found  $m/z$  583.2747  $[\text{M}+\text{H}]^+$  for  $\text{C}_{30}\text{H}_{38}\text{N}_4\text{O}_8$  requires 582.26896; Anal. Calc. for  $\text{C}_{30}\text{H}_{38}\text{N}_4\text{O}_8$ : C, 61.84%; H, 6.57%; N, 9.62%. Found: C, 61.67%; H, 6.56%; N, 9.32%;

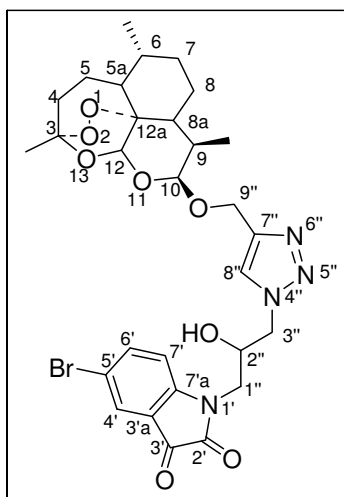
**Compound 5.5e (EXP57E)**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure M. However, 0.308 mmol of the azide **4.22d** was used, which gave **5.5e** as an orange foam (138.3 mg, 42%). m. p. 112-115°C;  $R_f$  (EtOAc:Hex 3:2) 0.13;  $IR_{\max}(\text{KBr})/\text{cm}^{-1}$  3458 (OH), 1743 ( $\text{C}_3=\text{O}$ ), 1605 ( $\text{C}_2=\text{O}$ ), 1470 (Ar C=C);  $\delta_{\text{H}}$  (400MHz,  $\text{DMSO-d}_6$ ) 8.05 (1H, s, H-8''), 8.01 (1H, dd,  $J$  1.6 and 8.4, H-6'), 7.85 (1H, d,  $J$  1.6, H-4'), 7.11 (1H, d,  $J$  8.8, H-7'), 5.50 (1H, d,  $J$  3.2, OH), 5.44 (1H, d,

$J$  5.6, H-12), 4.85 (1H, br. s, H-10), 4.79 (1H, d,  $J$  12.4, H-9''a/b), 4.61 (dd,  $J$  3.6 and 14.0,

H-3''a/b), 4.56 (1H, d, *J* 12.4, H-9''a/b), 4.42 (1H, dd, *J* 8.0 and 14.0, H-3''a/b), 4.24 (1H, m, H-2''), 3.75 (2H, d, *J* 6.8, H-1''), 2.45 (1H, m, H-9), 2.25-1.17 (11H, m, protons of the DHA nucleus), 1.34 (3H, s, H-14), 0.91 (3H, dd, *J* 2.0 and 6.4, H-16), 0.82 (3H, d, *J* 7.2, H-15);  $\delta_C$  (100MHz, DMSO- $d_6$ ) 182.5, 158.5, 151.3, 146.1, 144.3, 132.6, 125.3, 120.4, 114.4, 104.0, 100.6, 87.7, 86.5, 81.2, 67.4, 61.1, 60.4, 53.6, 52.8, 44.5, 37.3, 36.7, 34.8, 31.0, 26.3, 24.9, 24.7, 20.8, 13.4; HRMS(ESI) found *m/z* 695.1567  $[M+H]^+$  for  $C_{29}H_{35}IN_4O_8$  requires 694.14996; Anal Calc. for  $C_{29}H_{35}IN_4O_8$ : C, 50.15%; H, 5.08%; N, 8.07%. Found: C, 50.11%; H, 5.01%; N, 7.90%;

### Compound 5.5f (EXP57FB)



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure M. However, 0.474 mmol of the azide **4.22b** was used, which gave **5.5f** as an orange foam (103.8 mg, 52%); m. p. 104°C;  $R_f$  (EtOAc:Hex 3:2) 0.16;  $IR_{\max}(KBr)/cm^{-1}$  3464 (OH), 1744 ( $C_3=O$ ), 1609 ( $C_2=O$ ), 1472 (Ar  $C=C$ );  $\delta_H$  (400MHz, DMSO- $d_6$ ) 8.06 (1H, s, H-8''), 7.87 (1H, dd, *J* 2.0 and 8.8, H-6'), 7.74 (1H, d, *J* 2.0, H-4'), 7.24 (1H, d, *J* 8.8, H-7'), 5.50 (1H, d, *J* 3.6, OH), 5.44 (1H, d,

*J* 5.6, H-12), 4.85 (1H, br. s, H-10), 4.79 (1H, d, *J* 12.4, H-9''a/b), 4.61 (1H, dd, *J* 3.2 and 13.6, H-3''a/b), 4.55 (1H, d, *J* 12.0, H-9'' a/b), 4.43 (1H, dd, *J* 8.0 and 13.6, H-3''a/b), 4.24 (1H, m, H-2''), 3.76 (2H, d, *J* 6.8, H-1''), 2.45 (1H, m, H-9), 2.25-1.14 (11H, m, protons of the DHA nucleus), 1.34 (3H, s, H-14), 0.91 (3H, d, *J* 6.4, H-16), 0.82 (3H, d, *J* 6.8, H-15);  $\delta_C$  (100MHz, DMSO- $d_6$ ) 182.8, 158.8, 150.8, 144.3, 140.3, 127.1, 125.3, 120.1, 115.5, 114.1, 104.1, 100.6, 87.7, 81.2, 67.4, 61.1, 53.6, 52.8, 44.6, 44.5, 37.3, 36.7, 34.8, 31.0, 26.3, 24.9, 24.7, 20.8, 13.4; HRMS(ESI) found *m/z* 5647.1719  $[M+H]^+$  for  $C_{29}H_{35}BrN_4O_8$  requires 646.16383; Anal Calc. for  $C_{29}H_{35}BrN_4O_8$ : C, 53.79%; H, 5.45%; N, 8.65%. Found: C, 53.40%; H, 5.35%; N, 8.28%;

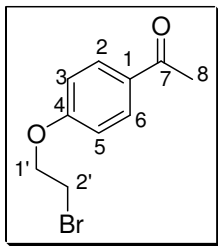
### N. General procedure for preparation of compounds 5.6a-c

Anhydrous  $K_2CO_3$  (44.0 mmol, 2.0 eq) was added to hydroxyacetophenone (22.0 mmol, 1.0 eq) dissolved in 5 mL anhydrous DMF. The appropriate dibromoalkane (66.0 mmol, 3.0 eq)



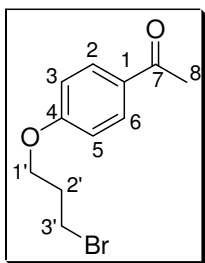
was added and the resulting mixture stirred for 48 hours at 25°C. The product mixture was extracted with DCM and the combined organic layer washed several times with water to remove DMF before being dried over anhydrous Na<sub>2</sub>SO<sub>4</sub>. Concentration of the organic layer *in vacuo* afforded a residue which was purified using column chromatography (0-30% EtOAc:Hex).

### 1-(4-(2-bromoethoxy)phenyl)ethanone, 5.6a



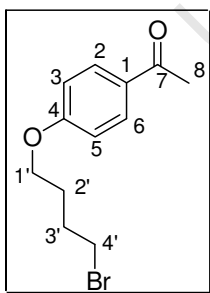
Colourless crystalline solid (3.90g, 73%); m. p. 58°C; R<sub>f</sub> (EtOAc:Hex 1:4) 0.32; δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 7.95 (2H, d, *J* 8.8, H-2 and H-6), 6.95 (2H, d, *J* 9.2, H-3 and H-5), 4.36 (2H, t, *J* 6.0, H-1'), 3.66 (2H, t, *J* 6.4, H-2'), 2.56 (3H, s, H-8); δ<sub>C</sub> (100MHz, CDCl<sub>3</sub>) 196.6, 161.9, 131.0, 130.6(x2), 114.3(x2), 67.9, 28.5, 26.3

### 1-(4-(3-bromopropoxy)phenyl)ethanone, 5.6b



Colourless liquid (3.84g, 68%); R<sub>f</sub> (EtOAc:Hex 1:4) 0.43; δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 7.92 (2H, d, *J* 9.2, H-2 and H-6), 6.94 (2H, d, *J* 8.4, H-3 and H-5), 4.18 (2H, t, *J* 6.0, H-1'), 3.59 (2H, t, *J* 6.4, H-3'), 2.54 (3H, s, H-8), 2.34 (2H, quint, *J* 6.4, H-2'); δ<sub>C</sub> (100MHz, CDCl<sub>3</sub>) 196.6, 162.6, 130.6(x2), 130.6, 114.2(x2), 65.6, 32.2, 29.6, 26.3

### 1-(4-(4-bromobutoxy)phenyl)ethanone, 5.6c



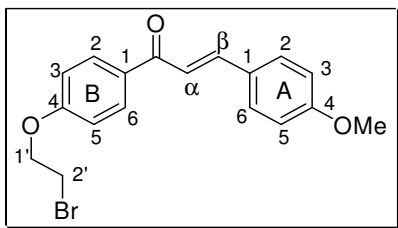
Colourless liquid (3.89g, 65%); R<sub>f</sub> (EtOAc:Hex 1:4) 0.45; δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 7.90 (2H, d, *J* 8.4, H-2 and H-6), 6.90 (2H, d, *J* 8.4, H-3 and H-5), 4.06 (2H, t, *J* 5.6, H-1'), 3.47 (2H, t, *J* 6.4, H-4'), 2.52 (3H, s, H-8), 2.06 (2H, quint, *J* 6.8, H-2'), 1.96 (2H, quint, *J* 6.4, H-3'); δ<sub>C</sub> (100MHz, CDCl<sub>3</sub>) 196.6, 162.8, 130.6(x2), 130.4, 114.1(x2), 67.1, 33.2, 29.4, 27.8, 26.3

### O. General procedure for preparation of compounds 5.7a-c

To the *O*-alkylated acetophenone **5.6** (2.06 mmol, 1.0 eq) in 5 mL MeOH was added 5 mL of methanolic NaOH (3% w/v). The resulting mixture was stirred at 25°C for 30 minutes. A

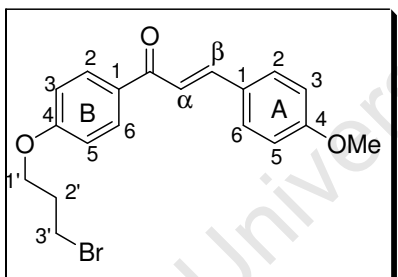
methanolic solution of the commercially available, 4-methoxybenzaldehyde (2.06 mmol, 1.0 eq) was added drop-wise and the mixture stirred for another 8 hours. A precipitate formed which was filtered and washed with cold MeOH. Recrystallization from MeOH afforded the pure product.

**(E)-1-(4-(2-bromoethoxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, 5.7a**

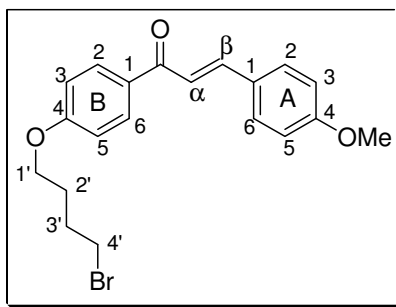


The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure O. However **5.6a** was used which gave **5.7a** as a white powder (563.2 mg, 76%); m. p. 90°C;  $R_f$  (EtOAc:Hex 3:7) 0.21;  $\delta_H$  (400MHz,  $CDCl_3$ ) 8.04 (2H, d,  $J$  9.2, H-2B, H-6B), 7.90 (1H, d,  $J$  15.6, H $\beta$ ), 7.61 (2H, d,  $J$  8.4, H-2A, H-6A), 7.42 (1H, d,  $J$  15.6, H $\alpha$ ), 7.00 (2H, d,  $J$  8.8, H-3B, H-5B), 6.95 (2H, d,  $J$  8.8, H-3A, H-5A), 4.38 (2H, t,  $J$  6.4, H-1'), 3.87 (3H, s, OMe), 3.68 (2H, t,  $J$  6.4, H-2');  $\delta_C$  (100MHz,  $CDCl_3$ ) 188.7, 161.7, 161.6, 144.0, 132.1, 130.2(x2), 130.1(x2), 127.8, 119.6, 114.4(x4), 67.9, 55.4, 28.6

**(E)-1-(4-(3-bromopropoxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, 5.7b**



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure O. However 1.94 mmol each of **5.6b** and 4-methoxybenzaldehyde were used which gave **5.7b** as a light yellow powder (510.4 mg, 70%); m. p. 51-52°C;  $R_f$  (EtOAc:Hex 3:7) 0.28;  $\delta_H$  (300MHz,  $CDCl_3$ ) 8.03 (2H, d,  $J$  9.0, H-2B, H-6B), 7.77 (1H, d,  $J$  15.6, H $\beta$ ), 7.60 (2H, d,  $J$  8.7, H-2A, H-6A), 7.42 (1H, d,  $J$  15.6, H $\alpha$ ), 6.98 (2H, d,  $J$  9.3, H-3B, H-5B), 6.93 (2H, d,  $J$  8.7, H-3A, H-5A), 4.20 (2H, t,  $J$  6.0, H-1'), 3.86 (3H, s, OMe), 3.61 (2H, t,  $J$  6.3, H-3'), 2.36 (2H, quint.,  $J$  6.3, H-2');  $\delta_C$  (75MHz,  $CDCl_3$ ) 188.7, 162.3, 161.6, 143.9, 131.6, 130.7(x2), 130.1(x2), 127.8, 119.6, 114.4(x2), 114.3(x2), 65.6, 55.4, 32.2, 29.6

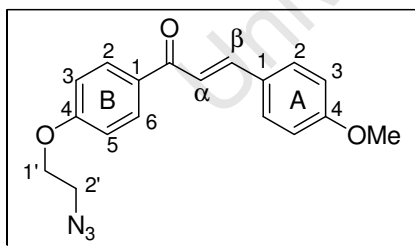
**(E)-1-(4-(4-bromobutoxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, 5.7c**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure O. However 1.84 mmol each of **5.6c** and 4-methoxybenzaldehyde were used which gave **5.7c** as a light yellow solid (852.6

mg, quant); m. p. 91-92°C;  $R_f$  (EtOAc:Hex 3:7) 0.29;  $\delta_H$  (400MHz,  $CDCl_3$ ) 8.03 (2H, d,  $J$  8.8, H-2B, H-6B), 7.78 (1H, d,  $J$  15.6, H $\beta$ ), 7.61 (2H, d,  $J$  8.8, H-2A, H-6A), 7.43 (1H, d,  $J$  15.6, H $\alpha$ ), 6.93-6.98 (4H, m, H-3A, H-5A, H-3B, H-5B), 4.09 (2H, t,  $J$  6.0, H-1'), 3.86 (3H, s, OMe), 3.51 (2H, t,  $J$  6.4, H-4'), 2.09 (2H, quint.,  $J$  6.4, H-2'), 2.01 (2H, quint.,  $J$  5.6, H-3');  $\delta_C$  (100MHz,  $CDCl_3$ ) 188.8, 162.6, 161.6, 143.8, 131.5, 130.7(x2), 130.1(x2), 127.9, 119.6, 114.4(x2), 114.3(x2), 67.1, 55.4, 33.2, 29.4, 27.8

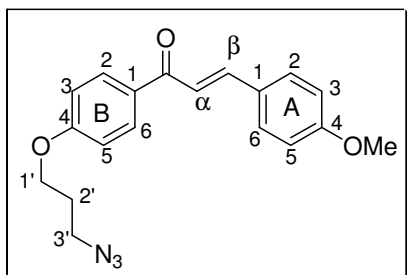
**P. General procedure for preparation of compounds 5.8a-c**

Sodium azide (2.78 mmol, 2.0 eq) was added to a solution of **5.11** (1.39 mmol, 1.0 eq) in 3 mL of anhydrous DMF. The reaction mixture was stirred at 25°C for 18 hours. The addition of ice-cold water to the product mixture resulted in the formation of a precipitate which was filtered and washed with copious amounts of water. Recrystallization from MeOH afforded the pure product.

**(E)-1-(4-(2-azidoethoxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, 5.8a**

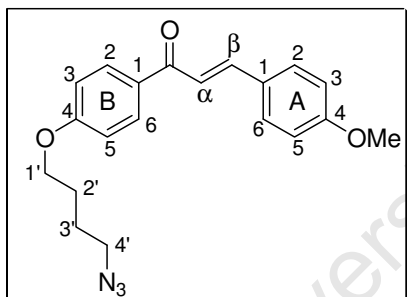
The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure P. However **5.7a** was used which gave **5.8a** as a light yellow solid

(448.4 mg, quant); m. p. 97-98°C;  $R_f$  (EtOAc:Hex 3:7) 0.19;  $\delta_H$  (400MHz,  $CDCl_3$ ) 8.04 (2H, d,  $J$  9.2, H-2B, H-6B), 7.79 (1H, d,  $J$  15.6, H $\beta$ ), 7.60 (2H, d,  $J$  8.4, H-2A, H-6A), 7.42 (1H, d,  $J$  15.6, H $\alpha$ ), 7.00 (2H, d,  $J$  9.2, H-3B, H-5B), 6.94 (2H, d,  $J$  8.8, H-3A, H-5A), 4.23 (2H, t,  $J$  5.2, H-1'), 3.86 (3H, s, OMe), 3.64 (2H, t,  $J$  5.2, H-2');  $\delta_C$  (100MHz,  $CDCl_3$ ) 188.8, 161.8, 161.6, 144.0, 132.0, 130.7(x2), 130.1(x2), 127.8, 119.6, 114.4(x4), 67.1, 55.4, 50.1

**(E)-1-(4-(3-azidopropoxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, 5.8b**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure P. However 1.33 mmol of **5.7b** and 2.66 mmol of sodium azide were used which gave **5.8b** as a light yellow solid (335.3 mg,

74%); m. p. 56°C;  $R_f$  (EtOAc:Hex 3:7) 0.30;  $\delta_H$  (400MHz,  $CDCl_3$ ) 8.04 (2H, d,  $J$  9.2, H-2B and H-6B), 7.79 (1H, d,  $J$  15.6, H $\beta$ ), 7.61 (2H, d,  $J$  8.4, H-2A, H-6A), 7.43 (1H, d,  $J$  15.6, H $\alpha$ ), 6.99 (2H, d,  $J$  9.2, H-3B, H-5B), 6.95 (2H, d,  $J$  8.8, H-3A, H-5A), 4.15 (2H, t,  $J$  6.0, H-1'), 3.87 (3H, s, OMe), 3.55 (2H, t,  $J$  6.4, H-3'), 2.10 (2H, quint,  $J$  6.0, H-2');  $\delta_C$  (100MHz,  $CDCl_3$ ) 188.8, 162.4, 161.6, 143.9, 131.6, 130.7(x2), 130.1(x2), 127.9, 119.6, 114.4(x2), 114.3(x2), 64.8, 55.4, 48.2, 28.7

**(E)-1-(4-(4-azidobutoxy)phenyl)-3-(4-methoxyphenyl)prop-2-en-1-one, 5.8c**

The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure P. However 1.28 mmol of **5.7c** and 2.57 mmol of sodium azide were used which gave **5.8c** as an off-white solid (461.0 mg, quant); m. p. 61°C;  $R_f$  (EtOAc:Hex 3:7) 0.45;  $\delta_H$

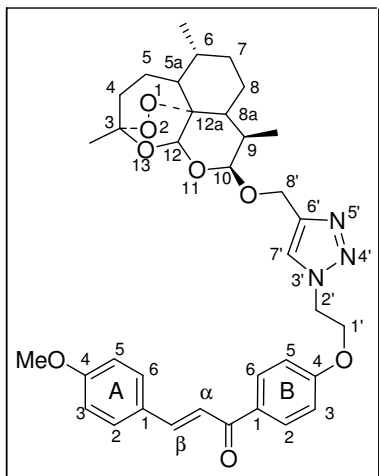
(300MHz,  $CDCl_3$ ) 8.01 (2H, d,  $J$  8.7, H-2B, H-6B), 7.77 (1H, d,  $J$  15.6, H $\beta$ ), 7.59 (2H, d,  $J$  8.7, H-2A, H-6A), 7.41 (1H, d,  $J$  15.6, H $\alpha$ ), 6.91-6.97 (4H, m, H-3A, H-5A, H-3B, H-5B), 4.07 (2H, t,  $J$  5.7, H-1'), 3.84 (3H, s, OMe), 3.37 (2H, t,  $J$  6.3, H-4'), 1.89 (2H, quint,  $J$  5.7, H-2'), 1.78 (2H, quint,  $J$  6.3, H-3');  $\delta_C$  (75MHz,  $CDCl_3$ ) 188.7, 162.5, 161.5, 143.8, 131.4, 130.7(x2), 130.1(x2), 127.8, 119.6, 114.4(x2), 114.2(x2), 67.4, 55.4, 51.1, 26.4, 25.7

**Q. General procedure for preparation of compounds 5.9a-c**

The azide **5.8** (0.464 mmol, 1.0 eq) and acetylene **5.4** (0.510 mmol, 1.1 eq) were dissolved in 3 mL of  $CH_2Cl_2:H_2O$  (1:1). Copper (II) sulphate pentahydrate (0.0232 mmol, 5 mol%) and sodium ascorbate (0.0696 mmol, 15 mol%) was added to the mixture. The resulting mixture was stirred for 6 hours at 25°C. Upon completion, the product mixture was diluted with water

and extracted with EtOAc. The combined organic layer was washed with water and brine, dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated under reduced pressure. The crude product so obtained was subjected to column chromatography (0.5-10% MeOH:DCM).

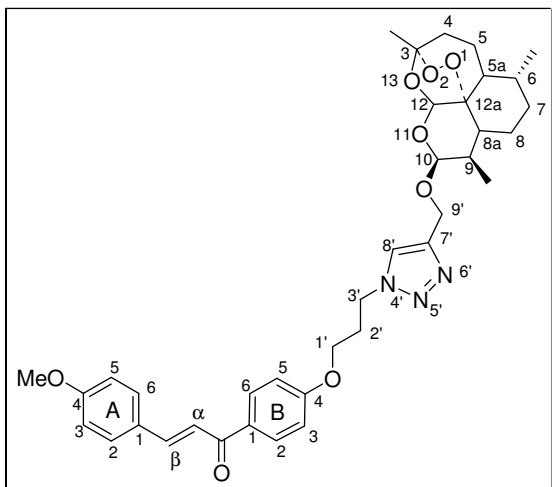
### Compound 5.9a (EXP66A)



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure Q. However **5.8a** was used which gave **5.9a** as a light brown gel (113.6 mg, 38%);  $R_f$  (MeOH:DCM 1:39) 0.13;  $\delta_H$  (300MHz,  $\text{CDCl}_3$ ) 7.99 (2H, d,  $J$  8.7, H-2B, H-6B), 7.74 (1H, d,  $J$  15.6, H $\beta$ ), 7.69 (1H, s, H-7'), 7.57 (2H, d,  $J$  8.7, H-2A, H-6A), 7.37 (1H, d,  $J$  15.6, H $\alpha$ ), 6.92 (2H, d,  $J$  8.7, H-3B, H-5B), 6.91 (2H, d,  $J$  9.0, H-3A, H-5A), 5.40 (1H, s, H-12), 4.93 (1H, d,  $J$  12.9,

H-8'a/b), 4.89 (1H, d,  $J$  3.9, H-10), 4.78 (2H, t,  $J$  5.4, H-1'), 4.66 (1H, d,  $J$  12.6, H-8'a/b), 4.43 (2H, t,  $J$  5.7, H-2'), 3.83 (3H, s, OMe), 2.62 (1H, m, H-9), 2.39-1.12 (11H, m, protons of the DHA nucleus), 1.41 (3H, s, H-14), 0.90 (3H, d,  $J$  6.3, H-16), 0.85 (3H, d,  $J$  7.5, H-15);  $\delta_C$  (100MHz,  $\text{CDCl}_3$ ) 188.6, 161.7, 161.3, 145.2, 144.2, 132.3, 130.8(x2), 130.2(x2), 127.7, 123.5, 119.4, 114.4(x2), 114.2(x2), 104.1, 101.6, 88.0, 81.1, 66.6, 61.7, 55.4, 52.6, 49.5, 44.4, 37.4, 36.4, 34.6, 30.8, 26.2, 24.7, 24.5, 20.3, 13.0; HRMS(ESI) found  $m/z$  646.3151  $[\text{M}+\text{H}]^+$  for  $\text{C}_{36}\text{H}_{43}\text{O}_8$  requires 645.30502; Anal Calc. for  $\text{C}_{36}\text{H}_{43}\text{O}_8$ : C, 66.96%; H, 6.71%; N, 6.51%. Found: C, 66.99%; H, 6.71%; N, 6.44%;

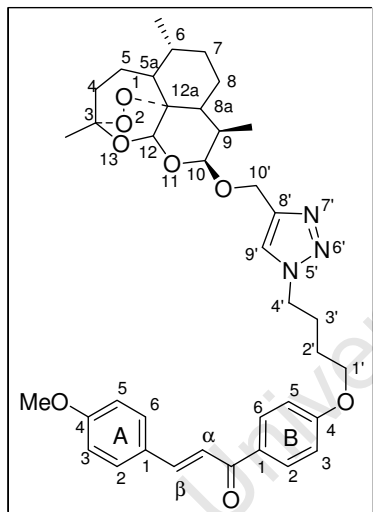
### Compound 5.9b (EXP66B)



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure Q. However 0.445 mmol of **5.8b**, 0.489 mmol of **5.4**, 0.0223 mmol of copper (II) sulphate pentahydrate and 0.0667 mmol of sodium ascorbate were used which gave

**5.9b** as a light yellow solid (186.1 mg, 65%); m. p. 145-146°C;  $R_f$  (MeOH:DCM 1:39) 0.15;  $\delta_H$  (300MHz,  $CDCl_3$ ) 8.00 (2H, d,  $J$  9.0, H-2B, H-6B), 7.76 (1H, d,  $J$  15.6, H $\beta$ ), 7.58 (2H, d,  $J$  9.0, H-2A, H-6A), 7.50 (1H, s, H-8'), 7.39 (1H, d,  $J$  15.6, H $\alpha$ ), 6.94 (2H, d,  $J$  9.3, H-3B, H-5B), 6.92 (2H, d,  $J$  8.7, H-3A, H-5A), 5.37 (1H, s, H-12), 4.90 (1H, d,  $J$  12.3, H-9'a/b), 4.88 (1H, br. s, H-10), 4.66 (1H, d,  $J$  12.6, H-9'a/b), 4.58 (2H, t,  $J$  6.3, H-1'), 4.04 (2H, t,  $J$  6.0, H-3'), 3.83 (3H, s, OMe), 2.61 (1H, m, H-9), 2.47-2.28 (3H, m, H-2' and a proton of the DHA nucleus), 2.08-1.12 (10H, m, protons of the DHA nucleus), 1.41 (3H, s, H-14), 0.89 (3H, d,  $J$  5.7, H-16), 0.83 (3H, d,  $J$  7.5, H-15);  $\delta_C$  (75MHz,  $CDCl_3$ ) 188.6, 162.0, 161.5, 145.2, 143.9, 131.8, 130.7(x2), 130.1(x2), 127.7, 122.8, 119.4, 114.4(x2), 114.1(x2), 104.0, 101.7, 88.0, 81.0, 64.3, 61.7, 55.3, 52.4, 46.8, 44.3, 37.3, 36.3, 34.5, 30.7, 29.7, 26.1, 24.6, 24.3, 20.2, 12.9; HRMS(ESI) found  $m/z$  660.3258  $[M+H]^+$  for  $C_{37}H_{45}O_8$  requires 659.32067; Anal Calc. for  $C_{37}H_{45}O_8$ : C, 67.36%; H, 6.87%; N, 6.37%. Found: C, 67.26%; H, 6.85%; N, 6.35%;

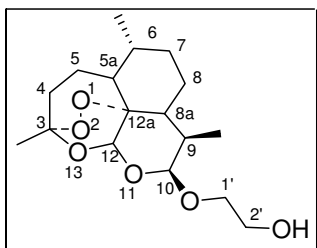
#### Compound 5.9c (EXP66C)



The conditions employed for the preparation and purification of this compound is the same as the conditions described in General Procedure Q. However 0.427 mmol of **5.8c**, 0.470 mmol of **5.4**, 0.0235 mmol of copper (II) sulphate pentahydrate and 0.0704 mmol of sodium ascorbate were used which gave **5.9c** as a light brown gel (161.4 mg, 55%);  $R_f$  (MeOH:DCM 1:39) 0.21;  $\delta_H$  (300MHz,  $CDCl_3$ ) 8.00 (2H, d,  $J$  9.3, H-2B, H-6B), 7.75 (1H, d,  $J$  15.6, H $\beta$ ), 7.58 (2H, d,  $J$  8.4, H-2A, H-6A), 7.53 (1H, s, H-9'), 7.40 (1H, d,  $J$  15.6, H $\alpha$ ), 6.92 (2H, d,  $J$  8.7, H-3B, H-5B), 6.91 (2H, d,  $J$  8.7, H-3A, H-5A), 5.39 (1H, s, H-12), 4.91 (1H, d,  $J$  12.0, H-10'a/b), 4.90 (1H, d,  $J$  4.8, H-10), 4.67 (1H, d,  $J$  12.3, H-10'a/b), 4.44 (2H, t,  $J$  7.2, H-1'), 4.05 (2H, t,  $J$  5.7, H-4'), 3.83 (3H, s, OMe), 2.63 (1H, m, H-9), 2.34 (1H, m, proton of the DHA nucleus), 2.12 (1H, quint,  $J$  7.2, H-2'), 1.99 (1H, m, proton of the DHA nucleus), 1.88-1.76 (3H, m, H-3' and a proton of the DHA nucleus), 1.75-1.13 (7H, m, protons of the DHA nucleus), 1.42 (3H, s, H-14), 0.90 (3H, d,  $J$  5.7, H-16), 0.86 (3H, d,  $J$  7.5, H-15);  $\delta_C$  (100MHz,  $CDCl_3$ ) 188.7, 162.4, 161.6, 143.9, 143.7, 131.6, 130.7(x2), 130.1(x2), 127.8, 122.4, 119.6, 114.4(x2), 114.2(x2), 104.1, 101.7, 88.0, 81.1, 72.3, 67.2, 61.7, 55.4, 52.6, 49.9, 44.4, 37.4, 36.4, 34.6, 30.8, 27.2, 26.2, 24.7,

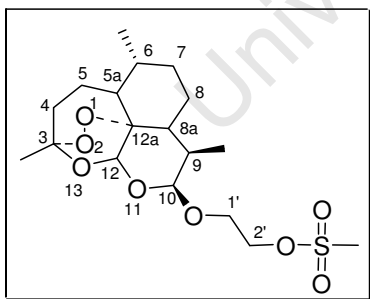
24.5, 20.3, 13.0; HRMS(ESI) found  $m/z$  674.3444  $[M+H]^+$  for  $C_{38}H_{47}O_8$  requires 673.33632; Anal Calc. for  $C_{38}H_{47}O_8$ : C, 67.74%; H, 7.03%; N, 6.24%. Found: C, 67.57%; H, 7.00%; N, 6.11%;

### Compound 5.10

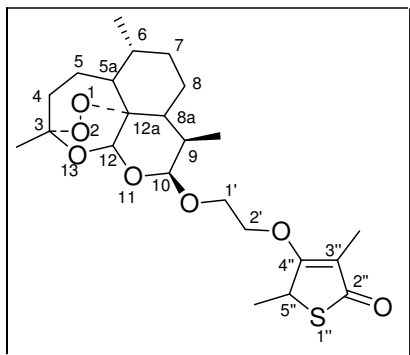


Boron trifluoride etherate (5 drops) was added to a solution of dihydroartemisinin **1.15** (1.00 g, 3.54 mmol, 1 eq) and ethylene glycol (439.0 mg, 7.08 mmol, 2 eq) in 10 mL of anhydrous DCM at 0°C. The resulting mixture was allowed to warm to 25°C and allowed to stir at this temperature for 19 hours. The product mixture was washed with saturated  $\text{NaHCO}_3$ , water and brine. The organic layer was dried over anhydrous  $\text{Na}_2\text{SO}_4$  and concentrated. Purification of the crude product using column chromatography (0-20% EtOAc:Hex) afforded the 10 $\beta$ -isomer as light green prisms (860.6 mg, 74%); m. p. 72-73°C;  $R_f$  (EtOAc:Hex 2:3) 0.4;  $\text{IR}_{\text{max}}(\text{KBr})/\text{cm}^{-1}$  3501 (OH);  $\delta_{\text{H}}$  (400MHz,  $\text{CDCl}_3$ ) 5.39 (1H, s, H-12), 4.95 (1H, d,  $J$  3.3, H-10), 4.29 (2H, d,  $J$  2.4, H-1'), 2.65 (1H, m, H-9), 2.41-1.20 (11H, m, protons of the DHA nucleus), 1.42 (3H, s, H-14), 0.93 (3H, d,  $J$  6.0, H-16), 0.91 (3H, d,  $J$  7.2, H-15);  $\delta_{\text{C}}$  (100MHz,  $\text{CDCl}_3$ ) 104.2, 102.6, 88.0, 81.0, 70.7, 62.3, 52.5, 44.4, 37.4, 36.4, 34.6, 30.9, 26.1, 24.7, 24.6, 20.3, 13.0; HRMS(ESI) found  $m/z$  351.1784  $[M+\text{Na}]^+$  for  $C_{17}H_{28}O_6$  requires 328.18859

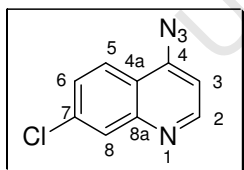
### Compound 5.11



To a solution of the alcohol **5.11** (643.7 mg, 1.96 mmol, 1 eq) in anhydrous DCM (3 mL) at 0°C under nitrogen was added  $\text{Et}_3\text{N}$  (218.2 mg, 2.16 mmol, 1.1 eq) and methanesulfonylchloride (336.8 mg, 2.94 mmol, 1.5 eq). The resulting mixture was stirred for two hours at 0°C. The reaction mixture was then quenched with water and extracted with DCM. The combine organic layer was dried over anhydrous  $\text{MgSO}_4$  and concentrated. The crude colourless product was used without further purification.

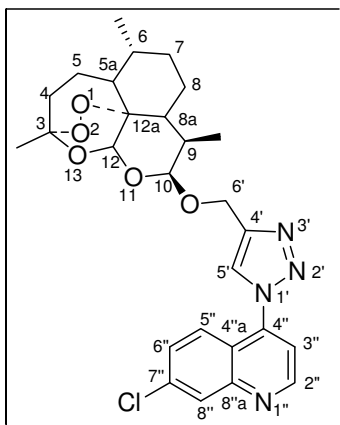
**Compound 5.12 (EXP51D)**

The potassium salt of thiolactone **3.5** (140.0 mg, 0.768 mmol, 1 eq) was added to a solution of **5.11** (375.0 mg, 0.922 mmol, 1.2 eq) in anhydrous DMF (2 mL). The resulting mixture was stirred at 60°C under a nitrogen atmosphere. Thin layer chromatography signalled the completion of the reaction after 20 hours whereupon water was added to the reaction mixture followed by extraction with ethyl acetate. The combined organic layer was washed with copious amounts of water to remove DMF before being dried over anhydrous MgSO<sub>4</sub>. Concentration *in vacuo* afforded the crude product which was subjected to column chromatography (5-40% EtOAc:Hex) to yield the pure product as a light yellow oil (175.5 mg, 49%); R<sub>f</sub> (EtOAc:Hex 3:7) 0.28; δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 5.36 (1H, s, H-12), 4.80 (1H, d, *J* 3.2, H-10), 4.33-4.41 (2H, m, H-2'), 4.20 (1H, qd, *J* 1.2 and 6.8, H-5''), 4.10 (1H, m, H-1'a/b), 3.66 (1H, m, H-1'a/b), 2.62 (1H, m, H-9), 2.34–1.23 (11H, m, protons of the DHA nucleus), 1.79/1.80 (3H, d, *J* 1.2, H-7''), 1.56/1.58 (3H, d, *J* 6.8, H-6''), 1.40 (3H, s, H-14), 0.88 (3H, d, *J* 7.2, H-16), 0.86 (3H, d, *J* 7.6, H-15); δ<sub>C</sub> (100MHz, CDCl<sub>3</sub>) 195.3 (195.5), 178.1, 114.7 (114.8), 104.1, 102.5 (102.6), 87.9, 80.8 (80.9), 70.0 (70.2), 67.5 (67.6), 60.3, 52.5, 44.3, 41.4 (41.6), 37.5 (37.6), 36.4, 34.5, 30.7, 26.1, 24.4 (24.7), 20.2 (20.3), 19.7 (19.8), 12.8 (12.9), 8.8 (8.9) Chemical shift values given in brackets represent those of the other diastereomer.

**4-Azido-7-chloroquinoline, 5.13**

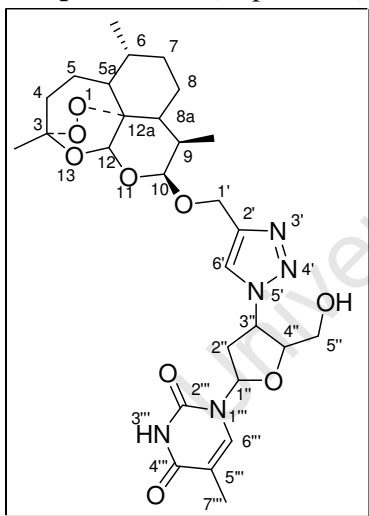
Sodium azide (1.33 g, 20.5 mmol, 2.0 eq) was added to a solution of 4,7-dichloroquinoline (2.00 g, 10.1 mmol, 1.0 eq) in 3 mL of anhydrous DMF. The reaction mixture was stirred at 60°C for 10 hours. The addition of ice-cold water to the product mixture resulted in the formation of a precipitate which was filtered and washed with copious amounts of water. Recrystallization from hot DCM afforded the product as light green needles (1.90 g, 92%); m. p. 115-116°C; R<sub>f</sub> (EtOAc:Hex 1:4) 0.26; δ<sub>H</sub> (400MHz, CDCl<sub>3</sub>) 7.09 (1H, d, *J* 5.2, H-3), 7.45 (1H, dd, *J* 2.0 and 8.8, H-6), 7.96 (1H, d, *J* 8.8, H-5), 8.04 (1H, d, *J* 2.0, H-8), 8.80 (1H, d, *J* 5.2, H-2); δ<sub>C</sub> (100MHz, CDCl<sub>3</sub>) 151.3, 149.6, 146.3, 136.6, 128.2, 127.5, 123.7, 120.0, 108.7



**Compound 5.14 (EXP61C/08)**

Brown gel (258.2 mg, 50%);  $R_f$  (MeOH:DCM 1:19) 0.16;  $\delta_H$  (300MHz,  $CDCl_3$ ) 9.03 (1H, d,  $J$  4.5, H-2''), 8.21 (1H, d,  $J$  1.5, H-8''), 7.96 (1H, s, H-5'), 7.97 (1H, d,  $J$  9.3, H-5''), 7.56 (1H, dd,  $J$  2.1 and 9.3, H-6''), 7.48 (1H, d,  $J$  4.2, H-3''), 5.29 (1H, s, H-12), 5.04 (1H, d,  $J$  13.2, H-6'a/b), 4.97 (1H, d,  $J$  4.5, H-10), 4.80 (1H, d,  $J$  13.2, H-6'a/b), 2.52 (1H, m, H-9), 2.26-1.19 (11H, m, protons of the DHA nucleus), 1.54 (3H, s, H-14), 0.94 (3H, d,  $J$  7.8, H-16), 0.84 (3H, d,  $J$  6.3, H-15);  $\delta_C$

(100MHz,  $CDCl_3$ ) 151.4, 150.2, 146.2, 141.0, 136.9, 129.4, 129.0, 124.6, 124.2, 120.7, 116.0, 108.1, 99.8, 93.9, 84.1, 69.5, 61.7, 42.4, 40.7, 34.9, 34.7, 30.4(x2), 25.1, 21.0, 18.8, 12.3; HRMS(ESI) found  $m/z$  527.2083  $[M+H]^+$  for  $C_{27}H_{31}ClN_4O_5$  requires 526.1983; Anal Calc. for  $C_{27}H_{31}ClN_4O_5$ : C, 61.53%; H, 5.93%; N, 10.63%. Found: C, 61.03%; H, 5.34%; N, 10.13%

**Compound 5.15 (Exp62A/08)**

Light green foam (199.9 mg, 45%); m. p. 122°C;  $R_f$  (MeOH:DCM 1:19) 0.11;  $\delta_H$  (400MHz,  $CDCl_3$ ) 9.27 (1H, s, H-3'''), 7.68 (1H, s, H-3'), 7.46 (1H, s, H-6'''), 6.24 (1H, br. s, H-1'''), 5.46 (1H, s, H-12), 4.93 (1H, br. s, H-10), 4.91 (1H, d,  $J$  12.8, H-1'a/b), 4.71 (1H, d,  $J$  12.8, H-1'a/b), 4.43 (1H, br. s, H-3''), 4.03 (1H, br. d,  $J$  11.2, H-5''a/b), 3.81 (1H, br. d,  $J$  11.6, H-5''a/b), 3.57 (1H, br. s, OH), 3.00 (2H, br. s, H-2''), 2.65 (1H, m, H-9), 2.40-1.16 (11H, m, protons of the DHA nucleus), 1.93 (3H, s, H-7'''), 1.44 (3H, s, H-14), 0.94 (3H, d,  $J$  5.6, H-16), 0.90 (3H, d,  $J$  7.2, H-15);  $\delta_C$

(100MHz,  $CDCl_3$ ) 163.8, 150.5, 145.6, 137.9, 122.8, 111.3, 104.2, 102.1, 88.8, 88.0, 85.4, 81.1, 61.8, 61.6, 59.3, 52.5, 44.4, 37.5, 37.4, 36.4, 34.6, 30.9, 26.1, 24.7, 24.5, 20.3, 12.3, 12.4; HRMS(ESI) found  $m/z$  590.2811  $[M+H]^+$  for  $C_{28}H_{39}N_5O_9$  requires 589.27478; Anal Calc. for  $C_{28}H_{39}N_5O_9$ : C, 57.03%; H, 6.67%; N, 11.88%. Found: C, 56.78%; H, 6.61%; N, 11.49%;

#### 7.4. Single Crystal X-ray Diffraction

X-ray single crystal intensity data were collected on a Nonius Kappa-CCD diffractometer using graphite monochromated MoK $\alpha$  radiation. Temperature was controlled by an Oxford Cryostream cooling system (Oxford Cryostat). The strategy for the data collections was evaluated using the Bruker Nonius "Collect" program. Data were scaled and reduced using DENZO-SMN software.<sup>3</sup> Absorption correction was made empirically by utilizing SADABS program.<sup>4</sup> The structure was solved by direct methods and refined employing full-matrix least-squares with the program SHELXL-97<sup>4</sup> refining on F<sup>2</sup>. Packing diagrams were produced using the program PovRay and graphic interface X-seed.<sup>5</sup>

#### 7.5. Procedures for Biological Assays

##### 7.5.1. *In vitro* Antiplasmodial Assay: D10 strain of *P. falciparum*

The test compounds were tested in duplicate against the chloroquine sensitive (CQS) strain of *P. falciparum* (D10). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen.<sup>6</sup> Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler.<sup>7</sup>

The compounds were prepared to a 2mg/ml stock solution in 10% DMSO or 10% methanol and were sonicated to enhance solubility. All compounds were tested as a suspension. Stock solutions were stored at -20°C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC<sub>50</sub> – value). Test compounds were tested at a starting concentration of 100  $\mu$ g/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 0.2  $\mu$ g/ml. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100 ng/ml. The highest concentration of solvent to which the parasites were exposed to, had no measurable effect on the parasite viability (data not shown). The IC<sub>50</sub>-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

##### 7.5.2. *In vitro* Antiplasmodial Assay: W2 strain of *P. falciparum*

Ring stage, W2-strain *P. falciparum* parasites (1% parasitaemia, 2% haematocrit) were cultured in 0.5 mL of medium in 48-well culture dishes.<sup>8</sup> Inhibitors from 10 mM stocks in

DMSO were added to cultured parasites to give a final concentration of 20  $\mu\text{M}$ . From 48-well plates, 125  $\mu\text{M}$  of culture was transferred to two 96 well plates (duplicates). Serial dilutions (1:5) of inhibitors were made to final concentrations of 10  $\mu\text{M}$ , 2  $\mu\text{M}$ , 0.4  $\mu\text{M}$ , 80 nM, 16 nM and 3.2 nM. Cultures were maintained at 37°C for 2 days after which the parasites were washed and fixed with 1% formaldehyde in PBS. After two days, parasitaemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival.<sup>8</sup>

### **7.5.3. Assay of Enzyme Inhibition: Falcipain-2 of *P. falciparum***

IC<sub>50</sub>s against falcipain-2 were determined as described previously.<sup>8</sup> Briefly, an equal amounts of recombinant falcipain-2 were incubated with different concentrations of inhibitors (added from 100x stocks in DMSO) in 100 mM sodium acetate (pH 5.5), –10 mM dithiothreitol for 30 min at room temperature before addition of the substrate benzoxycarbonyl-Leu-Arg-7-amino-4-methyl-coumarin (final concentration = 25  $\mu\text{M}$ ). Fluorescence was continuously monitored for 30 min at room temperature in a labsystem Fluoroskan<sup>®</sup> II spectrofluorometer. IC<sub>50</sub>s were determined from plots of activity over enzyme concentration with GraphPrism software.

### **7.5.4. BACTEC Assay: H<sub>37</sub>Rv strain of *M. tuberculosis***

All work was carried out in the Biosafety Level 3 laboratory of the Division of Molecular Biology and Human Genetics of the University of Stellenbosch. A clinical isolate of *M. tuberculosis* was selected from a bank of genetically well-characterized (according to their IS6110 fingerprint profile) strains located in the Division of Molecular Biology and Human Genetics of the University of Stellenbosch for the evaluations.<sup>9</sup> The strain was drug sensitive to isoniazid and rifampicin. Compounds to be tested for antimycobacterial activity were filtered and sterilized through 0.22 micron syringe filters (millex LG). The *M. tuberculosis* clinical isolate was grown on Lowenstein-Jensen culture medium for 4 weeks.<sup>10</sup> After incubation at 37°C, bacteria were collected, suspended in 7H9 mycobacterial growth medium (Middlebrook) and the bacterial suspension added to a BACTEC vial containing BACTEC 12B growth medium (Becton Dickinson, USA). Growth was monitored every 24 hours until a growth index (GI) value of 500 was reached. From this culture, 0.1 mL was added to a new BACTEC vial and the growth monitored every 24 hours until a GI value of 500 was reached.<sup>11</sup> This culture was the primary culture and was used for testing bacterial viability against a variety of inhibitors. The test compound was dissolved in DMSO and 0.1ml of this solution, was added to a BACTEC vial to give a final concentration of 1.0 $\mu\text{g/ml}$  or

10.0 µg/ml. BACTEC vials were incubated at 37°C and the growth index (GI) was monitored every 24 hours in a BACTEC 460TB system. A growth index below 10 was considered as negative growth and above 10 as positive growth. The growth index of the treated samples, were compared to a control culture containing sterile DMSO.

#### **7.5.5. MABA Assay: H<sub>37</sub>Rv strain of *M. tuberculosis***

In brief, the MICs of the test compounds were determined by the microplate Alamar blue assay (MABA).<sup>12</sup> Stock solutions were prepared in DMSO at a final concentration of 12.8 mM and the final testing concentrations ranged from 128 to 0.5 µM. *M. tuberculosis* H<sub>37</sub>Rv (ATCC 27294, Rockville, Md.) was grown to late log phase (70 to 100 Klett units) in a Middlebrook 7H9 broth supplemented with 0.2% v/v glycerol, 0.05% Tween 80 and 10% v/v oleic acid-albumin-dextrose-catalase. Cultures were centrifuged for 15 min at 4°C at 3.150 x g, washed twice and resuspended in phosphate-buffered saline. Suspensions were then passed through an 8 µm-pore-size filter to remove clumps and aliquots were frozen at -80°C. The number of CFU was determined by plating on 7H11 agar plates. Two-fold dilutions of compounds were prepared in Middlebrook 7H12 medium (7H9 broth containing 0.1% w/v casitone, 5.6 µg/mL palmitic acid, 5 mg/mL bovine serum albumin and 4 mg/mL catalase, filter-sterilized) in a volume of 100 µL in 96-well, black, clear-bottom microplates (BD Bioscience, Franklin Lakes, N.J.). *M. tuberculosis* H<sub>37</sub>Rv (100 µL inoculum of 2 x 10<sup>4</sup> CFU) was added, yielding a final testing volume of 200 µL. The plates were incubated at 37°C. On the 7<sup>th</sup> day of incubation 12.5 µL of 20% Tween 80 and 20 µL of Alamar blue were added to all wells. After incubation at 37°C for 16-24 hours, fluorescence of the wells was measured at an excitation of 530 nm and emission of 590 nm. The MICs were defined as the lowest concentration effecting a reduction in fluorescence of ≥90% relative to the mean of replicate bacteria-only controls.

#### **7.5.6. LORA Assay: H<sub>37</sub>Rv strain of *M. tuberculosis***

Recombinant H<sub>37</sub>Rv (pFCA-luxAB) was grown to NRP phase 2 in 300 mL Dubos Tween albumin broth (Becton Dickson) in a BiostatQ fermentor (B. Braun Biotech) to mimic the Wayne oxygen-limited culture with a headspace ratio of 0.5. The culture was agitated at a stir rate of 120 rpm with no detectable perturbation of the surface medium.<sup>13</sup> The fermentor culture was operated and maintained within a biosafety level 3 laboratory. The dissolved oxygen concentration (DOC) was continuously monitored with an Ingold oxygen sensor probe. The optical densities of the cultures at 570 nm (A<sub>570</sub> values), the numbers of relative

light units (RLUs) and the CFU levels were determined at 3-day intervals. Bacterial samples were removed through a silicone septum with a syringe in order to preclude the introduction of oxygen. The number of CFU was estimated by plating dilutions of aliquots on Dubos oleic-albumin agar plates in triplicate and incubating the cultures at 37°C. The colonies were enumerated every week for 5 weeks. The cells were harvested on the 22<sup>nd</sup> day, i.e. a time when the A<sub>570</sub> and DOC readings indicated achievement of the desired growth phase (NRP-2). Fifty millilitre aliquots of bacterial culture samples were centrifuged (2.700 x g, 30 min, 4°C), washed once with prechilled phosphate-buffered saline (PBS: pH 7.4) suspended in 1 mL of PBS and stored at -80°C.

Prior to use, the cultures were thawed, diluted in Middlebrook 7H12 broth and sonicated for 15s. The cultures were diluted to obtain an A<sub>570</sub> of 0.03-0.05 and 3000-7000 RLUs per 100 µL. This corresponds to  $5 \times 10^5$ – $2 \times 10^6$  CFU/mL. Two-fold serial dilution of test compounds were prepared in a volume 100 µL in black 96-well microtiter plates and 100 µL of the cell suspension was added. The microplate cultures were placed under anaerobic conditions by using an Anoxomat model WS-8080 (MART Microbiology) and three cycles of evacuation and filling with a mixture of 10% H<sub>2</sub>, 5% CO<sub>2</sub> and the balance N<sub>2</sub> (7.32). An anaerobic indicator strip was placed inside the chamber to visually confirm the removal of oxygen. The plates were incubated at 37°C for 10 days and then transferred to an ambient gaseous condition (5% CO<sub>2</sub>-enriched air) incubator for a 28 h “recovery”. Luminescence readings were obtained following the 28 h aerobic recovery. The data were analyzed graphically and the lowest concentration of test compound preventing metabolic recovery (90% reduction relative to untreated cultures) was determined as described previously.<sup>14</sup>

## 7.6. References

1. Perrin, D.D.; Armarego, W.L.F. *Purification of Laboratory Chemicals*, Pergamon Press, Oxford, **1988**
2. Wang, C-L. J.; Salvino, J.M. *Tetrahedron Lett.*, **1984**, 25, 5246
3. Otwinowski, Z.; Minor, W. *Methods in Enzymology, Macromolecular Crystallography*, Carter Jr., C. W., Sweet, R. M., (eds.), part A, vol. 276, Academic Press, **1997**, 307-326
4. (a) Sheldrick, G. M. *SHELXL-97 and SHELXS-97*, University of Göttingen, Germany, **1997**; (b) Sheldrick, G. M. *SADABS*, University of Göttingen, Germany, **1996**
5. Barbour, L.J. *J. Supramol. Chem.*, **2001**, 1, 189
6. Trager, W.; Jensen, J.B. *Science*, **1976**, 193, 673.
7. Makler, M.T.; Ries, J.M.; Williams, J.A.; Bancroft, J.E.; Piper, R.C.; Gibbins, B.L.; Hinrichs, D.J. *Am. Soc. Trop. Med. Hyg.*, **1993**, 48, 739
8. Greenbaum, D.C.; Mackey, Z.; Hansell, E.; Doyle, P.; Gut, J.; Caffrey, C.R.; Lehman, J.; Rosenthal, P.J.; McKerrow, J.H.; Chibale, K. *J. Med. Chem.*; **2004**, 47, 3212
9. Warren, R.; Richardson, M.; Sampson, S.; Hauman, J.H.; Beyers, N.; Donald, P.R.; Van Helden., P.D. *J. Clin. Microbiol.*, **1996**, 34, 2219
10. Somoskovi, A.; Magyar, P. *J. Clin. Microbiol.*, **1999**, 37, 1366
11. Siddiqi, S. H. *460TB system. Product and Procedure Manual*, Becton Dickinson and Company, Maryland, U.S.A., **1995**
12. Collins, L.A.; Franzblau, S.G. *Antimicrob. Agents Chemother.*, **1997**, 41, 1004
13. Cho, S.H.; Goodlet, D.; Franzblau, S.G. *Tuberculosis (Edinburg)*, **2006**, 86, 445
14. Pauli, G.F.R.; Case, R.J.; Inui, T.; Wang, Y.; Cho, S.; Fischer, N.H.; Franzblau, S.G. *Life Sc.*, **2005**, 78, 485