UNIVERSITY OF
LIVERPOOL

Targeting the Hemoglobin Degradation Pathway and the Cytochrome bc_1 Complex of *Plasmodium Falciparum* Malaria

Thesis submitted in accordance with the requirements of the University of Liverpool for the degree of Doctor in Philosophy by

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Contents

Abstract

Malaria is one of the world's most deadly diseases and is becoming an increasingly serious problem as malaria parasites develop resistance to drugs such as chloroquine and mefloquine. There is, therefore, considerable urgency to develop new classes of antimalarials. This study describes the synthesis of artemisinin analogues and novel quinolones which target the parasite food vacuole and cytochrome bc_1 complex respectively.

Artemisinin is an unusual 1,2,4-trioxane, which has been used clinically in China for the treatment of multidrug resistant *Plasmodium falciparum* malaria. The first goal of this study was to make some artemisinin analogues which combine both improved water solubility and metabolic stability with enhanced antimalarial activity. This involved the synthesis of alkylamino substituted pyrrole derivatives. An array of C-10 pyrrole derivatives of dihydroartemisinin were prepared using Mannich chemistry in two steps from dihydroartemisinin with good overall yield: It was proposed that the presence of the tertiary amine groups (attached to the pyrrole ring) of the target molecules would serve to aid localisation of the drug within the parasite food vacuole by an ion-trapping mechanism.

Initial antimalarial *in vitro* assessment (vs. K1 *P.falciparum*) demonstrates that these analogues are active in the low nanomolar region with a result that four out of the 18 prepared have been selected for further *in vivo* antimalarial assessment. Ultimately, one of the compounds is more active than artesunate from the *in vivo* studies. Further iron degradation studies suggest that these pyrrole derivatives generate both primary and secondary carbon centered radicals in a manner similar to artemisinin.

Attempt to synthesise heterocyclic sulfone analogues of artemisinin lead to an interesting rearrangement, which was reproduced with sugars.

Quinones and quinolones are highly efficient antimalarials as they inhibit the mitochondrial respiration process of *Plasmodium falciparum* by binding specifically to the cytochrome bc_1 complex. A library of naphthoquinone, 2- and 3-substituted quinolone analogues were synthesised and tested *in vitro* to study their structure-activity relationship.

Naphthoquinone derivatives were prepared in one-step via Mannich reaction. 2-Substituted-quinolones were made via a three-step synthesis employing a Copper or Suzuki coupling, followed by Ziegler alkylation and hydrolysis of quinoline. A four-step synthesis from 7-chloroquinol-4-one, with an extra step of parallel synthesis, gave a series of quinolone derivatives substituted at the 3-position.

In vitro results showed that 2-substituted quinolones were more potent with IC_{50} values between 30 and 185 nM (vs. 3D7 strains), which was validated by molecular modelling; it was observed that the higher the Goldscore is, the more active the quinolone is. Further *in vitro* tests revealed 1000 fold difference in sensitivity between parasite and mammalian bc_1 complex, indicating that these molecules should have good therapeutic indices.

Abbreviations

δ .....chemical shift

υ .....wavelength

abs. .....absolute

ACTs.....artemisinin based combinations therapies

AHA.....anhydroartemisinin

aq.....aqueous

Ar.....aryl

ATP.....adenosine triphosphate

^{o}C.....degree celsius

Calcd.....calculated

CAN.....Cerium(IV) Ammonium Nitrate

cat.....catalyst

Cbz.....carboxybenzyl

CsF.....cesium fluoride

$Cu(OAc)_2$.....copper (II) acetate

Cyt *c* .....cytochrome *c*

d.....doublet

DAPCy.....Diacetoxybis(dicyclohexylammonio) palladium

DCM.....dichloromethane

DEAD..... diethylazodicarboxylate

Dieth.....diethylamine

DHA.....dihydroartemisinin

DMAP.....4-dimethylaminopyridine

DMF.....dimethylformamide

EC_{50} .....half maximal effective concentration

ED_{50}.....50% effective dose

ED_{90}.....90% effective dose

eq.....molar equivalent

Et_2O.....diethyl ether

EtOAc.....ethyl acetate

EtOH.....ethanol

ES.....electrospray

FV.....food vacuole

g.....gram(s)

H_2O.....water

HRMS.....high resolution mass spectrometry

Hz.....hertz

hrs.....hours

IC_{50}.....half maximal inhibitory concentration

Imidz.....imidazole

IMM.....inner membrane of mitochondria

ISP..... iron sulfur protein

m .....*meta*

m.....multiplet

M.....molar

m-CPBA.....*meta*-chloroperoxybenzoic acid

MHz .....MegaHertz

mg .....milligram

mL .....milliliter

mmol .....millimoles

$MgSO_4$ .....magnesium sulfate

morph.....morpholine

mp.....melting point

MS.....mass spectra

NaBr .....sodium bromide

Na_2CO_3 .....sodium carbonate

NADH .....nicotinamide adenine dinucleotide

NaOH .....sodium hydroxide

ND.....non determinated

n-Hex..... *n*-hexane

nM .....nanomolar

NMO.....*N*-methylmorpholine-N-Oxide

NMR .....nuclear magnetic resonance

p .....*para*

Pd/C .....palladium on carbon

$Pd(OAc)_2$ .....palladium diacetate

$Pd(PPh_3)_4$ .....tetrakis(triphenylphosphine)palladium(0)

PE .....petroleum ether

p.f. .....*Plasmodium falciparum*

pipd.....piperidine

pipz.....piperazine

PPh_3 .....triphenylphosphine

ppm.....part per million

pyr.....pyrrole

pyrm.....pyrimidine

pyro.....pyrrolidine

R*f* .....retention factor

r.t..... room temperature

SAR.....structure activity relationship

SEM.....scanning electron microscopy

s .....singlet

TBD.....to be determinated

tetz.....tetrazole

TFAA 2,2,2-trifluoroacetamide

thiadz.....thiadiazol

THF..... tetrahydrofuran

thiomorph.....thiomorpholine

t.l.c. .....thin layer chromatography

TMSCl.....trimethylsilyl chloride

tol.....toluene

TPAP.....tetrapropylammonium perruthenate

UHP.....urea hydrogen peroxide

UQ.....ubiquinone

CHAPTER 1

General Introduction

1. Introduction

This chapter will present an introduction to malaria and the treatments used against this parasitic disease. The two principal biological targets will be introduced and a summary of inhibitors used against these targets will form the introduction to this thesis.

1.1. Introduction to malaria

1.1.1. The facts of Malaria

Malaria is one of the World's most deadly diseases as it affects more than 6% of the global population (300 million cases) and it has been estimated that every 30 seconds a child dies from malaria. It is an infectious disease caused by the protozoan parasite *Plasmodium*, and is transmitted through the bite of the female *Anopheles* mosquito.

41% of the world's population is exposed to malaria. Mortality is currently estimated at over a million people per year, this has risen in recent years, probably due to increasing resistance to antimalarial medicines,[1] also resistance to insecticides by mosquitoes.[2]

Malaria can cause headache, fever, nausea and vomiting, painful joints and muscles. If the infection is not treated, it can progress rapidly by infecting and destroying red blood cells (anemia) and by clogging small blood vessels carrying blood to the brain (cerebral malaria) and other vital organs.

1.1.2. A disease of the developing world

Malaria affects tropical and subtropical areas of the world, 90% of malaria deaths occur in sub Saharan Africa.

On these two maps below (**Figure 1**), you can see that malaria coincides almost exactly with the distribution of poverty in the world.[3] As a disease of the poorest nations, malaria remains a poor cousin of the major health problems of the developed world, and

funding for malaria control and research is dwarfed by that for heart disease, cancer, AIDS, and asthma.

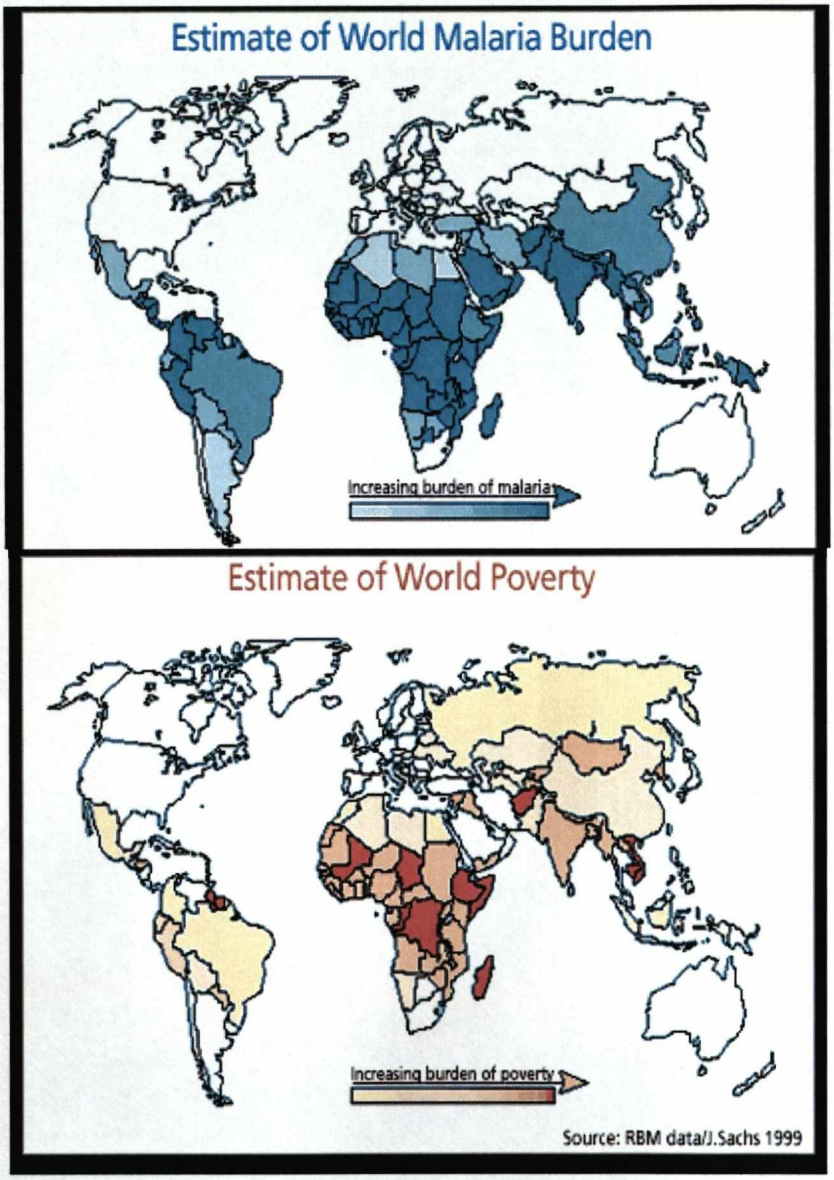


Figure 1. Comparison between Malaria burden and world poverty

Antimalarial drug development has been severely limited by the lack of interest shown by pharmaceutical companies in investing large sums for the development of drugs for a disease of a disadvantaged population. Indeed, nearly all available antimalarials have been developed through government (including military) research

programs (chloroquine, primaquine, mefloquine, Fansidar, halofantrine), the fortuitous identification of efficacy in natural product (quinine, artemisinins), or the identification of antimalarial potency in drugs marketed for other indications (folate antagonists, sulfanomides, antibiotics, atovaquone).

1.1.3. The malaria pathogen

Plasmodium, a unicellular eukaryotic cell of the protozoa group is the parasite responsible for malaria. Four main species cause human disease: *Plasmodium falciparum* (maligniant tertian), *vivax* (benign tertian malaria), *malariae* (quartan malaria) and *ovale* (Ovale tertian).

Plasmodium falciparum is by far the most important species, as it is responsible for nearly all severe malaria. This parasite is an enormous problem in Africa and is endemic in most malarious regions of the world. *P. falciparum* has demonstrated the ability to develop resistance to most available antimalarial drugs.[4]

Infection with *P.vivax* is also very common and although this infection causes relatively little severe disease, it is one of the most important causes of morbidity among parasitic infections, particularly in Southeast Asia, the Indian subcontinent, South and Central America and parts of Oceania. Drug resistance in *P. vivax* has been recognized only recently, but it is increasing, with vivax malaria resistance to chloroquine and other antimalarials noted in Southeast Asia, Oceania, India, and South America.

P. malariae and *P. ovale* are relatively uncommon causes of human malaria. These two parasites have a chronic liver phase (hypnozoite), in addition to the transient hepatic phase that precedes erythrocytic infection. Hypnozoites require specific therapy for eradication.

1.1.4. Parasite's life cycle

The female *Anopheles* mosquito is the vector responsible for transmitting the parasite. Life cycle of the protozoa is complex occurring in both man and in the

mosquito. The life cycle comprises of both the sexual and asexual forms. The sexual cycle is in the mosquito, while the asexual cycle is in man (**Figure 2**).

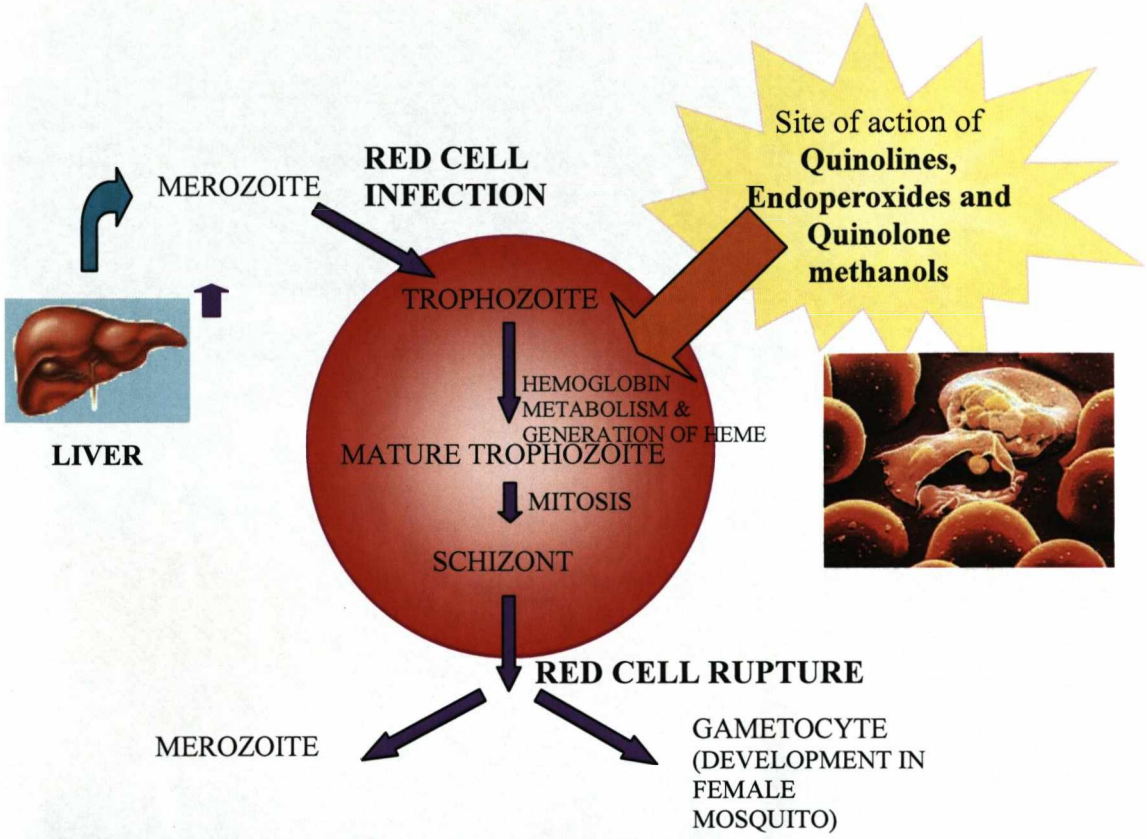


Figure 2. Parasite's life cycle

The parasite enters the host's blood stream when the anopheline mosquito, harboring plasmodial sporozoites, bites for a blood meal. Within thirty minutes of the parasite's sporozoites entering the bloodstream, they enter the parenchymal cells of the liver (pre-erythrocytic stage). This lasts 10-14 days, during which time they multiply. Hepatocytes rupture to release merozoites that enter red blood cells. These form motile intracellular parasites, known as trophozoites (erythrocytic stage). Mitotic divisions occur in the cells giving rise to schizonts. These red cells rupture, releasing mature merozoites, most of which go on to parasitise other red blood cells, with the release of merozoites and cells debris. Other sporozoites remain in liver cells in a resting stage (hypnozites) that can be activated in malaria relapses weeks or months later. Some

parasites fuse to form gametocytes in the red blood cells and their life cycle completes only in the mosquito.

Female and male come together within the mosquito to form zygote-oocyte (sporocyst). Division and multiplication of the sporocyst take place to produce many sporozoites. These then migrate to the salivary gland, waiting to infect again.

1.1.5. History of antimalarials

Malaria-like febrile illnesses (with names like "the ague" or "paludism") have been described since Hippocrates as fevers that are periodic and associated with marshes and swamps. The word "malaria" comes from the Italian "mal'aria" for "bad airs".

In 1880, Charles Louis Laveran first observed parasites in the blood of a patient suffering from malaria. Six years later Camillo Golgi, an Italian neurophysiologist observed that the parasites produced differing numbers of merozoites (new parasites) upon maturity and release of merozoites into the blood stream resulted in fever. During the 1890s, Giovanni Batista Grassi, Raimondo Filetti, William H. and Stephens named the four human malaria parasites. Ronald Ross later demonstrated that malaria parasites could be transmitted from infected patients to mosquitoes.

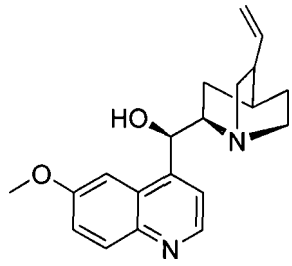
About 400 species of *Anopheles* mosquitoes exist; however, only about 70 of these are indicated in human malaria transmission at different levels and different areas.

Typically this age-long disease has been associated with difficulties in diagnosis and control. Although after long exposures some people become protected with acquired immunity, it is not possible to achieve a complete sterile immunity. It is thought that about 60% of people infected show no symptoms, making case studies difficult. *P. falciparum* malaria can lead to death and is influenced by other infectious diseases such as measles, malnutrition can also play a role. Vector control such as pesticides and mosquito nets have had limited success, so the main combat technique is through drug treatment. The development of a successful drug treatment is challenging due to the complexity of the protozoa's life cycle, both in man and in mosquito vector. Immunity would have been the next line of combat, but allelic diversity and antigenic variation makes it difficult to develop a suitable vaccine.

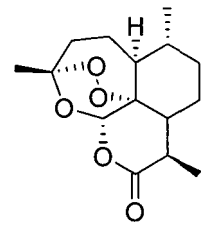
Several factors contribute to the persistence of the severe worldwide malaria problem. Efforts to control mosquito vectors, which were quite successful in some areas many years ago, have been limited by financial constraints and insecticide resistance. Current programs to treat and control malaria, especially in highly vulnerable young children and pregnant women, are severely limited in most endemic regions. An effective malaria vaccine is not yet available despite significant effort and is unlikely to be available to those who most in need it the near future. The problem is further exacerbated by malaria parasites who have consistently demonstrated the ability to develop resistance to available drugs. Although great strides have been made in the understanding of malaria in recent years, the development of new strategies to control the disease remains significantly limited by an incomplete understanding of the biology of the parasite and of the host response to parasite infection.

1.1.6. Malaria chemotherapy

What is remarkable about malarial fevers is that two herbal treatments, cinchona bark and qinghao, were used to treat malaria effectively for hundreds of years prior to the understanding of the mosquito cycle. Both quinine (derived from cinchona bark) and artemisinin (from qinghao), which remain of prime importance in the control of malaria, are natural products (**Figure 3**).



1, quinine

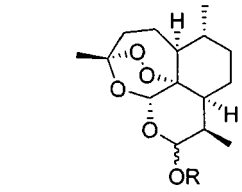


2, artemisinin

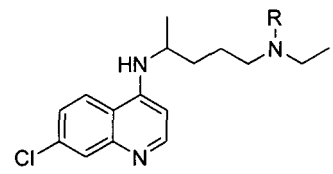
Figure 3

At present, important antimalarial drugs include a number of quinolines, inhibitors of enzymes required for folate metabolism, some antibiotics, a series of

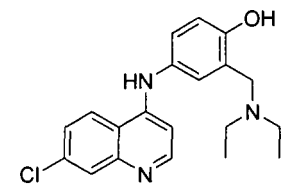
endoperoxides related to the natural product artemisinin, and the hydroxynaphthoquinone atovaquone (**Figure 4**). Notably among them were artemisinin and quinine derivatives, each with its own pharmaceutical limitations (**Table 1**).



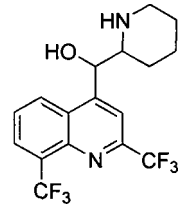
3, R(β)= Me, artemether
4, R(α)= $COCH_2CH_2CO_2H$, artesunate
5, R(α)= $CH_2Ph(CO_2H)$-*p*, artelinic acid



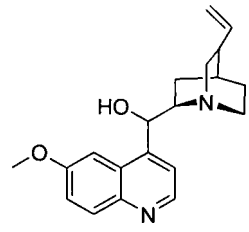
6, R= C_2H_5, chloroquine
7, R= H, desethyl chloroquine



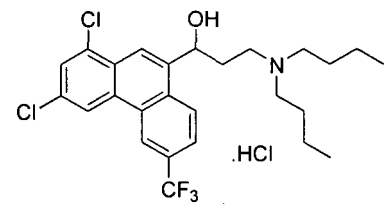
8, Amodiaquine



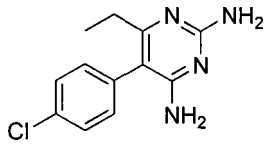
9, Mefloquine



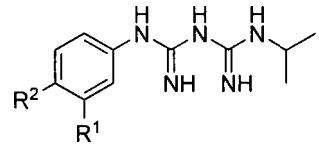
10, C8R; C9S, quinidine
11, C8S; C9R, quinine



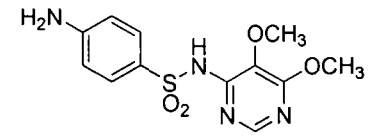
12, Halfan- halofantrine hydrochloride



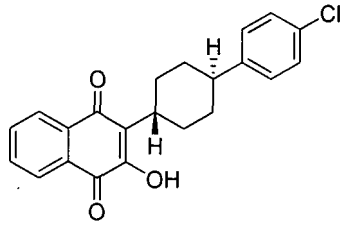
13, *Pyrimethamine*



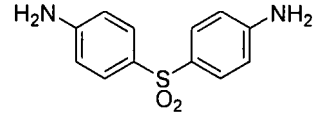
14, R^1= H, R^2= Cl, Proguanil
15, R^1= Cl, R^2= Cl, chloroproguanil



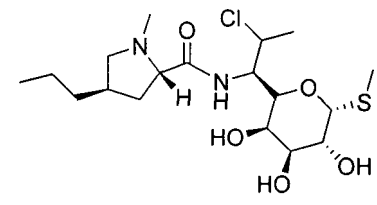
16, *Sulfadoxine*



17, Atovaquone



18, Dapsone



19, Clindamycin

Figure 4: Example of some existing antimalarials.

Drug	Class	Use	Side effects
Chloroquine	4-Aminoquinoline	Treatment and chemoprophylaxis of sensitive parasites	Cardiotoxicity, vomiting, rashes, itching and behavioral alterations.
Artemisinins	Sesquiterpene lactone endoperoxides	Treatment of multidrug-resistant *P.falciparum*	Neurotoxic in animal models embryotoxic
Amodiaquine	4-Aminoquinoline	Treatment of some chloroquine-resistant *P.falciparum*	Hepatitis and agranulocytosis
Quinine/ Quinidine	Quinoline methanol	Treatment of chloroquine resistant *P.falciparum*	Fever, confusion, respiratory arrest and arrhythmias, cinchonism (tinnitus, giddiness, blurred vision), hyperglycemia and hypertension.
Mefloquine (Lariam)	Quinoline methanol	Chemoprophylaxis and treatment of *P.falciparum*	Anxiety, depression, hallucinations, acute psychosis and seizures, transcient CNS toxicity, giddiness, convulsions, insomnia, neuropsychiatric reactions, gastrointestinal disturbances.
Primaquine	8-aminoquinoline	Radical cure and terminal prophylaxis of *P.vivax* and *ovale*	Methaemaglobinaemia with cyanosis hemolysis
Pyrimethamine/ sulfadoxine (Fansidar)	Folate antagonist, sulfa combination	Treatment of some chloroquine-resistant *P.falciparum*	Haemolytic anemia and agranulocytosis, skin rashes and megaloblastic anemia,[5] Headache, itching, insomnia, muscle aches, convulsions, nausea, shortness of breath, cough, rash, diarrhea, blood disorders

Proguanil	Folate antagonist	Chemoprophylaxis (with chloroquine)	Stomach upset and mouth sores, skin rush
Doxycycline	Tetracycline antibiotic	Treatment of *P.f.* (with quinine); chemoprophylaxis	Headache and sun sensitivity
Halofantrine	Phenanthrene methanol	Treatment of some chloroquine resitant *P.falciparum*	Abdominal pain, gastrointestinal disturbances, headache, cough, cardiac deaths, hemolytic anemia and convulsions[6]
Atovaquone (Malarone, combined with proguanil)	Quinone	Treatment and chemoprophylaxis of *P.falciparum* (with proguanil)	Rash, fever, vomiting, diarrhea and headache.

Table 1: Some existing antimalarials, their uses and their side effects.

Most of the antimalarials are used in combination for a total clearance of the parasite in the host's blood. For example, artemether/ lumefantrine (whose marketed name is Coartem), atovaquone/ proguanil (Malarone), artesunate/ amodiaquine (ASAQ) are such combinations.

1.1.7. Drug resistance

We are presently at a critical juncture in the history of the chemotherapy of malaria as an increasing drug resistance is leading to the need to rethink therapeutic approaches.

The World Health Organization (WHO) defines "drugs resistance" as the "ability of parasite strains to survive and/or multiply despite the administration and absorption of a drug given in doses equal to or higher than those usually recommended but within tolerance of the subject". This was modified to include the phrase "the form of the drug

active against the parasite must be able to gain access to the parasite or the infected erythrocyte for the duration of the time necessary for its normal action".[7-9]

Drug resistance involves mutations in the drug target so that the drug does not bind or inhibit the target, as well as expressing higher levels of the target, which can be accomplished either through increased transcription and translation or gene amplification, resulting in the requirement for higher levels of drugs to achieve the same level of inhibition.

Plasmodium falciparum became resistant to the antimalarial drug chloroquine through mutations in a single parasite gene which acts both as a shield and a chink in the armor of *P. falciparum* by making the parasite less susceptible to chloroquine, but more susceptible to some others antimalarials. Chloroquine interferes with the detoxification of haematin in the parasite's food vacuole. Resistance is associated with reduced accumulation of chloroquine in the vacuole, which results from reduced uptake of the drug, increased efflux, or a combination of the processes.[10]

It is becoming an increasingly serious problem as malaria parasites develop resistance to others drugs such as mefloquine. There is therefore considerable urgency to develop new classes of antimalarials.

1.1.8. New compounds, new approaches and new targets

Many approaches to antimalarial discovery are now available. Among important efforts that are currently ongoing are the optimization of therapy with available drugs, including use of combination therapy, the development of analogs of existing agents, the discovery of natural products, the use of compounds that were originally developed against other diseases, the evaluation of drug resistance reversers and the consideration of new chemotherapeutic targets.

To optimize therapy with existing drugs, new dosing and formulations are developed. Combinations therapies (e.g. artemisinin derivatives, atovaquone, amodiaquine/sulfadoxine/pyrimethamine,[11] chlorproguanil/ dapsone[12]) are under study as first-line therapies in areas with widespread resistance. Combining antimalarials are

very effective and slow the progression of parasite resistance to new drugs (e.g. artesunate/mefloquine).[13]

Another approach to antimalarial chemotherapy is to improve upon existing antimalarials by chemical modifications of these compounds. Many existing antimalarials have been developed through this approach. For example, chloroquine, primaquine and mefloquine were synthesized from quinoline which derived from quinine;[14] also new peroxides related to artemisinin[15] and new folate antagonists[16] are under study. Recent work has identified specific mutations in genes encoding target enzymes, which inhibits folate pathway enzymes.[17]

Natural products are the source of the most important drugs currently available to treat severe *falciparum* malaria, quinine and derivatives of artemisinin, therefore a plant product with specific clinical activity can be the starting point for a medicinal chemistry effort. Lately, it has been shown that curcumin, derived from turmeric (a yellow spice used in many Indian dishes) can be used against malaria, HIV and the virus that triggers cervical cancer.[18]

A fourth approach to antimalarials is to identify drugs that are developed or marketed as treatments for other diseases. The advantage is that the drug has already been given to humans, so will be quite inexpensive to develop as antimalarials.

Some drugs have been able to reverse the resistance of *P. falciparum*, which offers a new approach to chemotherapy. It appears that the inexpensive and efficient antimalarial may be resurrected by combination with effective resistance reversers.

Progress towards the characterization of the biology of malaria parasites has given rise to new targets for antimalarial therapy, which are considered based on their locations within the malaria parasite. The targets locations are the cytosol, the parasite membrane, the food vacuole, the mitochondrion and the apicoplast.

1.2. Artemisinin

1.2.1. The discovery of artemisinin

Artemisinin (2) (qinghaosu) is an unusual 1,2,4-trioxane (**Figure 5**), which has been used clinically in China for the treatment of multidrug resistant *Plasmodium falciparum* malaria.[19]

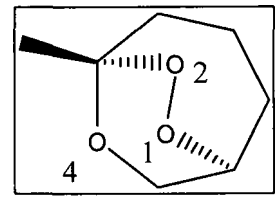


Figure 5

Artemisia annua –sweet wormwood or qinghao (pronounced "ching-how") – was also used by Chinese herbal medicine practitioners for at least 2000 years, initially to treat hemorrhoids. The earliest description of qinghao herb for treatment of malaria-related symptoms is found in the writings of Ge Hong (281-340 AD), because of the antipyretic activity of the tea-brewed leaves.

In 1596, Li Shizhen, a famous herbalist, recommended this herb for fever, and specified that the extract must be prepared in cold water.[20]

In 1967, the government of the people's Republic of China established a program to screen traditional remedies for drug activities[21] in an effort to professionalise traditional medicine. Qinghao was tested in this program and found to have potent antimalarial activity. In 1972, the active ingredient was purified and named qinghaosu (essence of qinghao). Qinghaosu and derivatives were then tested on thousands of patients. Artemisinin derivatives are now widely used in Southeast Asia and are starting to be used elsewhere.[22]

Research from various groups have established that the 1,2,4-trioxane peroxide linkage and the dialkylperoxide (figure 4) are essential or responsible for the

antimalarial activity of artemisinin and its derivatives because the deoxyartemisinin is completely devoid of biological activity.[23]

A direct comparison of *in vitro* values reveals that arteflene activity is about 10% that of qinghaosu against a battery of resistant lines of *P. falciparum.*[24] The carba-artemisinin analogue in which the non peroxidic oxygen atom in the trioxane pharmacophore in artemisinin is replaced by carbon has been constructed by an elegant total synthesis. This molecule has a simple peroxide pharmacophore, and displays an activity against *P. falciparum* that is about 4% of that of artemisinin.[25] Thus, the trioxane pharmacophore is essential for the expression of optimal antimalarial activity.

So far no clinical resistance has been found against the artemisinin class of antimalarial drugs, and they are effective against multidrug-resistant strains of *Plasmodium falciparum.*

1.2.2. Mechanism of action of artemisinins

There is no definite explanation of the mechanism of action of artemisinin and related peroxide-containing compounds.

1.2.2.1. Parasite-specific proposed mechanisms of action

In vitro antimalarial activity of artemisinin was shown to be sensitive to steric effects, based on this observation Krishna and co-workers suggested that the molecule undergoes activation after binding to a specific site.[26, 27] Eckstein-Ludwig and co-workers showed that artemisinin specifically inhibits PfATP6 (or parasite encoded Sarco/Endoplasmic Reticulum Ca^{2+}-ATPase),[28] which is responsible for the maintenance of calcium ion concentrations. It was suggested that artemisinin binds to the protein by hydrophobic interactions whilst leaving the peroxide bonds exposed (not covered by the binding pocket).[29] This allows cleavage of the peroxide bridge by iron to generate carbon-centered radicals (as discussed later in section 1.2.2.2), leading to enzyme inactivation and parasite death.

However, enantiomers of trioxanes structurally related to artemisinin showed equivalent levels of activity against chloroquine-sensitive, chloroquine-resistant, and multidrug-resistant strains of *P. falciparum*.[30] These results imply that activation of artemisinin does not depend on stereospecific interaction with a protein. In addition, introduction of bulky side groups to artemisinin may cause either a decrease in activity or increased activity, depending on which residues are added.[31] Both points weaken the theory that a protein-binding site is crucial for artemisinin activity.

1.2.2.2. Non-specific proposed mechanisms of action

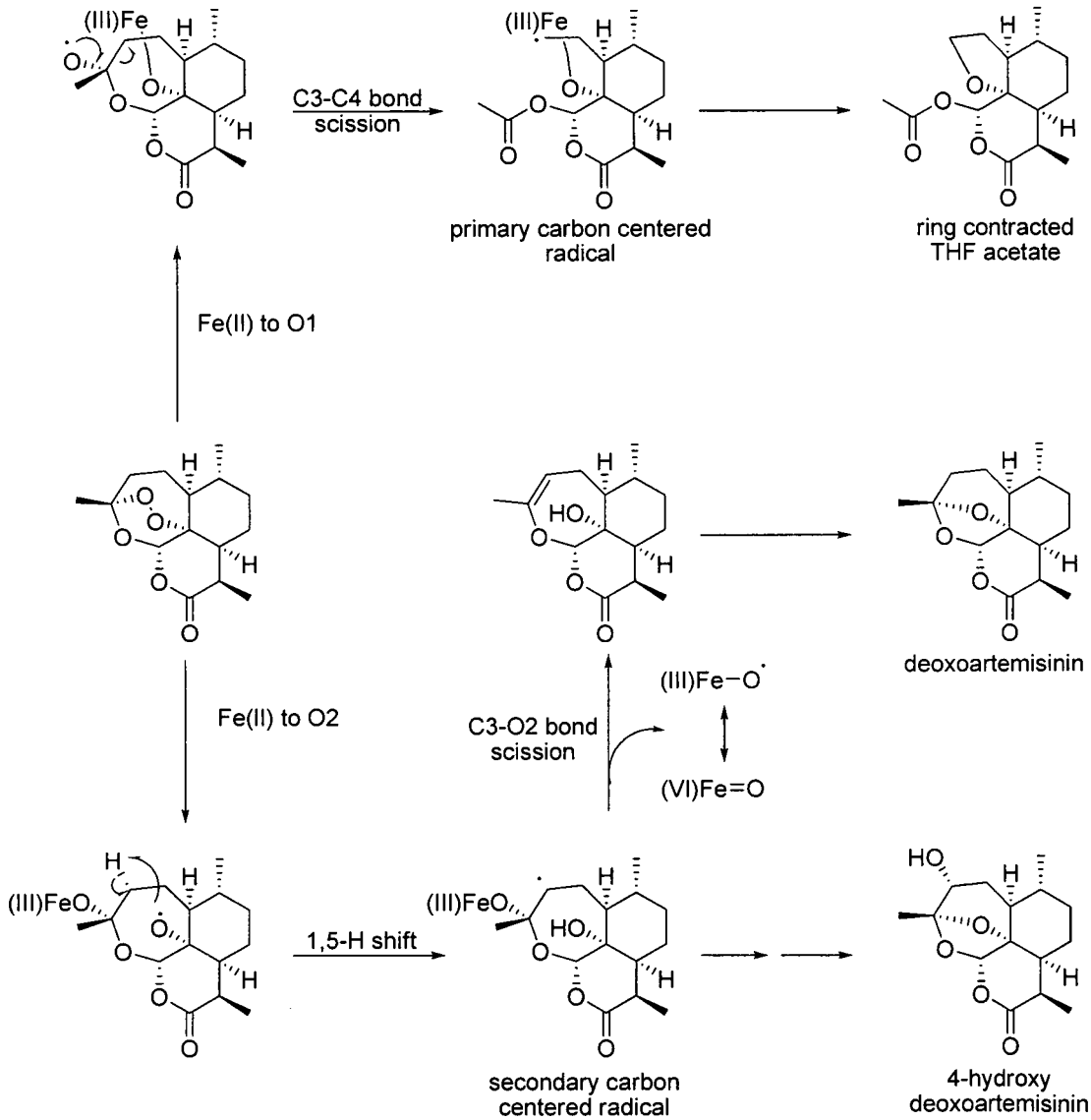
The endoperoxide bridge, present in artemisinin and all its derivatives, is essential for antimalarial activity. This was demonstrated by the lack of activity of deoxyartemisinin, a reduced form of artemisinin in which a single oxygen replaces the endoperoxide bridge.[32] Based on this finding, the mechanism of action of artemisinin is believed to involve an interaction with ferriprotoporphyrin IX ("heme"), or ferrous ion, in the acidic parasite food vacuole which results in the generation of cytotoxic radical species.[33]

Carbon radicals are formed by heme located within the lipid bilayer and mediate the production of allylic C-radicals on unsaturated lipids. These become lipid peroxides in the presence of O_2 going on to produce hydroxyl and superoxide radicals. These species can then cause oxidative damage to receptors and enzymes positioned in the vicinity of the lipid bilayer, which leads to vacuole rupture and parasite auto-digestion.[34] Treatment of erythrocytes with artemisinin caused a proportion of the cells to lyse dependent on the dose give though these experiments were performed at drug concentration up to 10^3-10^5 times higher than effective concentration tested *in vitro*.[35]

Endoperoxides are known to be unstable, especially in the presence of iron, and to breakdown to form free radicals. Studies using synthetic trioxanes and a magnesium centred synthetic metalloporphyrin were undertaken. These examined the nature of the coordination between the peroxide moiety and heme and the resulting cleavage of the peroxide bridge. It was found a close interaction between the peroxide bond and the metal center is required indicating that the activation of the peroxide moiety occurs

through an innersphere electron transfer. Also, the ability to carry out alkylation is essential for the antimalarial activity of artemsinin.[36, 37]

Two models of artemisinin antimalarial mechanism of action are suggested, both of which show formation of free radicals, mediated by iron (**Scheme 1**).



Scheme 1. Iron degradation suggested mechanism

1.2.3. Hemoglobin digestion in parasite's food vacuole

The *Plasmodium* food vacuole houses the specialized components of malarial hemoglobin catabolism (**Figure 6**).

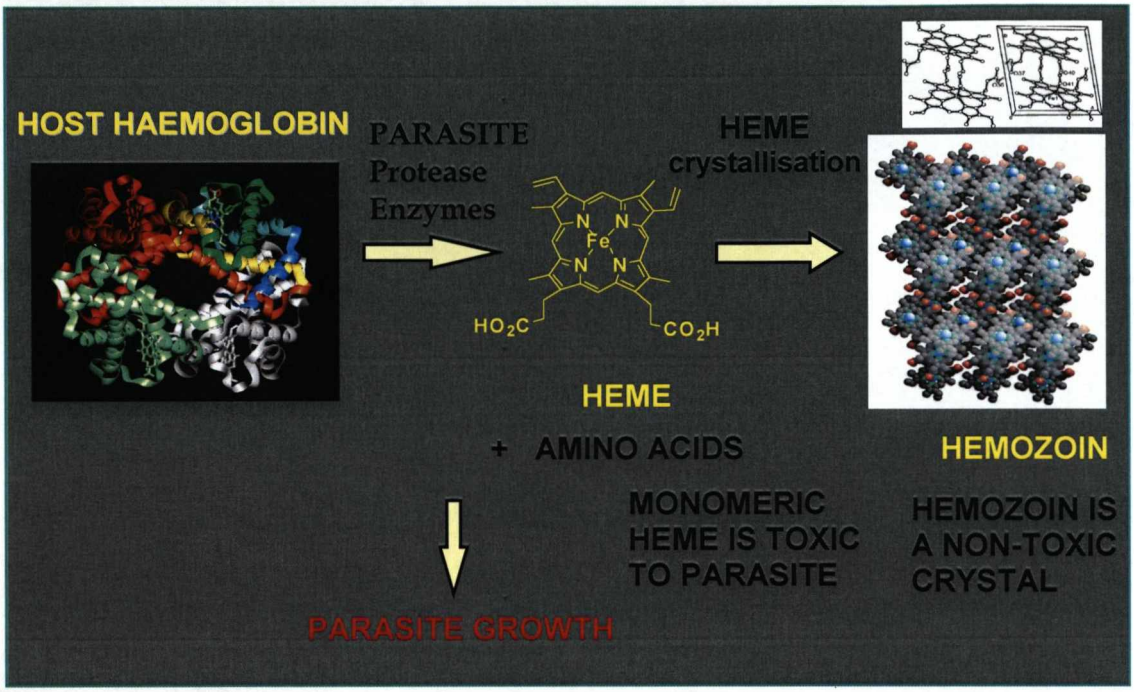


Figure 6: Metabolic processes within the *Plasmodium* food vacuole[38]

Hemoglobin is degraded by proteases, generating amino acids and free heme. The heme is crystallised to hemozoin,[39] possibly by the action of histidine-rich proteins. Free heme could also [1] react with molecular oxygen, generating reactive oxygen species that are scavenged by host or [2] be degraded, liberating iron, some of which is probably utilised by the parasite.

Free heme is a toxic by-product of hemoglobin degradation. Free heme can cause enzyme inhibition, peroxidation of membranes, production of free radicals, and impaired leukocytes function.[40, 41] *Plasmodium falciparum* has little or no heme oxygenase (the enzyme used by vertebrates to catabolize heme). All *Plasmodium* species have a unique capability to detoxify heme in the food vacuole by polymerizing it into a crystalline structure called hemozoin, or malarial pigment.[42-44]

Inhibiting hemoglobin degradation within the food vacuole is a valid approach to antimalarial chemotherapy. Multiple plasmepsins[45] and falcipains have been identified in the food vacuole. Plasmepsin-1 and plasmepsin-2 are able to cleave undenatured hemoglobin between phenylalanine and leucine residues; it is suggested that Falcipain-2[46] and falcipain-3[47] would digest native hemoglobin, therefore participate in the initial cleavage of hemoglobin.

1.2.4. Problems with artemisinin derivatives

Typical of most natural products, artemisinin is associated with limited availability, high cost, as well as poor oral bioavailability and short half life. For parent compounds, such as artesunate, which have very short half-lives (<10 min), the antimalarial effect is less important than that of their metabolite, DHA, whose half-live[27] is somewhat longer ($\sim$1 h). Due to high recrudescent rates resulting from their short plasma half lives as a monotherapy, it is recommended that artemisinin should be combined with more slowly eliminating drugs such as mefloquine or lumefantrine.[48-50]

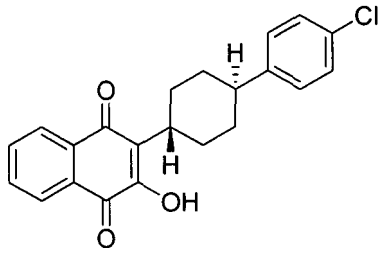
The main goal of research described in this thesis was the synthesis of new artemisinin analogues that combine both improved water solubility and metabolic stability with enhanced antimalarial activity.

The first part of the project was focused on the synthesis of alkylamino substituted pyrrole analogues. The second part involved the synthesis of sulfone derivatives

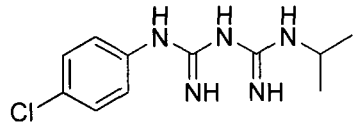
1.3. Inhibitors of parasite respiration

Atovaquone (**20**), a naphthoquinone antimicrobial agent, is classed as a quinone. Its combination with Proguanil (**21**), trademarked Malarone, has been developed during the 1990's and has been approved for treatment of falciparum malaria in more than 30 countries (**Figure 7**). Atovaquone affects parasite's mitochondrial functions selectively,[51] and is used to treat pneumonia and toxoplasmosis. Atovaquone couldn't be used as monotherapy because the parasite developed a mutation of a cytochrome *b* gene

localized in the mitochondrial genome.[52] Addition of proguanil solved the resistance problem and this synergistic agent has overcome the rate of treatment.[53]



20, Atovaquone



21, Proguanil

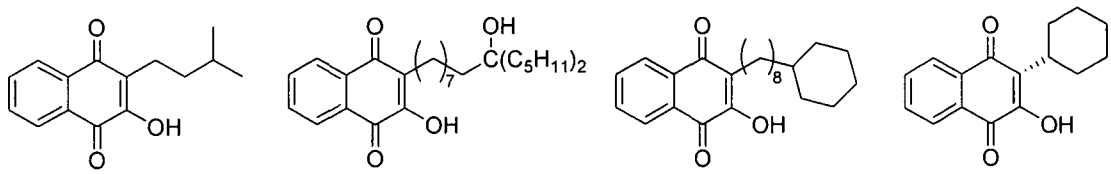
Figure 7

Malarone is the best current drug to treat resistant strains of Plasmodium falciparum. It is expensive due to the stereochemistry of atovaquone and a synthetic route that includes 6 steps.[54]

1.3.1. Development of Atovaquone

Atovaquone is a naphthoquinone belonging to a family of compounds that have been investigated as antimalarials for over 50 years. During World War II, research on new antimalarials (**Figure 8**), including investigations on naphthquinone, has been carried out, in order to find an alternative to quinine. Hydrolapachol, derived from lapachol,[55] was found to have an antimalarial activity. Hundreds of lapachol analogues were then synthesised and tested, which led into lapinone, needed in large doses to treat vivax malaria.

In the 1960s, coinciding with the emergence of chloroquine resistance, there was a renewed interest in developing hydroxynaphthquinone analogues, such as menoctone whose clinical trials were disappointing.[56] Parvaquone was first synthesized and was identified as a good anti theilerial as well as menoctone.[57] Modifications of the cyclohexyl moiety of parvaquone gave rise to atovaquone, which is metabolically stable and has a broad-spectrum against a number of encaryotic pathogens, including malaria parasites.[58]



22, Hydrolapachol **23**, Lapinone **24**, Menoctone **25**, Parvaquone

Figure 8

Cultured *Plasmodium falciparum* isolates from different parts of the world were inhibited by atovaquone at low molecular concentrations (IC_{50} 0.7-4.3 nM), although some strains of *Plasmodium falciparum* were relatively resistant. *In vivo* tests were carried on mice infected with *P. yoelii* and *P. berghei*, on Aotus monkeys infected with *P. falciparum*, the drug was found to be highly effective in curing malaria.[58] When atovaquone was given to patients with *P. falciparum* in the United Kingdom, a prompt clinical response with removal of the parasites from the blood was observed, however most patients developed recrudescent malaria.[59] Some extensive studies in Thailand,[53] and in Zambia,[60] demonstrated that the parasite was completely cleared of the blood for two thirds of the patients, while the other third developed recrudescent malaria. Sensitivity to atovaquone was assessed from several recrudescing patients. Although all of the parasites isolated upon admission of the patients were sensitive to atovaquone with an IC_{50} of ~ 3.3 ng/mL, the paired recrudescent parasites showed high level of resistance (IC_{50} of >3000 ng/mL).[53] Atovaquone as a stand-alone antimalarial, was found to be unacceptable.

A search for a partner drug with atovaquone was necessary to reduce the chance of drug resistance development. Atovaquone combined with a number of other antimalarials (tetracycline, doxycycline, pyrimethamine and proguanil) were investigated, few drugs had additive effects, while others had antagonistic effects.[61] Proguanil was found to have the best synergistic action when tested against three different *P. falciparum* isolates. Proguanil is cheap to produce, has a favorable safety profile and has been used as an antimalarial for almost 50 years, although it failed to clear the parasites on its own for 90% of the patients in Thailand.[53]

A mechanism explaining the synergy between atovaquone and proguanil has been recently proposed.[62]

1.3.2. Mechanism of Atovaquone action

Atovaquone is a substituted 2-hydroxy-naphthoquinone that is used therapeutically to treat *Plasmodium falciparum* malaria, *Pneumocystis carinii* pneumonia, and *Toxoplasma gondii* toxoplasmosis. It is thought to act on these organisms by inhibiting the cytochrome bc_1 complex,[63] atovaquone binds tightly and competitively to the ubiquinol oxidation site of the cytochrome bc_1 complex, between the cytochrome b and the iron-sulfur protein.[64]

Cytochrome bc_1 complex is found in the mitochondria of *Plasmodium falciparum.* Mitochondria's function is to generate an electrochemical gradient across the inner membrane, which is then used as an energy source for the myriad of synthetic and transport activities associated with motochondria. Atovaquone is a potent and selective inhibitor of the cytochrome bc_1 complex of mitochondria electron transport in *P. falciparum*, which leads to the death of the parasite.[65]

Electron transfer processes (**Equation 1**) are of great importance in many metabolic pathways of living organisms. They are essential for the parasite's respiration, in which energy gained by oxidation of nutrients is converted into energy of the anhydride bond of ATP. Energy conversion is achieved by coupling the transfer of electrons to the translocation of protons across a lipid membrane. The generated electrochemical proton gradient is used for ATP synthesis.

The mitochondrial respiratory chain consists of four large multisubunit membrane protein complexes embedded in the inner mitochondrial membrane that are linked by the freely diffusible electron carriers ubiquinone (UQ) and cytochrome *c* (Cyt *c*) (**Figure 9**).

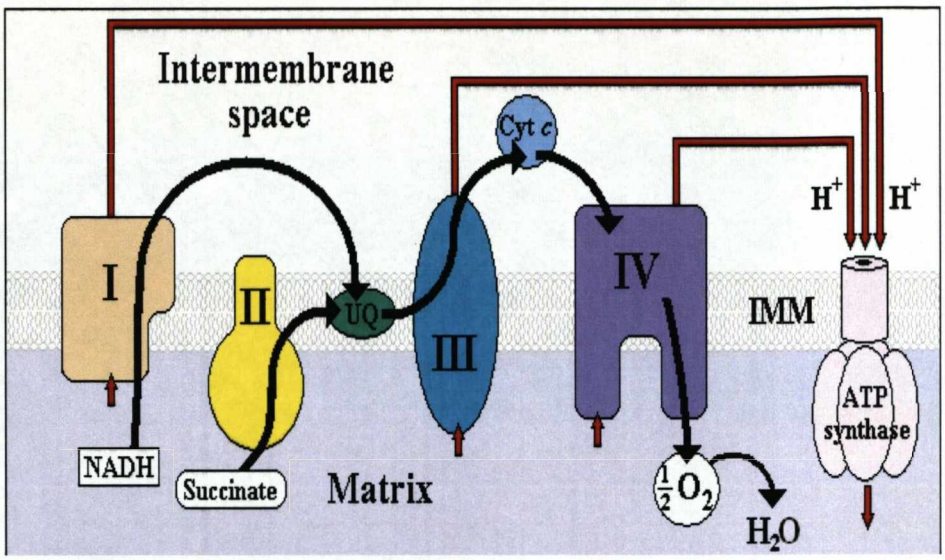
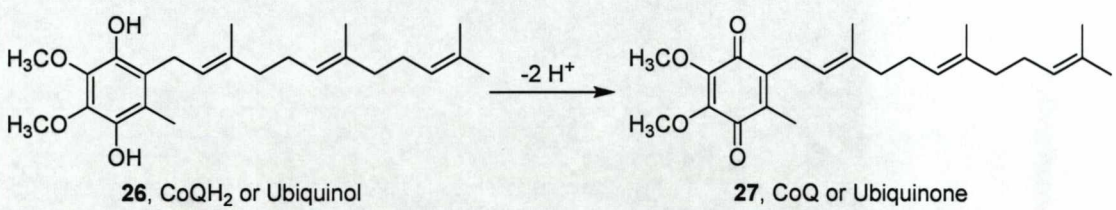


Figure 9

Ubiquinone, co-enzyme Q (**27**), is found in the inner membrane of the mitochondria and take part in the electron transport chain. Indeed, cytochrome bc_1 complex, also called ubiquinol-cytochrome *c* oxidoreductase or complex III, catalyses the respiration chain which takes place in the inner membrane of the mitochondria. Complex III included the Rieske iron-sulfur protein, cytochrome *b* and cytochrome c_1. The transfer of electrons from ubiquinol to cytochrome *c* and the associated proton translocation is highlighted in the following equations (**Equation 1 - Scheme 2**):

$$CoQH_2 + 2\ Fe^{III}\text{-cytochrome c} \longrightarrow CoQ + 2\ Fe^{II}\text{-cytochrome c}$$

Equation 1. Redox equation of ubiquinone with iron in cytochrome *c*



26, $CoQH_2$ or Ubiquinol **27**, CoQ or Ubiquinone

Scheme 2. Oxidation of Ubiquinol

To understand the mechanism of electron transfert in cytochrome bc_1 complex, several crystal structures of this complex have been obtained. The complex III, including cytochrome bc_1 and ubiquinol, was purified from beef heart mitochondria[66, 67] and from the yeast.[68] The chemical composition and spectrophotometric properties of the crystal have been described.

1.3.3. New lead molecules against cytochrome bc_1-complex

As the structure of the cytochrome bc_1 complex is well known, we can show by computational modelling the binding of atovaquone to the bc_1 complex. Some studies have screened a library of 2-hydroxy-naphthquinones substituted at position 3 with aromatic, cyclic, and non-cyclic alkyl chains for inhibition of bc_1 complex activity. Some compounds of this library have been tested in order to establish a quantitative structure/ activity relationship (SAR) based on side chain length. These comparisons allow a starting point to develop some new inhibitors of the cytochrome bc_1 complex, such as 2-hydroxy-naphthquinone with linear alkyl side-chain at position 3 (**Figure 10**).[69]

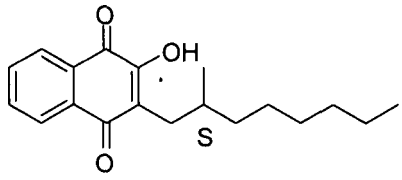


Figure 10

1.4. Conclusion.

As a result of parasite drug resistance, and lack of drug development, there are more people dying of malaria now than there were 20 years ago. Recognition of this problem by the international community and the engagement of the pharmaceutical industry and other key stakeholders, has catalysed the concerted search for new antimalarial drugs with novel targets.[70-72]

The two following chapters elaborate the synthesis of semi-synthetic analogues from dihydroartemisinin and synthetic quinolones to target the hemoglobin degradation pathway and the cytochrome bc_1 complex.

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CHAPTER 2

Synthesis of C-10 heterocyclic derivatives of dihydroartemisinin

2. Synthesis of C-10 heterocyclic derivatives of dihydroartemisinin (DHA)

2.1. Introduction

The artemisinins (**1-5**) are the most efficient and fast acting antimalarial drugs used in malaria chemotherapy. However, derivatives **2-5** are cleared from blood within 2 hours and parasites that are not all killed within this time can re-emerge resulting in recrudescence of the disease in the patient. In order to prevent recrudescence,[1] the artemisinins are used in combination therapies with drugs that have longer half lives (eg amodiaquine, mefloquine, sulfadoxine/pyrimethamine or lumefantrine).[2-5]

The therapeutic value of artemisinin (**1**), *qinghaosu*, is limited to a great degree by its low solubility in both oil and water. Therefore a number of more soluble derivatives have been developed, such as DHA (**2**), artemether (**3**),[6] arteether (**4**)[7] and sodium artesunate (**5**)[8, 9] to enhance their absorption (**Figure 1**).

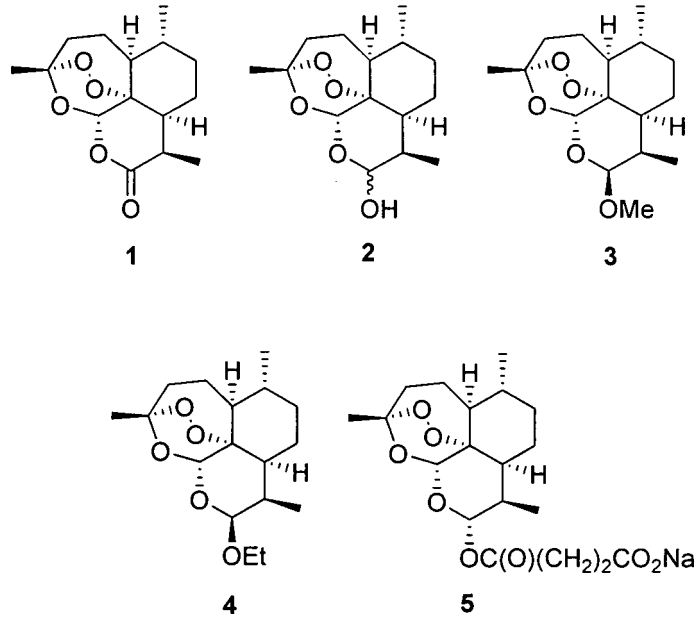


Figure 1. Existing artemisinin derivatives.

Lipophilic artemisinins, such as artemether and arteether, although they are more potent than artemisinin, produce fatal central nervous system toxicity in high dose in rats

and dogs.[10-12] Because of their lipophilicity, it is proposed that these derivatives get into the brain and form free radicals, which destroy neurones.[13]

To treat advanced cases of *P.falciparum* malaria, a water-soluble derivative of artemisinin is required, which can be delivered quickly by intramuscular injection.[8] The water-soluble sodium artesunate is currently the drug of choice[14] and is administered in combination therapy most often with mefloquine.[15]

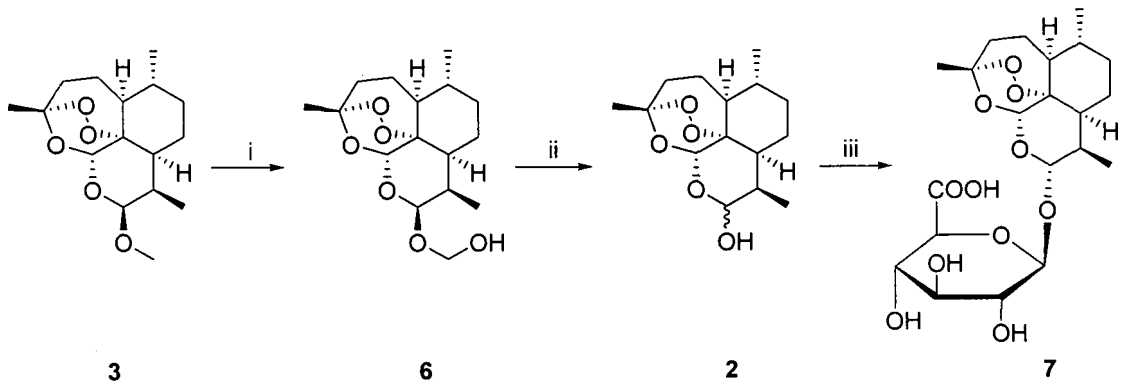
The major challenge in this field is to prepare a semi-synthetic analogue from DHA in only one or two high yielding steps to provide analogues with a log P of less than 3.25 and which is more metabolically stable than artesunate (**5**) or artemether (**3**).[16] In this project we have explored an approach to new, more water-soluble analogues of DHA based either on polar C-10 pyrrole derivatives or on carboxyl isosteres linked to the C-10 position of artemisinin.

2.2. Pyrrole analogues

2.2.1. Introduction

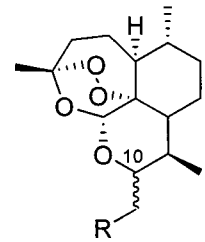
Several "mechanism-based approaches" have been investigated for improving the antimalarial activity of artemisinin derivatives. These include the incorporation of groups to enhance the stability of proposed "parasitisidal intermediates" and the covalent attachment of "iron chelator functionality" to enhance the interaction of the peroxide bridge with available "free iron" in the food vacuole of the parasite.[17]

A major drawback of many semi-synthetic analogues including artemether is that they undergo rapid metabolism *in vivo*, yielding initially DHA (**2**) *via* cytochrome P450-mediated dealkylation.[18] This results in a short half-life since DHA is rapidly glucuronidated to produce a glucuronide (**7**) that is excreted into the bile and the urine.[19] A recent synthesis by O'Neill and Stachulski has confirmed that the mammalian metabolite of DHA (**7**) has C-10α configuration (**Scheme 1**).[20]



Scheme 1. i) Cytochrome P450, monohydroxylation (Phase I metabolism); ii) elimination of CH_2O; iii) glucuronidation (Phase II metabolism).

The C-10 carba linkage (**Figure 2**) increases stability and is expected to produce a molecule with a longer half-life.



R= alkyl, aromatic

Figure 2. Artemisinin's analogue with C-10 carba linkage

It has been proposed that the incorporation of an amino functionality will enhance drug activity by increasing the cellular accumulation within the acidic (pH 4.7) parasite food vacuole by "ion trapping" (**Figure 3**).[21] The higher concentration of drug available for interaction with heme, which enhances generation of the required alkylating species, may have been responsible for the increased antimalarial activity observed in the study led by O'Neill and co-workers.[22] In this work, several amino alkyl analogues of artemisinin were shown to be more potent than artemisinin or artemether in *in vitro* cultures of *Plasmodium falciparum*.

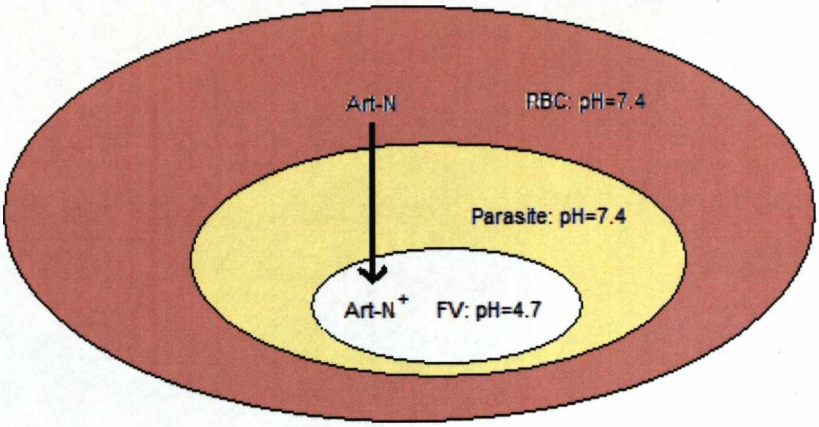


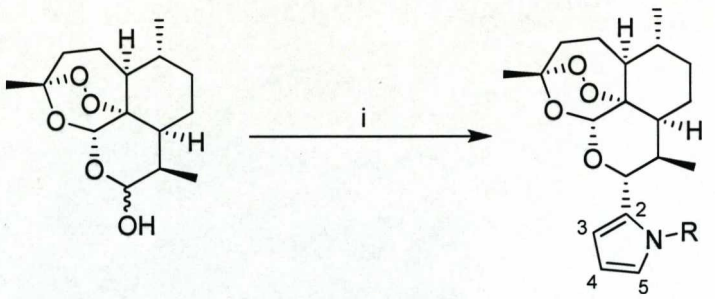
Figure 3. Protonation of amine functionality on artemisinin within the parasite Food Vacuole (FV). Once the drug has acquired a positive charge by protonation it is effectively "ion-trapped" within this organelle.

2.2.2. Synthesis of pyrrole analogues of artemisinin

This section focuses on the synthesis of C-10 pyrrole analogues of dihydroartemisinin. We chose the synthesis of C-10 pyrrole analogues with Mannich side chains as target molecules for the following reasons:

- C-10-aryl artemisinins or C-10-hetaryl systems cannot generate DHA by hydrolysis or metabolism.
- The Mannich side chain provides molecules that have the potential to be formulated as salts.[23]
- Amines should accumulate more in the acidic digestive vacuole of the parasite.[21, 24]

Three different approaches were explored for the synthesis; first, the C-10 heterocyclic analogues **8-9** were synthesised directly from **2** in the presence of a Lewis acid using the nucleophilic *H*-pyrrole and *N*-methylpyrrole (**Scheme 2**). This particular heterocycle was chosen as the electron rich ring provides scope for the introduction of electrophiles at the C-5 position following incorporation on to the artemisinin scaffold.



2

R= H: **8**, 77%
R= Me: **9**, 84%

Scheme 2. *Reagents and conditions*: i) $BF_3.Et_2O$, pyrrole or *N*-methylpyrrole, DCM, -50°C, 30 mins.

The stereochemistry at the C-10 position was determined by ^1H NMR spectroscopy. The signal due to H10 appears as a doublet at 4.49 ppm with a $^3J_{H10\text{-}H9}$ value of 10.8 Hz, which is indicative of a *trans-trans* diaxial relationship between H10 and H9.[25] This stereochemistry is believed to be purely steric as the bulky pyrrole ring prefers to attack the oxonium intermediate in an equatorial position and adopt an *anti* position to the methyl group (**Figure 4**).

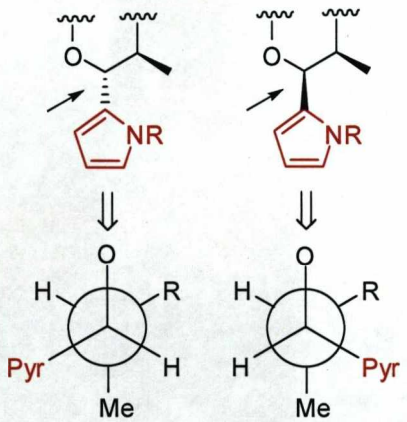
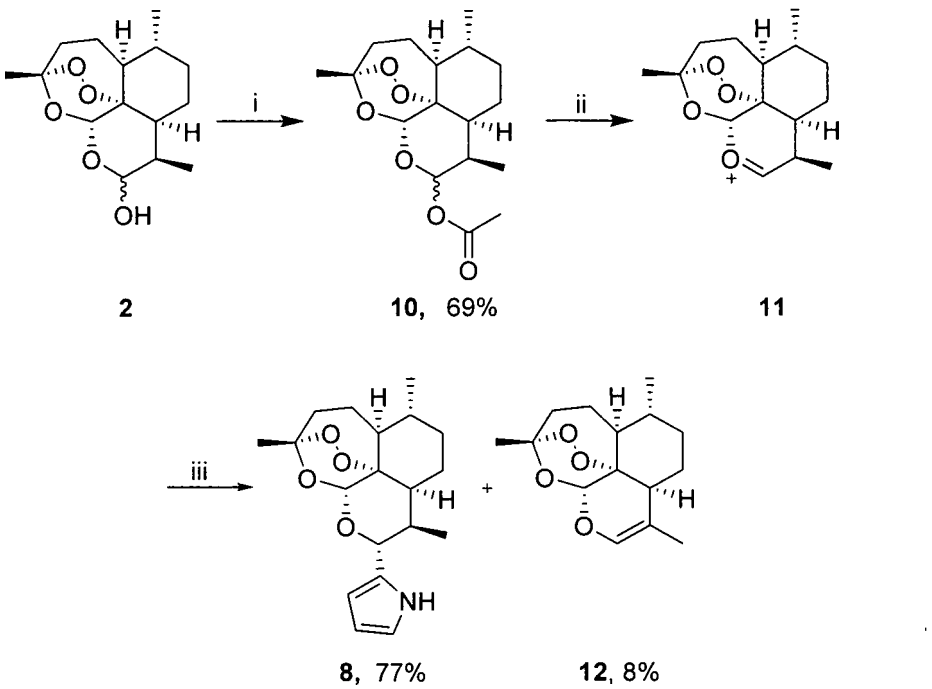


Figure 4. Newman projection of C-10α and β pyrrole analogue.

Secondly, the synthesis of **8** was made via an intermediate, **10** formed by treatment of DHA with acetic anhydride in DMAP.[22] The better anomeric leaving group allows smooth formation of the oxonium ion **11** which was easily intercepted with the nucleophilic pyrrole ring, with little formation of the by-product anhydroartemisinin **12**.

$BF_3.Et_2O$ catalysed reaction of **10** with the pyrrole led to the formation of the product **8** in good yield and some side-product anhydroartemisinin (**Scheme 3**).

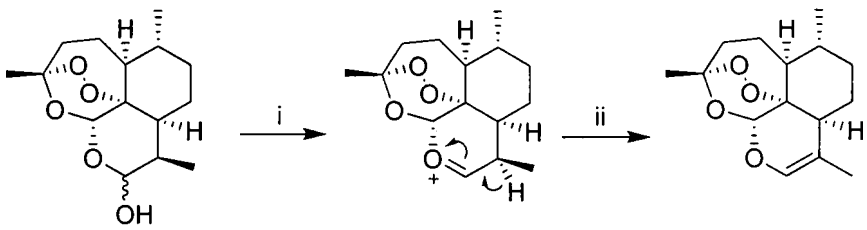


2 **10**, 69% **11**

8, 77% **12**, 8%

Scheme 3. *Reagents and conditions*: i) DMAP, pyridine, acetic anhydride, DCM; ii) $BF_3.Et_2O$, DCM, -50°C, 30 mins; iii) pyrrole, DCM, -50°C, 30 mins.

Formation of anhydroartemisinin (**12**) followed the mechanism below (**Scheme 4**). This by-product is formed by dehydration in the absence of a nucleophile.

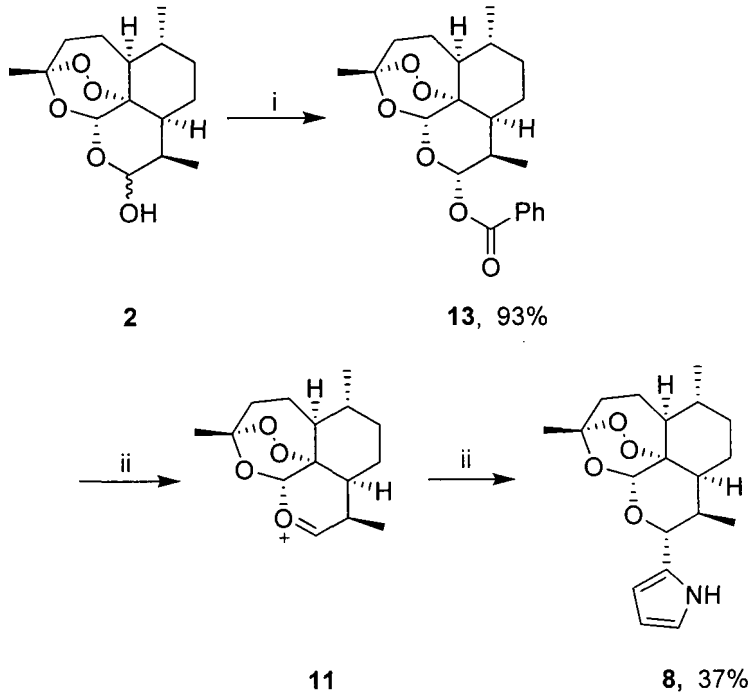


2 **11** **12**

Scheme 4. Mechanism of formation of AHA; i) $BF_3.Et_2O$, DCM, ii) Deprotonation.

Thirdly, acylation of **2** with benzoyl chloride with pyridine as the nucleophilic catalyst[22] gave **13**. Treatment of the pyrrole catalysed by $BF_3.Et_2O$ gave **8** via S_N1 with

the oxonium ion **11** as an intermediate. The α configuration is preferred for **8** because pyrrole is bulky, therefore it attacks the oxonium in equatorial (**Scheme 5**).



2 **13**, 93%

11 **8**, 37%

Scheme 5. *Reagents and conditions*: i) PhCOCl, pyridine, DCM, O°C; ii) $BF_3.Et_2O$, pyrrole, DCM, -50°C, 30 mins.

We observed that the α configuration is the major product for **10** and **13** because the C-10 position of artemisinin is analogous to a sugar anomeric center, with the anomeric hydroxyl predominantly equatorial,[26] therefore the acylation takes place mainly in equatorial position.

It can be concluded that there is no benefit from synthesis via 10α and β-acetate and 10α-benzoate derivatives as the overall yields were not as good as the direct route to obtain **8** with 77% yield.

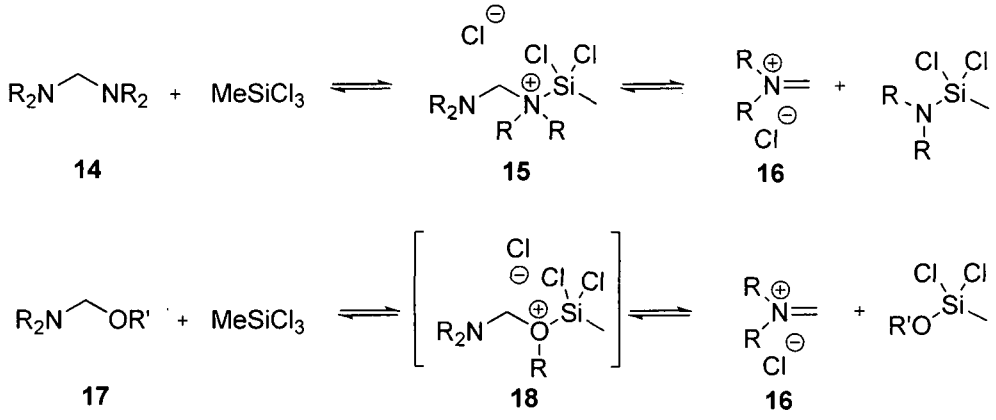
We were particularly interested in the pyrrole synthesis because the electron-rich ring is suitable for a wide range of electrophilic manipulations. We therefore attempted the synthesis of a variety of analogues from **8** and **9** based on Mannich chemistry to incorporate an amino-alkyl side-chain.

Due to the amino functionality in these analogues and improved water-solubility, we anticipated improved *in vitro* and *in vivo* antimalarial activities based on the observations made with other semi-synthetic amino-alkyl artemisinin derivatives.[22, 27, 28]

2.2.3. Analogues made via Mannich reaction

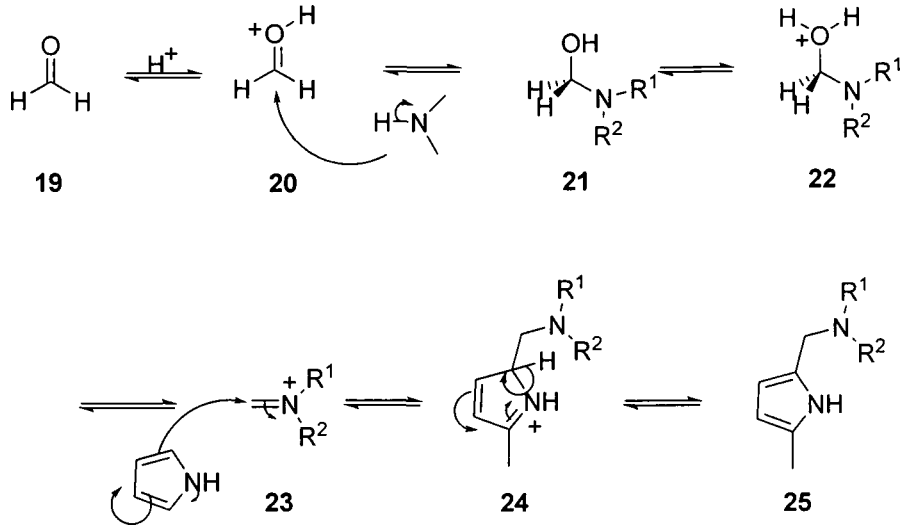
Mannich reactions carried out under classical aqueous reaction conditions may evolve depending on the nucleophilicity of the substrate and more particularly on the pH of the solvent system. The failure of an attempted Mannich reaction may be attributed either to the weak electrophilicity of the particular intermediate involved or conversely to the low nucleophilicity of the substrate.

The Mannich reaction can be performed in many ways.[29, 30] Heaney and co-workers formed iminium salts (**16**) from *in situ* reactions of aminals (**14**) or aminols ethers (**17**) with heterocycles and chlorosilanes (**Scheme 6**).[31]



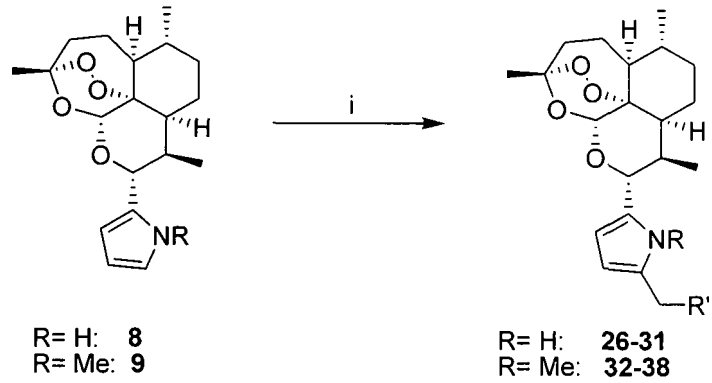
Scheme 6

We chose to carry out this reaction on the pyrrole ring in acidic conditions using formaldehyde and several secondary amines. The mechanism involves the preliminary formation of an iminium salt **23** from the amine and formaldehyde (**19**). Acid-catalysed elimination of water gave **23**. The electrophilic salt adds to the pyrrole ring's 2-position because the intermediate **24** is more stable due to its linear conjugated system to give **25** (**Scheme 7**).



Scheme 7. Mechanism of the Mannich reaction.

The application of Mannich reactions has been widely reported in literature.[32, 33] We performed this reaction by sequentially dissolving **8** or **9** at r.t. in ethanol, followed by addition of the secondary amine (3.2 equiv.), formaldehyde (3.2 equiv.) and acetic acid (1.0 mL in 5.0 mL EtOH). The reaction mixture was then left for half an hour and quenched with sodium hydroxide. The crude product was extracted with DCM and the organic phase washed with brine. Purification by flash chromatography gave the Mannich product (**Scheme 8**).

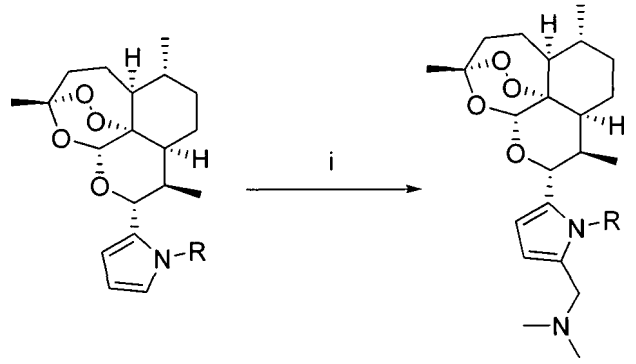


Scheme 8. *Reagents and conditions*: i) CH_2O, secondary amine, AcOH, EtOH, r.t., 30 mins.

The above conditions were used to prepare **26-31** from **8** in acceptable yields (**Table 1**). The Mannich reactions gave lower yields with the amines diethylamine, piperidine and pyrrolidine.

Similarly, **32-38** were synthesised from **9** in very good yields (**Table 1**). The high yields could be explained by the fact that the *N*-methyl group gives electronic density to the pyrrole ring by inductive effect, suggesting that the *N*-methylpyrrole ring is a better nucleophile than *H*-pyrrole. Due to the better nucleophilicity of *N*-methylpyrrole, the yields for the Mannich reactions from **9** are higher than reactions from **8**.

Repetition of the Mannich reaction with commercial Eschenmoser's salt (**Scheme 9**) gave **39** and **40** in 70% and 86% yields respectively.



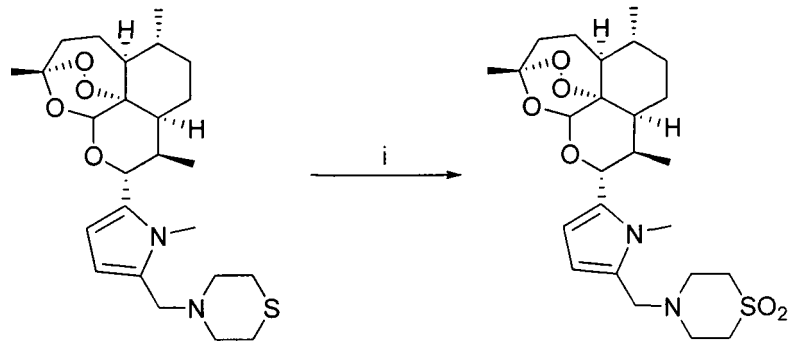
R= H: **8**
R= Me: **9**

R= H: **39**, 70%
R= Me: **40**, 86%

Scheme 9. *Reagents and conditions*: i) $[CH_2N(CH_3)_2]^+I^-$, acetonitrile, r.t., 24 hrs.

Sulfone formation

There are several ways to oxidise sulfides into sulfones, such as *m*CPBA,[34] oxone[35] and urea hydrogen peroxide.[36, 37] Thioether **37** was oxidized to its corresponding sulfone (**41**) by treatment with catalytic amount of TPAP and NMO in DCM in 35% yield (**Scheme 10**).



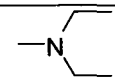
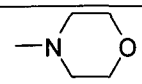
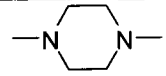
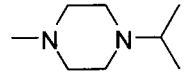
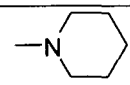
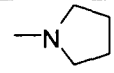
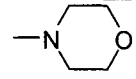
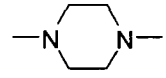
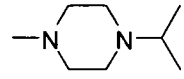
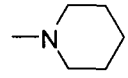
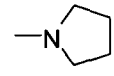
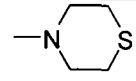
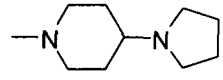
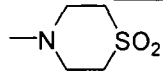
37 **41**, 35%

Scheme 10. *Reagents and conditions*: i) TPAP, NMO, DCM, r.t., 24 hrs.

Storage of the Mannich derivatives for a month at r.t. indicated some instability. They were re-purified by column chromatography. Although the Mannich derivatives were kept in the freezer, they would partly decompose after few months and had to be purified before antimalarial assessment. It was observed that the pyrrole derivatives containing either a piperazine, morpholine or thiomorpholine ring system were more stable than those having single nitrogen in the Mannich side-chain.

The table below shows the artemisinin derivatives prepared (**Figure 5**), their corresponding calculated log P and yields (**Table 1**). Solubility is expressed by log P (where P= partition coefficient between octanol and water). Ideally the log P must be lower than the neurotoxic artemether (3.3-3.5) or arteether (3.99) to resolve the problem of blood-brain barrier penetration. Log P values of no more than 3.25 are desirable.[16] Polar groups can be used to enhance aqueous solubility; these may be hydrogen-bond donor and/or acceptor groups. Therefore **41**, with calculated log P of 2.21, is the most water soluble compound; also the morpholine derivatives (**27** and **32**) appear to have lower log P.

Table 1: Semi-synthetic artemisinins prepared

Compound	Structure -R	Structure –R'	log P	Yield (%)
8	-H	-	3.56	77
9	-Me	-	3.80	84
26	-H		4.24	24
27	-H		3.16	70
28	-H		3.32	60
29	-H		3.97	70
30	-H		4.29	35
31	-H		3.88	24
39	-H		3.56	70
32	-Me		3.40	75
33	-Me		3.55	83
34	-Me		4.21	76
35	-Me		4.53	88
36	-Me		4.11	97
37	-Me		4.12	90
38	-Me		4.06	54
40	-Me		3.80	86
41	-Me		2.21	35

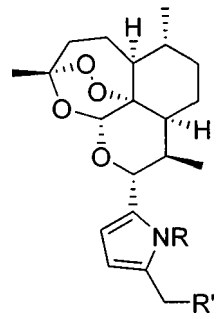
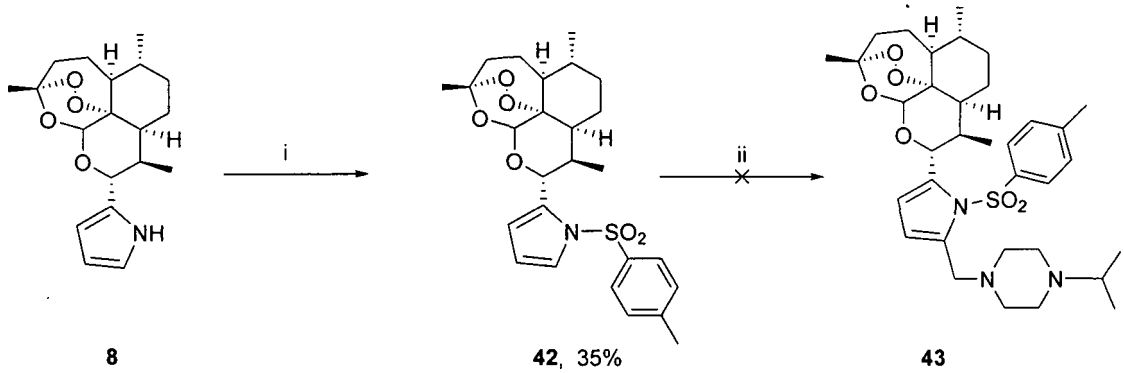


Figure 5. Artemisinin derivatives made.

2.2.4. Failed approach to *N*-sulfonyl pyrrole Mannich analogues

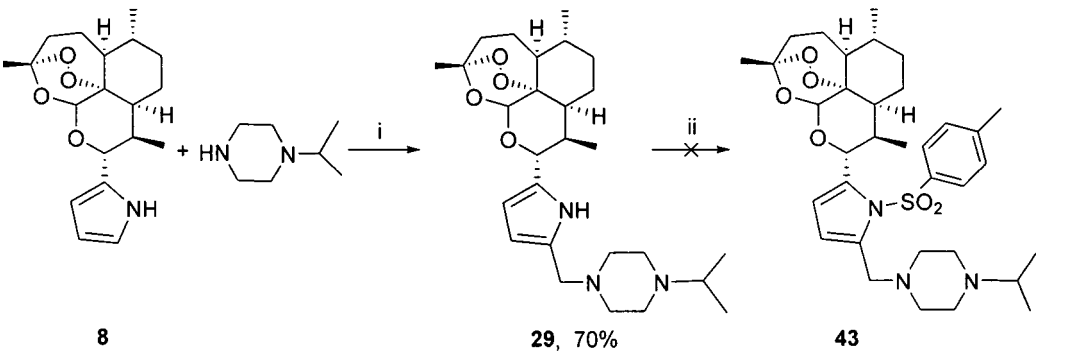
To increase the polarity of DHA derivatives, we attempted functionalisation of the nitrogen of the pyrrole ring with a polar group such as toluene sulfonyl. Deprotonation of **8** with NaH followed by addition of *para*-toluene sulfonyl chloride[38] gave **42**. However, the Mannich reaction on **42** failed possibly due to the fact that toluene sulfonyl, being an electron withdrawing substituent, takes the electronic density of the pyrrole ring, therefore preventing attack of the iminium salt (**Scheme 11**).



Scheme 11. *Reagents and conditions*: i) NaH, *para*-toluene sulfonylchloride, toluene, 0°C, 2 hrs. ii) *N*-isopropylpiperazine, formaldehyde, acetic acid, ethanol, r.t., 30 mins.

Alternatively, we prepared **29** by performing the Mannich reaction on **8** with isopropylpiperazine in 70% followed by treatment of **29** with *para*-

toluenesulfonylchloride in basic conditions. Again the second step failed, perhaps due to the fact that the nitrogen of pyrrole ring is too hindered to be functionalised (**Scheme 12**).



Scheme 12. *Reagents and conditions*: i) formaldehyde, acetic acid, ethanol, r.t., 30 mins, ii) NaH, *para*-toluene sulfonylchloride, toluene, 0°C, 2 hrs.

From the small library of artemisinin analogues made, a selection was screened for their antimalarial activity.

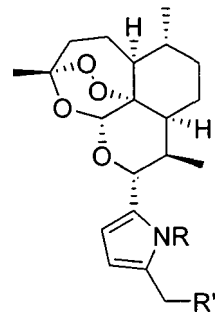
2.2.5. Antimalarial activity

2.2.5.1. *In vitro* testing

The antimalarial activity of the Mannich derivatives was firstly evaluated *in vitro* against chloroquine resistant K1 and then chloroquine sensitive 3D7 strains of *P. falciparum* by [3H]-hypoxanthine incorporation using some artemisinins and chloroquine as positive controls.

The activity *in vitro* of a drug is measured with its IC_{50}. The term IC_{50} represents the concentration of an inhibitor (one of the Mannich derivatives) that is required for 50% growth inhibition of the parasite *in vitro*. The analogues that were initially selected for *in vitro* evaluation were molecules, **27-29**, **32-34**, **38** and **41** where high yields were obtained. These compounds were also shown to be more stable in solution than analogues such as **26, 30 and 31** which showed a tendency to degrade in solution.

The *in vitro* tests' results against K1 (**Tables 2** and **3**) showed that there may be a correlation between measured IC_{50} and calculated log P as **41** is the most hydrophilic (log P= 2.21) and has the lowest relative IC_{50} (Rel. IC_{50}= 0.27). Several derivatives have remarkable activity against *P. falciparum*. They all displayed better activity than artemisinin; for example **32** displayed a superior activity than the clinically used semi-synthetic artemether.



R= H: **27-29**
R= Me: **32-34, 38, 41**

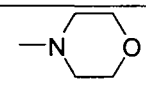
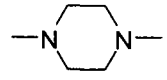
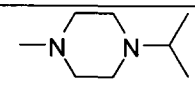
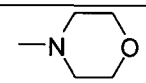
Compounds	Structure -R	Structure –R'	log P	IC_{50} (nM)	Rel. IC_{50}
Artemisinin (1)	-	-	3.17	0.98	1.00
Artemether (3)	-	-	3.51	0.53	0.54
27	-H		3.16	0.59	0.60
28	-H		3.32	0.91	0.93
29	-H		3.97	0.65	0.66
32	-Me		3.40	0.36	0.37

Table 2: *In vitro* results of artemisinin analogues, strain chloroquine-resistant K1

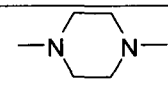
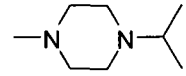
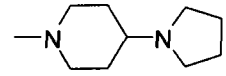
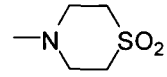
Compounds	Structure -R	Structure –R'	log P	IC_{50} (nM)	Rel. IC_{50}
Artemisinin (1)	-	-	3.17	2.40	1.00
Artemether (3)	-	-	3.51	1.27	0.53
33	-Me		3.55	0.77	0.32
34	-Me		4.21	1.44	0.60
38	-Me		4.06	1.58	0.66
41	-Me		2.21	0.65	0.27

Table 3: *In vitro* results of artemisinin analogues, strain chloroquine-resistant K1

The rest of the pyrrole analogues were tested against 3D7 strain (**Table 4**). Compounds **8**, **9**, **35-37** and **40** have better activity than artemisinin. Interestingly, this suggests that *N*-methylpyrrole analogues have a better activity than H-pyrrole analogues (**26**, **30**, **31** and **39**).

The low nanomolar activities observed with most of the analogues prepared suggests that incorporation of the polar side-chain is tolerated and does not reduce antimalarial potency.

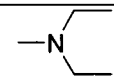
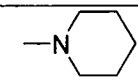
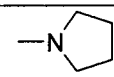
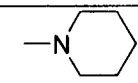
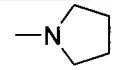
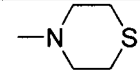
Compounds	Structure -R	Structure–R'	Log P	IC_{50} (nM)	Rel. IC_{50}
Artemisinin	-	-	3.17	12.8	1.00
Chloroquine	-	-	3.73	29.6	2.31
Artesunate	-	-	3.04	9.5	0.74
8	-H	-	3.56	7.4	0.58
26	-H		4.24	75.8	5.92
30	-H		4.29	59.4	4.64
31	-H		3.88	16.9	1.32
39	-H		3.56	20.5	1.60
9	-Me	-	3.80	5.2	0.41
35	-Me		4.53	6.1	0.47
36	-Me		4.11	3.7	0.29
37	-Me		4.12	7.2	0.56
40	-Me		3.80	11.6	0.91

Table 4: *In vitro* results of artemisinin analogues, strain chloroquine-sensitive 3D7

Cytotoxicity studies were also made measuring the *in vitro* activities against mammalian KB cells (**Table 5**). The observed IC_{50}s were in the micromolar which is good because the therapeutic index is really high. The therapeutic index is therefore high which proves the selectivity of 3D7 strain inhibition.

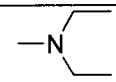
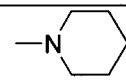
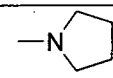
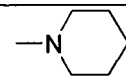
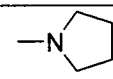
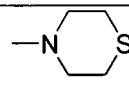
Compounds	Structure -R	Structure–R'	IC_{50} (nM) 3D7	IC_{50} (μM) KB	T.I. (KB/3D7)
Artemisinin	-	-	12.8	>354.19	>0.04
Chloroquine	-	-	29.6	112.52	0.26
Artesunate	-	-	9.5	39.29	0.24
8	-H	-	7.4	62.56	0.12
26	-H		75.8	48.16	1.57
30	-H		59.4	143.47	0.41
31	-H		16.9	47.01	0.36
39	-H		20.5	43.61	0.47
9	-Me	-	5.2	60.36	0.09
35	-Me		6.1	39.25	0.15
36	-Me		3.7	42.24	0.09
37	-Me		7.2	137.86	0.05
40	-Me		11.6	41.04	0.28

Table 5: *In vitro* results of artemisinin analogues, strains chloroquine-sensitive 3D7 and mammalian KB and therapeutic index (T.I.)

2.2.5.2. *In vivo* testing

A selection of the compounds were screened for their *in vivo* activity against *Plasmodium berghei*. First a single dose 4-days Peter's suppressive test was performed using 30mg/kg of the compound (**Table 6**). All the compounds tested displayed a good activity, with **32** and **33** completely eliminating the parasites.

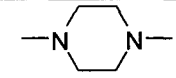
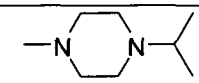
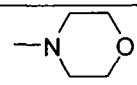
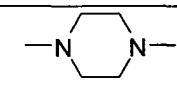
Compounds	-R	-R'	log P	% inhibition for 30 mg/kg
Artemether (**3**)	-	-	3.51	100
Artesunate (**5**)	-	-	3.04	100
28	-H		3.32	97.7
29	-H		3.97	90.5
32	-Me		3.40	100
33	-Me		3.55	100

Table 6: Clearance of parasites with 30 mg/kg dose

Encouraged by the single dose experiments, we carried out dose response on compound **32** to determine ED_{50} and ED_{90}, which are the point where a precise concentration of the drug given will respectively clear 50% and 90% of the parasites. In the case of **32**, 1.77 mg/kg cleared 50% of the parasite and 5.20 mg/kg was enough to kill 90% of the parasites (**Table 7**).

Mannich analogues	IC_{50}/nM	Clearance of parasites at 30 mg/kg	ED_{50} mg/kg	ED_{90} mg/kg
32	0.36	100%	1.77	5.20
Artemether	0.93	100%	5.88	10.57
Artesunate	ND	100%	3.23	>10

Table 7: Dose response results for **32**

Compound **32** is three times more potent than artemether and twice as active as artesunate in this *in vivo* experiment.

2.2.6. Iron degradation

Artemisinins act via mechanisms that are distinct from other antimalarial drugss. Antimalarial activity may arise from alkylation of vital intraparasitic biomolecules by free radicals generated within the malaria parasite through an iron (II)-induced degradation process.[39-41] The parasite's death in the presence artemisinin is more likely to involve specific radicals and targets rather than non specific cell damage caused by freely diffusing oxygen and carbon centred radical species. The following section details the investigation of specific "transitory" species that may be responsible for the antimalarial mechanism of action of artemisinin.

The peroxide within the 1,2,4-trioxane system of artemisinins is essential for antimalarial activity. Therefore carbaartemisinin analogue **44**, which has been constructed by an elegant total synthesis, displays an activity against *P. falciparum* that is ~4% of that of artemisinin.[42] Also artemisinins lacking a peroxidic oxygen atom such as the desoxy compounds **45** and **46**,[43] and the 1-carba analogue **47**, in which one oxygen of the peroxide bridge is replaced by carbon,[44] are also devoid of activity. However, 10-deoxo-10-dihydroartemisinin derivative **48** in which the peroxide is intact retains antimalarial activity, and in fact is more active than artemisinin against the malaria parasite *in vitro*[45] (**Figure 6**). Thus, the trioxane pharmacophore is essential for the expression of optimal antimalarial activity.

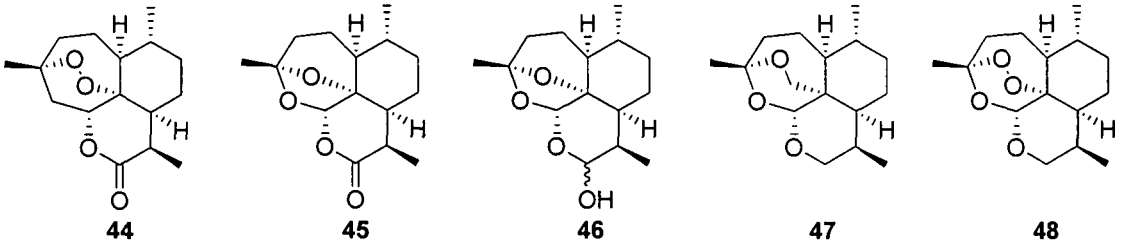
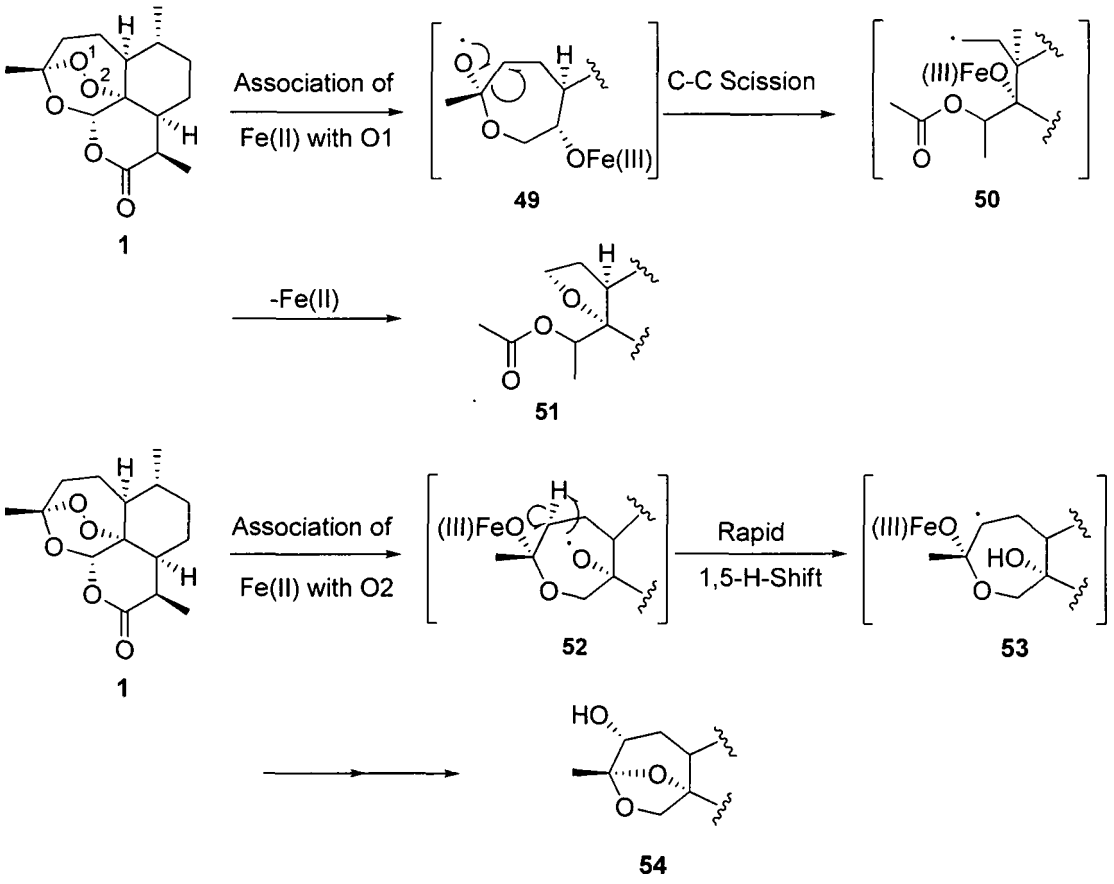


Figure 6. Analogues **44-47** with no antimalarial activity and compound **48** more active than artemisinin.

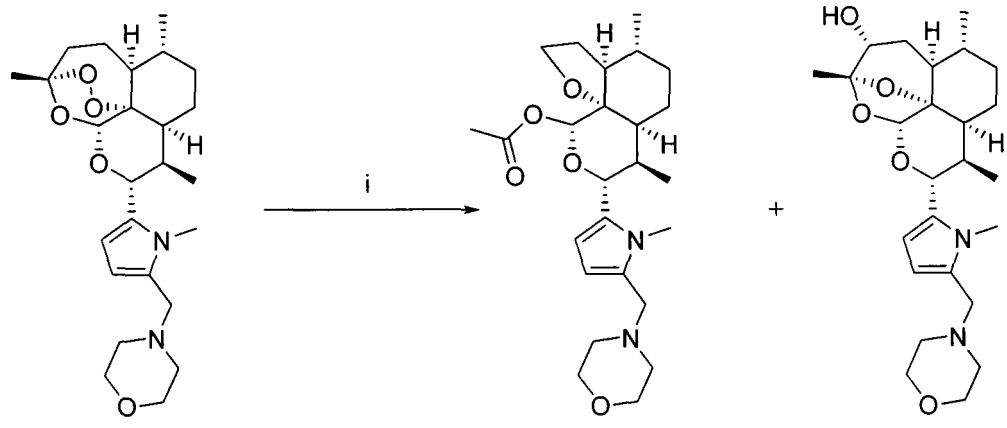
It is important to note that the antimalarial activity of artemisinin appears to be reduced by iron chelators, such as pyridoxal benzoylhydrazone and 1,2-dimethyl-3-hydroxypyrid-4-one.[46] This suggests that free or "chelatable" iron(II) is required for bioactivation, as opposed to ferrous haem, because the iron chelators would be unable to bond with iron(II) within the porphyrin ring system of heme.

Since artemisinin is an unsymmetrical endoperoxide, the oxygen atoms of the peroxide linkage can associate with reducing ferrous ions in two ways. Association of Fe (II) with oxygen 1 provides an oxy-radical (**49**) that goes on to produce a primary carbon-centered radical (**50**) to give furano acetate (**51**). Alternatively, association with oxygen 2 provides an oxy radical species (**52**) that, via a 1,5-H shift, can produce a secondary carbon-centered radical (**53**) to afford hydroxyl deoxo product (**54**) (**Scheme 13**).[47]



Scheme 13

The ferrous iron-mediated degradation of **32** was examined with iron (II) sulfate and iron (II) chloride. Degradation of **32** gave two main products (**Scheme 14**). Isolation of **55** and **56** point to the fact that carbon-centered radicals might be involved in the mechanism of these artemisinin derivatives.



32 **55** **56**

Scheme 14. *Reagents and conditions*: i) $FeSO_4$, acetonitrile/water: 1/1, 1 hr, r.t. or i) $FeCl_2.4H_2O$, acetonitrile, 30 mins, r.t.

Iron (II)	Yield for (**55**)	Yield for (**56**)
$FeSO_4$	72%	21%
$FeCl_2$	42%	23%

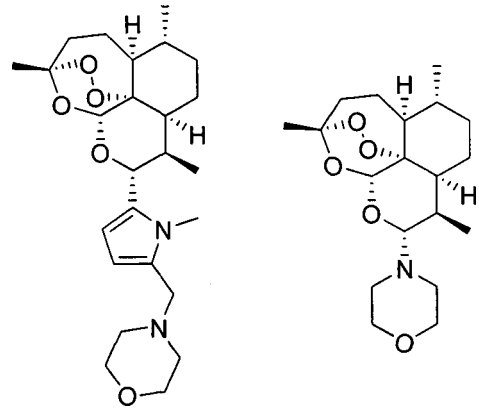
Table 8: Yields obtained for Iron (II)-Induced Degradation of Endoperoxide **32**.

The furano acetate was produced in higher yields (**Table 8**) than deoxohydroxyartemisinin, which would suggest Fe (II) associates mainly with O1. Thus, it is likely that these Mannich analogues behave in a manner similar to artemether and artemisinin, and we propose that their potent antimalarial activity may be mediated by the formation of carbon radical species in the parasite's food vacuole.

2.2.7. Summary on C-10 pyrrole Mannich analogues

A small library of weakly basic and polar C-10 pyrrole analogues has been prepared with modular chemistry amenable to parallel synthesis methods.

The antimalarial tests revealed that morpholine and *N*-methylpiperazine analogues have superior biological profiles to clinically used sodium artesunate. The measurements of ED_{90} and ED_{50} are encouraging for morpholine analogue (**32**), therefore further investigations, including pre-clinical toxicological evaluation need to be carried out to fully assess the potential of this compound. Lipophilic artemisinins are likely to be neurotoxic. With a log P of 3.05, the morpholine compound **57** has been found to be highly neurotoxic (**Figure 7**).[28] It would be interesting to evaluate the toxicity of **32** which is less lipophilic with an extra pyrrole ring.



32 **57**

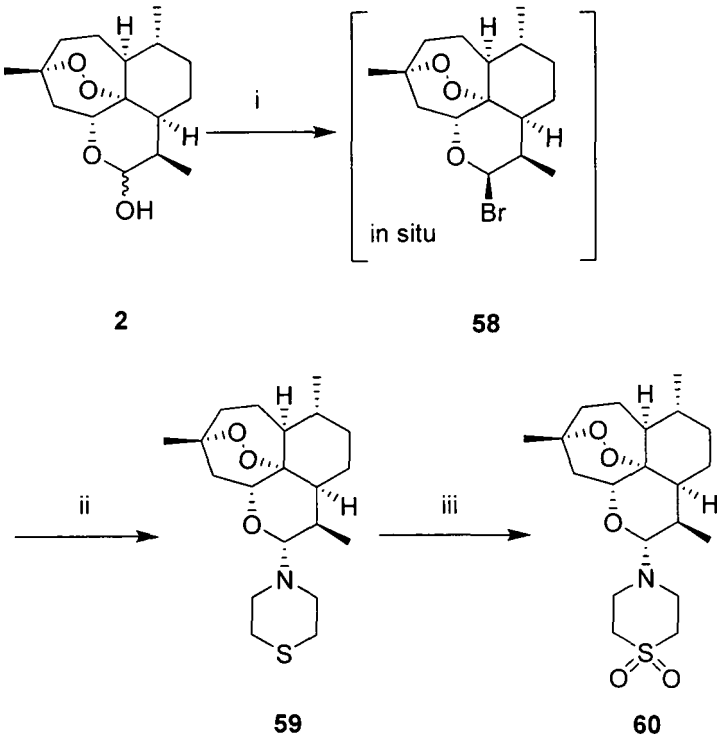
Figure 7

Initial studies employing ferrous (II) salts indicate that this class of semi-synthetic artemisinin generate both primary and secondary carbon centered radicals in a manner similar to artemisinin. Further work is required to establish the role of these intermediates in the mechanism of action.

2.3. Synthesis of sulfone analogues

2.3.1. Introduction

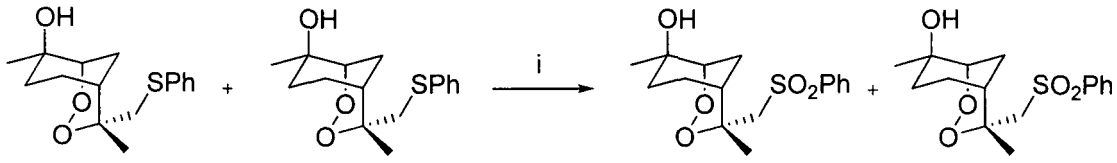
Artemisone (**60**) has been produced from DHA in multikilogram-scale reactions by Haynes and co-workers.[28] Treatment of DHA (**2**) first with a mixture of NaBr and TMSCl in toluene gave the intermediate (**58**) which was then treated *in situ* with thiomorpholine to provide the sulfide (**59**). **59** was finally oxidised to **60** using TPAP/NMO (**Scheme 15**).



Scheme 15. *Reagents and conditions*: i) TMSCl, NaBr, Toluene, 0°C, ii) thiomorpholine, Et_3N, CH_2Cl_2, iii) NMO, TPAP (cat.), CH_2Cl_2, 20°C.

Artemisone displays favorable physicochemical properties, such as log P 2.49, and negligible neuro- and cytotoxicities. In addition to enhanced bioavailability, artemisone has an ED_{90} of 1.5 mg/kg against *Plasmodium berghei* and 3.9 mg/kg against *Plasmodium yoelii* and is significantly more active than artesunate, chloroquine and pyrimethamine.

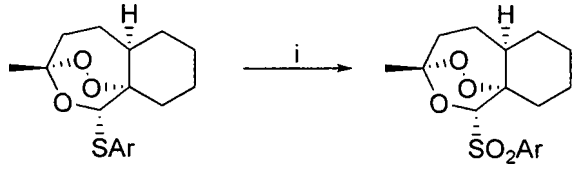
A number of groups have explored the introduction of a sulfonyl unit into synthetic endoperoxides. For example Bachi and co-workers[48] studied the effect of varying functional groups on bicyclic endoperoxides and it was found that compounds bearing a sulfide group, in this case thiophenol, showed very poor antimalarial activity. Upon oxidation to a sulfone functional group considerably enhanced activity was observed (**Scheme 16**).



61, IC_{50}> 2500 nM **62**, IC_{50}> 2500 nM **63**, IC_{50}= 55 nM **64**, IC_{50}= 89 nM

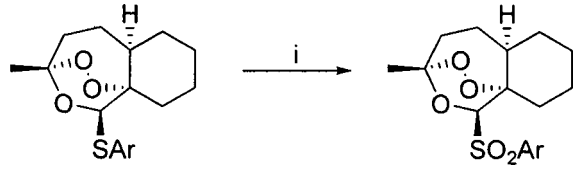
Scheme 16. *Reagent and conditions*: i) *m*CPBA, r.t.

Also, Posner and co-workers reported that various sulfone endoperoxides have higher antimalarial activity than the corresponding sulfides (**Scheme 17**).[49]



65α, Ar= Ph, > 2500 nM
66α, Ar= *p*-MeOPh, > 2500 nM
67α, Ar= *p*-ClPh, 2500 nM

68α, Ar= Ph, 56 nM
69α, Ar= *p*-MeOPh, 89 nM
70α, Ar= *p*-ClPh, 110 nM



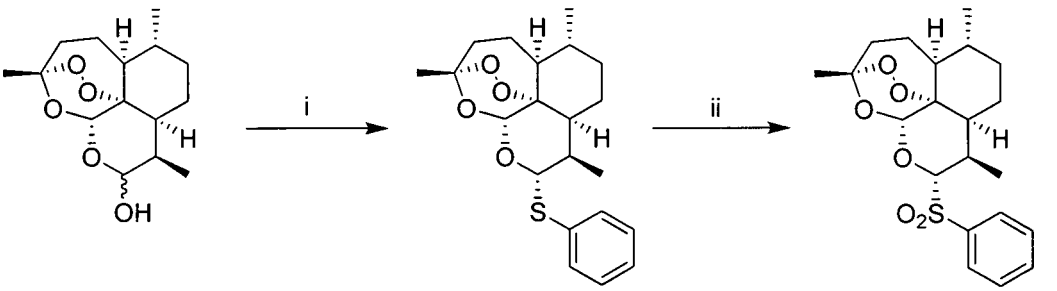
65β, Ar= Ph, 59 nM
66β, Ar= *p*-MeOPh, 43 nM
67β, Ar= *p*-ClPh, 25 nM

68β, Ar= Ph, 33 nM
69β, Ar= *p*-MeOPh, 30 nM
70β, Ar= *p*-ClPh, 23 nM

Scheme 17. Sulfur containing 1,2,4-trioxanes and their *in vitro* activity.

We were interested in preparing analogues with both sulfide and sulfone groups at the anomeric position. The first target molecule was the C-10-thiophenol derived sulphide **71**.

Oh and co-workers[50] have reported a two step synthesis of this compound from DHA. Thioacetal **71** was prepared by allowing **2** to react with thiophenol in the presence of $BF_3.Et_2O$. The thioacetal product was oxidised to produce **72** in good yields (**Scheme 18**).

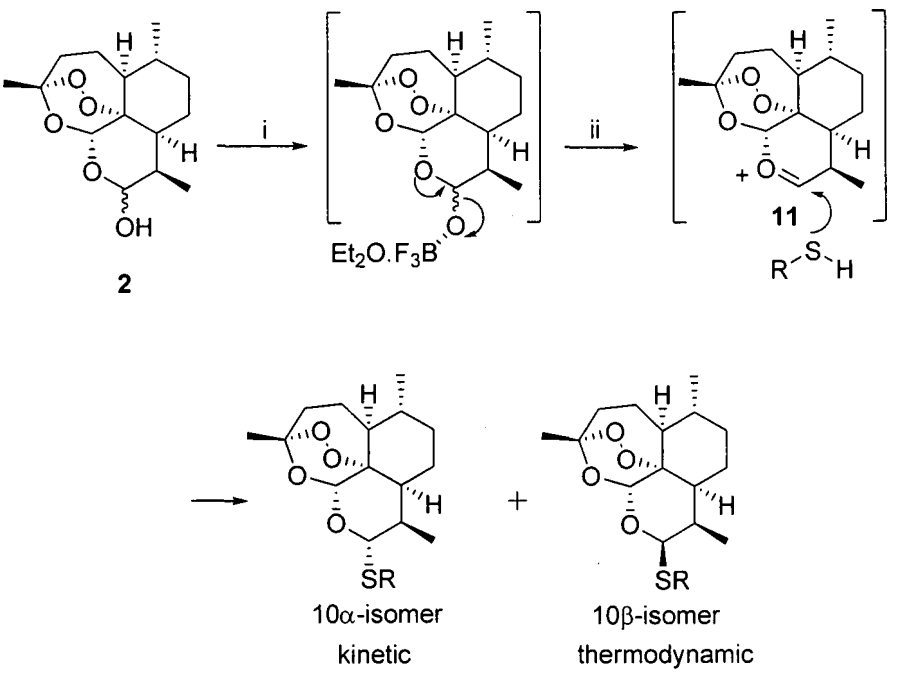


2 **71**, 85% **72**, 95%

Scheme 18. *Reagents and conditions*: i) Thiophenol, $BF_3.Et_2O$, DCM, r.t., 20 mins, ii) H_2O_2/UHP, TFAA, $NaHCO_3$, acetonitrile, r.t., 10 mins.

2.3.2. Mechanism of thioacetal formation

The synthesis of thioacetal derivatives involves the use of Lewis acid such as boron trifluoride. This Lewis acid catalyses oxonium generation from DHA and interception by a nucleophilic thiol produces predominantly the kinetic alpha product in equatorial configuration and the thermodynamic beta product in axial configuration (**Scheme 19**).



Scheme 19. *Reagents and conditions*: i) $BF_3.OEt_2$, DCM; ii) RSH, DCM, r.t., 10 mins.

As previously noted by several groups,[51, 52] the stereochemistry of the α and β isomers is determined by the chemical shift of H-10 and coupling constant between H-9 and H-10. The major product obtained in this reaction is the α-isomer as indicated by a chemical shift at 4.7 ppm and a large coupling constant (J = 11.0 Hz) indicating a *trans* diaxial relationship, while minor product is the β-isomer with H-10 at 5.6 ppm and J= 5.3 Hz. As noted, the β-isomer is the thermodynamic product, any tetrahydropyran bearing an electronegative substituent in the 2-position will prefer that substituent to be axial, this is known as the anomeric effect. The low-lying antibonding orbital C-S σ* is stabilised with the oxygen lone pair overlapping, which can only take place if the substituent is axial (**Figure 8**).

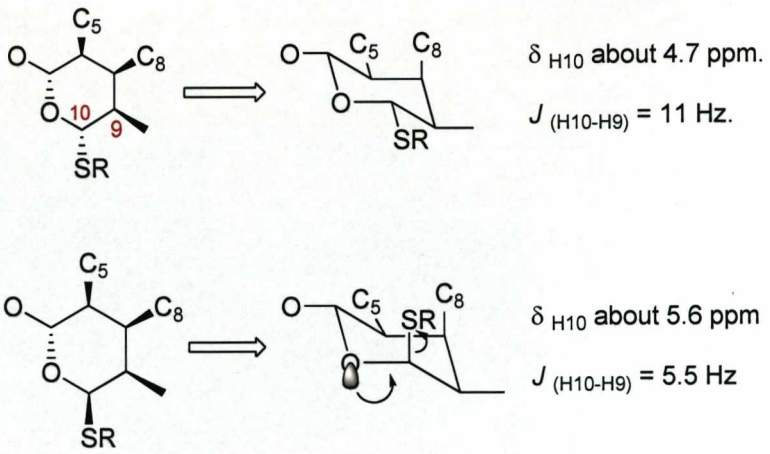
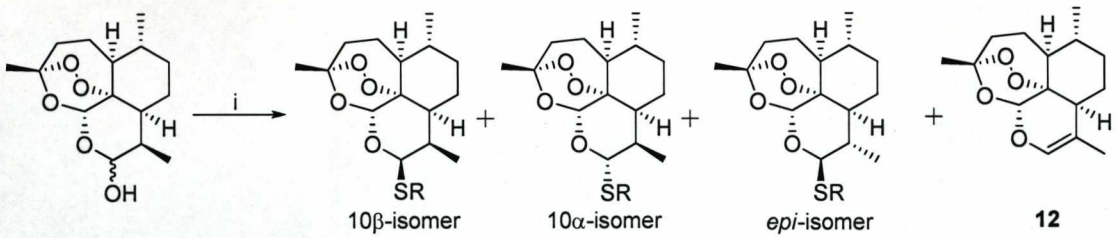


Figure 8. Chair conformation of α and β-isomers

The *S*-acetalisation on DHA was carried on with several heterocycles. We were expecting only α and β-isomers, however we observed other side products such as the *epi*-isomer and anhydroartemisinin (**12**) (**Scheme 20**).



71, R= thiophenol
73, R= 5-mercapto-1-methyl-1*H*-tetrazole
74, R= 3-mercapto-4-methyl-4*H*-1,2,4-triazole
75, R= 2-mercaptopyrimidine
76, R= 2-mercapto-5-methyl-1,3,4-thiadiazole
77, R= 2-mercaptoimidazole

Scheme 20. *Reagents and conditions*: i) $BF_3.Et_2O$, RSH, various solvents, 30-60 mins.

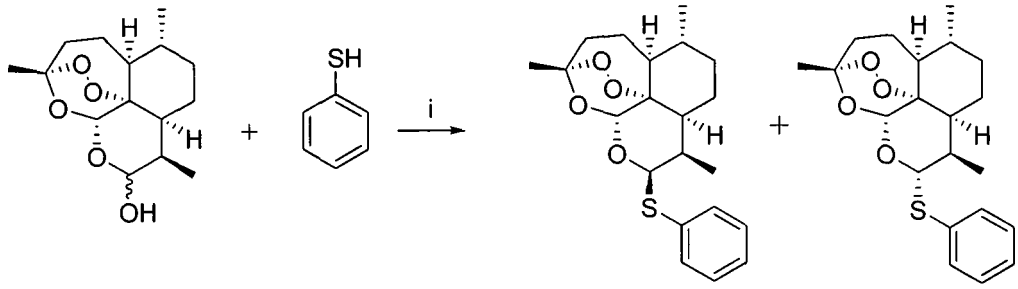
The following table (**Table 9**) presents the products obtained for reactions with the mercapto-heterocycles employed:

-R	10α-isomer	10β-isomer	*epi*-isomer	AHA	DHA	Solvents	Time mins
71	75%	20%	-	-	-	DCM	30
73	45%	8%	22%	19%	-	DCM	30
74	-	-	37%	56%	-	Acetonitrile +DCM	30
75	20%	6%	10%	55%	-	Acetonitrile	30
76	7%	20%	8%	10%	20%	Diethylether	60
77	-	-	86%	6%	-	Acetonitrile	60

Table 9: Yields obtained in each configuration with each heterocycle.

Most of the mercapto heterocycles are insoluble in DCM, therefore we tried the nucleophilic substitution in other aprotic solvents (**Table 9**):

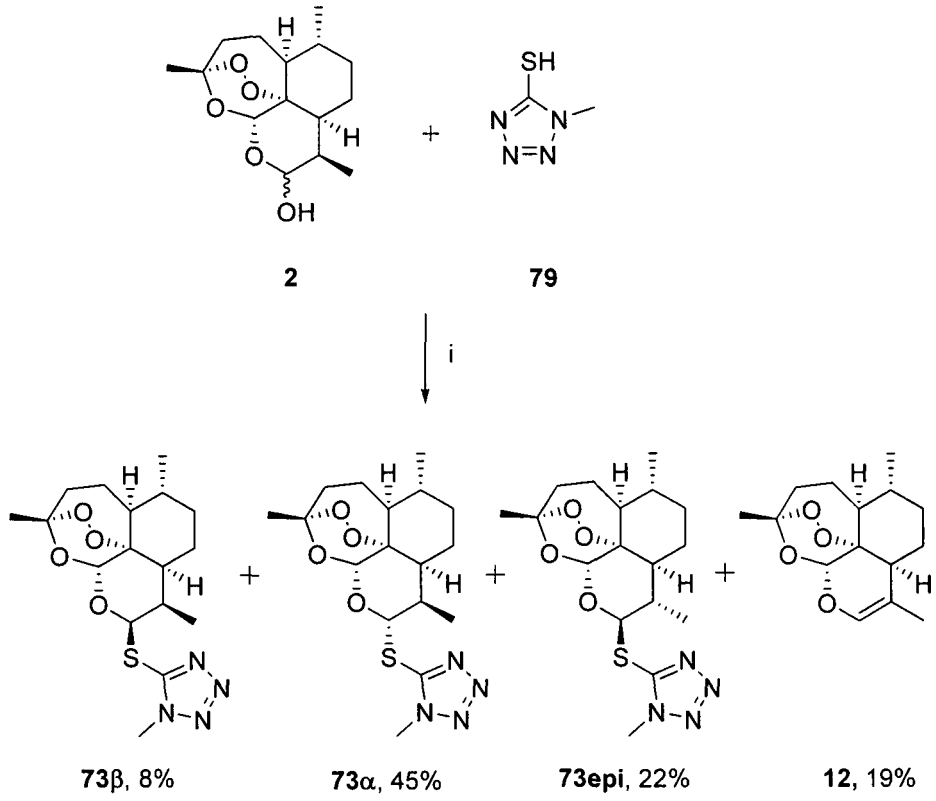
DHA was first reacted with thiophenol in DCM to give 25% of the thermodynamic product (**71β**) and 75% of the kinetic product (**71α**), which is expected as the reaction was quenched after 30 mins (**Scheme 21**).[50]



2 **78** **71β**, 20% **71α**, 75%

Scheme 21. *Reagents and conditions*: i) $BF_3.Et_2O$, PhSH, DCM, 30 mins.

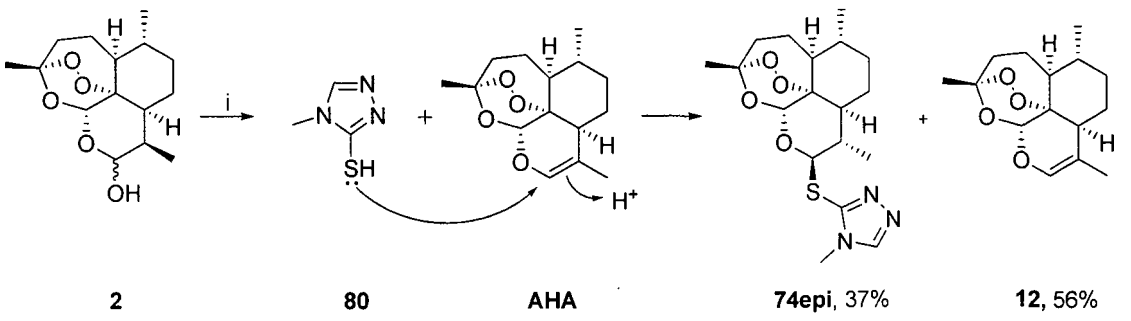
Due to the electrowithdrawing nitrogens on the tetrazole **79**, the nucleophilic substitution with DHA is slower. This is why some side products *epi*-isomer and AHA were obtained for the reaction between DHA and **79** (**Scheme 22**).



Scheme 22. *Reagents and conditions*: i) $BF_3.Et_2O$, DCM, 30 mins.

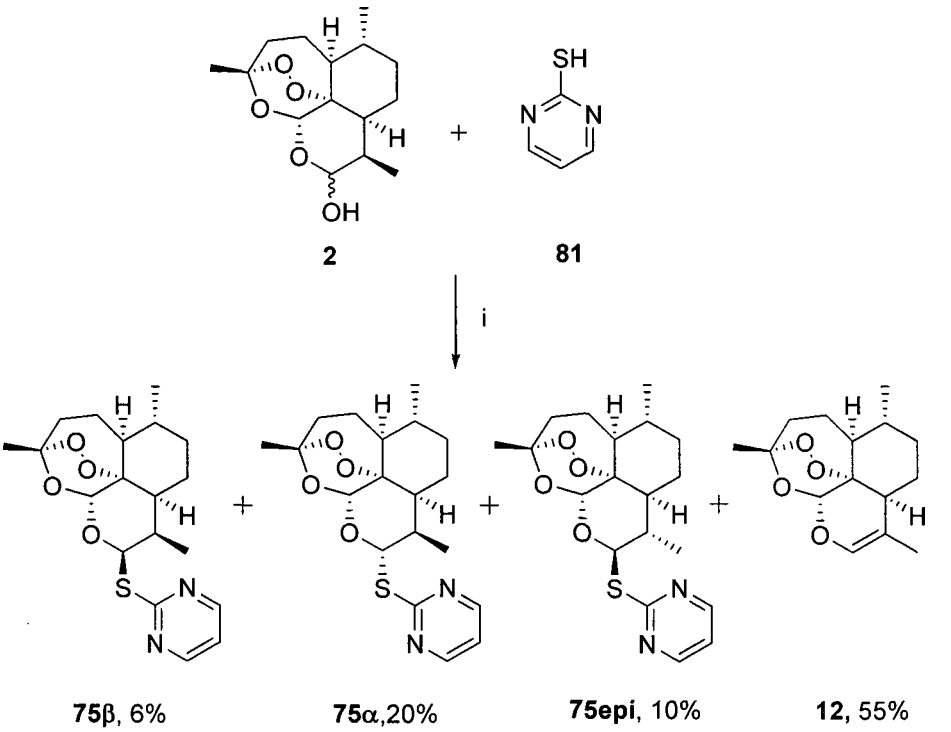
The mercapto heterocycles **74-77** are insoluble in DCM, therefore the reactions were carried out in other aprotic solvents in the presence of the Lewis acid $BF_3.Et_2O$ (**Table** 7).

3-mercapto-4-methyl-4H-1,2,4-triazole (**80**) was reacted with DHA in a mixture 1/1: DCM/acetonitrile to give 56% AHA as major product and 37% of *epi*-isomer (**Scheme 23**). This result could be explained by the poor solubility of **80**, therefore the competing dehydration reaction predominates without nucleophile to give the product anhydroartemisinin (**12**).[53] The *epi*-isomer is formed with nucleophilic attack of **80** on the axial position of AHA, followed by inverson of the methyl group on C-9 (**Scheme 23**).



Scheme 23. *Reagents and conditions*: i) $BF_3.Et_2O$, DCM/acetonitrile, 30 mins.

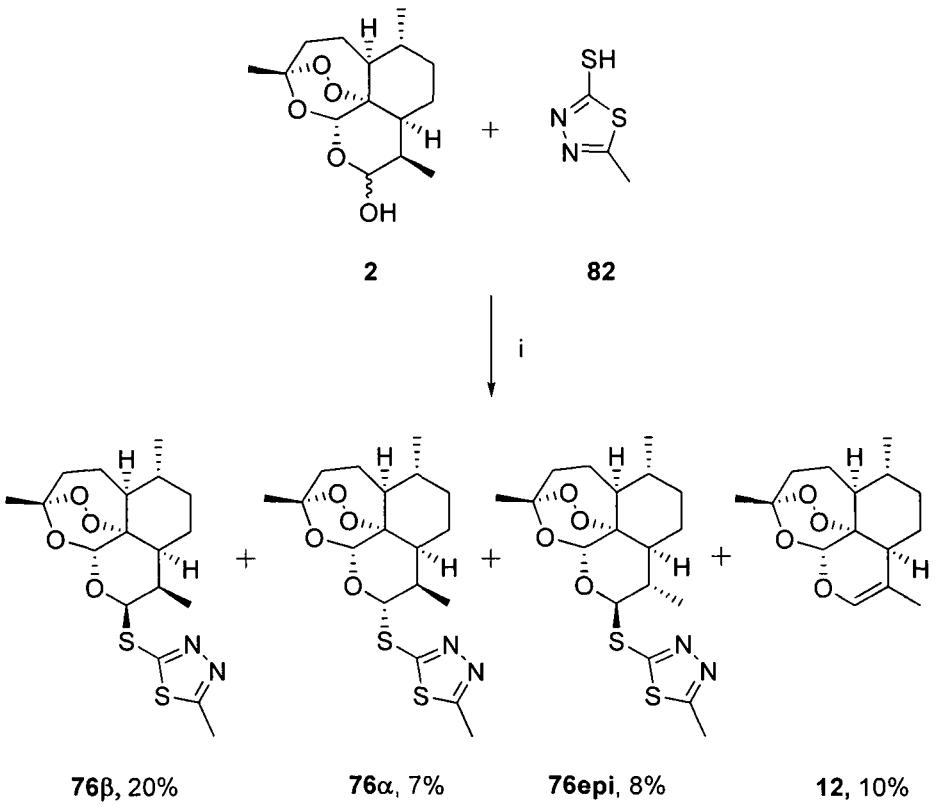
The similar reaction with mercaptopyrimidine (**81**) gave a mixture of the 4 products. The fact that AHA is the major product means that the mercapto heterocycle **81** is not completely solubilised in acetonitrile (**Scheme 24**).



Scheme 24. *Reagents and conditions*: i) $BF_3.Et_2O$, acetonitrile, 30 mins.

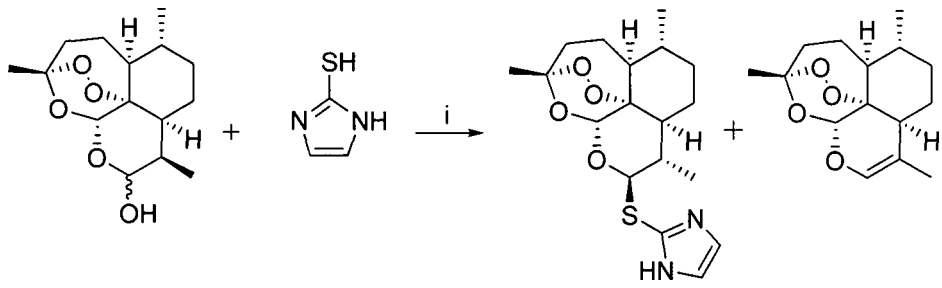
2-Mercapto-5-methyl-1,3,4-thiadiazole (**82**) reacted with DHA for an hour because the reaction in diethylether was slower. This explains why the β-isomer is the major

product (20%) (**Scheme 25**). Also 20% of the starting material DHA was recovered. Diethyl ether seems to slow the reaction, $BF_3.Et_2O$ is possibly chelating with the O of the reaction solvent. More polar solvent might stabilise the oxonium intermediate, which is observed for S_N1 reactions.



Scheme 25. *Reagents and conditions*: i) $BF_3.Et_2O$, diethylether, 60 mins.

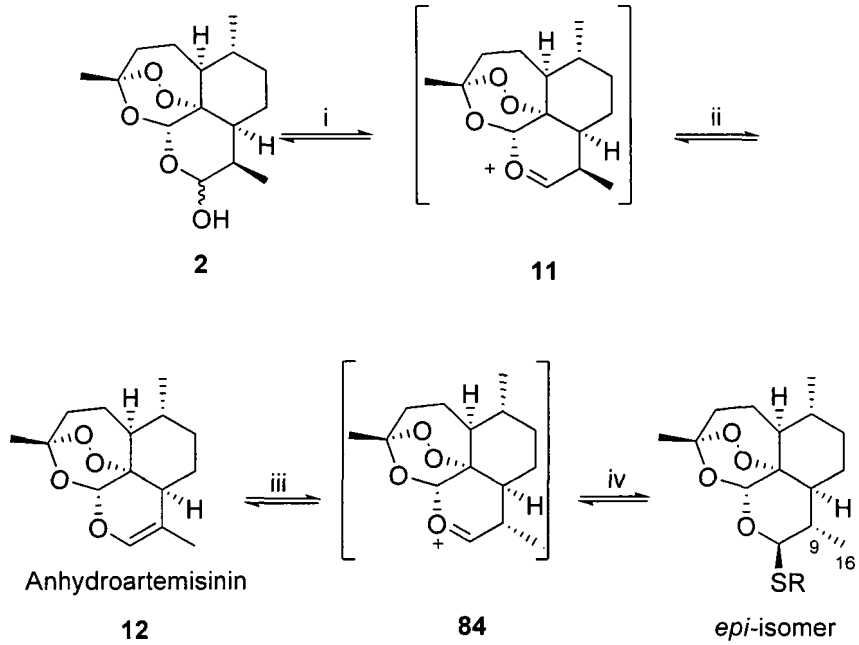
In the reaction between imidazole (**83**) and DHA, mostly the *epi*-isomer was observed as a product (**Scheme 26**), which means that AHA is formed at first followed by the nucleophilic attack (**Scheme 27**). This result suggests that **83** is less nucleophilic than **78** and **79**. Beside **81** and **83** have similar molecular structures, which imply that if **81** would have reacted with DHA for an extra 30 mins, the *epi*-isomer may have been obtained in higher yield.



2 **83** **77epi**, 86% **12**, 6%

Scheme 26. *Reagents and conditions*: i) $BF_3.Et_2O$, **83**, acetonitrile, 30 mins.

The epimerisation of the C-9 centre may result from a rapid equilibration between oxonium ions **11** and **84**, through the anhydroartemisinin (**12**) which can be protonated by the β-face, before the *S*-acetalisation (**Scheme 27**). The stereochemistry of 9-*epi*-isomer was determined with ^1H NMR: the signal of the methyl 16 at C-9 is strongly deshielded in (example with **73epi**: δ 1.03 ppm instead of 0.73 (α)-0.87(β) ppm), which is typical of the *epi*-artemisinin series (configuration α of Me-16).[54]



2 **11**

Anhydroartemisinin

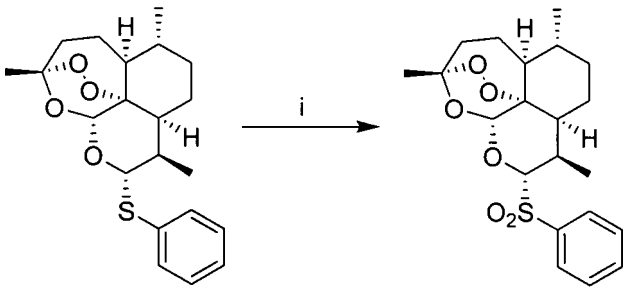
12 **84** *epi*-isomer

Scheme 27. *Mechanism of epimerisation:* i) Reaction with $BF_3.Et_2O$; ii) Deprotonation; iii) epimerisation; iv) *S*-acetalisation with RSH.

DHA is very sensitive to the strong Lewis acid, BF_3, and this leads to rapid E1 elimination of water from DHA. We thought using a catalytic amount of $BF_3.Et_2O$ would form the oxonium intermediate in smaller amounts to allow the mercapto heterocycles to intercept more efficiently, but this method didn't give any product at all. Therefore the method of choice was using one equivalent of $BF_3.Et_2O$, and the key to higher yields included solubilisation and a mercapto heterocycle with good nucleophilicity.

2.3.3. Oxidation of C-10 anomeric sulfide derivatives and discovery of a new rearrangement reaction

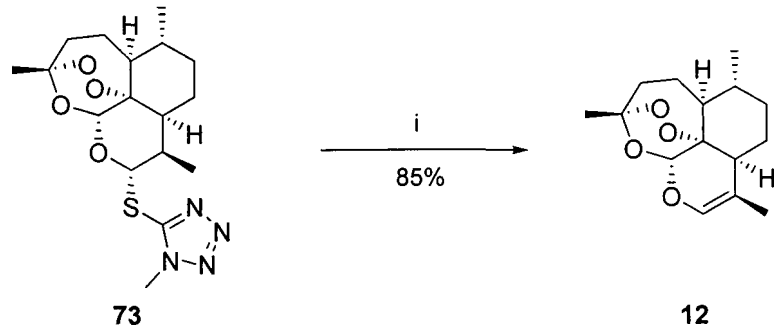
The thioacetal product **71** can be oxidised to **72** by oxidation with H_2O_2/urea complex (UHP), trifluoroacetic anhydride (TFAA) and $NaHCO_3$ in good yields (**Scheme 28**) [50].



71 **72**, 95%

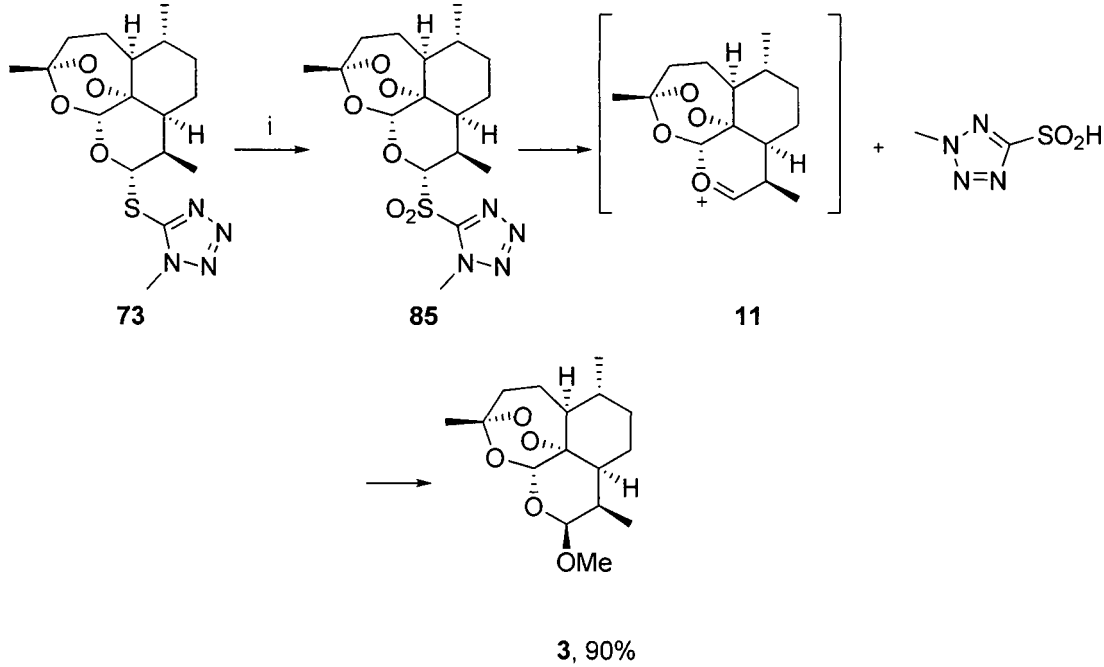
Scheme 28. *Reagents and conditions*: i) UHP (3 equiv.), TFAA (3 equiv.), $NaHCO_3$ (5 equiv.), CH_3CN, -30°C, 10 mins.

We tried the conversion of the α-isomer of **73** into its sulfone analogue through several ways. Oxidation with UHP and TFAA didn't give any product, whereas oxidation with *m*CPBA gave several products none of which was the desired product. Alternatively, we tried the oxidation with oxone using water as the solvent[35] but we exclusively formed anhydroartemisinin (**12**) (**Scheme 29**).



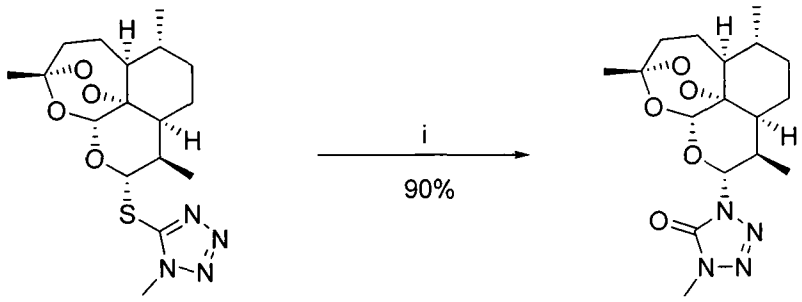
Scheme 29. *Reagents and conditions*: i) oxone, NaOH, water, r.t., 1 hr.

Repeat of the oxidation with oxone in methanol/ water gave β-artemether in 90% (**Scheme 30**). We assumed that the sulfone (**85**) may have been formed and then lost in an S_N1 reaction.



Scheme 30. *Reagents and conditions*: i) oxone, NaOH, water/MeOH: 1/1, r.t., 1 hr.

Further, we attempted the oxidation with TPAP (**Scheme 31**) but observed an unusual rearrangement product as shown in **Scheme 32**. The single-crystal X-ray of **86** (**Appendix 1**) obtained by slow evaporation of solvent (hexane /DCM) is shown in **Figure 9** below.



73 **86**

Scheme 31. *Reagents and conditions*: i) TPAP, NMO, molecular sieves, DCM, r.t., overnight.

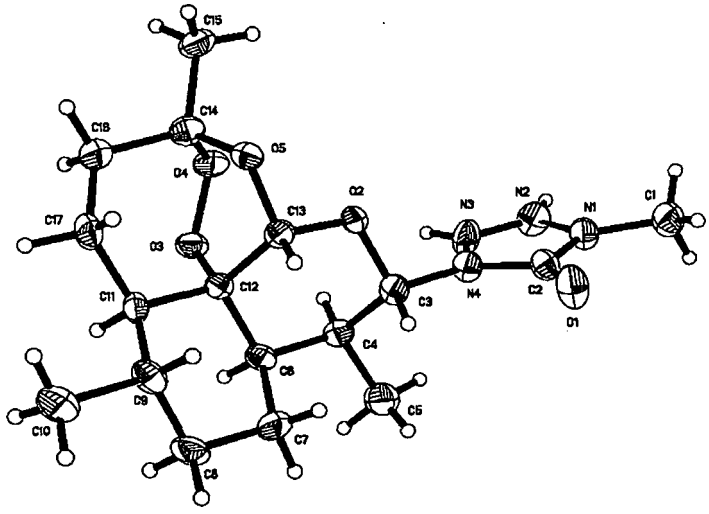
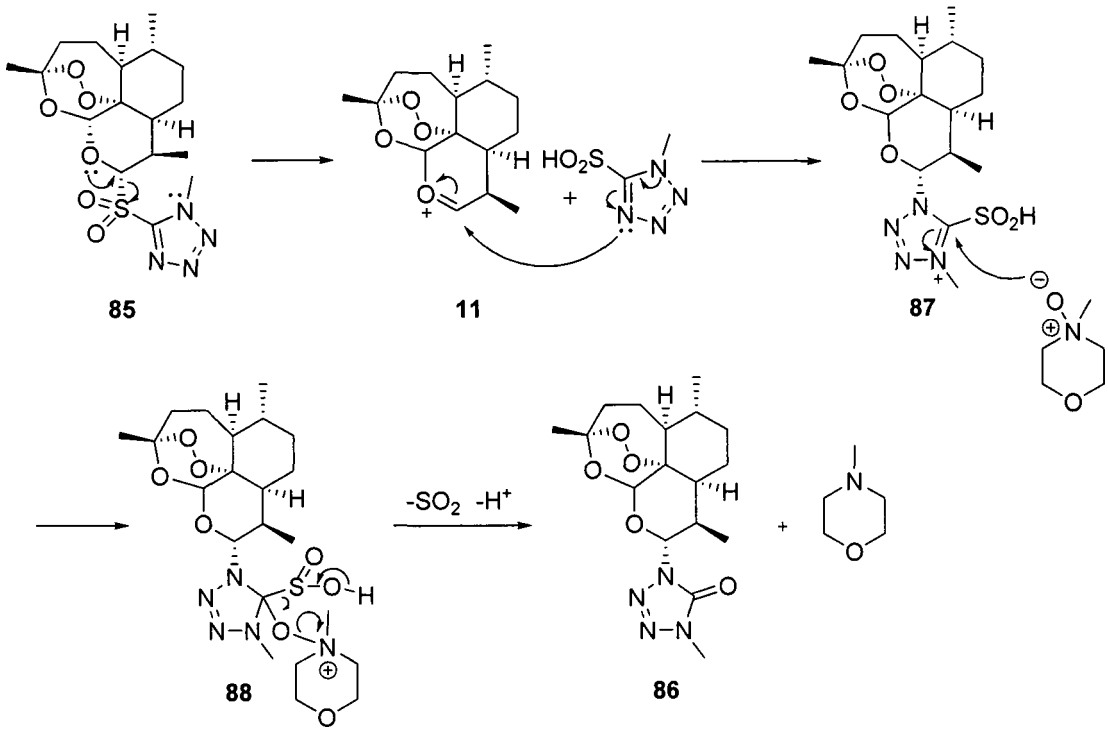


Figure 9. Crystallographic structure of **86.**

From the x-ray crystal structure, we explained the formation of **86** by the mechanism shown in **Scheme 32**. First, the oxonium ion **11** was formed, after elimination of the sulfone group. The nitrogen of the heterocycle then attacked the oxonium followed by hydration to give **86** (**Scheme 32**).

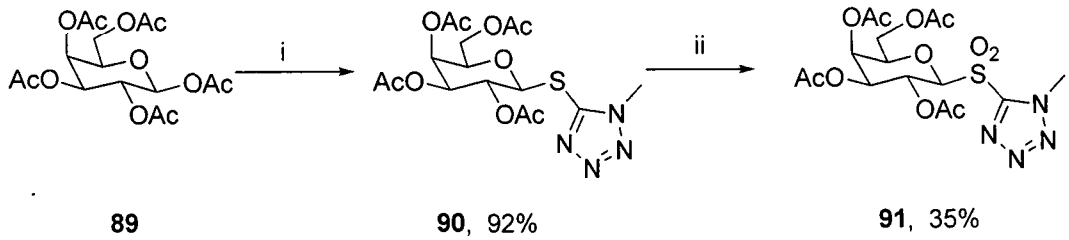


Scheme 32. Proposed mechanism of rearrangement.

Since this reaction is unprecedented in the literature we decided to investigate the generality of this type of rearrangement and set out to perform the same reaction on sugar derivatives as described below.

2.3.4. Sugar chemistry

D-Galactose β-pentaacetate (**89**) was first converted into its sulfide (**90**) by treatment with 5-mercapto-1-methyl-1*H*-tetrazole. However the oxidation of sulfide (**90**) did not give the expected rearrangement product, but the sulfone **91** (**Scheme 33**). Due to the neighbouring group participation of the acetate next to the anomeric carbon, the sulphide has β-configuration. Neighbouring group participation can occur because the acetate substituent on the 2 position is in equatorial, therefore the acetate oxygen lone pair can reach the σ* orbital of the sulphide C-O bond.

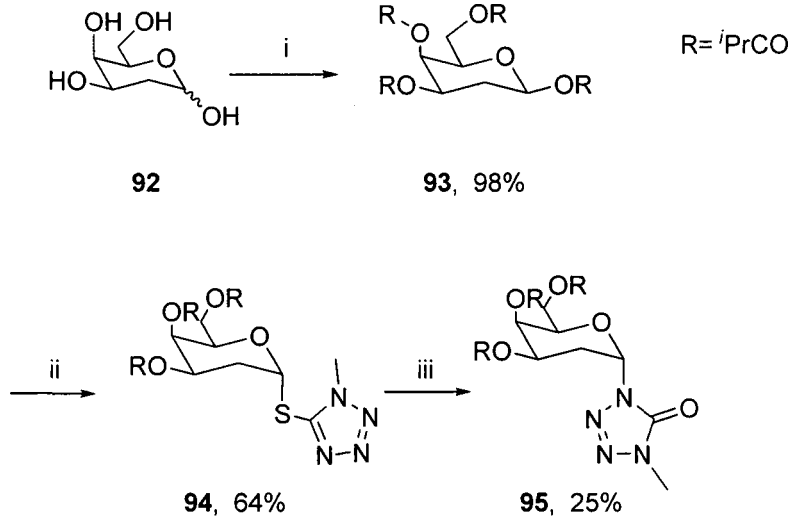


89 **90**, 92% **91**, 35%

Scheme 33. *Reagents and conditions*: i) $BF_3.Et_2O$, Mercaptotetrazol, DCM, r.t., 20 mins, ii) TPAP, NMO, molecular sieves, DCM, r.t., overnight.

We anticipated the β-configuration of **90/91** was not suitable for the tetrazole moiety to behave as a leaving group, hence we attempted the same reactions with 2-deoxy-D-galactose (**92**).

The sugar (**92**) was reacted with isobutyryl chloride in pyridine to give protected 2-deoxy-D-galactose (**93**), exclusively as the β-isomer in quantitative yield.[55] Lewis acid catalysed reaction of **93** with mercaptotetrazole gave **94** in axial configuration in 64% yield. Finally TPAP oxidation of **94** gave the rearranged compound **95** in 25% yields, 48% of the starting material was recovered (**Scheme 34**).



92 **93**, 98%

94, 64% **95**, 25%

Scheme 34. *Reagents and conditions*: i) ^{i}PrCOCl, pyridine, -12°C, 30 mins, ii) $BF_3.Et_2O$, (32), DCM, r.t., 20 mins, iii) TPAP, NMO, molecular sieves, DCM, r.t., overnight.

We assumed the sulfone group in the equatorial position (eg **91**) doesn't leave because of its stability. However, **94** has its anomeric substituent in axial, the C-S bond is longer and weaker and breaks more easily. Therefore when it gets oxidised into sulfone, the sulfone being a good leaving group led to the rearrangement.

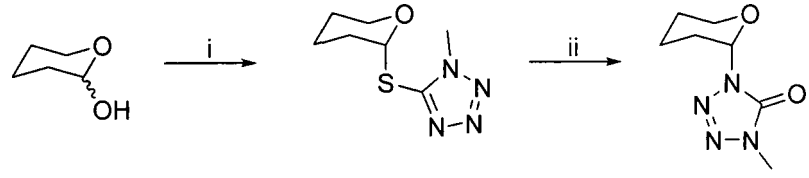
2.3.5. Conclusion and future work

A small number of sulfide derivative of artemisinin were prepared. The reactions led to a mixture of isomers; this is a serious drawback, because it requires laborious column chromatography to obtain the individual pure isomers.

Oxidation of sulfides into sulfones results in elimination and formation of anhydroartemisinin with heteroaryl analogues; it appears that the C-10 anomeric sulfones cannot be considered as drug development candidates due to their instability.

An interesting rearrangement was observed upon oxidation of **73**. We repeated the experiment with a sugar and observed the same rearrangement with the product **95**.

To investigate the generality of this behaviour, it would be interesting to react tetrahydro-2*H*-pyran-2-ol (**96**) with 5-mercapto-1-methyl-1H-tetrazole (**79**) and oxidise the corresponding sulfide (**97**) into sulfone (**98**) (**Scheme 35**).



96 **97** **98**

Scheme 35. *Reagents and conditions*: i) $BF_3.Et_2O$, heterocycle **79**, DCM, 30 mins.; ii) TPAP, NMO, molecular sieves, DCM, r.t., overnight.

2.4. Experimental

Air- and moisture-sensitive reactions were carried out in oven-dried glassware sealed with rubber septa under a positive pressure of dry nitrogen or argon from a manifold or balloon. Similarly sensitive liquids and solutions were transferred via syringe. Reactions we stirred using Teflon-coated magnetic stir bars. Organic solutions were concentrated using a Buchi rotary evaporator with a diaphragm vacuum pump.

Purification of reagents and solvents

Anhydrous were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. DCM was distilled from CaH_2. All other reagents were used as received from commercial sources unless otherwise indicated.

Purification of products

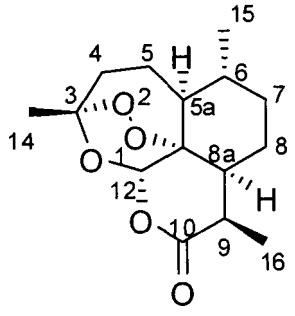
Analytical thin layer chromatography was performed with 0.25 mm silica gel 60F plates with 254nm fluorescent indicator coated aluminium sheets from merck. Plates were visualised by ultraviolet light or by treatment with iodine, p-anisaldehyde, ninhydrin or potassium permanganate followed by gentle heating. Chromatographic purification of products was accomplished by flash chromatography, as described by Still and co-workers.[56]

Analysis

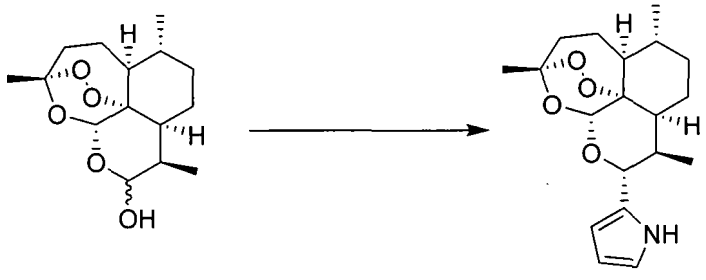
Melting points were determined in open tubes in a Gallenkamp, Melting Point Apparatus, and are uncorrected. NMR spectra were recorded on a brucker AC 200 (1H, 200 MHz) and a Brucker AMX 400 (1H, 400 MHz; 13C, 100 MHz) spectrometer. Chemicals shifts are described in parts per million (ppm) downfield from an internal standard of trimethylsilane. Multiplicities are recorded as broad peaks (br), singlet (s), doublets (d), triplets (t), quartets (q), doublet of doublets (dd), doublet of triplets (dt) and multiplets (m). Coupling values are in Hz. Mass spectra were recorded on a VG analytical 7070E machine and Frisons TRIO spectrometers using electron ionisation

(EI), chemical ionisation (CI) or electron spray (ES). Infrared spectra were recorded on a PerkinElmer RX1 FT-IR spectrometer and are reported in wavenumbers (cm^{-1}). Microanalyses (%C, %H, %N) were performed in the University of Liverpool Microanalysis laboratory. Reported atomic percentages are within error limits ± 0.5%. In instances where purity was not determined by elemental analysis, compounds displayed only one observable spot by t.l.c. at the reported R_f.

Artemisinin numbering scheme used throughout this analysis:[50]



10α-(1*H*-Pyrrol-2-yl)artemisinin (8).

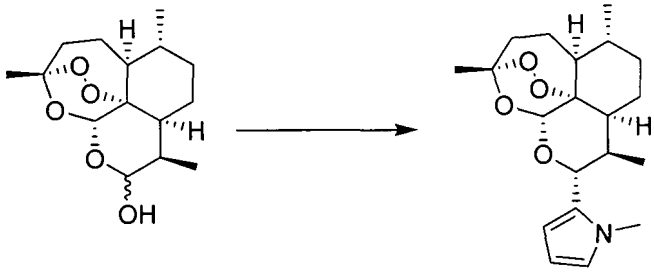


2 **8**

A solution of DHA (**2**) (250 mg, 0.88 mmol) in DCM (15 mL) -50 °C was treated sequentially with pyrrole (295 mg, 4.40 mmol) and $BF_3.Et_2O$ (187 mg, 1.32 mmol) and stirred at -50 °C for 1 hour. The mixture was quenched with sat $NaHCO_3$, extracted with DCM and washed with brine, then dried with $MgSO_4$ and concentrated in vacuum. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give a clear oil (125 mg, 77%): R_f= 0.36 (25% EtOAc/ *n*-Hex); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.64 (1H, s, NH), 6.76 (1H, dd, J = 2.5, 1.6 Hz, N-CH), 6.07 (1H, dd, J = 2.5, 5.5 Hz, pyr CH), 6.03 (1H, m, pyr CH), 5.40 (1H, s, H12), 4.49 (1H, d, J = 10.8 Hz, H10), 2.57 (1H, m, H9), 2.39 (1H, dt, J = 13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, J = 6.3 Hz, H15) and 0.63 (3H, d, J = 7.1 Hz, H16) ppm; ^{13}C NMR (100

MHz, $CDCl_3$) δ_C 130.2, 117.7, 107.39, 106.8, 104.3, 92.1, 80.7, 72.1, 51.9, 45.9, 37.4, 36.4, 34.2, 33.1, 31.6, 26.1, 24.8, 22.6, 21.3, 20.3, 14.1 and 14.0 ppm; MS (CI), $[M-2CH_2-OH]^+$ (100) 288; HRMS calcd for $C_{19}H_{28}NO_4$ $[M+H]^+$ 334.2018, found 334.2012; IR υ_{max}= 3264 ($\upsilon_{N-H, Pyrrole}$), 2922 (υ_{C-H}), 880, 828 (υ_{O-O}) cm^{-1}; Anal. $C_{20}H_{29}NO_4$ requires C 68.44%, H 8.16%, N 4.20% found C 68.02%, H 8.24%, N 4.14%.

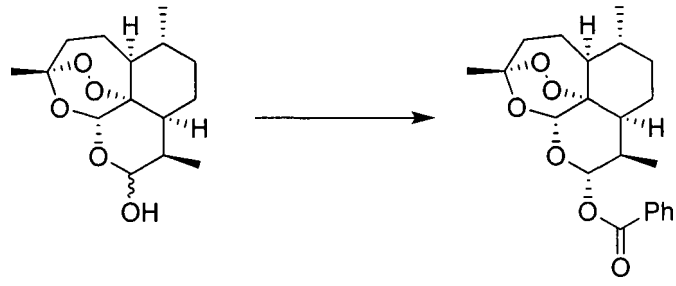
10α-(1-Methyl-pyrrol-2-yl)artemisinin (9).



2 **9**

A solution of dihydroartemisinin (300 mg, 1.05 mmol) in DCM (25 mL) at room temperature was treated sequentially with *N*-methylpyrrole (0.47 mL, 5.29 mmol) and $BF_3.Et_2O$ (0.19 mL, 1.51 mmol) and stirred for 10 min at r.t. and then cooled at -50 °C for 20 min. The mixture was quenched with sat $NaHCO_3$, extracted with DCM and washed with brine, then dried with $MgSO_4$ and concentrated in vacuum. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give a colourless crystal (378 mg, 84%): Rf=0.42 in 25% EtOAc/ *n*-Hex; ^1H NMR (400 MHz, $CDCl_3$) δ_H 6.54 (1H, t, J = 2.2 Hz, N-CH), 5.90 (2H, m, pyr CH), 5.38 (1H, s, H12), 4.50 (1H, d, J = 11.3 Hz, H10), 3.84 (3H, s, N-CH_3), 2.83 (1H, m, H9), 2.39 (1H, dt, J= 14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, J= 6.3 Hz, H15) and 0.61 (3H, d, J= 7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.2, 124.2, 109.9, 106.6, 104.6, 92.3, 81.1, 72.9, 52.4, 46.3, 37.8, 36.7, 35.4, 34.6, 31.3, 26.4, 25.2, 21.3, 20.7and 14.8 ppm; IR υ_{max}= 2926, 1732, 1498, 1457, 1376, 1272, 1114, 1100, 880 (O-O) and 828 (O-O) cm-1; MS (CI), $[M-2CH_2-OH]^+$ (100) 302; HRMS calcd for $C_{20}H_{30}NO_4$ $[M+H]^+$ 348.2175, found 348.2174; Anal. $C_{20}H_{29}NO_4$ requires C 69.14%, H 8.41%, N 4.03% found C 69.27%, H 8.45%, N 3.99%.

10α-(Benzoate)artemisinin (13).



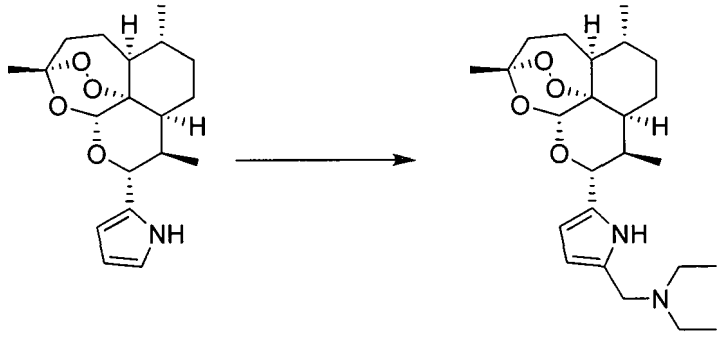
2 **13**

DHA (500 mg, 1.76 mmol) was dissolved in DCM (20 mL) and stirred for 30 minutes. Anhydrous pyridine (0.9 mL, 11.13 mmol) was added and the reaction vessel cooled to 0°C under nitrogen for 15 minutes. Benzoyl chloride (0.3 mL, 2.58 mmol) was added and the reaction mixture was allowed to warm to room temperature and left stirring for 20 hours. The reaction mixture was then dissolved in ethyl acetate (100 mL) and washed with citric acid (7%, 100 mL), sat $NaHCO_3$ and H_2O. The organic extracts were dried over $MgSO_4$ and concentrated in vacuum. The crude product was then purified by flash chromatography (10% EtOAc/ *n*-Hex) to give a white solid (**13**) (0.63g, 94%): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.12 (2H, dd, J = 7.0, 1.2 Hz, Ph), 7.54 (1H, m, Ph), 7.41 (2H, m, Ph), 6.03 (1H, d, J = 9.8 Hz, H10), 5.54 (1H, s, H12), 2.77 (1H, m, H9), 2.40 (1H, td, J = 14.4, 4.0 Hz, H4α), 2.05 (1H, m), 1.88 (1H, m), 1.77-1.57 (3H, m), 1.51-1.35 (5H, m), 1.43 (3H, s, H14), 0.99 (3H, d, J = 6.1 Hz, H15) and 0.94 (3H, d, J=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 133.3, 130.6, 130.1, 129.6, 128.29, 104.4, 92.5, 91.6, 81.9, 80.2, 51.6, 45.3, 37.3, 36.2, 34.12, 31.0, 26.0, 24.6, 22.1, 20.2, 12.2 ppm; IR υ_{max}= 2924 (C-H), 1737 (C=O), 877, 831 (O-O) cm^{-1}; HRMS (CI) $C_{23}H_{32}NO_6$ $[M+NH_4]^+$ requires 406.2230, found 406.2232; Anal. $C_{22}H_{28}O_6$ requires C 68.04%, H 7.22%, found C 67.79%, H 7.30%.

General procedure of the Mannich reaction with (8) and (9). Formaldehyde (0.1 mL, 3.2 equiv.) and a secondary amine (3.2 equiv.) solution were added to **(8)** or **(9)** (150 mg, 1 equivalent) in anhydrous ethanol (5 mL). Then glacial acetic acid (1.0 mL) was added to the reaction mixture, which was left at r.t. for 30 min. The reaction was basified (pH 8) with 2M sodium hydroxide solution (5 mL). The mixture was extracted with

EtOAc (3×25 mL) and combined organic extracts washed with brine. The organic phase was dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude product that was purified by flash chromatography using 5% methanol/ dichloromethane.

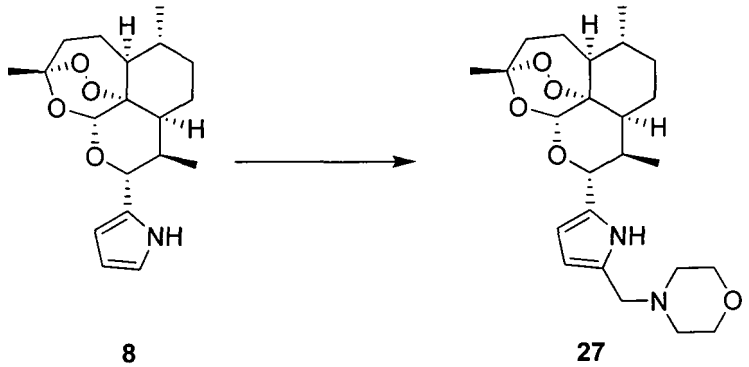
10α-(5-((Diethylamino)methyl)-1*H*-pyrrol-2-yl)artemisinin (26).



8 **26**

See general procedure for Mannich reaction above. Colourless sticky solid (24%); R_f=0.04 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.50 (1H, s, NH), 5.99 (1H, t, *J*= 2.8 Hz, pyr CH), 5.93 (1H, t, *J*=2.8 Hz, pyr CH), 5.37 (1H, s, H12), 4.42 (1H, d, *J*=11.0 Hz, H10), 3.69 (2H, AB quartet, *J*= 13.5 Hz, CH_2-N), 2.62 (4H, m, N-CH_2), 2.57 (1H, m, H9), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 1.09 (6H, t, *J*= 7.2 Hz, dieth CH_3), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 123.3, 106.8, 104.2, 92.1, 80.6, 72.0, 52.0, 46.1, 45.9, 37.4, 37.4, 36.3, 34.2, 33.0, 29.7, 29.7, 26.0, 24.8, 22.7, 21.4, 20.3, 14.1, 10.7 ppm; MS (ES+), $[M+H]^+$ (100) 419; HRMS calcd for $C_{24}H_{39}N_2O_4$ $[M+H]^+$ 419.2910, found 419.2927.

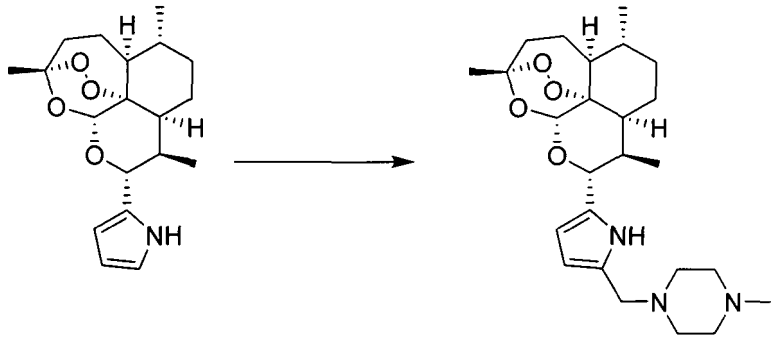
10α- (5-(Morpholinomethyl)-1*H*-pyrrol-2-yl)artemisinin (27).



8 27

See general procedure for Mannich reaction above. Orange solid (60%): mp = 33 °C; R_f=0.75 in 10% MeOH/ DCM;^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.85 (1H, s, NH), 5.93 (1H, t, *J*=3.2 Hz, pyr CH), 5.89 (1H, t, *J*=3.2 Hz, pyr CH), 5.38 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.70 (4H, t, *J*=4.6 Hz, morph CH_2-O), 3.47 (2H, s, morph CH_2-N), 2.57 (1H, m, H9), 2.43 (4H, m, N-CH_2), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.3, 127.5, 107.6, 107.0, 104.2, 92.3, 80.7, 72.1, 67.0, 55.8, 53.3, 52.0, 45.9, 37.4, 36.3, 34.2, 33.0, 26.0, 24.8, 21.4, 20.3 and 14.1 ppm; IR, υ_{max}= 3372, 1650, 1456, 1376, 1303, 1152, 1120, 1057, 926, 880, 865, 849, 828, 770, 722 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 455; HRMS calcd for $C_{24}H_{36}N_2O_5Na$ $[M+Na]^+$ 455.2522, found 455.2536; Anal. Calcd for $C_{24}H_{36}N_2O_5$: C, 66.64%; H, 8.39%; N, 6.48%; Found C, 66.44%; H, 8.44%; N, 6.45%.

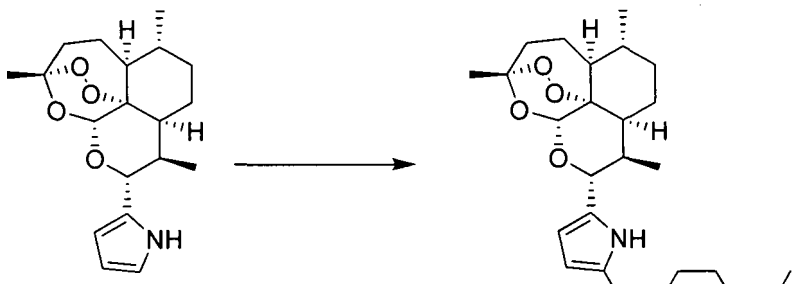
10α- (5-((4-Methylpiperazin-1-yl)methyl)-1*H*-pyrrol-2-yl)artemisinin (28).



8 28

See general procedure for Mannich reaction above. Yellow crystal (60%): mp = 36 °C; R_f=0.32 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.07 (1H, s, NH), 5.94 (1H, d, *J*=3.2 Hz, pyr CH), 5.90 (1H, d, *J*=3.2 Hz, pyr CH), 5.38 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.51 (2H, s, CH_2), 2.57 (1H, m, H9), 2.55 (8H, m, pipz CH_2), 2.39 (1H, dt, *J*=13.7, 4.1 Hz), 2.31 (3H, s, N-CH_3), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, *J*=6.2 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.8, 127.8, 108.1, 107.4, 104.6, 92.5, 81.1, 72.5, 55.6, 55.0, 52.9, 52.4, 46.3, 37.8, 36.7, 34.5, 33.4, 30.0, 26.4, 25.1, 21.7, 20.7 and 14.4 ppm; MS (ES+), $[M+H]^+$ (100) 446; HRMS calcd for $C_{25}H_{40}N_3O_4$ $[M+H]^+$ 446.3019, found 446.3004; IR υ_{max}= 3268 ($\upsilon_{N-H, Pyrrole}$), 2926 (υ_{C-H}), 1705 ($\upsilon_{C=N}$), 880, 826 (υ_{O-O}) cm^{-1}.

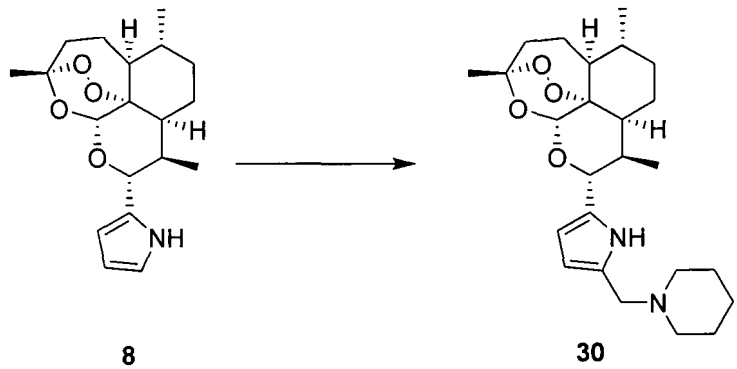
10α- (5-((4-Isopropylpiperazin-1-yl)methyl)-1*H*-pyrrol-2-yl)artemisinin (29).



8 **29**

See general procedure for Mannich reaction above. Colourless crystal (70%): mp = 45 °C; Rf=0.17 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.26 (1H, s, NH), 5.96 (1H, t, *J*=2.7 Hz, pyr CH), 5.92 (1H, t, *J*=2.7 Hz, pyr CH), 5.38 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.57 (2H, s, CH_2), 2.88 (1H, m, ^{i}Pr CH), 2.72 (8H, m, pipz CH_2), 2.57 (1H, m, H9), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 1.12 (6H, d, *J*=6.5 Hz, ^{i}Pr CH_3), 0.97 (3H, d, *J*=6.2 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.3, 130.1, 126.6, 108.7, 107.3, 104.6, 92.5, 81.1, 72.5, 55.4, 55.3, 52.3, 48.15, 46.3, 37.8, 36.7, 34.5, 33.4, 26.4, 25.1, 21.7, 20.7, 18.7, 18.5, 18.4 and 14.5 ppm; MS (ES+), $[M+H]^+$ (100) 474; HRMS calcd for $C_{27}H_{44}N_3O_4$ $[M+H]^+$ 474.3332, found 474.3318.

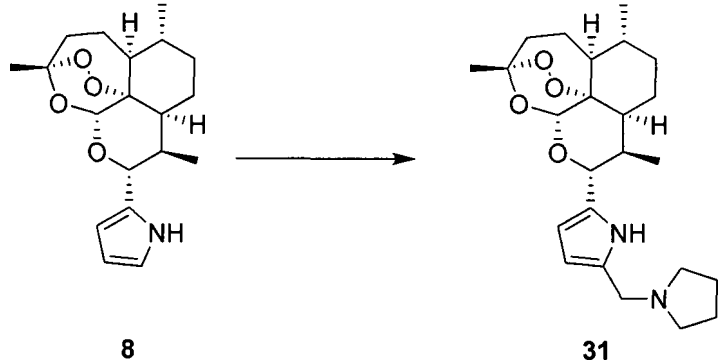
10α- (5-(Piperidin-1-ylmethyl)-1*H*-pyrrol-2-yl)artemisinin (30).



8 30

See general procedure for Mannich reaction above. Colourless sticky solid (65%); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.30 (1H, s, NH), 5.99 (1H, d, *J*=2.8 Hz, pyr CH), 5.94 (1H, d, *J*=2.8 Hz, pyr CH), 5.37 (1H, s, H12), 4.42 (1H, d, *J*=10.8 Hz, H10), 3.64-3.58 (2H, AB quartet, *J*= 14.4 Hz, CH_2), 2.59 (1H, m, H9), 2.52 (4H, m, pipd CH_2), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.67 (4H, m, pipd CH_2), 1.48 (2H, d, *J*=3.9 Hz, pipd CH_2), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 0.97 (3H, d, *J*=6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.7, 109.1, 107.1, 104.6, 92.5, 81.0, 72.4, 60.7, 56.0, 54.0, 52.4, 46.3, 37.8, 36.7, 34.6, 33.5, 26.4, 25.1, 24.1, 21.7, 21.4, 20.7 and 14.4 ppm; IR υ_{max}= 3250 ($\upsilon_{N\text{-}H,\ Pyrrole}$), 2933 ($\upsilon_{C\text{-}H}$), 1707 ($\upsilon_{C=N}$), 880, 827 ($\upsilon_{O\text{-}O}$) cm^{-1}.

10α-(5-(Pyrrolidin-1-ylmethyl)-1*H*-pyrrol-2-yl)artemisinin (31).

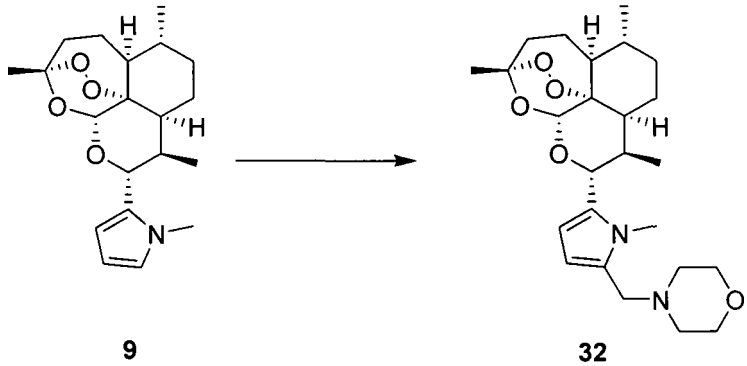


8 31

See general procedure for Mannich reaction above. Orange oil (70%); R_f=0.5 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 10.54 (1H, s, NH), 6.08 (1H, t, *J*=2.8 Hz, pyr CH), 6.04 (1H, t, *J*=2.8 Hz, pyr CH), 5.31 (1H, s, H12), 4.35 (1H, d, *J*=10.6 Hz,

H10), 4.04-4.14 (2H, AB quartet, J= 14.9 Hz, CH_2), 3.10 (4H, m, pyro CH ^iPr CH_2), 2.57 (1H, m, H9), 2.39 (1H, dt, J=13.7, 4.1 Hz, H4α), 2.00 (4H, m, pyro CH_2), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 0.97 (3H, d, J=6.3 Hz, H15) and 0.63 (3H, d, J=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 134.1, 120.2, 111.4, 106.6, 104.2, 92.2, 80.6, 71.9, 52.2, 52.0, 51.4, 50.5, 45.9, 37.3, 36.3, 34.1, 33.3, 26.0, 24,7, 23.2, 23.0, 21.4, 20.3, 14.1 ppm; MS (ES+), $[M+H]^+$ (100) 417; HRMS calcd for $C_{24}H_{37}N_2O_4$ $[M+H]^+$ 417.2753, found 417.2739.

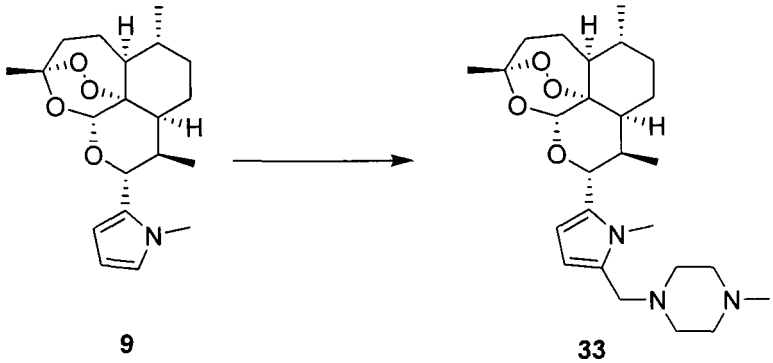
10α- (1-Methyl-5-(morpholinomethyl)-pyrrol-2-yl)artemisinin (32).



9 **32**

See general procedure for Mannich reaction above. Orange solid (89%); mp= 126 °C; R_f= 0.48 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.93 (2H, m, pyr CH), 5.43 (1H, s, H12), 4.53 (1H, d, J=11.1 Hz, H10), 3.88 (3H, s, N-CH_3), 3.72 (4H, t, J=4.4 Hz, morph CH_2), 3.52-3.45 (2H, AB quartet, J=12.8 Hz, CH_2), 2.90 (1H, m, H9), 2.47 (4H, m, morph CH_2), 2.39 (1H, dt, J=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, J=6.3 Hz, H15) and 0.61 (3H, d, J=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.1, 129.6, 108.9, 108.4, 104.6, 92.3, 81.1, 73.2, 67.3, 55.1, 53.4, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 26.4, 25.2, 21.9, 21.3 and 14.8 ppm; IR, υ= 1757, 1458, 1376, 1347, 1263, 1226, 1207, 1151, 1120, 1100, 1084, 1054, 1042, 1004, 979, 940, 926, 895, 880, 864, 852, 828, 800, 743 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 469; HRMS calcd for $C_{25}H_{38}N_2O_5Na$ $[M+Na]^+$ 469.2678, found 469.2664; Anal. Calcd for $C_{16}H_{26}N_2O_3$: C, 67.24%; H, 8.58%; N, 6.27%; Found C, 67.03%; H, 8.71%; N, 6.13%.

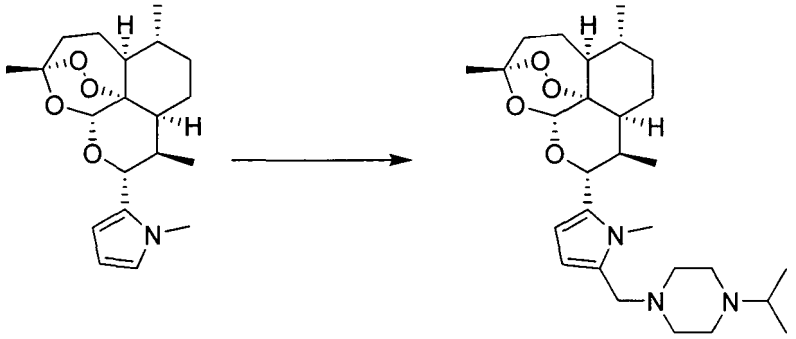
10α- (1-Methyl-5-((4-methylpiperazin-1-yl)methyl)-pyrrol-2-yl)artemisinin (33).



9 33

See general procedure for Mannich reaction above. Yellow crystal (83%); mp = 115 °C; R_f= 0.25 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.89 (1H, d, *J*=3.5 Hz, pyr CH), 5.86 (1H, d, *J*=3.5 Hz, pyr CH), 5.39 (1H, s, H12), 4.47 (1H, d, *J*=11.1 Hz, H10), 3.82 (3H, s, pyr N-CH_3), 3.45-3.38 (2H, AB quartet, *J*= 13.5 Hz, CH_2), 2.85 (1H, m, H9), 2.35-2.70 (8H, m, pipz CH_2), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.34 (3H, s, pipz N-CH_3), 1.41 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.2 Hz, H15) and 0.56 (3H, d, *J*=7.1 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 130.9, 130.5, 108.5, 108.3, 104.5, 92.3, 81.1, 73.1, 55.4, 54.8, 52.5, 52.4, 46.3, 45.9, 37.8, 36.7, 34.5, 32.1, 31.2, 26.4, 25.1, 21.3, 20.7 and 14.8 ppm; IR, υ_{max}= 2670, 1456, 1376, 1302, 1159, 1100, 1057, 1041, 975, 890, 849, 828, 762, 722 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 460; HRMS calcd for $C_{26}H_{42}N_3O_4$ $[M+H]^+$ 460.3175, found 460.3176; Anal. Calcd for $C_{26}H_{41}N_3O_4$: C, 67.94%; H, 8.99%; N, 9.14%; Found C, 68.08%; H, 9.05%; N, 9.08%.

10α- (5-((4-Isopropylpiperazin-1-yl)methyl)-1-methyl-pyrrol-2-yl)artemisinin (34).

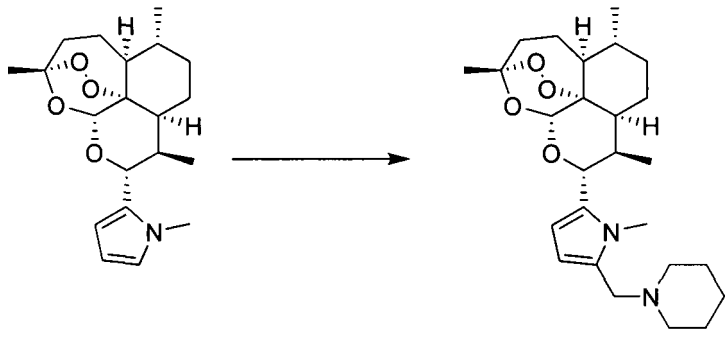


9 34

See general procedure for Mannich reaction above. Colourless solid (76%); mp= 68

°C; Rf= 0.21 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.89 (1H, d, J= 3.5 Hz, pyr CH), 5.87 (1H, d, J= 3.5 Hz, pyr CH), 5.39 (1H, s, H12), 4.48 (1H, d, J=11.3 Hz, H10), 3.81 (3H, s, N-CH_3), 3.51-3.42 (2H, AB quartet, J=13.5, CH_2), 3.05 (4H, m, pipz CH_2), 2.85 (1H, m, H9), 2.73 (5H, m, pipz CH_2), 2.39 (1H, dt, J=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 1.25 (6H, d, J=6.5 Hz, iPr CH_3), 2.08-1.20 (10H, m), 0.98 (3H, d, J=6.3 Hz, H15) and 0.61 (3H, d, J=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.1, 130.0, 108.8, 108.5, 104.6, 92.3, 81.1, 73.2, 56.5, 54.4, 53.8, 48.7, 46.3, 37.8, 36.7, 34.5, 32.1, 31.2, 26.4, 25.1, 21.3, 20.7, 18.0 and 14.8 ppm; IR, υ_{max}= 3894, 3816, 3710, 3544, 3024, 2929, 2856, 2360, 1610, 1460, 1232, 1029 and 756 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 488; HRMS calcd for $C_{28}H_{46}N_3O_4$ $[M+H]^+$ 488.3488, found 488.3507; Anal. Calcd for $C_{28}H_{45}N_3O_4$: C, 68.96%; H, 9.30%; N, 8.62%; Found C, 68.53%; H, 9.42%; N, 8.40%.

10α-(1-Methyl-5-(piperidin-1-ylmethyl)-pyrrol-2-yl)artemisinin (35).

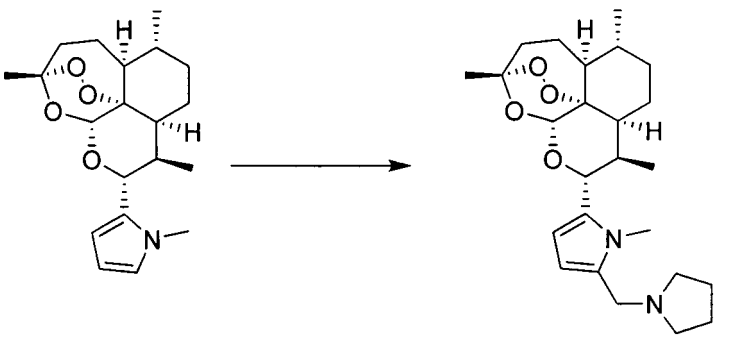


9 **35**

See general procedure for Mannich reaction above. Yellow solid (88%); R_f= 0.34 in 10% MeOH/ DCM; mp= 80 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.95 (1H, m, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, J=11.2 Hz, H10), 3.84 (3H, s, N-CH_3), 3.60 (2H, m, ppd CH_2), 2.85 (1H, m, H9), 2.52 (4H, m, pipd CH_2), 2.39 (1H, dt, J=14.0, 4.1 Hz, H4α), 1.67 (4H, m, pipd CH_2), 1.46 (2H, m, pipd CH_2), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, J=6.2 Hz, H15) and 0.58 (3H, d, J=7.1 Hz, H16) ppm; IR, υ_{max}= 3948, 3836, 3710, 3589, 3539, 2931, 2343, 1462, 1122, 1037, 827, 758 and 679 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 445; HRMS calcd for $C_{26}H_{41}N_2O_4$ $[M+H]^+$ 445.3066, found 445.3058; Anal. $C_{26}H_{40}N_2O_4$ requires C 70.24%, H 9.14%, N 6.22% found C 70.10%, H

9.14%, N 6.22%.

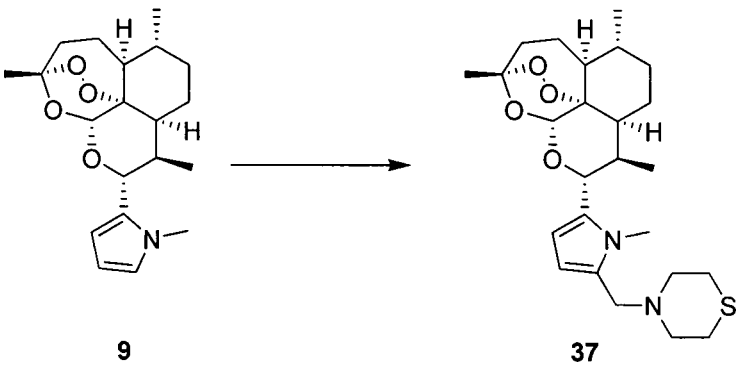
10α-(1-Methyl-5-(pyrrolidin-1-ylmethyl)-pyrrol-2-yl)artemisinin (36).



9 36

See general procedure for Mannich reaction above. Pale yellow dry foam (97%); mp= 70 °C; R_f= 0.29 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.03 (1H, d, *J*=3.7 Hz, pyr CH), 5.97 (1H, d, *J*=3.6 Hz, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J*=11.2 Hz, H10), 3.87 (3H, s, N-CH_3), 3.85 (2H, s, CH_2), 2.86 (1H, m, H9), 2.82 (4H, m, pyro CH_2), 2.39 (1H, dt, *J*= 14.0, 4.1 Hz, H4α), 1.89 (4H, m, pyro CH_2), 1.39 (3H, s, H14), 1.2-2.1 (10H, m), 0.98 (3H, d, *J*=6.4 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 109.0, 104.6, 92.3, 81.0, 72.9, 53.8, 53.2, 52.4, 50.3, 46.3, 37.8, 36.7, 34.5, 32.4, 31.2, 26.4, 25.2, 23.6, 21.3, 20.6 and 14.8 ppm; IR, υ_{max}= 3834, 3759, 3323, 2970, 2345, 1658, 1458, 1377, 1321, 1199, 1124, 1095, 1049, 881 (O-O), 825 (O-O), 762 and 681 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 431; HRMS calcd for $C_{25}H_{39}N_2O_4$ $[M+H]^+$ 431.2910, found 431.2907.

10α-(1-Methyl-5-(thiomorpholinomethyl)-pyrrol-2-yl)artemisinin (37).

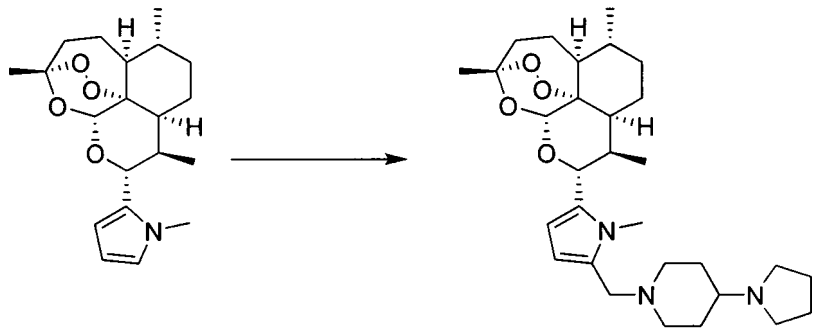


9 37

See general procedure for Mannich reaction above. Yellow oil (90%); R_f= 0.92 in

10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J*=3.5Hz, pyr CH), 5.86 (1H, d, *J*=3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.46 (1H, d, *J*=11.1 Hz, H10), 3.81 (3H, s, N-CH_3), 3.46 and 3.41 (2H, AB quartet, *J*=12.8 Hz, CH_2), 2.85 (1H, m, H9), 2.69 (4H, m, thiomorph), 2.64 (4H, m, CH_2-S), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 1.2-2.1 (10H, m), 0.98 (3H, d, *J*=6.3 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 108.9, 108.4, 104.5, 92.3, 81.0, 73.1, 55.5, 54.8, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 28.3, 26.4, 25.2, 21.3, 20.6 and 14.8 ppm; IR, υ= 3892, 3759, 3712, 3356, 2972, 2814, 2370, 2331, 1658, 1460, 1414, 1371, 1333, 1279, 1203, 1124, 1099, 1051, 948, 880, 823 and 762 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 485; HRMS calcd for $C_{25}H_{38}N_2O_4SNa$ $[M+Na]^+$ 485.2450, found 485.2460; Anal. Calcd for $C_{24}H_{36}N_2O_4S$: C, 64.25%; H, 8.09%; N, 6.24%; Found C, 64.19%; H, 8.12%; N, 6.22%

10α-(1–Methyl–5-((4-(pyrrolidin–1–yl)piperidin–1–yl)methyl)-pyrrol-2-yl)artemisinin (38).

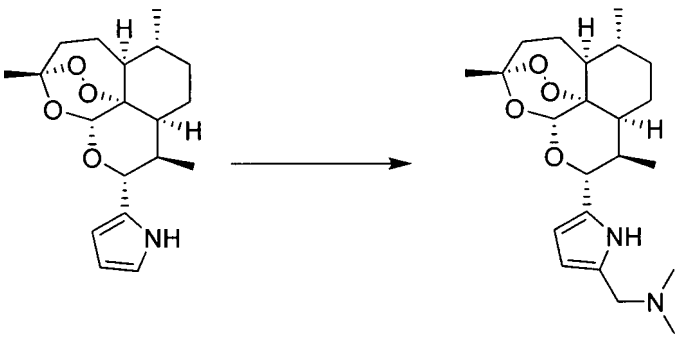


9 **38**

See general procedure for Mannich reaction above. Colourless sticky solid (54%); R_f= 0.10 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J*= 3.5 Hz, pyr CH), 5.84 (1H, d, *J*= 3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.46 (1H, d, *J*=11.1 Hz, H10), 3.78 (3H, s, N-CH_3), 3.42-3.36 (2H, AB quartet, *J*=13.5, CH_2), 3.17 (4H, m, pipd CH_2), 2.99 (4H, m, pyro CH_2), 2.85 (1H, m, H9), 2.82 (1H, m, pipd CH), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.08 (4H, m, pyro CH_2), 1.92 (4H, m, pipd CH_2), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*= 6.3 Hz, H15) and 0.61 (3H, d, *J*= 7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 130.9, 108.1, 108.0, 104.5, 92.3, 81.0, 73.1, 62.6, 55.0, 52.6, 52.3, 51.7, 46.4, 37.8, 36.7, 34.6, 32.0, 31.6, 31.3, 26.4,

25.2, 23.6, 21.3, 20.6 and 14.8 ppm; IR, υ_{max}= 1703, 1458, 1376, 1263, 1151, 1041, 965, 880, 828, 743 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 514; HRMS calcd for $C_{30}H_{48}N_3O_4$ $[M+H]^+$ 514.3645, found 514.3657.

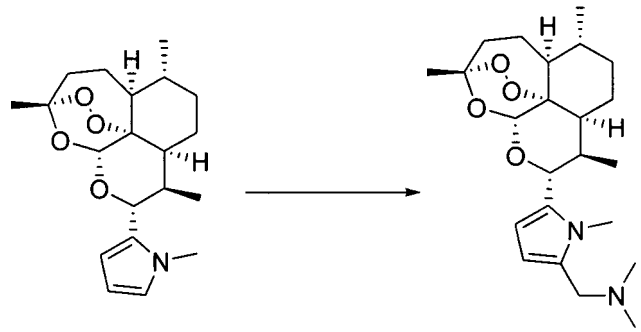
10α-(5-((Dimethylamino)methyl)-1*H*-pyrrol-2-yl)artemisinin (39).



8 **39**

Eschenmoser's salt (124 mg) was dissolved in the minimum amount of anhydrous acetonitrile and added drop wise over a period of 30 mins to a solution of **(8)** (140 mg) in anhydrous acetonitrile (10 mL). The mixture was left to stir at room temperature for 24 h. The mixture was then basified (pH 8) with 2M NaOH solution (3.0 mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (3×25 mL). The combined organic layers were washed with saturated H_2O and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude product that was purified by silica gel chromatography using 10 to 30% MeOH/ DCM as eluent: This gave **39** (70%); Colourless sticky solid, R_f= 0.01 in 10% MeOH/ DCM; ^1H NMR (400 MHz, $CDCl_3$) δ_H 9.70 (1H, s, NH), 6.02 (2H, m, pyr CH), 5.37 (1H, s, H12), 4.43 (1H, d, *J*=10.8 Hz, H10), 3.78-3.69 (2H, AB quartet, *J*= 13.4 Hz, CH_2), 2.63 (1H, m, H9), 2.46 (6H, s, N-$(CH_3)_2$), 2.39 (1H, dt, *J*=13.7, 4.1 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.97 (3H, d, *J*= 6.3 Hz, H15) and 0.63 (3H, d, *J*=7.1 Hz, H16) ppm; IR, υ_{max}= 3461, 2918, 2360, 1588, 1456, 1376, 1278, 1226, 1196, 1151, 1127, 1098, 1085, 1058, 1043, 940, 925, 894, 880, 848, 826, 772, 723 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 391; HRMS calcd for $C_{22}H_{35}N_2O_4$ $[M+H]^+$ 391.2597, found 391.2598.

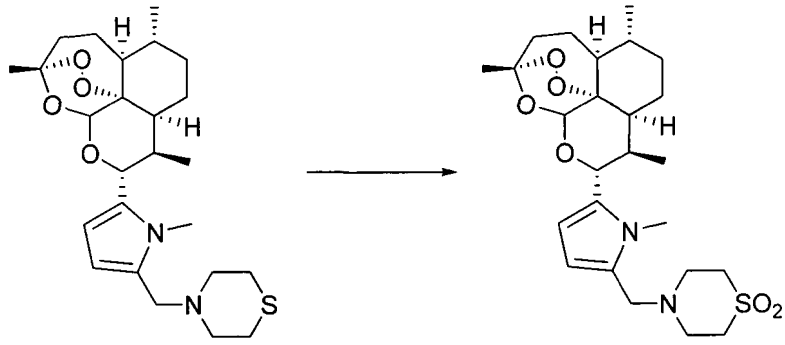
10α-(5-((Dimethylamino)methyl)-1-methyl-pyrrol-2-yl)artemisinin (40).



9 **40**

Eschenmoser's salt (124 mg) was dissolved in the minimum amount of anhydrous acetonitrile and added dropwise over a period of 30 mins to a solution of **(9)** (140 mg) in anhydrous acetonitrile (10 mL). The mixture was left to stir at r.t. for 24 h. The mixture was then basified (pH 8) with 2 M NaOH solution (3mL). The organic layer was then separated and the aqueous layer extracted with EtOAc (3×25 mL). The combined organic layers were washed with saturated H_2O and brine. The organic phase was then dried over $MgSO_4$, filtered and concentrated under reduced pressure to afford a crude product that was purified by silica gel chromatography using 10 to 30% MeOH/ DCM as eluent: This yielded **40** (70%); yellow sticky solid, R_f= 0.01 in 10% MeOH/ DCM; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.93 (1H, d, *J*=3.5 Hz, pyr CH), 5.90 (1H, d, J=3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J*=11.2 Hz, H10), 3.81 (3H, s, N-CH_3), 3.4 (2H, s, CH_2), 2.7 (1H, m, H9), 2.37 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.22 (6H, s, N-$(CH_3)_2$), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.4 Hz, H15) and 0.61 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100MHz, $CDCl_3$) δ_C 131.2, 130.6, 108.5, 104.5, 92.3, 81.0, 72.9, 55.7, 52.4, 50.7, 46.3, 45.0, 37.8, 36.7, 34.5, 31.9, 31.1, 26.3,25.1, 21.3, 20.6 and 14.7ppm; IR υ= 3366 (N-H), 2924 (C-H), 2360, 1708 (C=N), 1498, 1458, 1376, 1320, 1297, 1278, 1248, 1227, 1196, 1151, 1128, 1100, 1085, 1055, 1043, 976, 940, 927, 880 (O-O), 851, 828 (O-O), 775, 721, 708 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 427; HRMS calcd for $C_{23}H_{36}N_2O_4Na$ $[M+Na]^+$ 427.2581, found 427.2573; Anal. Calcd for $C_{23}H_{36}N_2O_4$: C, 68.29%; H, 8.97%; N, 6.92%; Found C, 67.88%; H, 9.06%; N, 6.82%.

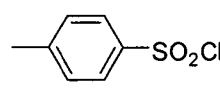
10α-(1-Methyl-5-(sulfonylmorpholinomethyl)-pyrrol-2-yl)artemisinin (41).



37 **41**

To a solution of **(37)** (100 mg, 0.22 mmol), prepared as previously described, in DCM at r.t. under nitrogen was added NMO (76 mg, 0.65 mmol), powered molecular sieves (500mg) and TPAP (10 mg, cat.). The mixture was stirred at r.t. over night after which it was filtered through a pad of silica and the residue was washed with EtOAc (3×15mL). The filtrate was concentrated in vacuum. The residue was then purified by flash chromatography (SiO_2; 35% EtOAc/ *n*-Hex) to give **41** as a yellow solid (38 mg, 35%); mp= 77 °C; R_f= 0.92 in 10% methanol/ dichloromethane; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.90 (1H, d, *J*=3.5 Hz, pyr CH), 5.88 (1H, d, *J*=3.5 Hz, pyr CH), 5.38 (1H, s, H12), 4.48 (1H, d, *J*=11.3 Hz, H10), 3.82 (3H, s, N-CH_3), 3.59-3.49 (2H, AB quartet, *J*=13.7 Hz, CH_2), 3.00 (4H, m, thiomorph CH_2), 2.95 (4H, m, thiomorph CH_2), 2.85 (1H, m, H9), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.3 Hz, H15) and 0.57 (3H, d, *J*=7.2 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 131.2, 129.8, 109.2, 108.6, 104.6, 92.3, 81.0, 73.2, 55.5, 54.8, 52.4, 46.3, 37.8, 36.7, 34.6, 32.1, 31.2, 28.3, 26.4, 25.2, 21.3, 20.6 and 14.8 ppm; MS (ES+), $[M+Na]^+$ (100) 517; HRMS calcd for $C_{25}H_{38}N_2O_6Na$ $[M+Na]^+$ 517.2348, found 517.2344.

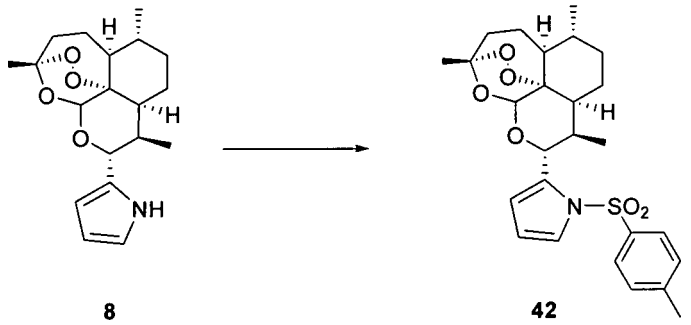
Purification of *para*-toluene sulfonyl chloride.[56]



The reagent is placed in the thimble of a soxhlet apparatus containing dry petroleum ether. After several hours of extraction under an inert atmosphere, the chloride will have dissolved in the solvent and the unwanted acid will be left behind in the soxhlet thimble.

On cooling the solvent mixture, the acid chloride crystallises and can be collected by filtration.

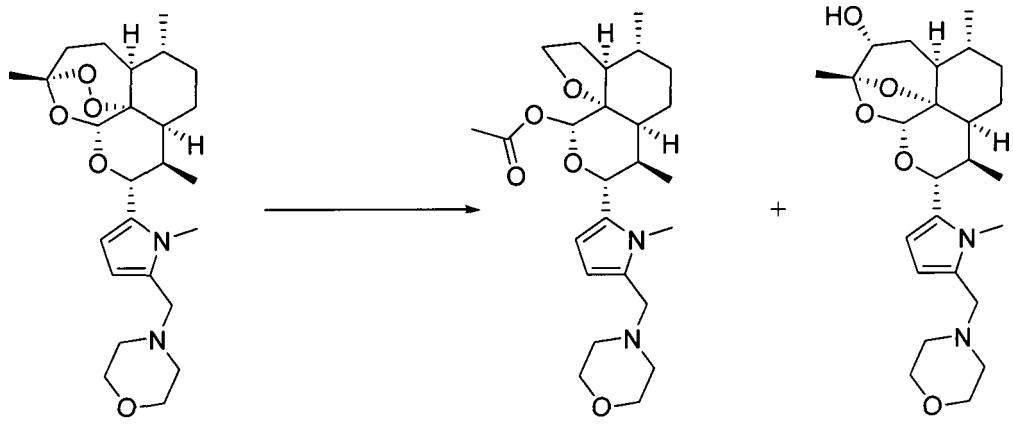
10α-(1-(Phenylsulfonyl)-1*H*-pyrrol-2-yl)artemisinin (40).



8 42

To a solution of **(8)** (227 mg, 0.68 mmol) in anhydrous THF at 0°C was added sodium hydride (41 mg, 1.02 mmol). The reaction mixture was stirred for 40 mins. Then the purified *para*-toluene sulfonyl chloride (259 mg, 1.36 mmol) was added to the reaction mixture. After 2 hrs the reaction was quenched with a sat $NaHCO_3$. The aqueous layer was extracted with EtOAc (3×25mL). The combined organic extracts were washed with brine (3×25 mL). The organic phase was then dried over $MgSO_4$ and concentrated under reduced pressure to afford a crude product that was purified by flash chromatography using 5% EtOAc/ *n*-Hex as eluent. This yielded **42** as a yellow oil (50 mg, 15%); R_f= 0.47 in 25% EtOAc/ *n*-Hex.; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.81 (2H, d, *J*=8.5 Hz, tol CH), 7.22 (2H, m, tol CH), 6.44 (1H, m, pyr CH), 6.26 (2H, m, pyr CH), 5.37 (1H, s, H12), 5.13 (1H, d, *J*=10.7 Hz, H10), 2.69 (1H, m, H9), 2.39 (1H, dt, *J*=14.0, 4.1 Hz, H4α), 2.37 (3H, s, tol CH_3), 1.39 (3H, s, H14), 2.08-1.20 (10H, m), 0.98 (3H, d, *J*=6.0 Hz, H15) and 0.61 (3H, d, *J*=7.1 Hz, H16) ppm; MS (ES+), $[M+Na]^+$ (100) 510; HRMS calcd for $C_{26}H_{33}NO_6S$ $[M+Na]^+$ 510.1926, found 510.1949.

$FeSO_4$-mediated degradation of 10α-(1-methyl-5-(morpholinomethyl)-1H-pyrrol-2-yl)artemisinin with iron (II) sulphate.



32 **55** **56**

To a solution of **32** (0.11 g, 0.25 mmol) in acetonitrile (5 mL) and water (5 mL) was added $FeSO_4.7H_2O$ (86 mg, 0.31 mmol). The reaction was left stirring at r.t. for 1 hour before being filtered through celite and washed with acetonitrile. Concentration under reduced pressure and flash column chromatography using DCM: MeOH / 9 :1 as eluent yielded the products **55** as yellow oil (0.08 g, 72%) and **56** as yellow oil (0.05 g, 21%).

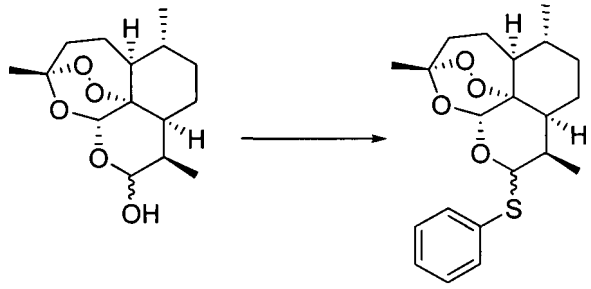
$FeCl_2$-mediated degradation of 10α-(1-methyl-5-(morpholinomethyl)-1H-pyrrol-2-yl)artemisinin with iron (II) chloride. To a solution of **32** (0.15 g, 0.34 mmol) in acetonitrile (13 mL) was added $FeCl_2.4H_2O$ (74 mg, 0.34 mmol) under nitrogen atmosphere. The reaction was left stirring at room temperature for 30 min before being filtered through Celite and washed with acetonitrile. Concentration under reduced pressure and flash column chromatography using EtOAc/*n*-Hex: 5/95 as eluent yielded **55** (0.09 g, 42%) and **56** (0.03 g, 23%).

Furano acetate (55): Yellow oil, R_f= 0.58 in 9:1/ DCM: MeOH; ^{1}H- NMR (400 MHz) δ_H 6.15 (1H, s, H12), 6.05 (1H, d, *J*=3.6 Hz, pyr CH), 5.88 (1H, d, *J*=3.6 Hz, pyr CH), 4.59 (1H, d, *J*=11.1 Hz, H10), 4.27 (1H, t, *J*=9.5 Hz, H4), 3.91 (1H, q, *J*=8.0 Hz, H4), 3.68 (4H, m, morph CH_2), 3.61 (3H, s, N-CH_3), 3.36 (2H, AB quartet, *J*=13.5 Hz, CH_2), 2.74 (1H, m, H9), 2.38 (4H, m, morph CH_2), 2.09 (3H, s, H14), 2.00-1.00 (9H, m), 0.95 (3H, d, *J*=6.6 Hz, H15) and 0.87 (3H, d, *J*=7.0 Hz, H16) ppm; ^{13}C-NMR (400MHz) δ_C 169.8, 130.8, 108.8, 106.9, 92.9, 80.6, 71.8, 68.9, 67.5 (2C), 55.7, 55.5, 53.7 (2C), 48.5,

35.9, 32.4, 31.0, 30.9, 30.4, 28.1, 22.5, 22.0, 21.0, 15.0 ppm; MS (ES+), $[M+Na]^+$ (100) 469; HRMS calc for $C_{25}H_{38}N_2O_5Na$ $[M+Na]^+$ 469.2678, found 469.2687.

3α-Hydroxydeoxyartemisinin (56): Yellow oil, R_f=0.48 in 9:1/ DCM: MeOH; ^{1}H-NMR (400 MHz) δ_H 5.92 (1H, d, *J*= 3.5 Hz, pyr CH), 5.87 (1H, d, *J*=3.5 Hz, pyr CH), 5.33 (1H, s, H12), 4.53 (1H, d, *J*=10.8, H10), 3.72 (3H, s, N-CH_3), 3.66 (4H, m, morph CH_2), 3.57 (1H, brs, –OH), 3.38 (2H, AB quartet, *J*=16.4 Hz, CH_2), 2.79 (1H, m, H9), 2.37 (4H, m, morph CH_2), 2.1-1.1 (9H, m), 1.55 (3H, s, H14), 0.90 (3H, d, *J*=6.44 Hz, H15) and 0.65 (3H, d, *J*=7.2 Hz, H16); ^{13}C-NMR (400MHz) δ_C 131.8, 130.0, 108.8, 108.6, 107.7, 95.7, 84.5, 77.1, 71.8, 70.1, 67.5 (2C), 55.5, 53.7 (2C), 42.9, 35.3, 34.8, 31.8, 30.8, 30.3, 22.5, 21.4, 21.0 and 14.6 ppm; MS (ES+), $[M+Na]^+$ (100) 469; HRMS calc for $C_{25}H_{38}N_2O_5Na$ $[M+Na]^+$ 469.2678, found 469.2691.

10α-Phenylthiodihydroartemisinin (71α) and 10β-phenylthiodihydroartemisinin (71β).



2 **71**

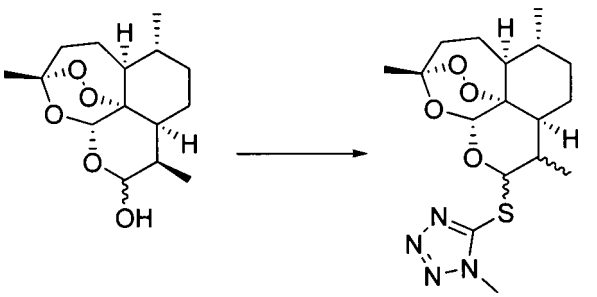
Thiophenol (698 mg, 6.34 mmol) and $BF_3.Et_2O$ (500 mg, 3.52 mmol) were added to a stirred solution of DHA (1g, 3.52 mmol) in DCM (50 mL) at r.t.. The solution was stirred for 10min, after which it was diluted with DCM (100 mL), washed with sat $NaHCO_3$ and brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (2% EtOAc/ *n*-Hex) to give 1.13g (85%) of **71α** and 140mg (11%) of **71β**.

71α: white solid; R_f= 0.18 in 5% EtOAc/ *n*-Hex; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.69 (2H, dd, *J*= 8.3, 1.8 Hz, Ph), 7.27 (3H, m, Ph), 5.34 (1H, s, H12), 4.74 (1H, d, *J*= 10.8 Hz, H10), 2.57 (1H, m, H9), 2.37 (1H, td, *J*= 4.0, 13.3 Hz, H4α), 1.41 (3H, s, H14), 2.08-1.20 (10H, m), 1.03 (3H, d, *J*= 7.0 Hz, H16) and 0.98 (3H, d, *J*= 5.7 Hz, H15) ppm;

^{13}C NMR (100 MHz, $CDCl_3$) δ_C 133.9, 132.6, 128.9, 127.6, 104.7, 92.6, 83.9, 77.7, 52.2, 46.4, 37.8, 36.6, 34.5, 31.5, 26.3, 25.2, 21.8, 20.6 and 15.4 ppm; MS (ES+), $[M+Na]^+$ (100) 399; HRMS calc for $C_{21}H_{28}O_4SNa$ $[M+Na]^+$ 399.1600, found 399.1606.

71β: white solid; R_f=0.25 in 5% EtOAc/ *n*-Hex; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.54 (2H, dd, *J*= 8.6, 1.5 Hz, Ph), 5.35 (1H, s, H12), 4.73 (1H, d, *J*= 5.3 Hz, H10), 3.12 (1H, m, H9), 2.39 (1H, td, *J*=4.0, 13.3 Hz, H4α), 1.40 (3H, s, H14), 2.08-1.20 (10H, m), 1.03 (3H, d, *J*= 7.0 Hz, H16) and 0.98 (3H, d, *J*= 5.7 Hz, H15) ppm.

10α-Tetrazolthiodihydroartemisinin (73α), 10β-tetrazolthiodihydroartemisinin (73β) and 10*epi*-tetrazolthiodihydroartemisinin (73*epi*).



2 **73**

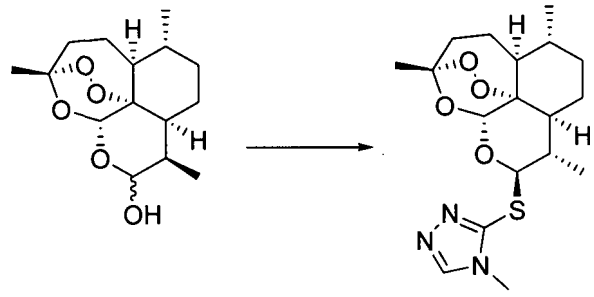
5-Mercapto-1-methyl-1H-tetrazol (368 mg, 3.17 mmol) and $BF_3.Et_2O$ (0.2 mL, 1.76 mmol) were added to a stirred solution of DHA (500 mg, 1.76 mmol) in DCM (20 mL) at r.t.. The solution was stirred for 10min, after which it was diluted with DCM (100 mL), washed with sat $NaHCO_3$ and brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (5% EtOAc/ *n*-Hex) to give **73α** (302 mg, 45%), **73β** (54 mg, 8%) and ***73epi*** (141 mg, 21%).

73α: white-yellow sticky solid; R_f= 0.23 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.99 (1H, d, *J*= 10.8 Hz, H10), 5.56 (1H, s, H12), 3.90 (3H, s, N-CH_3), 3.31 (1H, m, H9), 2.40 (1H, td, *J*=4.0, 13.3 Hz, H4α), 1.40 (3H, s, H14), 2.08-1.20 (10H, m), 0.99 (3H, d, *J*= 6.0 Hz, H15) and 0.73 (3H, d, *J*= 7.0 Hz, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 165.8, 105.0, 92.8, 82.4, 80.2, 60.8, 51.9, 45.8, 37.6, 36.5, 34.9, 34.3, 31.4, 26.1, 25.0, 21.9, 20.6 and 12.6 ppm; MS (ES+), $[M+H]^+$ (100) 383; HRMS calcd for $C_{17}H_{27}N_4O_4S$ $[M+H^+]$ 383.1753, found 383.1752.

73β: white sticky solid; R_f= 0.35 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 6.62 (1H, d, *J*= 6.3 Hz, H10), 6.05 (1H, s, H12), 3.89 (3H, s, N-CH_3), 3.28 (1H, m, H9), 2.40 (1H, td, *J*= 4.0, 13.3 Hz, H4α), 1.40 (3H, s, H14), 2.08-1.20 (10H, m), 0.99 (3H, d, *J*= 12.1 Hz, H15) and 0.87 (3H, d, *J*= 12.9 Hz, H16) ppm; MS (CI) for $C_{17}H_{26}N_4O_4SNa$ $[M+Na]^+$ 405, $[M+K]^+$ 421.

73*epi*: white-yellow sticky solid; R_f= 0.28 in 25% EtOAc/ *n*-Hex); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.72 (1H, d, *J*= 10.5 Hz, H10), 5.56 (1H, s, H12), 3.90 (3H, s, N-CH_3), 2.52 (1H, m, H9), 2.39 (1H, td, *J*=4.0, 13.3 Hz, H4α), 1.40 (3H, s, H14), 2.08-1.20 (10H, m), 1.03 (3H, d, *J*= 7.0 Hz, H16) and 0.98 (3H, d, *J*= 5.7 Hz, H15) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 166.1, 102.8, 90.9, 83.4, 82.1, 51.0, 47.3, 38.6, 37.2, 36.2, 34.4, 34.0, 31.6, 30.9, 25.6, 24.7, 19.8 and 18.0 ppm.

10-*epi*-Triazolthiodihydroartemisinin (74*epi*).

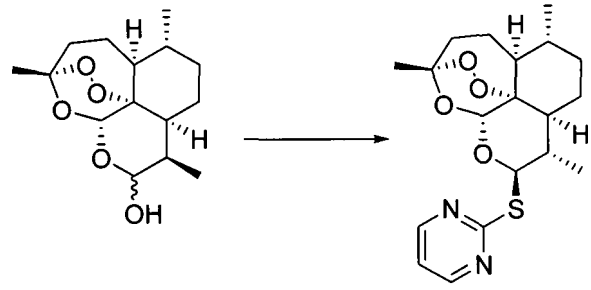


2 **74**

3-Mercapto-4-methyl-4H-1,2,4-triazol (183 mg, 1.76 mmol) and $BF_3.Et_2O$ (0.1 mL, 0.88 mmol) were added to a stirred solution of DHA (250 mg, 0.88 mmol) in DCM (10 mL) mix with acetonitrile (10 mL) at r.t.. The solution was stirred for 1 hr, after which it was diluted with DCM (100 mL), washed with sat-$NaHCO_3$ and brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (5% EtOAc/ *n*-Hex) to give 250 mg (37%) of *epi* as a white solid; R_f= 0.15 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.79 (1H, s, CH [triazol]), 6.83 (1H, d, *J*= 10.2 Hz, H10), 5.50 (1H, s, H12), 3.56 (3H, s, N-CH_3), 2.31 (1H, m, H9), 2.39 (1H, td, *J*=4.0, 13.3 Hz, H4α), 1.40 (3H, s, H14), 1.2-2.1 (10H, m) and 0.94 (6H, m, H15-16) ppm; IR, υ_{max}= 2924, 2724, 1458, 1376, 1222, 1159, 1140,

1104, 1082, 1050, 1011, 958, 931, 901, 888, 864, 847, 825, 723, 643 cm^{-1}; MS (CI), $[M+H]^+$ (100) 382; HRMS calc for $C_{18}H_{28}NO_4S$ $[M+H]^+$ 382.1800, found 382.1808.

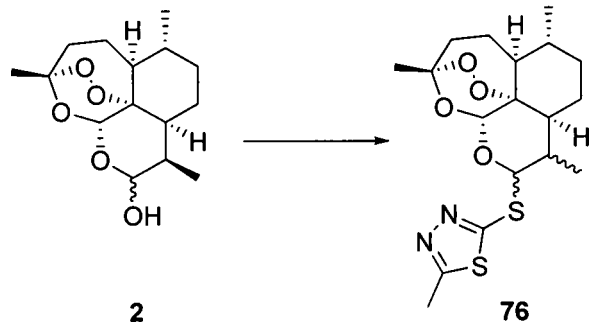
10*epi*-Pyrimidinthiodihydroartemisinin (75*epi*).



2 **75**

2-Mercaptopyrimidin (200 mg, 1.78 mmol) and $BF_3.Et_2O$ (0.1 mL, 0.89 mmol) were added to a stirred solution of DHA (250 mg, 0.89 mmol) in DCM (10 mL) mixed with acetonitrile (10 mL) at r.t.. The solution was stirred for 15 min, after which the reaction was quenched with sat-$NaHCO_3$, extracted with DCM and washed with brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give a white sticky solid (20 mg, 6%): R_f= 0.64 in 25% EtOAc/ *n*-Hex; ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.50 (2H, d, *J*= 4.8 Hz, pyrm CH), 6.95 (1H, t, *J*=4.8 Hz, pyrm CH), 5.89 (1H, d, *J*= 11.4 Hz, H10), 5.44 (1H, s, H12), 2.77 (1H, m, H9), 2.37 (1H, td, *J*=4.0, 13.5 Hz, H4α), 1.41 (3H, s, H14), 2.08-1.20 (10H, m), 1.00 (3H, d, *J*= 7.3 Hz, H15) and 0.98 (3H, d, *J*=6.4 Hz, H16); ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.6, 157.7, 117.2, 104.7, 92.9, 80.8, 80.7, 77.1, 52.3, 46.7, 37.7, 36.7, 34.4, 32.1, 26.4, 25.1, 21.7, 20.6 and 15.4 ppm; IR υ_{max}= 1565, 1551, 1461, 1377, 1303, 1263, 1228, 1190, 1151, 1084, 1059, 1054, 1049, 1044, 1039, 1035, 1002, 977, 926, 893, 879, 743 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 379; HRMS calcd for $C_{19}H_{27}N_2O_4S$ $[M+H]^+$ 379.1691, found 379.1702.

10α-Thiadiazolthiodihydroartemisinin (76α), 10β - thiadiazolthiodihydroartemisinin (76β) and 10*epi*-thiadiazolthiodihydroartemisinin (76*epi*).



2 **76**

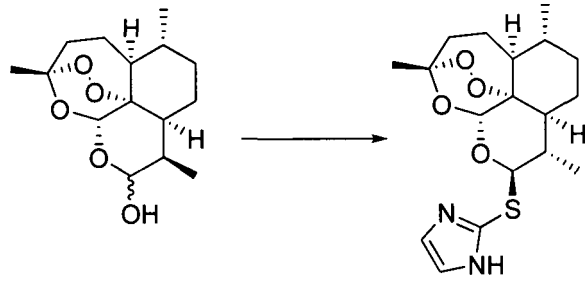
2-Mercapto-5-methyl-1,3,4-thiadiazol (232 mg, 1.76 mmol) and $BF_3.Et_2O$ (0.1 mL, 0.88 mmol) were added to a stirred solution of DHA (250 mg, 0.88 mmol) in anhydrous Et_2O (60 mL) at r.t.. The solution was stirred for 1 hr, after which it was diluted with DCM (100 mL), washed with sat $NaHCO_3$ and brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (2% EtOAc/ *n*-Hex) to give **76α** (24.3 mg, 7%), **76β** (69.5 mg, 20%), **76*epi*** (26.1 mg, 8%) and DHA (50.5 mg, 20%).

76α: White sticky solid, R_f= 0.35 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.36 (1H, d, *J*= 10.4 Hz, H10), 5.55 (1H, s, H12), 2.62 (1H, m, H9), 2.50 (3H, s, thiadz CH_3), 2.40 (1H, td, *J*= 4.0, 13.3 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.99 (3H, d, *J*=6.3 Hz, H15) and 0.72 (3H, d, *J*= 7.2 Hz, H16) ppm.

76β: White sticky solid, R_f= 0.35 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.42 (1H, s, H12), 5.05 (1H, d, *J*= 3.29 Hz, H10), 2.62 (1H, m, H9), 2.48 (3H, s, thiadz CH_3), 2.40 (1H, td, *J*= 4.0, 13.3 Hz, H4α), 1.42 (3H, s, H14), 2.08-1.20 (10H, m), 0.93 (3H, d, *J*=6.3 Hz, H15) and 0.86 (3H, d, *J*= 7.2 Hz, H16) ppm.

76*epi*: White sticky solid, R_f= 0.39 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.93 (1H, d, *J*= 6.6 Hz, H10), 6.07 (1H, s, H12), 3.22 (1H, m, H9), 2.46 (3H, s, thiadz CH_3), 2.40 (1H, td, *J*=4.0, 13.3 Hz, H4α), 1.42 (3H, s, H14), 1.2-2.1 (10H, m), 0.99 (3H, d, *J*=6.1 Hz, H16) and 0.95 (3H, d, *J*= 7.4 Hz, H15) ppm; MS (ES+), $[M+H]^+$ (100) 399; HRMS calcd for $C_{18}H_{27}O_4N_2S_2$ $[M+H]^+$ 399.1412, found 399.1414.

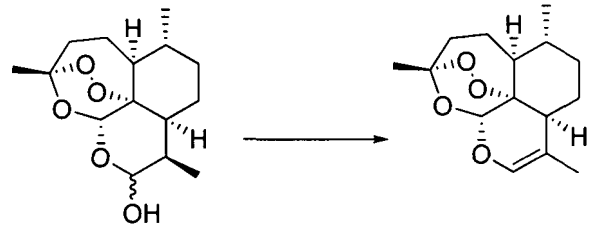
10*epi*-Imidazolthiodihydroartemisinin (77*epi*).



2 **77**

2-Mercaptoimidazole (176 mg, 1.76 mmol) and $BF_3.Et_2O$ (0.1 mL, 0.88 mmol) were added to a stirred solution of DHA (250 mg, 0.88 mmol) in acetonitrile (60 mL) at r.t.. The solution was stirred for 1 hr, after which it was diluted with DCM (100 mL), washed with sat $NaHCO_3$ and brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated EtOAc/ *n*-Hex) to give **77*epi*** (250 mg, 86%) as a white sticky solid; R_f= 0.05 in 25% EtOAc/ *n*-Hex; ^{1}H NMR (200 MHz, $CDCl_3$) δ_H 7.03 (2H, s, imidz CH), 5.44 (1H, s, H12), 4.73 (1H, d, *J*= 11.0 Hz, H10), 3.10 (1H, m, H9), 2.40 (1H, td, *J*= 4.0, 13.3 Hz, H4α), 1.49 (3H, s, H14), 2.08-1.20 (10H, m), 0.96 (3H, d, *J*=6.3 Hz, H15) and 0.92 (3H, d, *J*= 7.0 Hz, H16) ppm.

Anhydroartemisinin (12).[53]

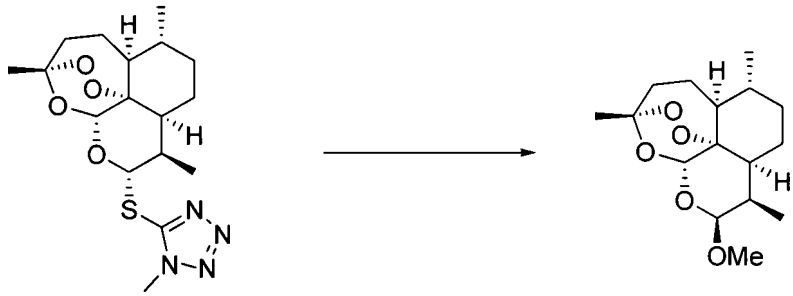


2 **12**

A vigorously stirred solution of NaOH (0.22 mmol) and deionized water (15 mL) was treated with sulphide (0.18 mmol). The resulting suspension was stirred at ambient temperature for 20 mins. To this was added sodium bicarbonate (1.44 mmol) and acetone (5 mL). The oxone solution (145 mg in 0.54 mL of 4.10^{-4} M EDTA) was added over 5 min. The suspension was vigorously stirred for 1 hr at r.t. The reaction was quenched with sodium bisulfite (90 mg in 2 mL of deionized water) and stirred for 15 minutes. The aqueous phase was isolated and extracted with EtOAc (10 mL). The

organic layers were combined and washed with deionized water (15 rnL), washed with brine (15 mL), dried over anhydrous $MgSO_4$, filtered and concentrated via rotary evaporation. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give a white solid (41 mg, 85%); R_f= 0.50 in 25% EtOAc/ *n*-Hex; mp= 96°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.18 (1H, q, *J*= 1.3 Hz, H10), 5.54 (1H, s, H12), 2.45-2.35 (1H, m), 2.10-2.00 (2H, m), 1.95-0.98 (17H, m), including 1.59 (3H, d, *J*=1.1 Hz, H16), 1.42 (3H, s, H16) and 0.98 (3H, d, *J*=5.8 Hz, H15) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 135.6, 108.8, 105.2, 90.3, 79.6, 52.1, 45.1, 38.1, 36.9, 34.8, 30.6, 26.5, 25.1, 20.9 and 16.8 ppm; IR υ_{max}= 2931, 2857, 2358, 1686, 1652, 1461, 1376, 1280, 1251, 1198, 1177, 1158, 1141, 1112, 1079, 1029, 1016, 992, 954, 904, 879 (O-O), 848 (O-O), 828 and 722; MS (ES+), $[M+H]^+$ (100) 267; HRMS calcd for $C_{15}H_{23}O_4$ $[M+H]^+$ 267.1596, found 267.1604; Anal. Calcd for $C_{15}H_{22}O_4$: C, 67.64%; H, 8.33%; Found C, 67.48%; H, 8.35%.

10β-Artemether (3).

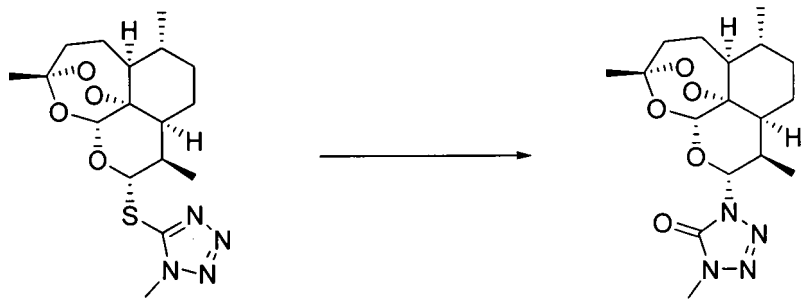


73 **3**

To a solution of **73** (145 mg, 0.38 mmol) prepared as described before in THF/ MeOH/ H_2O (1/1/1) at r.t. was added oxone (654.1 mg, 1.1 mmol). The mixture was stirred for 1 hour at r.t. then quenched with sat $NaHCO_3$, extracted with EtOAc and washed with brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give **3** as a white solid (110 mg, 90%): R_f= 0.50 in 25% EtOH/ hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.38 (1H, s, H12), 4.69 (1H, d, J=3.3 Hz, H10), 3.43 (3H, s, OCH_3), 2.63 (1H, m, H9), 2.37 (1H, td, *J*= 3.8, 13.52 Hz, H4α), 1.44 (3H, s, H14), 1.2-2.1 (10H, m), 0.95 (3H, d, *J*= 6.4 Hz, H15) and 0.90 (3H, d, *J*=7.5 Hz, H16) ppm; ^{13}C

NMR (100 MHz, $CDCl_3$) δ_C 104.1, 103.4, 87.7, 81.1, 56.0, 52.5, 44.5, 37.4, 36.4, 34.6, 30.9, 26.2, 24.7, 24.5, 20.4 and 13.0 ppm; MS (CI), $[M+NH_4-OCH_3-O_2]^+$ (100) 253; HRMS calcd for $C_{16}H_{30}O_5N$ $[M+NH_4]^+$ 316.2124, found 316.2128; Anal. Calcd for $C_{16}H_{26}O_5$: C, 64.43 %; H, 8.72 %; Found C, 63.91 %; H, 8.19 %.

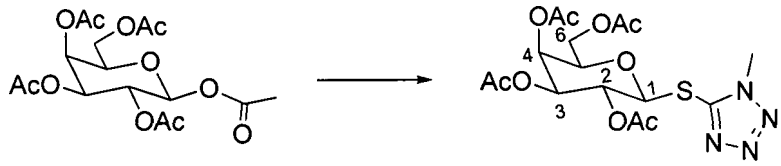
10α-Tetrazoloxodihydroartemisinin (86).



73 **86**

To a solution of **73** (70 mg, 0.18 mmol) in DCM (10 mL) at r.t. in nitrogen was added NMO (64 mg, 0.54 mmol), activated powdered molecular sieve (500 mg, 4Å), and TPAP (6 mg, cat.). The mixture was stirred at r.t. overnight after which it was filtered through a pad of SiO_2 and the residue was washed with EtOAc. The filtrate was concentrated in vacuum. The residue was then purified by flash chromatography (SiO_2; 35% EtOAc/ *n*-Hex) to give **86** as a white crystal (60 mg, 91%); R_f= 0.10 in 25% EtOAc/ *n*-Hex; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.51 (1H, s, H12), 5.39 (1H, d, *J*= 10.8 Hz, H10), 3.63 (3H, s, NCH_3), 3.31 (1H, m, H9), 2.39 (1H, td, *J*= 4.0, 13.3 Hz, H4α), 1.40 (3H, s, H14), 2.08-1.20 (10H, m), 0.99 (3H, d, *J*= 5.9 Hz, H16) and 0.98 (3H, d, *J*=7.0 Hz, H15) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 151.1 (C=O), 104.6 (C-O), 92.1 (C-O), 79.7 (C-O), 76.7 (C-O), 51.6, 45.4, 37.3, 36.2, 34.0, 31.6, 31.2, 30.1, 25.8, 24.6, 23.2, 21.4, 20.2 and 12.5 ppm; MS (ES+), $[M+Na]^+$ (100) 389; HRMS calcd for $C_{19}H_{26}N_4O_5Na$ $[M+Na]^+$ 389.1801, found 389.1783.

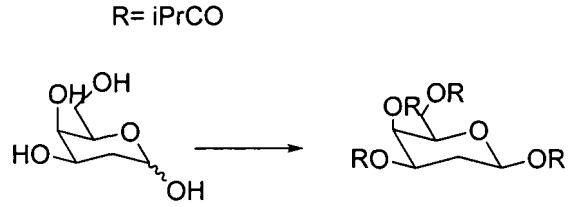
2,3,4,6-Tetra-O-acetate-1-tetrazolthio-β-D-Galactose (90).



89 **90**

5-Mercapto-1-methyl-1*H*-tetrazol (138 mg, 1.20 mmol) and $BF_3.Et_2O$ (0.13 mL, 1.02 mmol) were added to a stirred solution of **89** (260 mg, 0.66 mmol) in anhydrous DCM (20 mL) at r.t.. The solution was stirred for 10min, after which it was diluted with DCM (100 mL), washed with sat $NaHCO_3$ and brine. The organic layer was separated, dried with $MgSO_4$, filtered and evaporated to dryness. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give **90** as a yellow oil (272 mg, 92%): R_f= 0.80 in 10% MeOH/ DCM.; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 5.42 (1H, d, *J*=3.5 Hz, H1), 5.37 (1H, m, H3), 5.30 (1H, m, H2), 5.09 (1H, ddd, *J*= 1.1; 3.6 and 8.8 Hz, H4), 4.06 (3H, m, H5, H6), 3.98 (3H, s, tetz CH_3), 2.13 (3H, s, acetate CH_3), 2.08 (3H, s, acetate CH_3), 1.97 (3H, s, -CH_3 of acetate) and 1.96 (3H, s, acetate CH_3) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 170.6, 170.4, 170.2, 169.2 (C=O), 150.7 (C-S), 85.4, 75.6, 72.1, 71.2, 68.2, 66.8, 61.4, 34.4 (N-C), 21.2, 21.1 and 21.0 (CH_3 of acetate) ppm; MS (ES+), $[M+Na]^+$ (100) 469; HRMS calcd for $C_{16}H_{22}N_4O_9SNa$ $[M+Na]^+$ 469.1005, found 469.1008.

1,3,4,6-Tetra-O-isobutyryl-2-deoxy-β-D-Galactose (93).[55]

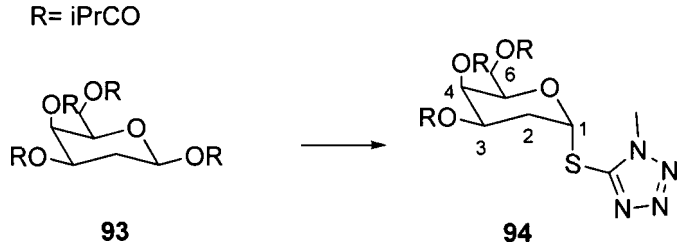


92 **93**

2-Deoxy-D-galactose (0.25g, 1.5 mmol) was stirred at -12 °C (ice-methanol) in pyridine (1.25 mL, 15.3 mmol) and $CHCl_3$ (2 mL). A solution of isobutyryl chloride (1mL, ~9 mmol) in $CHCl_3$ (2mL) was added dropwise over 30 minutes. The reaction was judged complete by t.l.c. 30 minutes after addition of isobutyryl chloride solution. The reaction mixture was diluted in Et_2O, washed with water, with 1 M HCl solution and with brine.

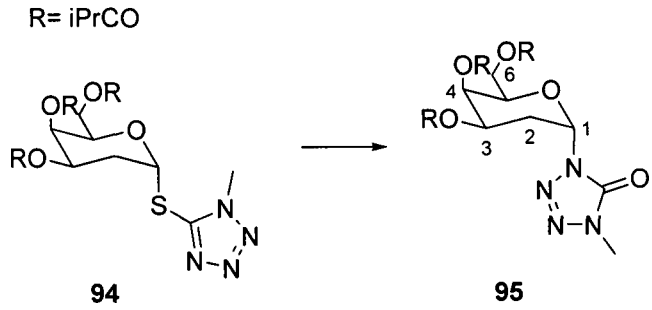
The organic layer was dried over $MgSO_4$, filtered and concentrated to give **93** as yellow oil (810 mg, 99%). R_f= 0.90 in 5:3:1/ EtOAc:*i*PrOH:H_2O; ^1H NMR (400 MHz, $CDCl_3$) δ_H 5.72 (1H, m, H1), 5.26 (1H, dt, *J*= 1.1 and 2.9 Hz, H4), 5.02 (1H, m, H3), 4.05 (2H, m, H6), 3.97 (1H, dt, *J*= 1.1 and 6.8 Hz, H5), 2.65-2.36 (4H, m, -CH of isobutyryl), 1.96 (2H, m, H2)) and 1.22-1.03 (12H, s, -CH_3 of isobutyryl) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.4, 174.9, 174.6, 173.9 (C=O), 90.6(C1), 71.0 (C4), 67.1, 63.5, 60.1, 33.0, 32.9, 32.8, 32.7, 29.8 (C2), 18.2, 18.1, 17.9, 17.9, 17.8, 17.8, 17.7 and 17.6 ppm; MS (ES+), $[M+Na]^+$ (100) 467; HRMS calcd for $C_{22}H_{36}O_9Na$ $[M+Na]^+$ 467.2257, found 467.2257; Anal. Calcd for $C_{22}H_{36}O_9$: C, 59.44%; H, 8.16%; Found C, 59.58%; H, 8.18%.

1-Tetrazolthio-3,4,6-tri-O-isobutyryl-2-deoxy-β-D-Galactose (94).



A solution of 2-deoxy-D-Galactose-tetraisobutyryl (421 mg, 0.95 mmol) in DCM (20 mL) -50°C was treated sequentially with 5-mercapto-1-methyl tetrazole (198 mg, 1.71 mmol) and $BF_3.Et_2O$ (0.14 mL, 1.14 mmol) and stirred at -50°C for 1 hr. The mixture was quenched with sat $NaHCO_3$, extracted with DCM and washed with brine, then dried with $MgSO_4$ and concentrated on rotary evaporator under vacuum. The crude product was purified by flash chromatography (10% EtOAc/ *n*-Hex) to give **94** (288 mg, 64%): R_f= 0.47 in 25% EtOAc/ *n*-Hex; ^1H NMR (400 MHz, $CDCl_3$) δ_H 6.54 (1H, d, *J*= 5.7 Hz, H1), 5.83-5.78 (1H, m, H4), 5.52 (1H, m, H3), 4.24 (1H, m, H5), 4.08 (2H, m, H6), 3.91 (3H, s, -CH_3 of tetrazol), 2.71-2.46 (3H, m, -CH of isobutyryl), 2.26 (2H, m, H2), 1.25-1.22 (3H, m, -CH_3 of isobutyryl) and 1.13-1.09 (6H, m, -CH_3 of isobutyryl) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.3, 176.2, 176.0 (C=O), 165.2 (C=N), 81.0, 71.0, 65.8, 65.4, 61.4, 34.9, 34.5, 34.4, 34.4 (-CH iPr), 28.3 (-CH_3 tetrazol), 19.5, 19.3, 19.3, 19.2, 19.1 and 19.0 (-CH_3 of iPr) ppm; MS (ES+), $[M+Na]^+$ (100) 495; HRMS calcd for $C_{20}H_{31}O_7N_4Na$ $[M+Na]^+$ 495.1889, found 495.1910.

1-Tetrazol-oxo-3,4,6-tri-O-isobutyryl-2-deoxy-β-D-Galactose (95).



To a solution of (48) (288 mg, 0.61 mmol) in dichloromethane (15 mL) at r.t. in nitrogen was added NMO (214 mg, 1.83 mmol), activated powdered molecular sieve (500 mg, 4Å), and TPAP (20 mg, cat.). The mixture was stirred at r.t. overnight after which it was filtered through a pad of SiO_2 and the residue was washed with ethyl acetate. The filtrate was concentrated on rotary evaporator under vacuum. The residue was then purified by flash chromatography (SiO_2; 10% ethyl acetate/hexane to 50% ethyl acetate/hexane) to give a yellow oil (25%); Rf= 0.14 in 25% ethylacetate/ hexane; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 6.02 (1H, d, J=5.9 Hz, H1), 5.83 (1H, m, H4), 5.49 (1H, m, H3), 4.06 (2H, m, H6), 3.64 (3H, s, -CH_3 of tetrazol), 2.52-2.44 (3H, m, -CH of iPr), 2.22 (2H, m, H2) and 1.30-1.10 (18H, s, -CH_3 of iPr) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 176.7, 176.2, 176.0 (C=O), 150.5 (N-C=O), 78.4, 70.6, 66.1, 65.6, 61.5, 34.5, 34.3, 34.1(CH, -iPr), 31.7, 28.4, 19.5, 19.3, 19.3, 19.2, 19.1 and 19.1 (CH_3, iPr) ppm; MS (ES+), $[M+Na]^+$ (100) 479; HRMS calcd for $C_{20}H_{32}N_4O_8Na$ $[M+Na]^+$ 479.2118, found 479.2165.

1.5. Literature

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CHAPTER 3

Targeting the cytochrome bc_1 complex

3. Targeting the cytochrome bc_1 complex

3.1. Introduction

Quinones and quinolones are highly efficient antimalarials as they inhibit the mitochondrial respiration process of *Plasmodium falciparum*. By binding specifically to the cytochrome bc_1 complex, they stop ubiquinone, also called co-enzyme Q, from entering the enzyme's active site. They are reversible enzyme inhibitors that do not form covalent linkages to the active site.

The cytochrome bc_1 complex is an enzyme catalyzing the transfer of electrons from ubiquinol to cytochrome c_1 enabling the transfer of protons across the inner mitochondrial membrane.[1] The catalytic core of the enzyme is organised in redox prosthetic groups which are located within three subunits: cytochrome c_1 and the iron-sulphur protein (ISP) which are membrane proteins with large, hydrophilic domains, and cytochrome *b*, a predominantly hydrophobic protein consisting of eight transmembrane helices which contains two hemes *b* of differing redox potential (low potential b_l and high potential b_h) and forms the two quinol binding sites Qo (site of quinol oxidation) and Qi (site of quinol reduction). A quinol molecule binds at the Qo-site, is deprotonated, transfers one electron through the iron-sulphur protein and cytochrome c_1 to cytochrome *c* and forms a highly unstable semiquinone species which immediately reduces b_l. The electron is then transferred to b_h, then to a quinol bound at the Qi site, forming a stable semiquinone species. A second quinol oxidation event at the Qo site completes the Q-cycle with the formation of fully reduced quinol at the Qi-site. Overall, two molecules of quinol are oxidised to quinone at the Qo site and one molecule of quinone is reduced to quinol at the Qi, site with the concerted transfer of two protons per quinol oxidised from the N (negative)- to the P (positive)-side of the inner mitochondrial membrane. The reduced ISP delivers electrons to cytochrome c_1 by macroscopic movement of its soluble cytoplasmic domain (alternately occupying the '*b*-' and'c_1-proximal' positions).[2] The **Figure 1** was drawn using co-ordinates of yeast enzymes (1EZV.pdb).

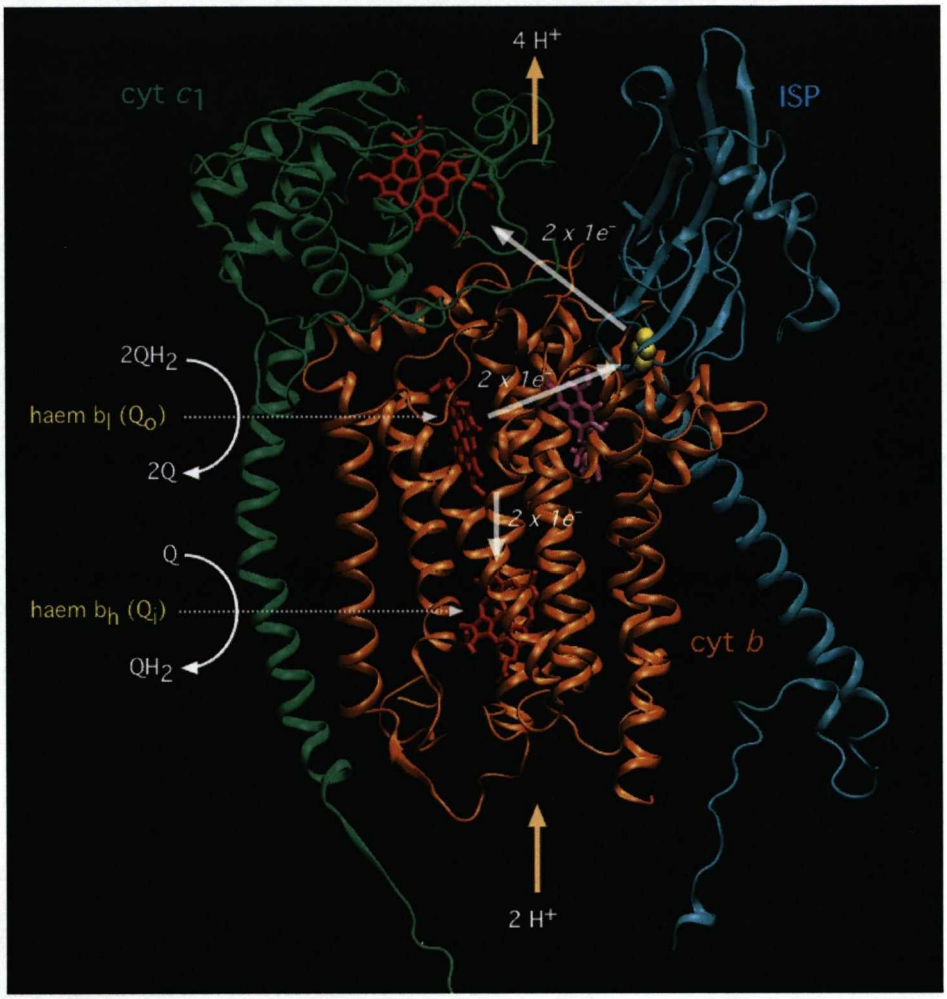
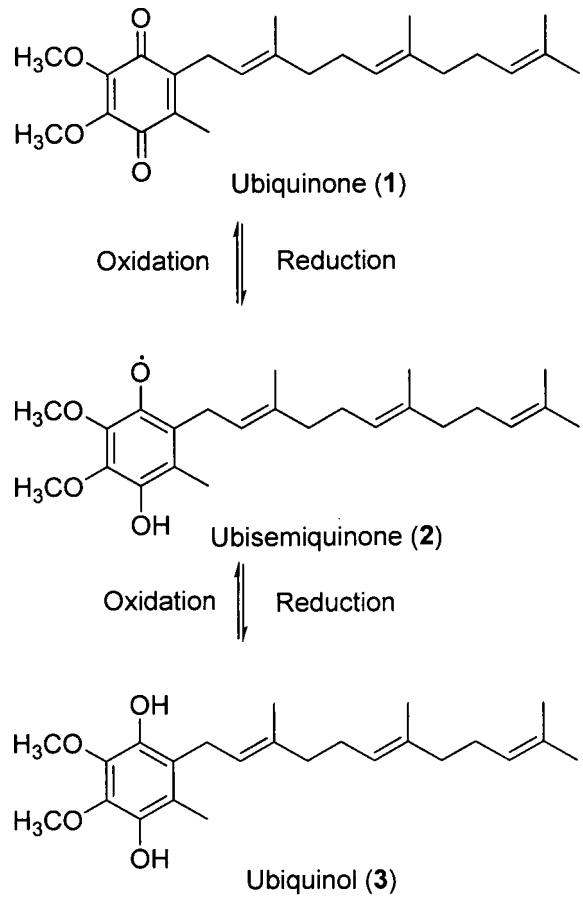


Figure 1. Cytochrome bc_1 complex: yeast enzyme, PDB co-ords 1EZV

In 1983 Ozawa and co-workers were the first to crystallise the cytochrome bc_1 complex from beef heart mitochondria.[3] In 2002, Lange and co-workers described the electron transfer pathway between cytochrome bc_1 complex and cytochrome *c*.[4]

Two successive one-equivalent reductions and two successive one-equivalent oxidations take place in the sites Q_0 and Q_i via the radical semiquinone $QH^{\cdot}$ or its depronated form Q^{-} (**Scheme 1**). These two sites are situated on opposite sides of the membrane linked by a transmembrane electron pathway.[1]



Scheme 1. Redox equation for ubiquinone

Inhibition of the cytochrome bc_1 complex leads to a collapse of the mitochondrial membrane potential ($\Delta\Psi_m$) resulting in cell death. A number of inhibitors selective for *bc*1 Q_o and Q_i sites have been developed over recent years.

3.1.1. Known Inhibitors of cytochrome bc_1 complex

3.1.1.1. Naphthoquinones

Some quinines (e.g. ubiquinones) have important roles in the biochemistry of energy production and serve as vital links in electron transport. Other quinines have been attributed a defense role as a result of their effectiveness at inhibiting the growth of bacteria, fungi or parasites.[5, 6]

The 1,4-naphthoquinone structure is common in various natural products[7] and is associated with biological activities including enzyme inhibition, anti-inflammatory, anticancer, antimicrobial activity and antimalarial activities.[8-11] The

biological activity imparted by 1,4-naphthoquinones in most cases relies upon their ability to accept one and/or two electrons to form radical anion or dianion species.[12]

Atovaquone (**Figure 2**) is a unique hydroxynaphthoquinone derivative with broad-spectrum activity against numerous protozoan parasites.[13] It is structurally similar to ubiquinone and its mechanism of action has been completely elucidated for *Plasmodium*.[14, 15]

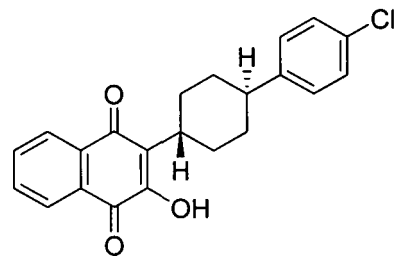
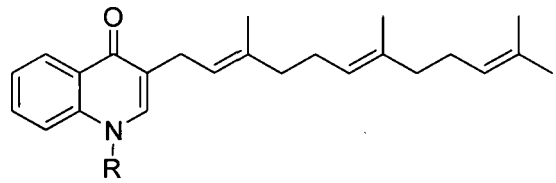


Figure 2. Atovaquone.

A disadvantage is its bioavailability, although this has been improved with a suspension formulation. High cost is another disadvantage, because the synthesis of atovaquone results in a mixture of diastereoisomers.[16]

3.1.1.2. Quinolones

Quinolones are widely used antibacterial agents. They were first developed in the early 1960s with the non-fluorinated drug nalidixic acid and proceeded in the 1980s to the first 6-fluorinated derivatives with enhanced activity against Gram-negative bacteria (e.g. norfloxacin, ofloxacin, ciprofloxacin). [17-19]Quinolones are also inhibitors of the cytochrome bc_1 complex, for example Aurachin C and D (**Figure 3**).[20]



5, Aurachin A, R= OH
6, Aurachin D, R= H

Figure 3. Quinolones, bc_1 complex's inhibitors.

3.1.1.3. Pyridinone

GSK pharmaceuticals have developed a novel class of antimalarial compounds; the non-chiral 4(1H)-pyridone derivative (GW844520) is a potent and selective inhibitor of the cytochrome bc_1 complex of mitochondrial electron transport in *P. falciparum* (**Figure 4**).[21]

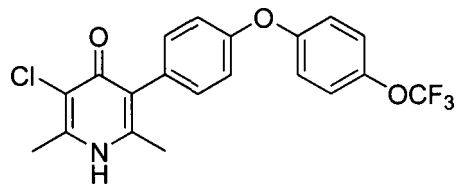


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Figure 4. GW844520

3.1.1.4. Acridones

The acridone skeleton is present in natural products and has been studied for its biomedical activities as a treatment for cancer,[22] viral infections[23] and parasitic diseases including malaria.[24, 25]

Riscoe and co-workers synthesised and tested acridone derivatives to develop a better understanding of the anti-malarial structure-activity relationships.[26] The activity was optimal (IC_{50}= 1.5 pM) with a long side chain terminated by a trifluoromethyl group (**Figure 5**). It is interesting to note that this side chain resembles the long relatively flexible isoprenyl side chain of ubiquinone.

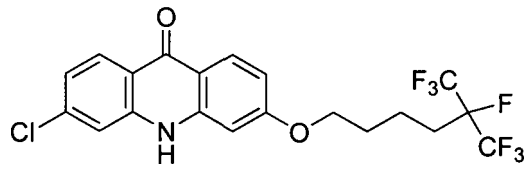

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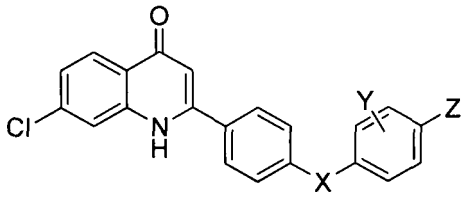
Figure 5. 3-(5,6,6,6-tetrafluoro-5-trifluoromethylhexyloxy)-6-chloroacridone (IC_{50}=1.5 pM)

3.2. Synthesis

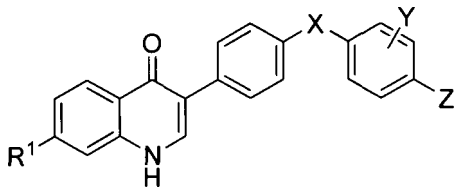
The aim of this study was to explore the SAR of a series of 2- and 3-aryl quinolones and naphthoquinone and to determine the optimal position for substitution with a bi-aryl side-chain (**Figure 6**).

2-QUINOLONE TEMPLATE



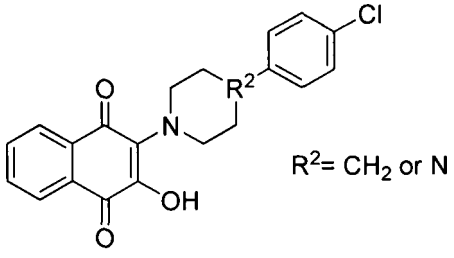
X= O, CH_2
Y= H, Cl, OCF_3
Z= H, F, CF_3, OCF_3

3-QUINOLONE TEMPLATE



R^1= H, Cl
X= O, CH_2
Y= H, Cl, OCF_3
Z= H, F, CF_3, OCF_3

HYDROXYNAPHTHQUINONE SERIES



R^2= CH_2 or N

Figure 6. Target molecules.

The study sets out to create more drug-like templates than the quinolone[27] or acridone[26] analogues produced by Riscoe and co-workers, where the heterocyclic ring system is substituted with a long aliphatic or perfluorinated alkyl chain. It was proposed that the phenoxy or benzyl aryl side-chain may have the ability to occupy the same hydrophobic binding site such as inhibitors like stigmatellin. Stigmatellin is another inhibitor of the co-enzyme Q, which is the cytochrome *c* reductase (**Figure 7**).

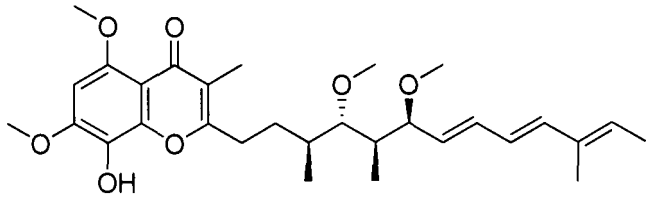


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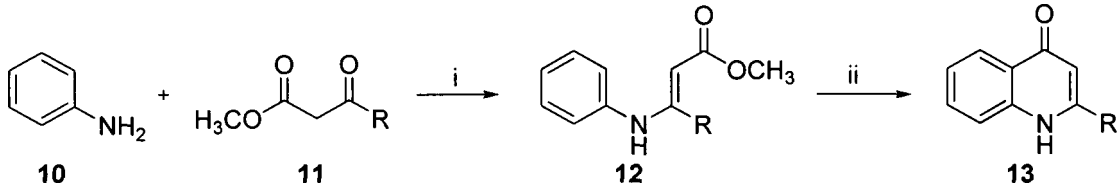
Figure 7. Stigmatellin

3.2.1. Quinolones substituted on the 2-position

Quinolones can be made by a variety of cyclisation reactions.[28, 29] A classical method for preparation of 2-substituted-4-quinolones (**13**) is the *Conrad-Limpach* reaction.[30] This is the best method when R is an alkyl (**Scheme 2**).



10 **11** **12** **13**

R= CH_3, C_3H_7, C_5H_{11}, C_7H_{15}

Scheme 2. *Reagent and conditions*: i) Benzene; ii) Heating.

The aim of this synthesis was to obtain a bi-aryl at the 2-position of the quinolone. A retrosynthesis study suggests we can start from the cheap commercially available 4-chloroquinoline or quinol-4-one which would react with an organolithium or a magnesium halide. The organolithium can be made from the corresponding bromide species with *n*-butyl lithium (**Figure 7**).

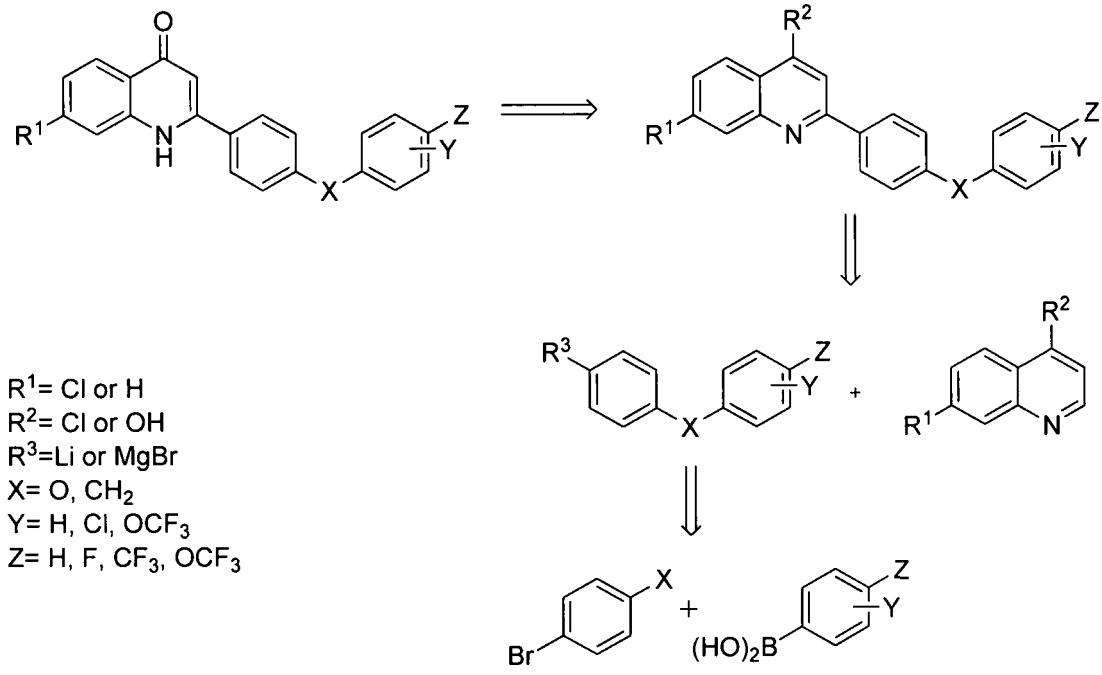
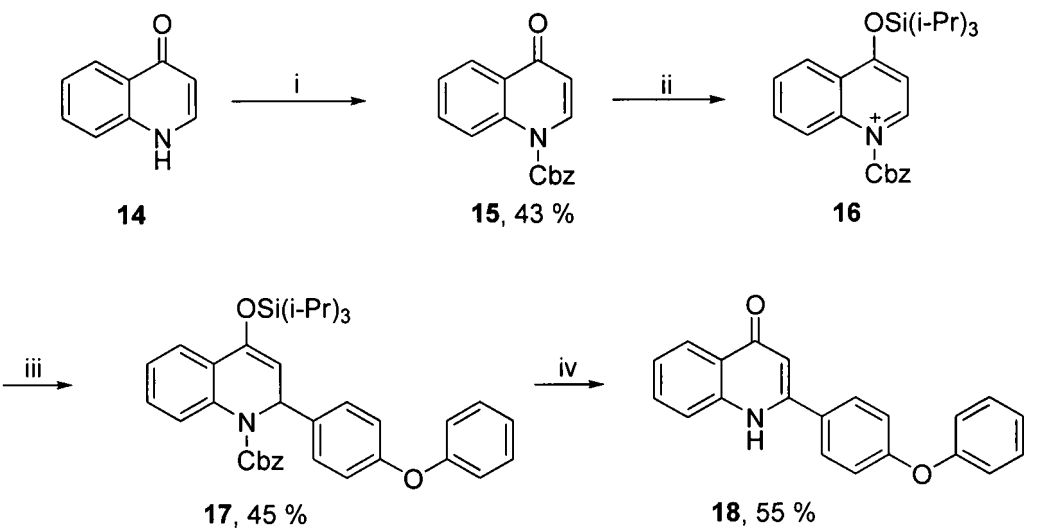


Figure 7. Disconnection

2-(4-Phenoxyphenyl)quinolin-4(1H)-one (**18**) was first prepared by the regioselective addition of a Grignard reagent to the *N*-protected 4-silyloxyquinolonium triflate (**16**), followed by transformation to the quinolone derivative (**17**).[31] The *N*-protected 4-quinolone (**15**) was readily prepared in acceptable yields by the protection of 4-hydroxyquinoline (**14**) with benzyl chloroformate under basic conditions. Conversion to the 4-silyloxyquinolonium triflates (**16**) was performed *in situ* by reaction of the *N*-protected 4-quinolone with TIPSOTf at 20°C for 1hr. Reaction of the Cbz-protected 4-silyloxyquinolinium triflate (**16**) with the aryl magnesium halide gave the corresponding adduct (**17**) in 45% yield. Transformation of the silyl enol ether (**17**) to the corresponding α,ß-unsaturated ketone and removal of the Cbz protecting group was performed *via a* novel method. Treatment of the Cbz-protected silyl enol ether with H_2 and catalytic amounts of 10% Pd/C, afforded the corresponding quinolone alkaloid (**18**) in a single transformation in good yield. The transformation is initially believed to occur by reductive cleavage of the Cbz group followed by oxidation of the silyl enol ether (**Scheme 3**).[31]



Scheme 3. *Reagents and conditions*: i) Benzylchloroformate, NaH, THF, r.t., overnight; ii) TIPSOTf, r.t., 1 hr; no stirring; iii) 2,6-lutidine, PhOPhMgBr, DCM/THF, r.t., 2 hrs; iv) H_2, 10% Pd/C, MeOH, r.t., 3 hrs.

The mechanism of the final step of deprotection occurs with a cis-addition of H_2 onto **17**, followed by the elimination of toluene and carbon dioxide. Oxidative addition of the allyl anion to a Pd(0) complex forms the (π-allyl)-palladium (**20**) *in situ*. Palladium enolate (**20**) undergoes reductive elimination to afford allyl ketone (**18**) and regenerate the Pd(0) complex (**Figure 8**).[32]

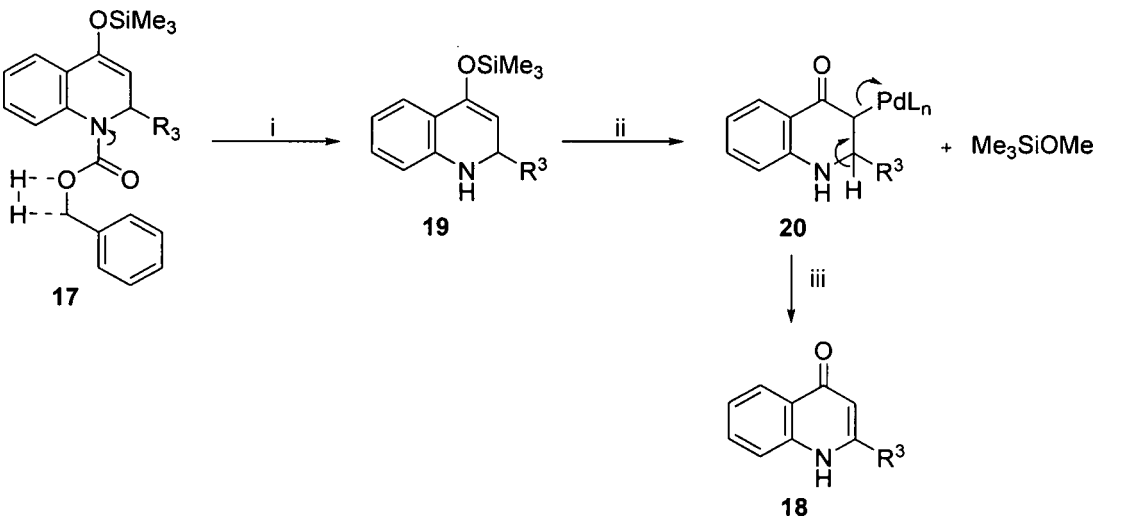
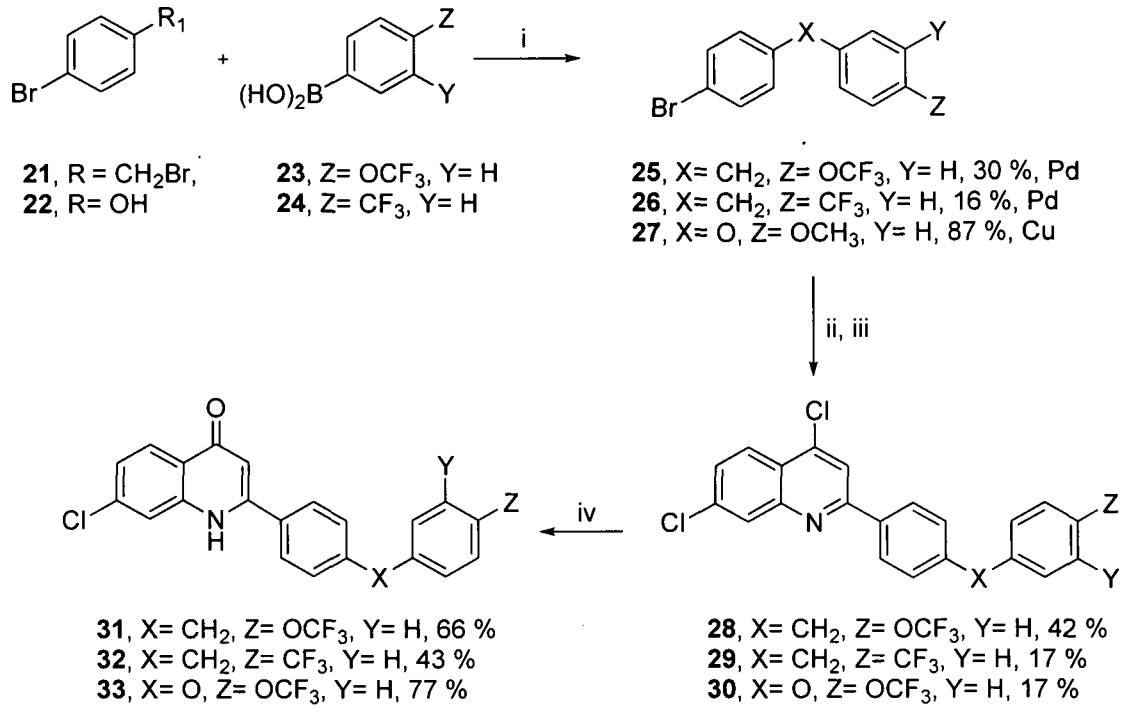


Figure 8. *Reagents and conditions*: i) elimination of CO_2 and toluene; ii) $MeOPdL_n$; iii) β-elimination.

2-Substituted-quinolones were also prepared via a three-step synthesis employing a Copper or Suzuki coupling to make the bromide species, followed by Ziegler alkylation[33] and hydrolysis of a 4-substituted quinoline derivative to give the target quinolone (**Scheme 4**).



Scheme 4. *Reagents and conditions*: i) Suzuki (**25-26**) or Copper (**27**) coupling; ii) *n*-BuLi, 4,7-dichloroquinoline, THF, -78°C, 4 hrs; iii) CAN, acetone, r.t., 30 mins; iv) 85 % $HCOOH/H_2O$, DMF, reflux, 4 hrs.

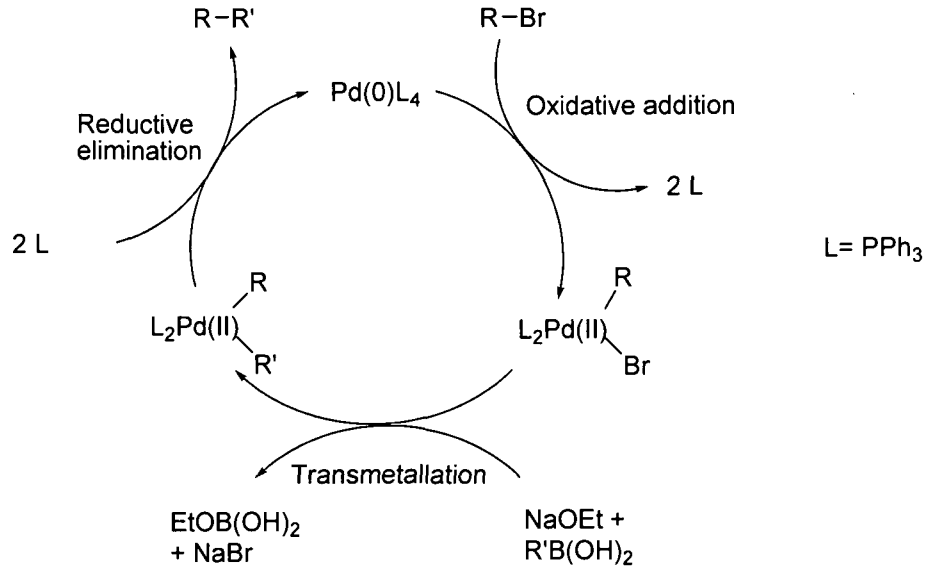
Details of step i;

Synthesis of the bromide derivatives **25** and **26** involves a Suzuki coupling.[34]

The Suzuki reaction, first published in 1979, is a palladium-catalysed cross coupling between a boronic acid and a halide or triflate. The Suzuki reaction is one of the most versatile and often used reactions for the selective construction of carbon-carbon bonds, in particular for the preparation of biaryl-containing molecules.[35, 36]

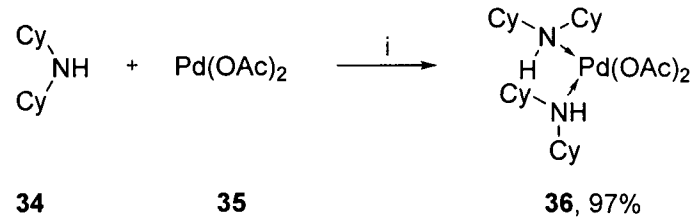
The mechanism starts with the oxidative addition of the less hindered halide to the palladium(0) complex, which generates a palladium (II) intermediate. This is followed by transmetallation with the phenyl boronic acid, to release the product via reductive elimination, reducing the complex to catalyst palladium(0). An additional

base, such as sodium carbonate, is required during the transmetallation step to capture the boronic acid (**Scheme 5**).



Scheme 5. Catalytic cycle for Suzuki reaction.

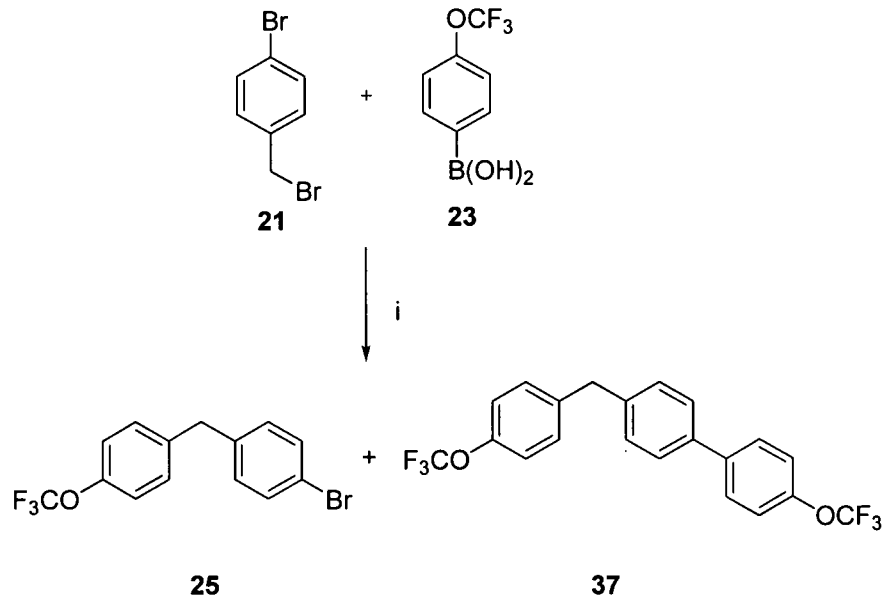
The choice of the catalyst is one of the keys to success for this reaction. Three palladium catalysts were used for this coupling in this chapter. A new palladium catalyst DAPCy (**36**) can be used for Suzuki coupling in aerobic conditions (**Scheme 6**).[37]



34 **35** **36**, 97%

Scheme 6. *Reagents and conditions*: i) dioxane, r.t., 3 hrs.

Coupling between 4-trifluoromethoxyphenyl boronic acid and 4-bromobenzybromide with DAPCy as catalyst gave the expected product, also recovery of unreacted bromide (**21**) and disubstituted side-product (**37**) (**Scheme 7**). Only 10% of the desired product (**25**) was obtained.



Scheme 7. *Reagents and conditions*: i) DAPCy, K_3PO_4, EtOH, aerobic conditions, r.t. or i) $PdCl_2$, dppf, K_3PO_4, dioxane, 100°C.

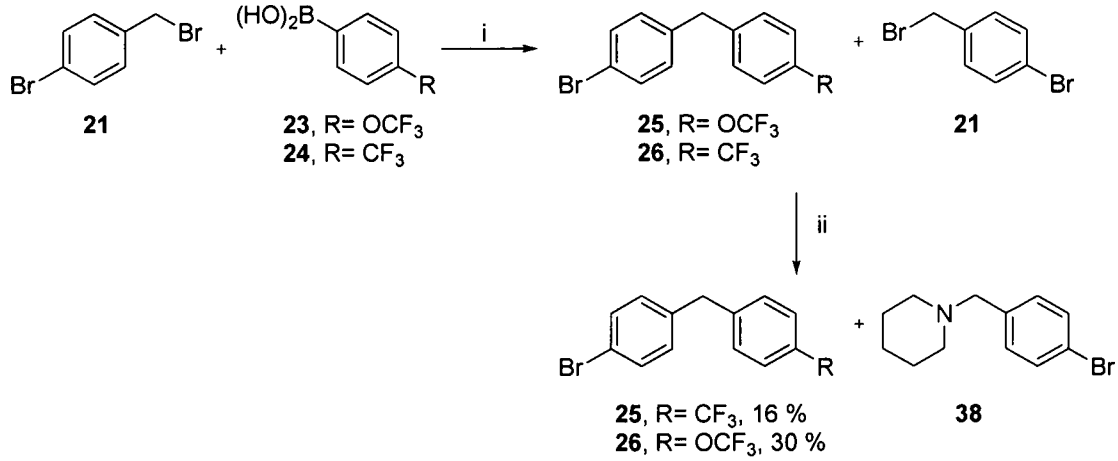
Similar reaction with palladium dichloride ($PdCl_2$) with 1,1'-bis(disphenyldiphenylphosphino)ferrocene (dppf) as ligand and potassium phosphate tribasic gave similar mixture (**25-37**).[38]

The Suzuki reaction conditions using DAPCy and $PdCl_2$/dppf led to no selectivity with both bromide groups of 4-bromobenzylbromide, giving 2 products which were difficult to isolate. These results led to the use of another palladium catalyst, $Pd[PPh_3]_4$, which was used for the same coupling. The synthesis of this catalyst is obtained in quantitative yield in one step from palladium dichloride (**Scheme 8**).[39]

$$2\ PdCl_2 + 8\ PPh_3 + 5\ NH_2NH_2.H_2O \longrightarrow 2\ Pd(PPh_3)_4 + 4\ NH_2NH_2.HCl + N_2 + 5\ H_2O$$

Scheme 8. *Reagents and conditions*: i) DMSO, argon, 140°C, 3hrs.

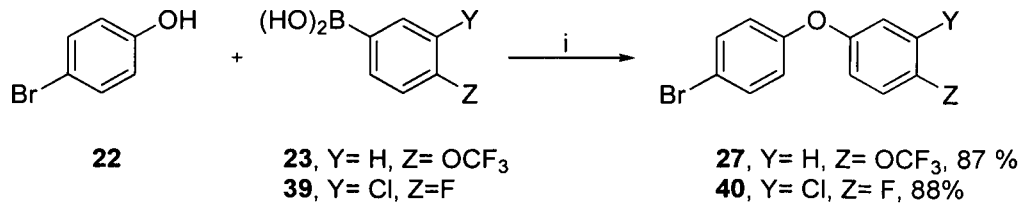
The synthesis[34] of **25-26** via Suzuki coupling with $Pd[PPh_3]_4$ gave a mixture of 4-bromobenzylbromide (**21**) and the desired product, since both these products are non-polar, they cannot be separated with column chromatography. An additionnal reaction of the mixture with piperidine in toluene was required to give the amine **38**, which is much more polar, allowing **25-26** to be easily purified via column chromatography (**Scheme 9**).



Scheme 9. *Reagents and conditions:* i) Na_2CO_3, $Pd[PPh_3]_4$, EtOH/ toluene, reflux, 16 hrs; ii) piperidine, toluene, reflux, 2 hrs.

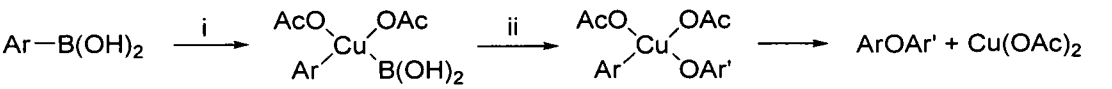
Although $Pd(PPh_3)_4$ seems to be a better catalyst than DAPCy because of no side-products, the yields obtained for Suzuki reaction modified by Langle and co-workers[34] with 4-bromobenzylbromide catalysed by $Pd(PPh_3)_4$ are low. This suggests 4-bromobenzylbromide may not be a good substrate for Suzuki coupling.

Copper coupling was also used as part of synthesis[40, 41] to make the phenoxyphenyl bromide species **27-40** (**Scheme 10**).



Scheme 10. *Reagents and conditions*: i) $Cu(OAc)_2$, NEt_3, DCM, mol.sieves, r.t., 48 hrs.

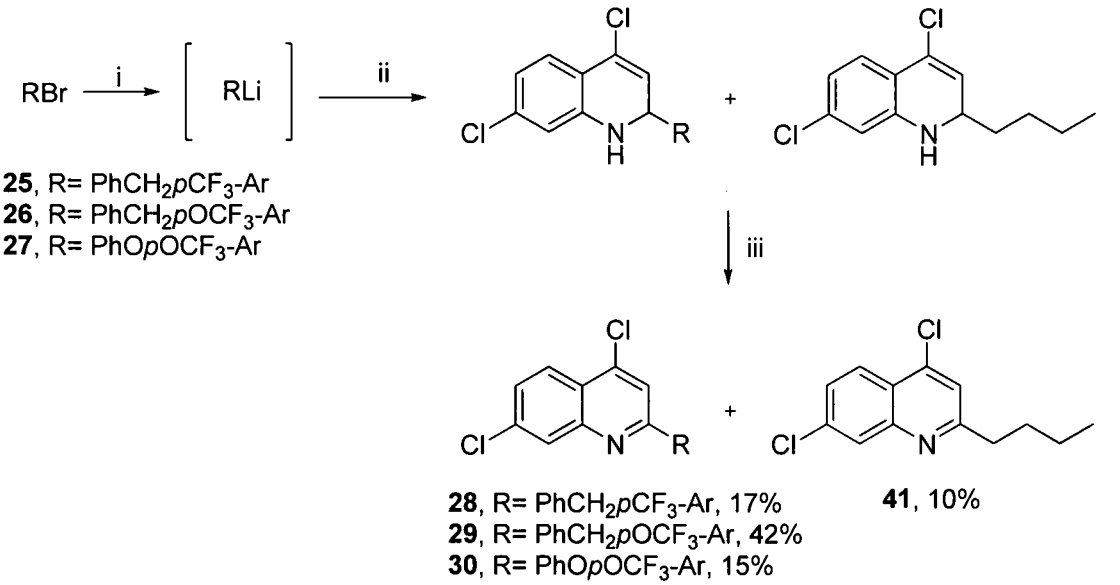
Evans and co-workers have speculated the plausible arylcopper phenoxide intermediate which undergo reductive elimination to the diaryl ether (**Scheme 11**). [40] Copper coupling with pyridine[42] as a base did not give good yields, triethylamine gave better yields.



Scheme 11. *Reagents and conditions*: i) $Cu(OAc)_2$; ii) Ar'OH, base.

Details of step ii, iii)

4,7-Dichloroquinoline undergoes a Ziegler-alkylation with organolithium reagents at -78°C with high regioselectivity.[43] 2-Butylquinoline has been observed as a side product, which indicates the remaining *n*-butyllithium reacts also with 4,7-dichloroquinoline (**Scheme 12**). CAN, also called ammonium cerium (IV) nitrate, oxidise an amine to an imine, and becomes reduced to cerium (III) in the process.



25, R= $PhCH_2pCF_3$-Ar
26, R= $PhCH_2pOCF_3$-Ar
27, R= $PhOpOCF_3$-Ar

28, R= $PhCH_2pCF_3$-Ar, 17%
29, R= $PhCH_2pOCF_3$-Ar, 42%
30, R= $PhOpOCF_3$-Ar, 15%

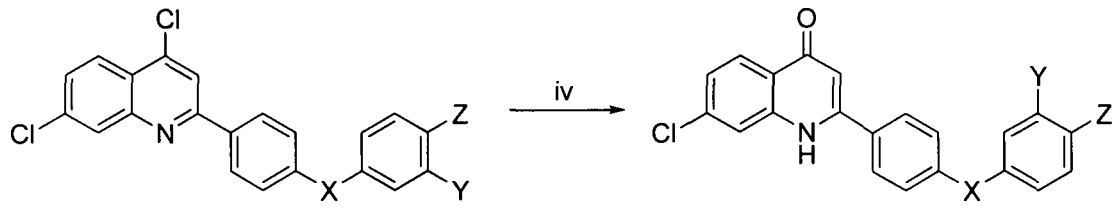
41, 10%

Scheme 12. *Reagents and conditions*: i, ii) *n*-BuLi, 4,7-dichloroquinoline, THF, -78°C, 4 hrs; iii) CAN, acetone, r.t., 30 mins.

Wolf and Lerebours obtained similar 2-substituted-4-chloroquinoline in 67-90% yields with MeLi, *n*-BuLi, *tert*-BuLi and PhLi. It is assumed that the decrease in yield is mainly a result of enhanced halogen-metal exchange under less cryogenic conditions since halogen-directed *ortho*-metalation in position 3 of quinoline was not observed.[44] Our organolithiums which are prepared *in situ* have additional halogens and this could explain why our yields are lower than Wolf's and Lerebours'.[43]

Development of step iv.

Quinolones were obtained via hydrolysis of 4-chloroquinolines **28-30** and filtration of the insoluble product (**Scheme 13**). It has been observed quinolones are very insoluble: **31** and **32** are only soluble in DMSO and **33** is insoluble. This solubility issue could reduce their activity profiles both *in vitro* and *in vivo*.

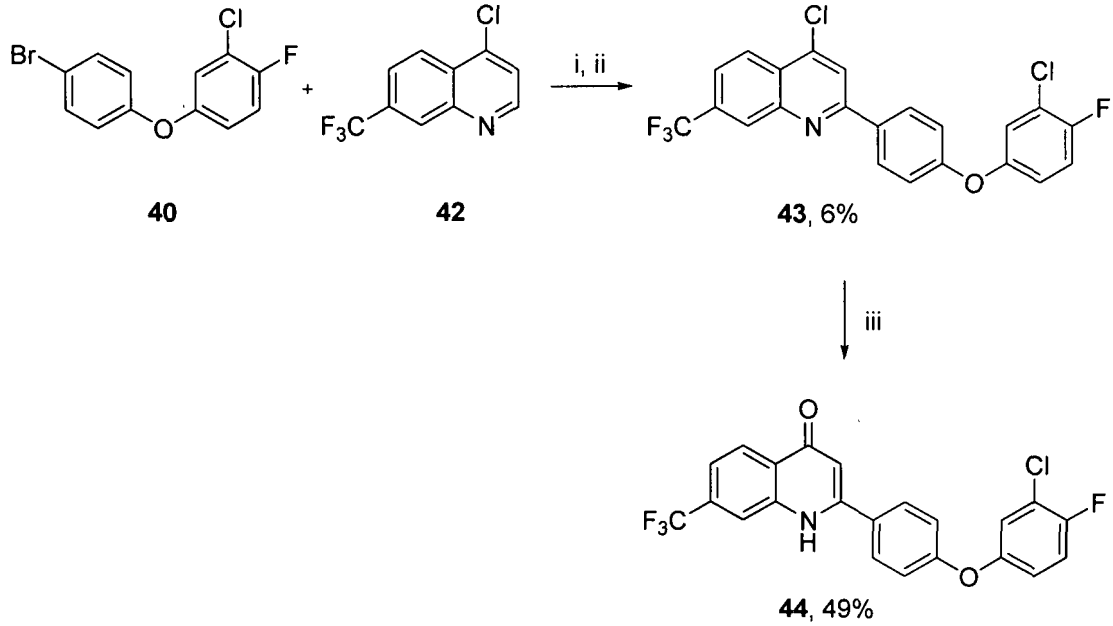


28, X= CH_2, Z= OCF_3, Y= H
29, X= CH_2, Z= CF_3, Y= H
30, X= O, Z= OCF_3, Y= H

31, X= CH_2, Z= OCF_3, Y= H, 66 %
32, X= CH_2, Z= CF_3, Y= H, 43 %
33, X= O, Z= OCF_3, Y= H, 77 %

Scheme 13. *Reagents and conditions*: iv) 85 % $HCOOH/H_2O$, DMF, reflux, 4 hrs.

In an attempt to increase the solubility of quinolones analogues we performed a similar synthesis from 4-chloro-7-trifluoromethylquinoline. Many side products were obtained for the Ziegler-alkylation, explaining the low yield of 6% (**Scheme 14**). *n*BuLi could deprotonate *ortho* to the chlorine or fluorine atom leading to a benzyne intermediate by elimination of chloride or fluoride.[45]



40 **42** **43**, 6%

44, 49%

Scheme 14. *Reagents and conditions*: i) *n*BuLi, THF, -78°C, 4 hrs; ii) CAN, acetone, r.t., 30 mins; iii) 85 % $HCOOH/H_2O$, DMF, reflux, 4 hrs.

3.2.2. 3-Substituted quinolones

A retrosynthetic analysis shows that quinolones substituted at the 3-position could be made from 3-bromoquinolone and a boronic acid via Suzuki coupling (**Figure 9**).

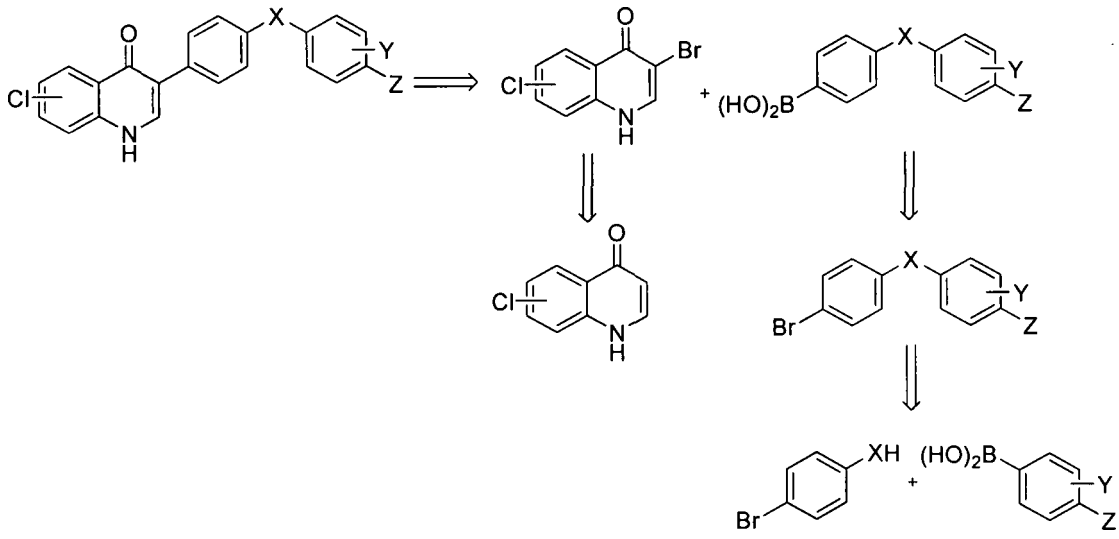


Figure 9. Disconnection.

As quinolones are insoluble, we either protected the 4-hydroxy function as a methyl or benzyl ether or converted the quinolone into a 4-chloroquinoline. A four-step synthesis from 7-chloroquinol-4-one gave a series of quinolone derivatives substituted at the 3-position. The boronic acid used in the third step had to be prepared via parallel synthesis (**Figure 10**).

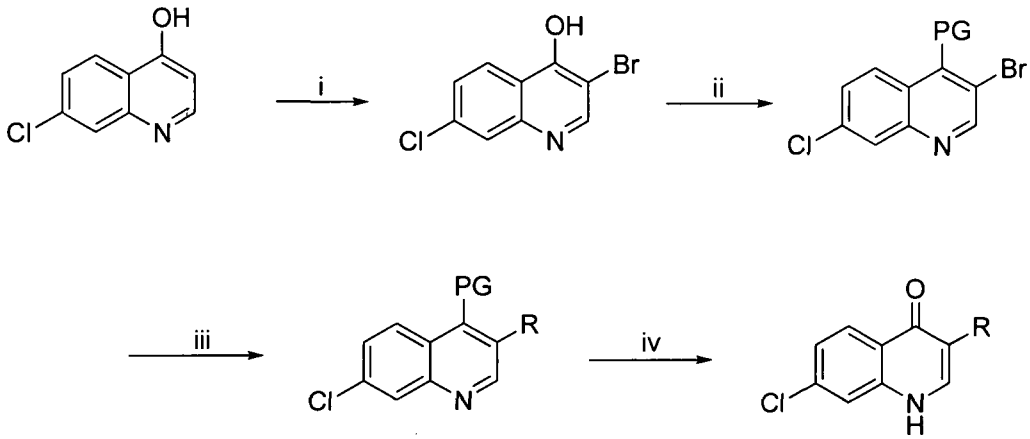
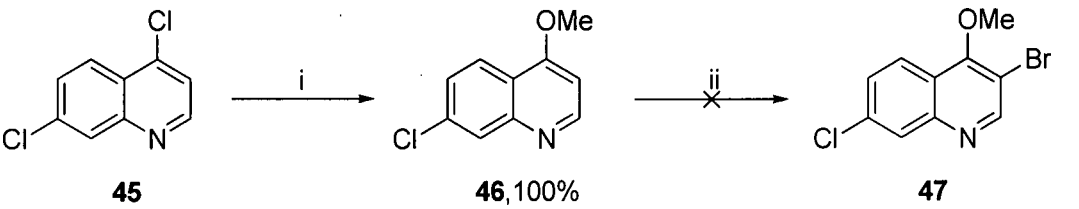


Figure 10. *Steps*: i) Bromination; ii) Protection of hydroxyl group/ chlorination; iii) Suzuki coupling with boronic acid; iv) Hydrolysis.

Details of step i (from **Figure 10**):

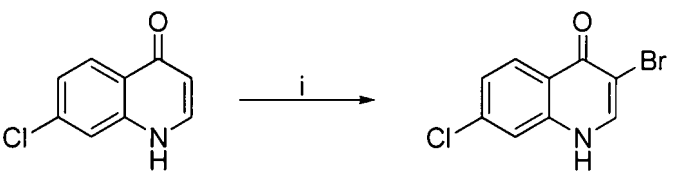
Bromination at the 3-position is essential for a further substitution on this position. We cannot brominate 4-chloroquinoline because it is not nucleophilic enough, therefore 7-chloro-4-methoxyquinoline (**46**) was synthesized from the corresponding 4,7-dichloroquinoline (**45**).[46] However, inspite of the addition of an electron releasing methoxy group in **46** bromination did not give the expected 3-bromo-4-methoxyquinoline (**47**)[47] (**Scheme 15**).



45 **46**,100% **47**

Scheme 15. *Reagents and conditions*: i) NaOMe, MeOH, reflux, 2.5 days; ii) Br_2, I_2 (cat), CH_3COOH, r.t., 2 hrs.

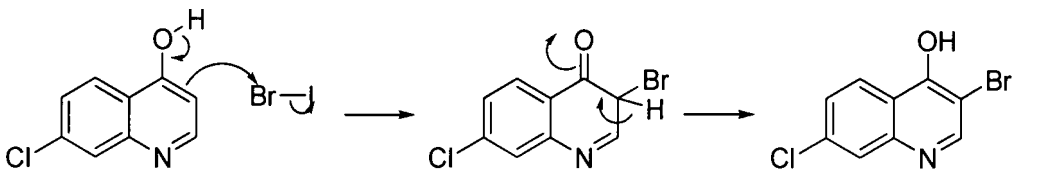
In contrast 7-chloro-quinol-4-one was successfully brominated in quantitative yield (**Scheme 16**).[47]



48 **49**, 100 %

Scheme 16. *Reagents and conditions*: i) Br_2, I_2 (cat.), CH_3COOH, r.t., 2 hrs.

We reasoned that for this reaction to occur the 4-position needs to be an hydroxyl group and a plausible mechanism is depicted in **Scheme 17**.



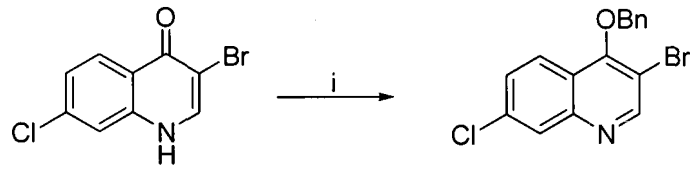
48 **50** **49**

Scheme 17. Suggested mechanism for bromination.

Details of step ii (from **Figure 10**):

3-Bromo-7-chloroquinol-4-one is highly insoluble in many organic solvents. Therefore, we protected the hydroxyl group to increase solubility (**Scheme 18**); the high acidity of the hydroxyl group affords better yields for its protection.

Protection of hydroxyl groups as esters or ethers are the most used methods. Fluoride is a catalyst for base-catalysed reactions and has a high capacity for hydrogen-bond formation. However due to its hygroscopic property, this catalyst is not easy to handle. To be less hygroscopic, caesium fluoride is absorbed on celite.[48]



49 **51**, 60%

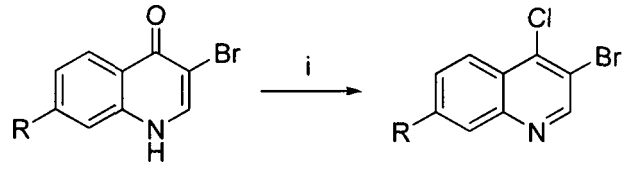
Scheme 18. *Reagents and conditions*: i) BnBr, CsF, DMF, r.t., overnight.

An alternative way to protect the hydroxyl group uses the Mitsonobu reaction, using benzyl alcohol as a nucleophile (**Scheme 19**).

49 **51**, 87%

Scheme 19. *Reagents and conditions*: i) BnOH, PPh_3, DEAD, DMF, reflux, 16 hrs.

The chlorination of 3-bromo-7-chloroquinol-4-one gave 3-bromo-4,7-dichloroquinoline in very good yields which could be used for further Suzuki coupling (**Scheme 20**).[49, 50]



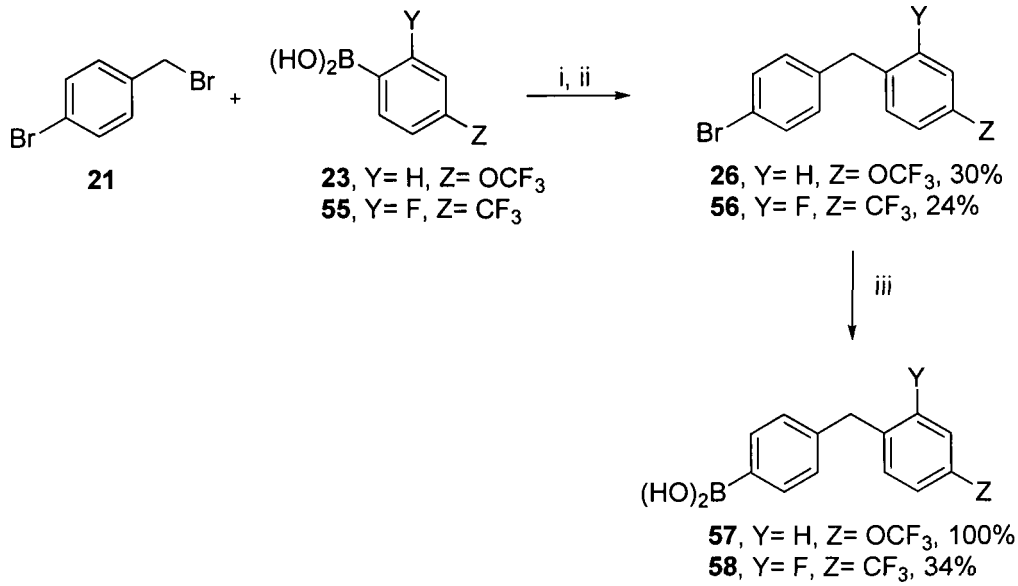
R= Cl: **49**
R= H: **52**

R= Cl: **53**, 77%
R= H: **54**, 85%

Scheme 20. *Reagents and conditions*: i) $POCl_3$, reflux, 30 mins.

Explanation of step iii (from **Figure 10**):

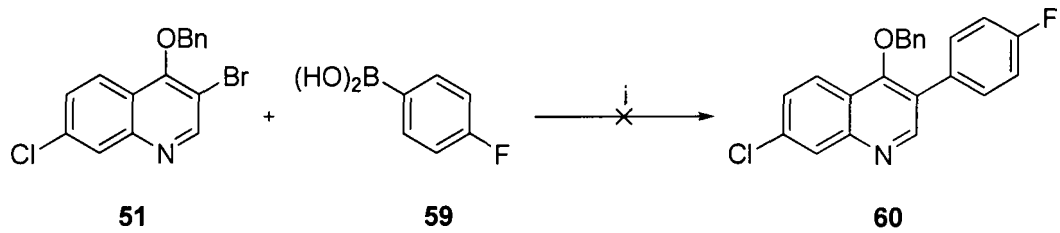
Boronic acids required for Suzuki coupling were synthesised via lithiation and boronation (**Scheme 21**).[51]



Scheme 21. *Reagents and conditions*: i) Na_2CO_3, $Pd[PPh_3]_4$, EtOH/ toluene, reflux, 16 hrs; ii) piperidine, toluene, reflux, 2 hrs; iii) *n*-BuLi, $B(OiPr)_3$, THF, -78°C to r.t., 16 hrs, H_3O^+/H_2O.

Lithiation-boronation didn't work as well as expected, this might be explained by the fact that the extra fluoride can be a good leaving group, which makes the proton in the β-position more acidic; *n*BuLi can take an aromatic proton and this is followed by the fluoride group leaving to form a benzyne.[45]

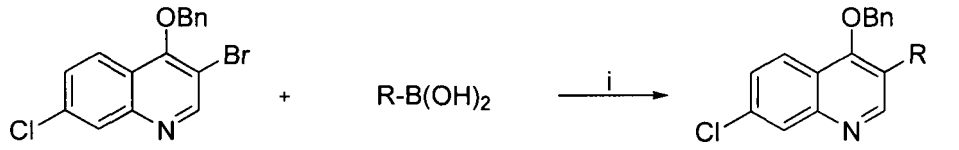
As already noted several palladium catalysts can be used for Suzuki coupling. Firstly, palladium (II) acetate with triphenyl phosphine was used as catalyst to attempt the coupling. As the starting material was recovered, we reasoned it may be due to the base, triethylamine, being too weak (**Scheme 22**).[52, 53]



51 **59** **60**

Scheme 22. *Reagents and conditions*: i) $Pd(OAc)_2$, PPh_3, NEt_3, DME, reflux, 16 hrs.

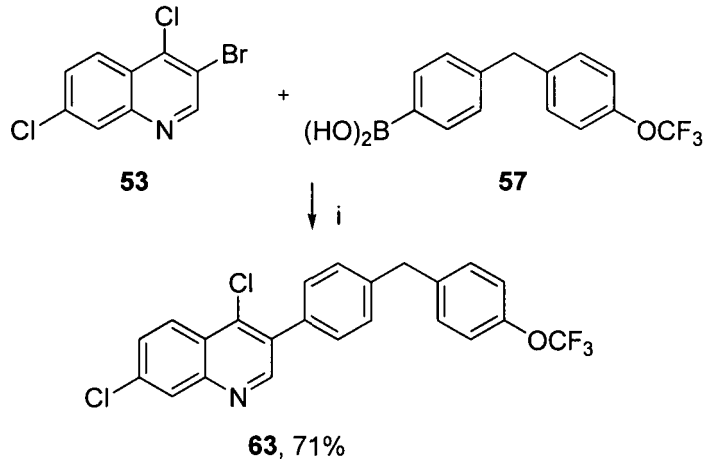
Preformed $Pd(PPh_3)_4$ was then tried as a catalyst (**Scheme 23**). This proved successful however, if more than 1 equivalent of boronic acid is added, substitution of the chloride group at the 7-position also takes place.



51 **59**, R= PhF; **61**, R= PhOPh; **60**, R= PhF, 80 %; **62**, R= PhOPh, 67 %

Scheme 23. *Reagents and conditions*: i) Na_2CO_3, $Pd(PPh_3)_4$, DME, reflux, 16 hrs.

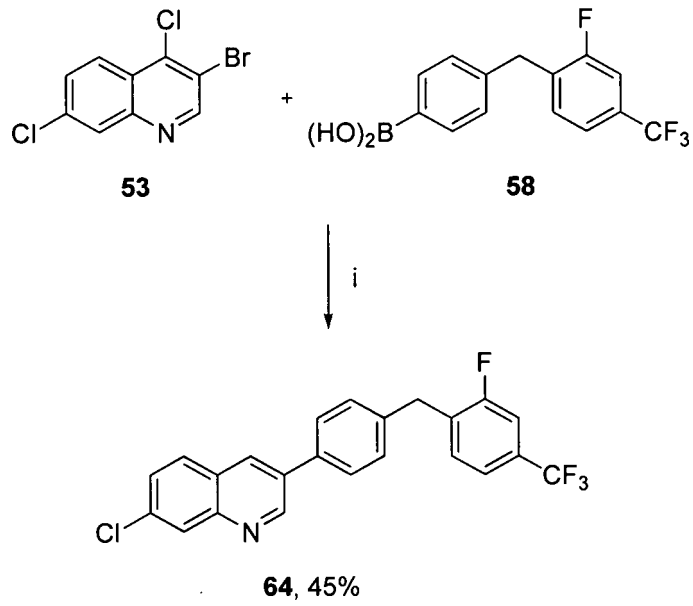
Suzuki coupling with a chloride group at the 4-position of quinoline also gave good yields (**Scheme 24**).



53 **57** **63**, 71%

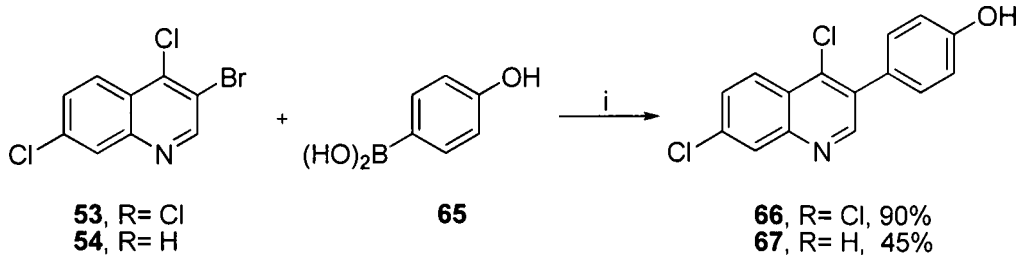
Scheme 24. *Reagents and conditions*: i) Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH, reflux, 16 hrs.

When an excess of catalyst was used in order to increase the yield of the reaction, C-Cl bond at the 4-position was broken, which gives the product **64** (**Scheme 25**).



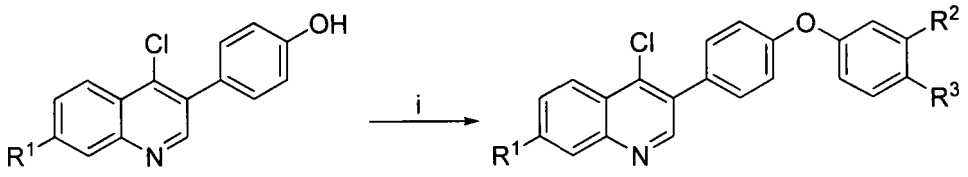
Scheme 25. *Reagents and conditions*: i) Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH, reflux, 16 hrs.

Suzuki coupling with 4-hydroxyphenyl boronic acid gave a lower yield, which can be explained by the hydroxyl group on the boronic acid chelating with the Pd catalyst. **66** and **67** can be used for further copper coupling (**Scheme 26**).



Scheme 26. *Reagents and conditions*: i) Na_2CO_3, $Pd(PPh_3)_4$, toluene, EtOH, reflux, 16 hrs.

A modular approach for the synthesis of 3-biaryletherquinoline (**68-73**) was used via $Cu(OAc)_2$-mediated[40, 42] arylation of phenols **66** and **67** with aryl boronic acid in the presence of triethylamine in good yields (**Scheme 27**).

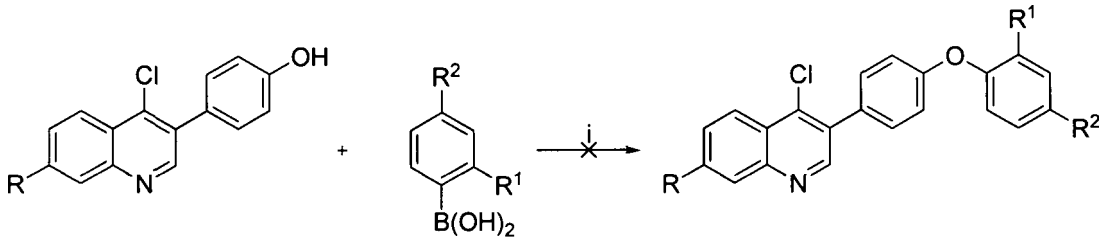


66, R^1= Cl, 45%
67, R^1= H, 90%

68, R^1= Cl, R^2= H, R^3= F, 63%
69, R^1=Cl, R^2= H, R^3= OCF_3, 68%
70, R^1=Cl, R^2= H, R^3= CF_3, 95%
71, R^1=Cl, R^2= H, R^3= tBu, 80%
72, R^1=H, R^2= OCF_3, R^3= H, 75%
73, R^1=H, R^2= Cl, R^3= F, 50%

Scheme 27. *Reagents and conditions*: i) boronic acid, $Cu(OAc)_2$, NEt_3, DCM, mol. sieves, r.t., 24-48 hrs.

It was observed that the copper couplings left for 48 hours gave better yields than after 24 hours. In addition copper coupling doesn't take place with substituents at the *ortho* position (**Scheme 28**).[40]



66, R= H
67, R= Cl

74, R^1= Cl, R^2= CF_3
75, R^1=F, R^2= F

76, R^1= Cl, R^2= CF_3
77, R^1=F, R^2= F

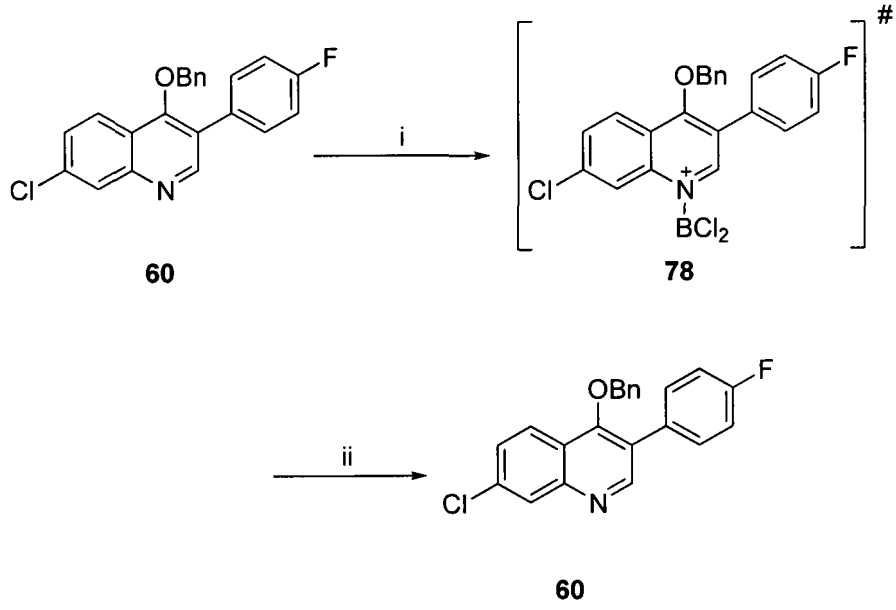
Scheme 28. *Reagents and conditions*: i) $Cu(OAc)_2$, NEt_3, DCM, mol. sieves, r.t., 24-48 hrs.

Details of step iv (see **Figure 10**):

The final step was to obtain the quinolone analogues via deprotection of the benzyloxy or displacement of the chloride group at the 4-position.

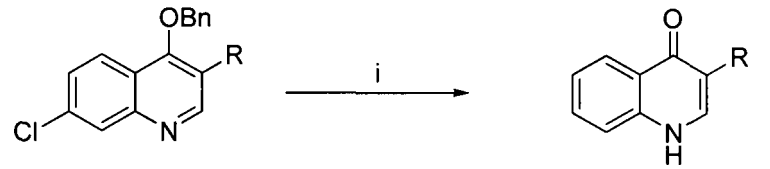
Deprotection of benzyl ether protected quinolone:

To deprotect 4-benzyloxyquinoline, BCl_3 was used as a Lewis acid.[54] During the reaction, we observed a new spot by t.l.c. indicating the formation of a new product. However, following work up only the starting material was obtained. A possible explanation for this was that the lone pair on **60** is complexing to produce **78** which is then recovered following work-up (**Scheme 29**).



Scheme 29. *Reagents and conditions*: i) BCl_3, DCM, r.t., 48 hrs; ii) MeOH, acetone.

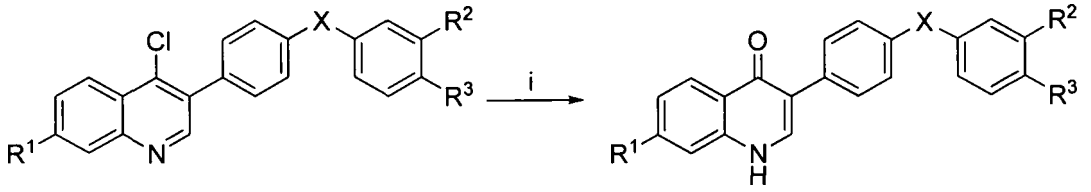
Treatment of the benzyloxyquinolines **60** and **62** with H_2 and catalytic amounts of 10% Pd/C afforded the corresponding quinolone targets **79** and **80** in a single transformation.[55] Although the expected hydrogenation took place, the C-Cl bond was also cleaved by Pd (**Scheme 30**).



Scheme 30. *Reagents and conditions*: i) H_2, Pd/C, MeOH, r.t., 30 mins.

Hydrolysis of 4-chloroquinolones:

To hydrolyse the chloride group at the 4-position, formic acid/water in refluxing DMF was used (**Scheme 31**).



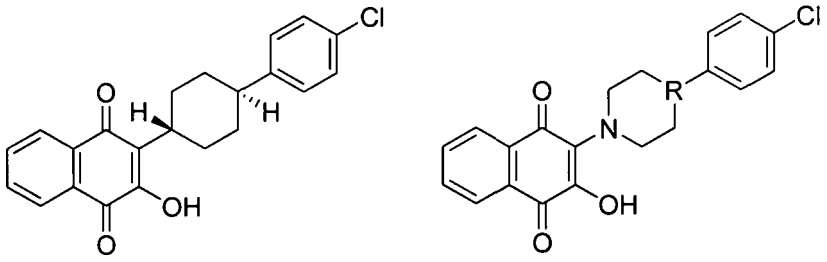
63, R^1=Cl, X=CH_2, R^2=H, R^3=OCF_3
68, R^1=Cl, X=O, R^2=H, R^3=F
69, R^1=Cl, X=O, R^2=H, R^3=OCF_3
70, R^1=Cl, X=O, R^2=H, R^3=CF_3
71, R^1=Cl, X=O, R^2=H, R^3=tBu
72, R^1=H, X=O, R^2=OCF_3, R^3=H
73, R^1=H, X=O, R^2=Cl, R^3=F

81, R^1=Cl, X=CH_2, R^2=H, R^3=OCF_3, 64 %
82, R^1=Cl, X=O, R^2=H, R^3=F, 73 %
83, R^1=Cl, X=O, R^2=H, R^3=OCF_3, 77 %
84, R^1=Cl, X=O, R^2=H, R^3=CF_3, 59 %
85, R^1=Cl, X=O, R^2=H, R^3=tBu, 56 %
86, R^1=H, X=O, R^2=OCF_3, R^3=H, 89 %
87, R^1=H, X=O, R^2=Cl, R^3=F, 60 %

Scheme 30. *Reagents and conditions*: i) 85 % $HCOOH/H_2O$, DMF, reflux, 4 hrs.

3.2.3. Naphthquinone

Atovaquone is an expensive drug because its synthesis requires separation of the diastereoisomers. We set out to make a similar molecule to Atovaquone with nitrogen instead of carbon to remove the chiral centers (**Figure 11**).

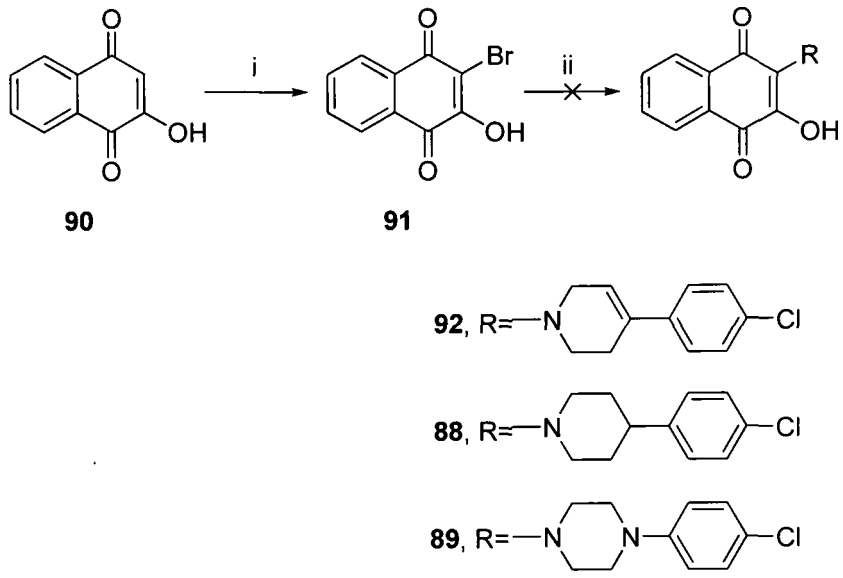


4

88, R= CH
89, R= N

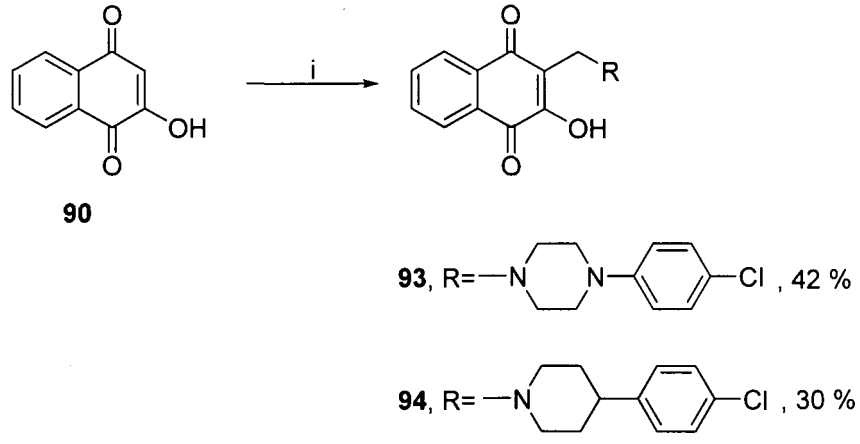
Figure 11. Atovaquone and target molecules.

Tandon and co-workers describe the synthesis of 2-*N*,*N*-dialkylamino-1,4-naphthoquinones in one or two steps; the reaction of 1,4-naphthoquinone (**90**) and its bromo derivative (**91**) with some aliphatic secondary amines give the desired naphthoquinones derivatives. It is stated that better yields (although yields were not specified) were obtained from **91**.[56] The same way, naphthoquinone was brominated and substitution of the bromide with a variety of amines was attempted, unfortunately only starting material was isolated (**Scheme 31**).



Scheme 31. *Reagents and conditions*: i) Br_2, I_2 (cat), AcOH, r.t., 2 hrs; ii) amine, abs. EtOH, reflux, 5 hrs.

The Mannich reaction was used as an alternative to functionalise at the 3-position (**Scheme 32**). The yields observed were moderate but unoptimised.



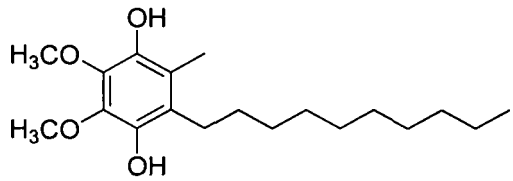
Scheme 32. *Reagents and conditions*: i) CH_2O, amine, CH_3COOH, abs EtOH, r.t., 2 hrs.

3.3. Antimalarial Activity and SAR Studies

EC_{50}, or the half maximal effective concentration, measure the drug potency for which 50% of the population exhibits a response. For agonist/stimulator assays the most common measure is the EC_{50}. We evaluated the *in vitro* activity of 2-phenoxyphenylquinolone (**18**), 3-phenoxyphenylquinolone (**80**) and atovaquone (as a reference) against bc_1 complex of *Plasmodium falciparum* (EC_{50}); for all quinolones, bc_1 complex activity was determined by monitoring the reduction of cytochrome *c* with decylubiquinol (QH_2) (**Scheme 33**) as electron donor. EC_{50} values were calculated using the four-parameter logistic method (Grafit).

For parasite assays IC_{50} is the most common measure of the *in vitro* activity, we measured the IC_{50}s of **18**, **93** and atovaquone against chloroquine-sensitive (3D7) strains.

Atovaquone has a much better activity than our two synthetic quinolones and naphthoquinone, we hoped the addition of functional groups on the quinolones analogues would increase their *in vitro* activities. The isomeric quinolones substituted on the 2 and 3 position show similar *in vitro* activities (**Table 1**). The *in vitro* activity observed for **93** was similar than the quinolones analogues, but many difficulties were observed with its synthesis due to the low solubility of naphthquinone and some instability, therefore we decided to concentrate on novel quinolone synthesis.



Scheme 33. Decylubiquinol

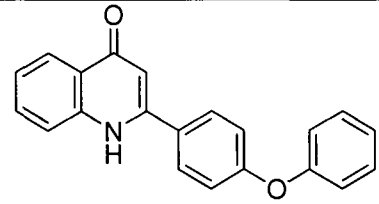
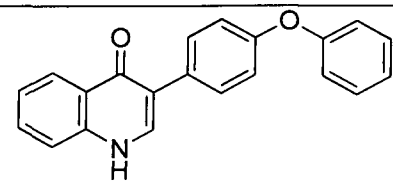
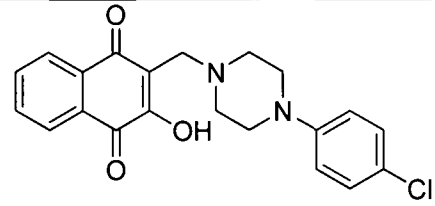
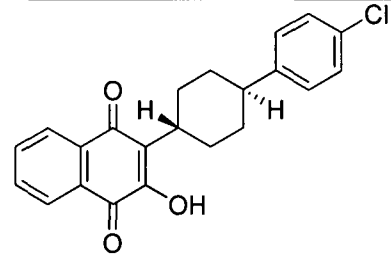
Inhibitor	Structure	EC_{50} ± SEM (nM)	IC_{50} ± SEM (nM)
2-(4-phenoxy-phenyl)quinol-4-one (**18**)		41 ± 8	244 ± 55
3-(4-phenoxy-phenyl)quinol-4-one (**80**)		56 ± 8	ND
2-((4-(4-chlorophenyl)piperazin-1-yl)methyl)-3-hydroxynaphthalene-1,4-dione (**93**)		ND	350 ± 62
Atovaquone		3 ± 2	1 ± 0.2

Table 1. *In vitro* activities: EC_{50} measures against enzyme bc_1 complex of *P.falciparum* and IC_{50}s against chloroquine-sensitive (3D7) strains.

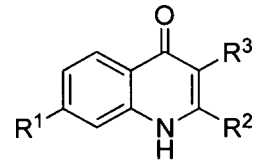
To develop a better understanding of the antimalarial structure-activity relationships of novel quinolones, we tested the ten analogues synthesised against *P. falciparum* to obtain their IC_{50} (**Table 2**).

There is a difference between IC_{50} against parasite and EC_{50} for inhibiting the enzyme bc_1 complex. As we did not obtain the EC_{50} of the quinolones tested, we assumed, in our analysis, that parasite inhibition is directly proportional to bc_1 complex inhibition.

Comparison of *in vitro* activities for **31** and **33** demonstrates the bridge between the two phenyl rings is optimal with $-CH_2-$. **31** is more active than **32**,

therefore the functional group $-OCF_3$ may have a better interaction with the cytochrome bc_1 complex than $-CF_3$. The analogues substituted at the 2-position, **31-33**, have better activities than these substituted at the 3-position **81-87**. Looking at the *in vitro* results for the 3-substituted quinolones **81-87**, the functional groups of choice would be $-OCF_3$ at the *meta* position, or –Cl *meta* and –F *para*. Fluoro and *tert*-butyl analogues (**82** and **85**) are insoluble in DMSO, which could explain their lack of activity, also **84** has a bad solubility and shows no activity either.

The most potent compound, **31**, shows an activity of 30 nM. It appears that the best profile for a quinolone derivative is substitution at 2-position (**Table 2**).



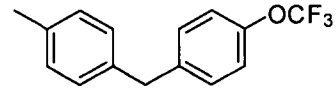
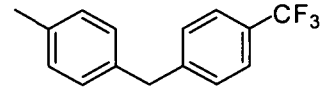
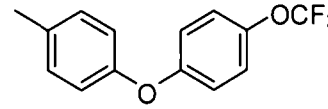
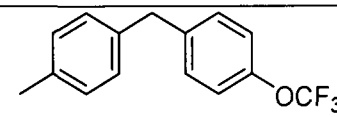
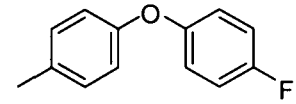
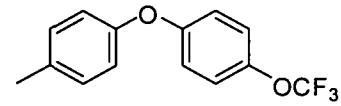
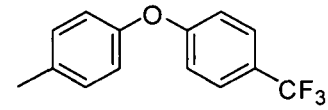
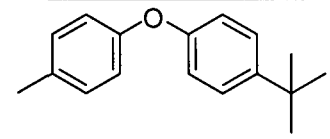
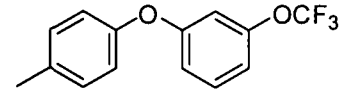
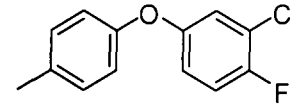
Compounds	R^1	R^2	R^3	IC_{50} (nM)
31	-Cl		-H	30
32	-Cl		-H	139
33	-Cl		-H	185
81	-Cl	-H		> 1000
82	-Cl	-H		> 1000
83	-Cl	-H		> 1000
84	-Cl	-H		> 1000
85	-Cl	-H		> 1000
86	-H	-H		387
87	-H	-H		455

Table 2. *In vitro* antimalarial activity of library of 10 novel quinolones against *P. falciparum* chloroquine-sensitive 3D7.

Further *in vitro* tests have been carried on quinolones **31-33**, **81** and **83** against *P. falciparum* bc_1 complex, yeast (wildtype and mutant) and the bc_1 of rat liver preparations.

All quinolones tested have low *in vitro* activities in the nM against parasite and in the μM against mammalian bc_1 which means that the quinolones inhibit selectively the parasite; thus the therapeutic index (TI) are very high (almost 1000 as the values are nM compared to μM), this is good as it should reduce the chances of toxicity.

2-substituted quinolone **31** looks very promising given the therapeutic index against the enzyme. Also the IC_{50} of 3.5 nM against the bc_1 complex of *P.falciparum* is really low, which means the drug is a good inhibitor of the targeted enzyme (**Table 3**).

Code	**Growth inhibition IC_{50}, *P. f.* 3D7 (nM)**	**P.f. IC_{50} bc_1 (nM)**	**IC_{50} Yeast wildtype (μM)**	**IC_{50} Yeast (PF3 mutant) (μM)**	**IC_{50} bc_1 Rat Liver (μM)**
31	30.3	3.5	No effect (14)	No effect 28	At 7.2, 21% inhibition of activity
32	138	TBD	TBD	TBD	TBD
33	185	TBD	2.36	1.30	At 7.2, 18% inhibition of activity
81	>1000	TBD	No effect (2.5)	TBD	At 7.2, 31% inhibition of activity
83	>1000	TBD	No effect (2.5)	TBD	At 7.2, 10% inhibition of activity

Table 3. IC_{50}s for selected compounds versus *P.f.* and bc_1 complex.

3.4. Molecular modelling

In addition to the synthesis and antimalarial assessment of analogues we were also interested in performing modelling studies in yeast bc_1 model (a surrogate of *Plasmodium falciparum* bc_1 complex).

These docking models illustrate that 2 and 3 phenoxyphenylquinolones are predicted to bind to the same area of the protein as atovaquone (**Figure 12-15**). The initial molecular modelling does not include the Rieske protein and the bc_1 complex was considered as rigid whilst the target molecules were considered fully flexible.

We have looked at both tautomeric forms of all three compounds. In general (considering both isomeric forms) the 2-isomer is predicted to be bound more strongly than the 3 isomer, with the 2-isomer being slightly less strongly bound than atovaquone. However, the differences are really rather small and probably within the error of the function used to estimate binding strength.

The potential antiplasmodial activity is supported by favourable binding energies for *in silico* docking of naphthoquinone and quinolones to Q_o.

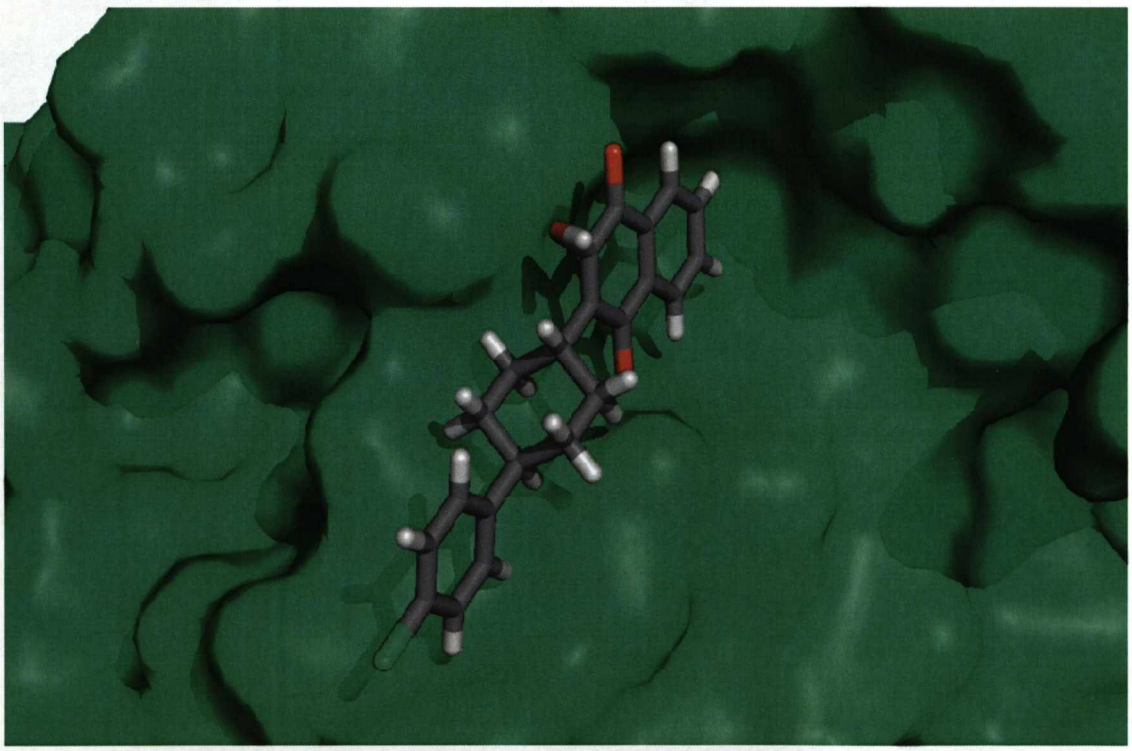


Figure 12. *In silico* docking mode for atovaquone to *P. falciparum* bc_1 Q_0 complex

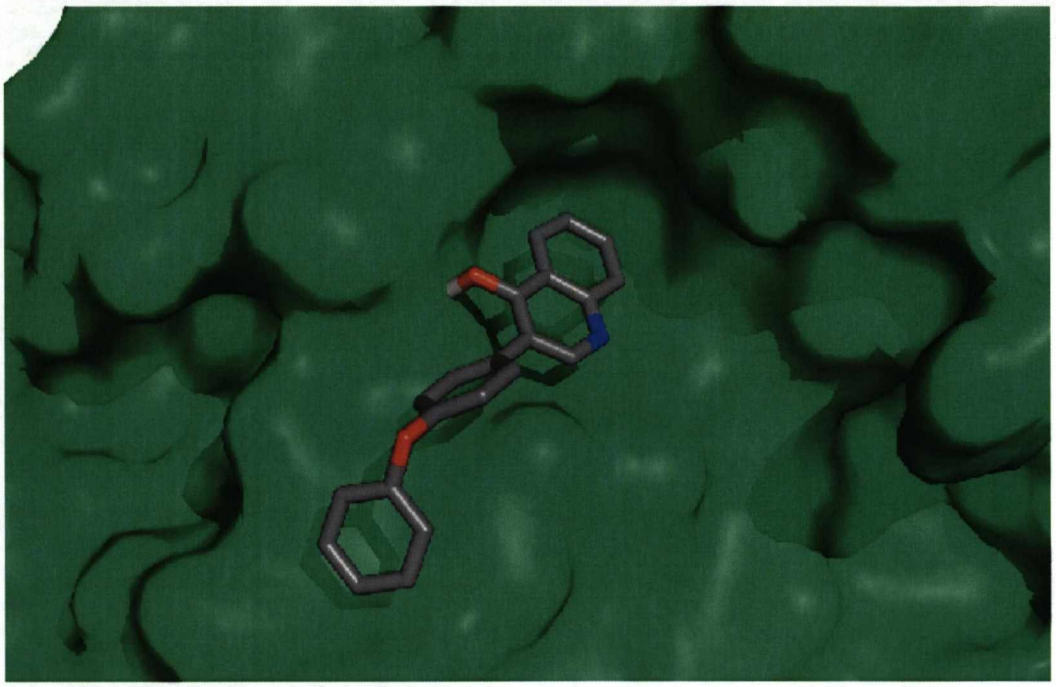


Figure 13. *In silico* docking mode for 3-phenoxyphenylquinolone to *P. falciparum* bc_1 Q_0 complex

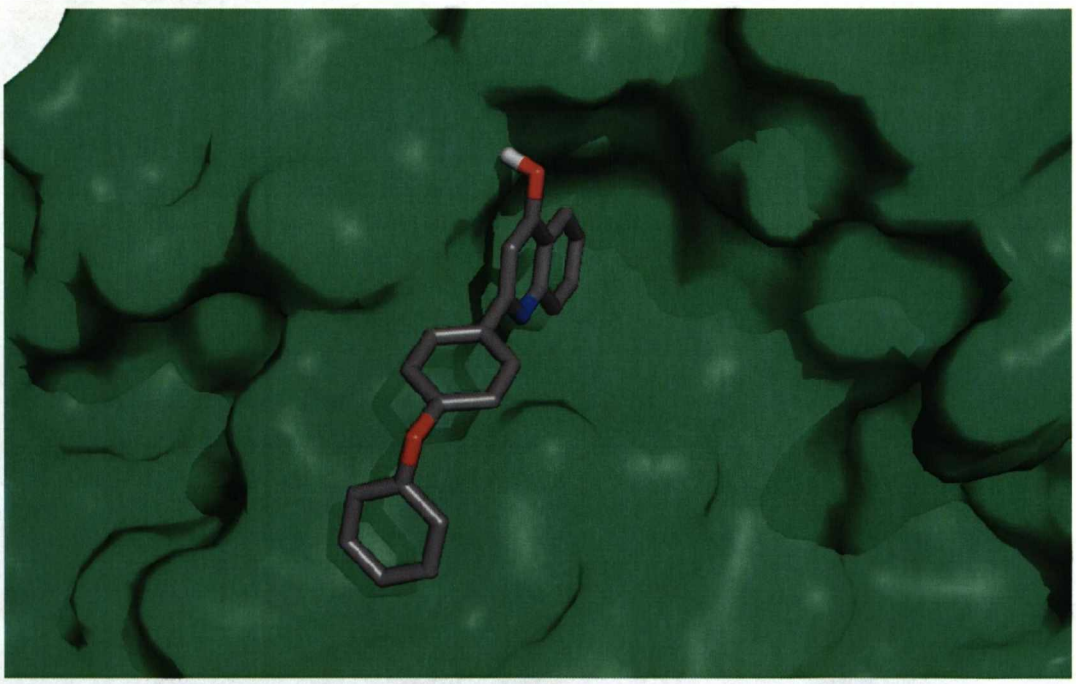


Figure 14. *In silico* docking mode for 2-phenoxyphenylquinolone to *P. falciparum* bc_1 Q_0 complex

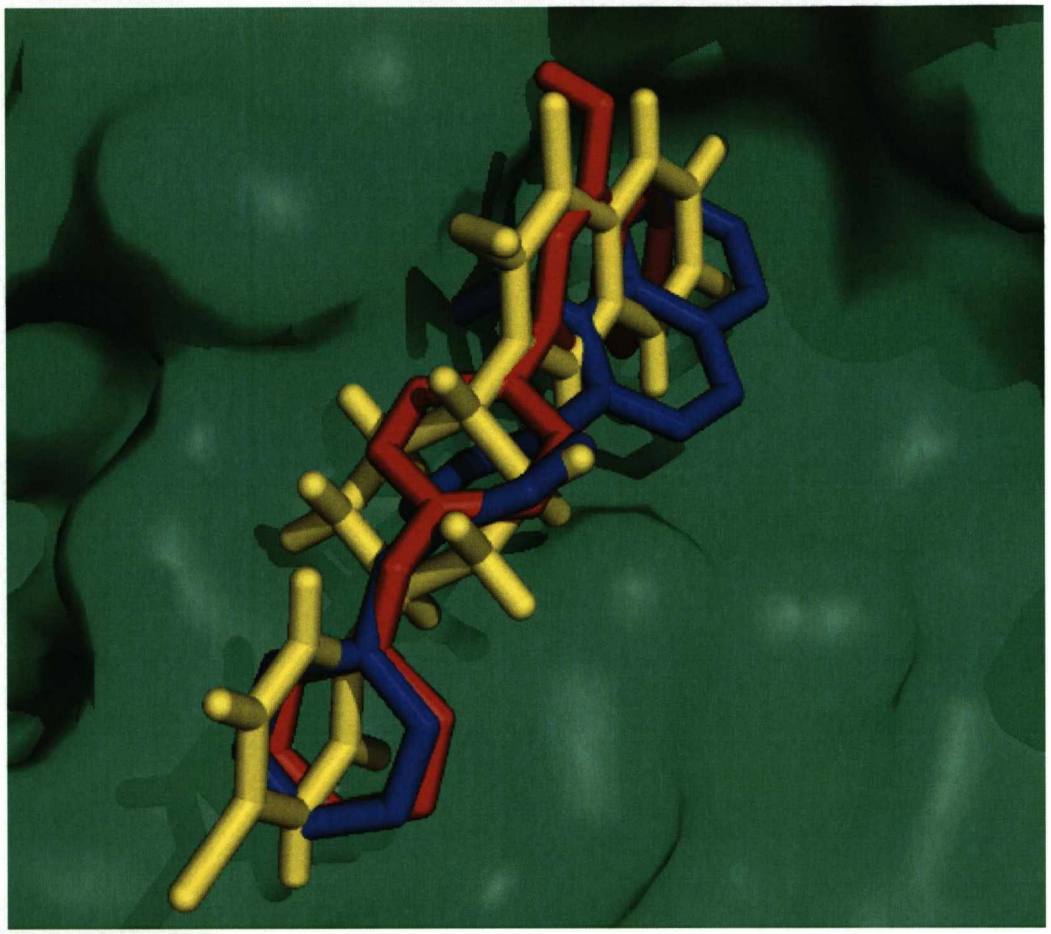


Figure 15. Superposed *in silico* docking for atovaquone, 3-phenoxyphenylquinolone and 2-phenoxyphenylquinolone

Since this study was based on a homology model that lacks the Rieske protein we decided to carry out molecular modelling using GOLD and the yeast bc_1 complex.

GOLD (Protein-Ligand Docking) is a program for calculating the docking modes of small molecules in protein binding sites. It is very highly regarded within the molecular modelling community for its accuracy and reliability.[57]

The starting point for this modelling was the stigmatellin-yeast crystal structure (pdb code 1KYO) which was downloaded from the database. The use of a yeast model serves as a surrogate for Plasmodium bc_1 complex.

Figure 16. Yeast bc_1 complex crystal structure (pdb code 1KYO).

When atovaquone binds to the ubiquinol oxidation pocket of the yeast cytochrome bc_1 complex, it binds specifically to H181 in the Rieske protein and by a water mediated hydrogen bond to glutamate 272 (E272) of cytochrome *b*. According to the molecular modelling, quinolone analogues interact in a similar way to atovaquone.[58]

Quinolone analogues **31, 32, 33, 85, 86** present the best fit docking pose with the quinolone inside the binding pocket and the C2 / C3 side chain in the pocket channel. In **31, 32** and **33** (lowest IC_{50}s) the carbonyl from the quinolyl moiety lies close to H181, whereas the NH faces E272. All three structures are superimposed. As for **85** and **86** the quinolyl moiety is inverted in the binding pocket when compared to the previous three, i.e. the carbonyl nearer to E272 and NH closer to H181.

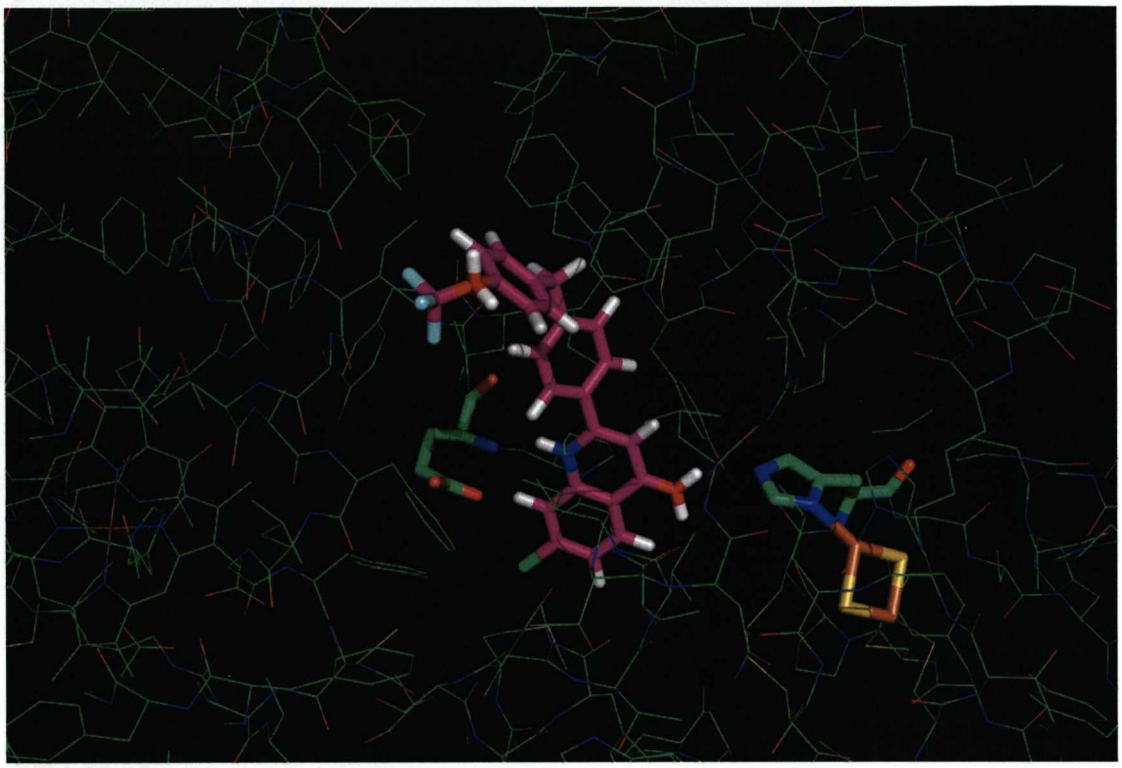


Figure 17. Binding of Quinolone **31** yeast bc_1 complex (pdb code 1KYO)

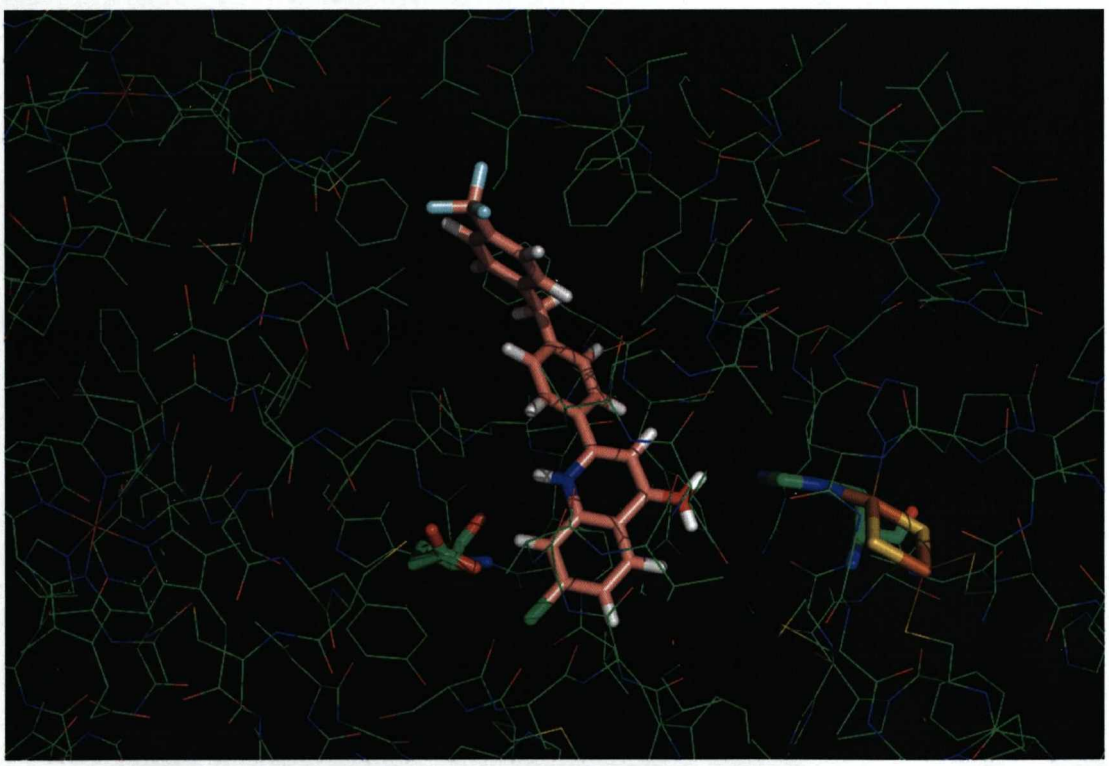


Figure 18. Binding of Quinolone **32** yeast bc_1 complex (pdb code 1KYO)

Conversely, quinolones **81, 82, 83, 84, 85** present the side chain inside the pocket and the quinolyl group in the channel. All five structures bind similarly in the Q_o site.

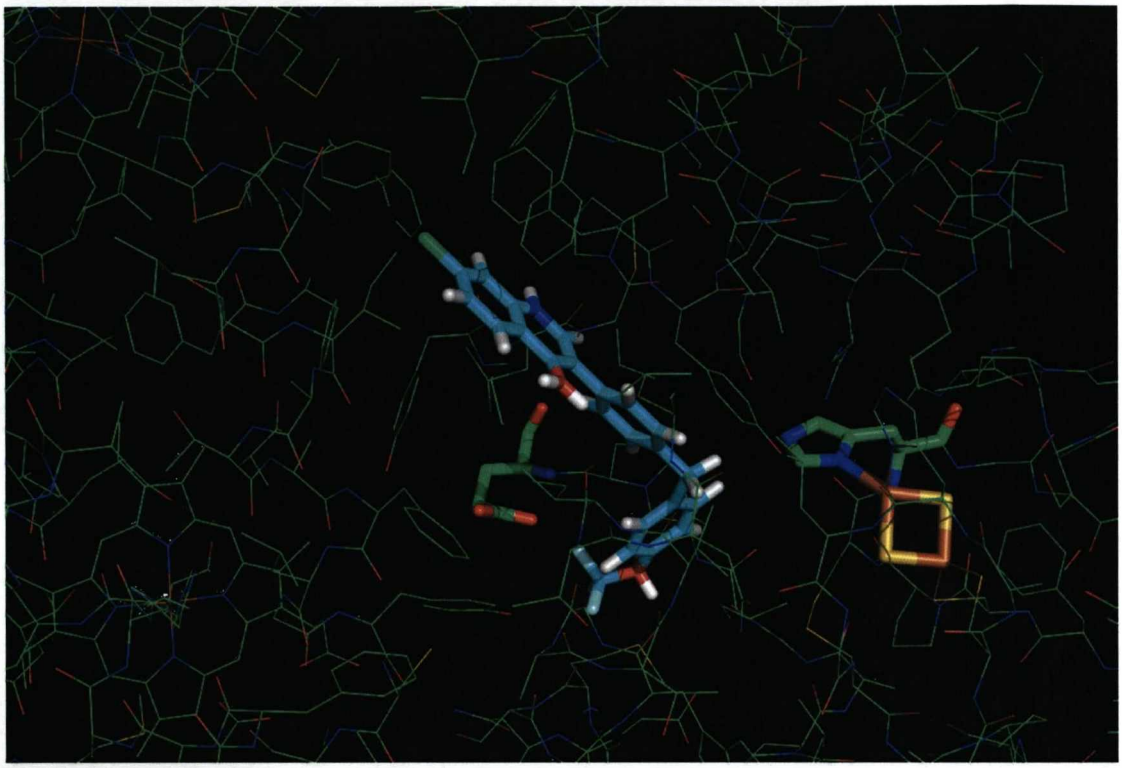


Figure 19. Binding of Quinolone **81** yeast bc_1 complex (pdb code 1KYO)

Substitution at C2 appears to be preferential rather than at C3. **32** displayed a higher GOLDscore than its counterpart **81** (65.39 and 59.61 respectively) as did **33** when compared to **83** (64.60 and 60.56 respectively). This docking study also favours *p*-OCF_3 (**31**) at the terminal aryl instead of *p*-CF_3 (**32**).

Additionally, despite the C3 substitution, **86** binds in a similar way to **33**, except for the quinolyl moiety, which is inverted. The side chains dock in the same way.

A long side chain at C2 (or other that docks in an identical way) appears to be crucial for good antimalarial activity. The preferential orientation of the quinolyl moiety should be when the carbonyl lies closer to H181. The major E-I enzyme interactions should arise from hydrophobic stacking. Hydrogen bonds cannot be

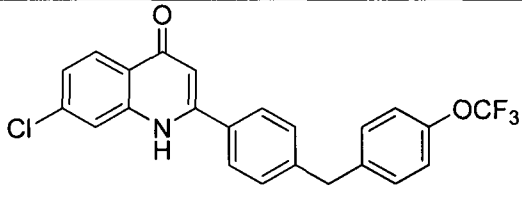
excluded but the good GOLDscore vs IC_{50} correlation should be indicative that this is a good model for this class of compounds (**Table 4-5**).

According to the distance to the glutamate E272, we can see quinolones **81-84** have no interaction, which could explain why their IC_{50}s are over 1 uM showing they have no activity. Quinolone **85** is close from E272 (3.63 Å) and H181 (4.26 Å), although its lack of activity could be due to its insolubility.

Quinolones	Distance to E272 / Å	Distance to H181 / Å
31	4.00 from quinolyl NH	3.07 from C=O
32	4.00 from quinolyl NH	3.04 from C=O
33	3.95 from quinolyl NH	3.00 from C=O
81	-	-
82	-	4.10 from spacer oxygen
83	-	3.46 from spacer oxygen
84	-	3.86 from spacer oxygen
85	3.63 from C=O	4.26 from quinolyl NH
86	3.29 from C=O	4.24 from quinolyl NH
87	-	3.92 from spacer oxygen

Table 4. Distance to glutamate 272 and H181.

A correlation between GOLDScore and IC_{50}s measured for the quinolones with an antimalarial activity against parasite (**31-33, 86-87**) is made. We can observe the lower is the GOLDScore, the lower the IC_{50} measured, which confirms the utility of the molecular modelling (**Table 5-Graphe 1**).

Name	Structure	IC_{50} (nM) 3D7	GOLDscore
31		30	65.39

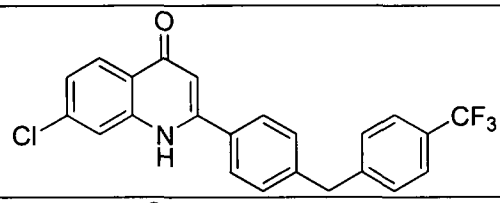
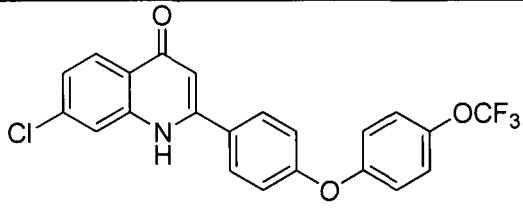
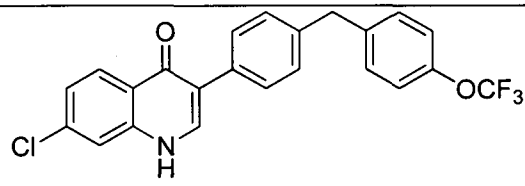
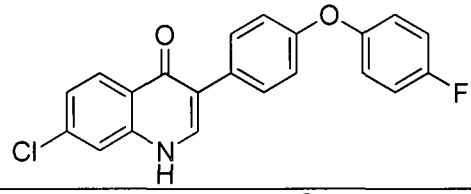
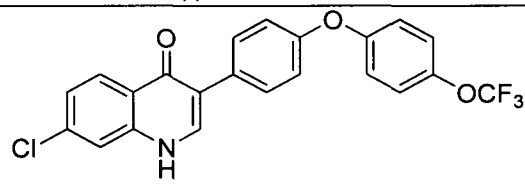
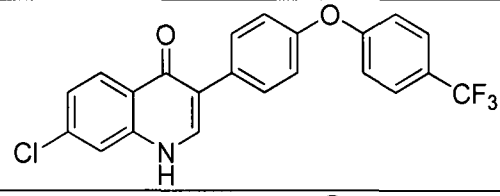
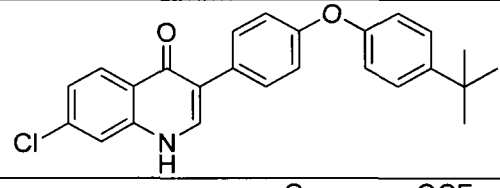
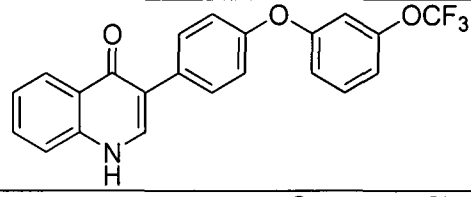
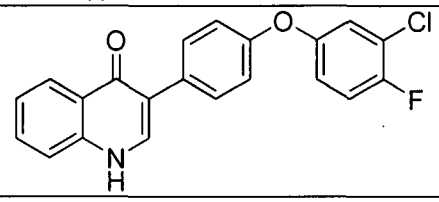
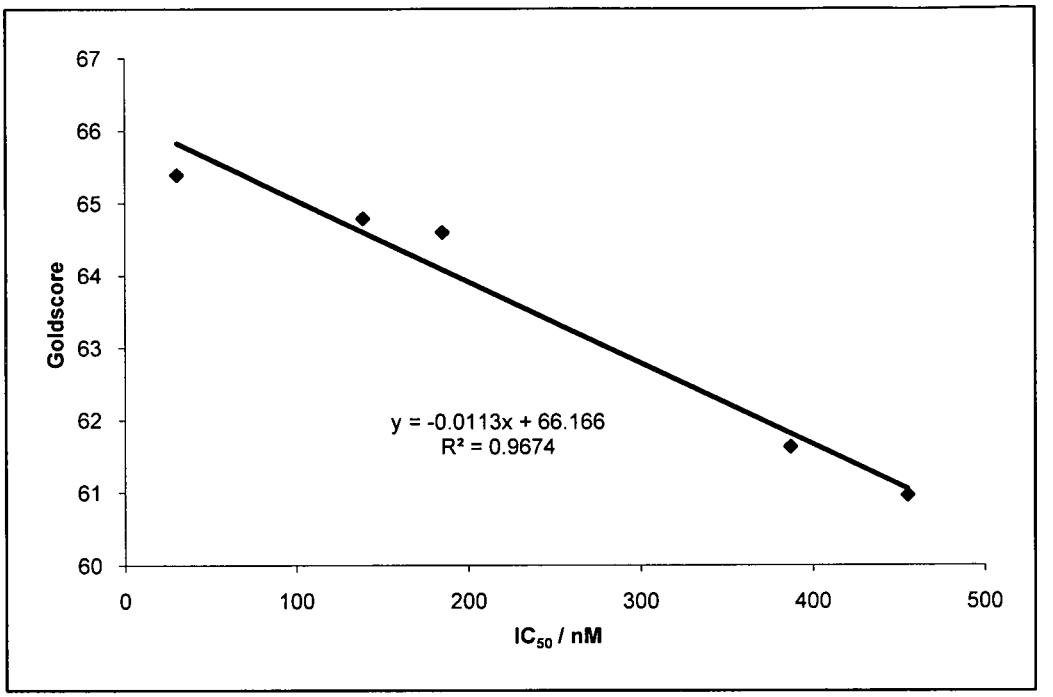
32		139	64.79
33		185	64.60
81		>1000	59.61
82		>1000 Insoluble	57.01
83		>1000	60.56
84		>1000 Insoluble	62.49
85		>1000 Insoluble	54.26
86		387	61.63
87		455	60.96

Table 5. IC_{50}s and GOLDscore of quinolone analogues.

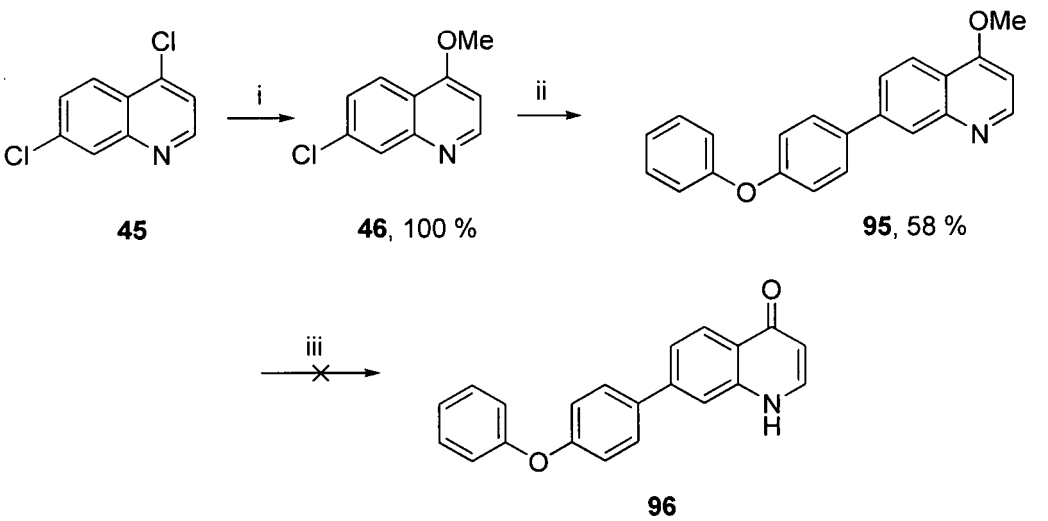


Graphe 1: GOLDScore = f(IC_{50}s).

3.5. Conclusion

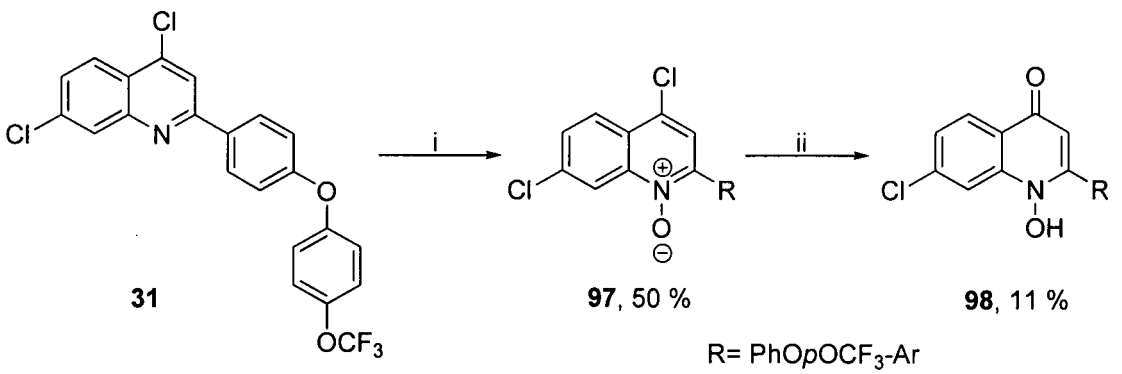
Novel synthetic routes to 2- and 3-aryl quinolone derivatives have been developed. Biological assessment of these quinolones has identified the 2-aryl series as potent antimalarials.

As we observed some disubstituted quinoline on the 3- and 7-position following Suzuki coupling with $Pd(PPh_3)_4$, we tried the following three-step synthesis via methoxylation of 4,7-dichloroquinoline (**45**), followed by Suzuki-type coupling to afford **95** and hydrolysis to produce quinolone analogue (**96**). Unfortunately, the final hydrolysis step in the synthesis was unsuccessful (**Scheme 34**).



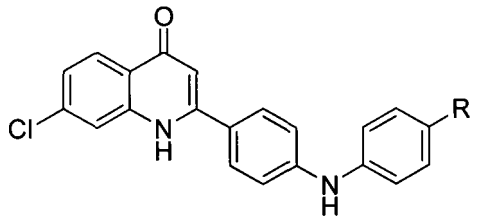
Scheme 34. *Reagents and conditions*: i) NaOMe, MeOH, reflux, 2.5 days; ii) $Pd(PPh_3)_4$, Na_2CO_3, $PhOPhB(OH)_2$, DMF, reflux, 16 hrs; iii) 85 % $HCOOH/H_2O$, DMF, reflux, 4 hrs.

Solubility is one of the main issues for quinolone analogues and some of the analogues (**82**, **84** and **85**) are not soluble in DMSO which could explain their poor activity. To increase the solubility, we would like to synthesise some *N*-hydroxyquinolone analogues. With an IC_{50} of 30 nM, analogue **31** has the best activity and we attempted to increase its activity with the addition of a hydroxyl group on the Nitrogen. Although we obtained some product it is clear that the route will require optimisation, particularly at step ii of the synthesis (**Scheme 35**).



Scheme 35. *Reagents and conditions*: i) *m*-CPBA, DCM, r.t., 1 day; ii) 85 % $HCOOH/H_2O$, DMF, reflux, 16 hrs.

It would also be interesting to synthesise some bi-aryl side-chains with an NH bridge (**Figure 20**), to complete the Structure Activity Relationship studied so far. We could synthesise the NH bridge of these quinolone analogues using (π-allyl)palladium complex,[59] copper-catalysed *N*-arylation[60] for example.



R= OCF_3, CF_3, F, etc.

Figure 20. Quinolone analogues with NH bridge.

Initial molecular modelling predicted superior binding for quinolone analogues substituted at the 2-position, which was confirmed by the *in vitro* results showing that this template leads to inhibition of the target enzyme and the lowest IC_{50}s observed for the 3-substituted quinolone analogues.

Further molecular modelling, using the reliable docking program GOLD, shows 2-substituted quinolone **31** had the highest GOLDscore, which is supported by the best *in vitro* activity result. The other quinolone analogues have high GOLDscore, unfortunately some of them have *in vitro* activities over 1000 nM, which could be the consequence of their poor solubility in DMSO.

3.6. Experimental

Melting points were determined in open tubes in a Gallenkamp, Melting Point Apparatus, and are uncorrected. NMR spectra were recorded on a brucker AC 200 (1H, 200 MHz) and a Brucker AMX 400 (1H, 400 MHz; 13C, 100 MHz) spectrometer. Chemicals shifts are described in parts per million (ppm) downfield from an internal standard of trimethylsilane. Multiplicities are recorded as broad peaks (br), singlet (s), doublets (d), triplets (t), quartets (q), doublet of doublets (dd), doublet of triplets (dt) and multiplets (m). Coupling values are in Hz. Mass spectra were recorded on a VG analytical 7070E machine and Frisons TRIO spectrometers using electron ionisation (EI), chemical ionisation (CI) or electron spray (ES). Microanalyses (%C, %H, %N) were performed in the University of Liverpool Microanalysis laboratory. Reported atomic percentages are within error limits ± 0.5%. IR spectrums were run with a laser Fourier Transform Infra Red Spectrometer (Jasco-FT/IR-4100), solids were run in the solid state. In instances where purity was not determined by elemental analysis, compounds displayed only one observable spot by t.l.c. at the reported R_f.

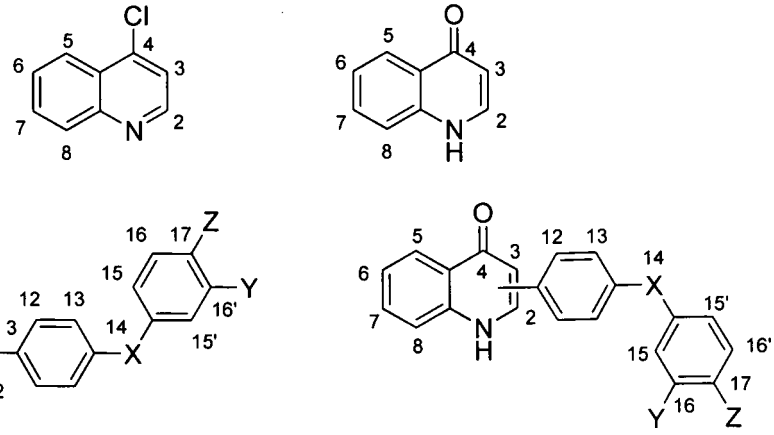
Purification of solvents

Anhydrous solvents were either obtained from commercial sources or dried and distilled immediately prior to use under a constant flow of dry nitrogen. THF was distilled with Na and benzophenone. All other reagents were used as received from commercial sources unless otherwise indicated.

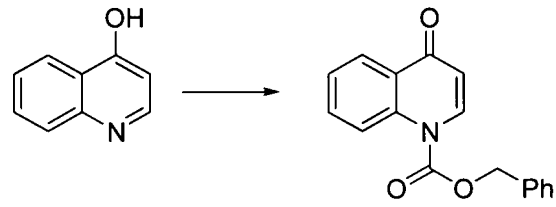
Purification of reagents.

meta-Chloroperbenzoic acid (ca. 77% pure as supplied by the Aldrich Chemical Company) was purified prior to use. Di-sodium hydrogen phosphate (4.32 g, 30.0 mmol) and sodium dihydrogen phosphate (1.18 g, 8.7 mmol) were dissolved in distilled water (1000mL). Commercial *m*CPBA (25.0 g) was washed with the buffer (2×500 mL), filtered and dissolved in DCM (250 mL). The organic extracts were dried over anhydrous magnesium sulfate and the solvent was removed *in vacuo*, furnishing *m*CPBA (ca. 90-100% pure). This was dried under reduced pressure, over P_2O_5 for 1 day. The product was obtained as a white flocculent solid (ca. 15 g).

Quinolone numbering scheme used throughout this analysis:



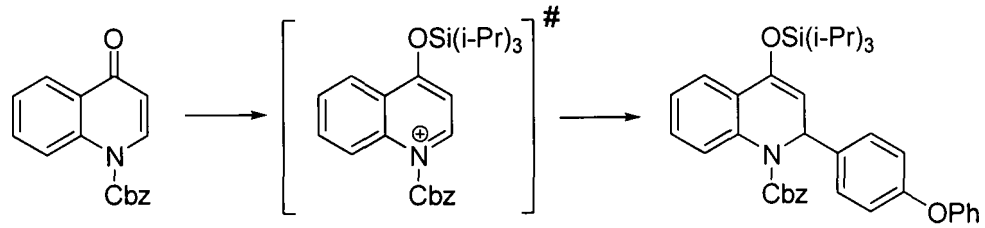
Benzyl 4-oxoquinoline-1(4H)-carboxylate.[31]



14 **15**

4-Hydroxyquinoline (1 g, 6.89 mmol) in THF was added to a stirred suspension of NaH (766 mg, 19.16 mmol, 60% in mineral oil) at r.t.. The resulting mixture was heated at 55 °C for 15 min. Benzyl chloroformate (1.6 mL, 10.25 mmol) was added and the mixture stirred at r.t. overnight (17 hr). T.l.c. indicated the presence of starting material and the reaction was warmed to 55 °C for 2hr. After this time the reaction mixture was poured into water and extracted with EtOAc. The organic layer was washed with water, brine and dried $MgSO_4$. Removal of solvent gave a dark yellow oil which was purified by column chromatography eluting with 40 % EtOAc/*n*-Hexane affording a translucent oil which was crystallized from Et_2O/ *n*-Hexane, giving the title compound as a colourless solid (832 mg, 43%); ^1H NMR ($CDCl_3$, 400 MHz) δ_H 8.67 (d, 1H, J = 9.0 Hz, H2), 8.37 (d, 2H, J = 8.5 Hz, CH), 7.67 (m, 1H, CH), 7.49-7.42 (m, 6H, CH), 6.25 (d, 1H, J = 8.5 Hz, CH), 5.47 (s, 2H, CH_2); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 179.4, 151.7, 138.9, 138.6, 134.4, 133.3, 129.7, 129.4, 129.3, 126.9, 125.9, 120.4, 112.9, 70.9; MS (CI), $[M+H\text{-}Cbz]^+$ (100) 146, $[M+H]^+$ (35) 280.

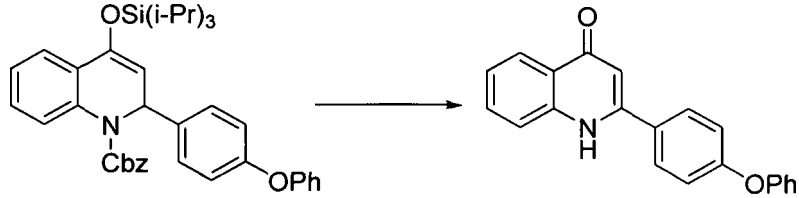
Benzyl 2-(4'-phenoxyphenyl)-4-(triisopropylsilyloxy)quinoline-1(2H)-carboxylate.[31]



15 **16** **17**

Benzyl 4-oxoquinoline-1(4H)-carboxylate (500 mg, 1.79 mmol) was treated with TIPSOTf (962 μL, 3.58 mmol) without stirring under argon for 1 hr. DCM (9 mL), 2,6-lutidine (415 μL, 3.58 mmol) and the freshly prepared Grignard reagent (8.95 mmol) in THF (10 mL) were added and the solution stirred at r.t. for 2 hrs. On completion the reaction mixture was quenched into iced water and extracted with DCM (3×10 mL). The organic extracts were washed with water, brine and dried Na_2SO_4. Purification by column chromatography eluting with hexane increasing to 10 % EtOAc gave the title compound as colourless crystals (485 mg, 45%); mp= 78 °C; ^{1}H NMR ($CDCl_3$, 400 MHz) δ_H 7.61 (d, 1H, J = 7.5 Hz, CH), 7.35-7.04 (m, 14H, CH), 6.94 (d, 1H, J = 7.5 Hz, CH), 6.82 (d, 2H, J = 8.5 Hz, CH), 6.19 (d, 1H, J = 5.5 Hz, CH), 5.34-5.23 (m, 3H, CH, CH_2), 1.28 (3, 3H, 3 x CH), 1.13 (d, 9H, J = 7.0 Hz, 3 x CH_3), 1.05 (s, 9H, 3 x CH_3); ^{13}C NMR ($CDCl_3$, 100 MHz) δ_C 157.4, 157.2, 154.7, 147.2, 136.5, 135.7, 135.5, 130.1, 129.0, 128.9, 128.6, 128.5, 128.4, 127.1, 124.6, 123.7, 122.9, 119.5, 118.9, 104.8, 68.4, 55.4, 18.5, 18.2, 13.2, 12.7; MS (ES+), $[M+Na]^+$ (100) 628; HRMS calcd for $C_{38}H_{43}NO_4SiNa$ $[M+Na]^+$ 628.2859, found 628.2830.

2-(4'-Phenoxyphenyl)quinolin-4(1H)-one.

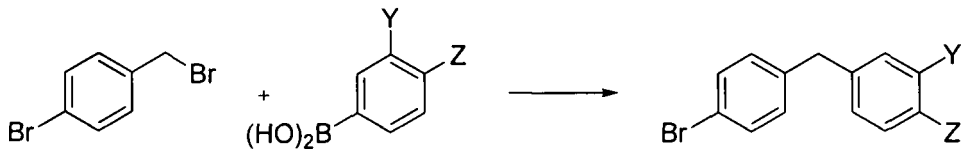


17 **18**

The silyl enol ether (380 mg, 0.63 mmol) and Pd/C (10 mol %) were suspended in anhydrous MeOH (30 mL) under an atmosphere of H_2 and stirred at r.t. for 3 hrs. $CHCl_3$ was added to the metallic looking suspension until all the precipitate had

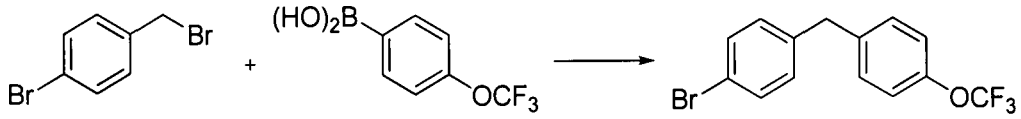
dissolved (~30 mL). The mixture was filtered through celite. Removal of solvent gave a pale solid which was filtered and washed with Et_2O to afford the title compound (109 mg, 55%); mp= 263 °C; ^{1}H NMR (DMSO-d_6, 400 MHz) δ_H 11.7 (bs, 1H, NH), 8.10 (d, 1H, J = 7.0 Hz, CH), 7.87 (d, 2H, J = 8.0 Hz, CH), 7.76 (d, 1H, J = 8.5 Hz, CH),7.67 (t, 1H, J = 7.0 Hz, CH), 7.47 (t, 1H, J = 7.5 Hz, CH), 7.34 (t, 1H, J = 7.5 Hz, CH), 7.23 (t, 1H, J = 7.5 Hz, CH), 7.17 (d, 2H, J = 8.5 Hz, CH), 7.13 (d, 1H, J = 8.0 Hz, CH), 6.33 (s, 1H, CH); ^{13}C NMR (DMSO-d_6, 100 MHz) δ_C 159.3, 156.1, 132.1, 130.6, 129.7, 125.1, 124.7, 123.6, 119.8, 119.0, 118.7, 107.3, 99.5; MS (CI), $[M+H]^+$ (100) 314; HMRS calcd for $C_{21}H_{16}NO_2$ $[M+H]^+$ 314.1181, found 314.1192; Anal. Calcd for $C_{21}H_{15}NO_2$: C, 80.50 %; H, 4.83 %; N, 4.47 %; Found C, 80.52 %; H, 4.83 %; N, 4.44 %.

General procedure 1.[34]



1-Bromo-4-(bromomethyl)benzene (1.05 eq.) and arylboronic acid (1.00 eq.) were dissolved in EtOH and toluene and aqueous Na_2CO_3 (1M) was added and the resulting mixture was deoxygenated with a stream of argon. After 20 min, $Pd(PPh_3)_4$ (0.05 equiv) was added, and mixture was brought to reflux, allowed to stir under argon for 12 hrs and cooled to r.t. . The solution was filtered through celite, washed with Et_2O (50 mL). The aqueous layer was extracted with Et_2O (3×30mL), and the organic phases were combined and washed with 1 M. NaOH (20mL) followed by brine (20 mL). The ethereal solution was dried over $MgSO_4$ and evaporated. Purification of the crude product by flash chromatography with pur PE gave a mixture of starting material and product. The mixture was dissolved in toluene (20 mL) and reacted for 2 hrs with piperidine (2.00 eq. of the estimated starting material left) under reflux. The reaction was judged completed and the solvent was evaporated. Purification of the crude product by flash chromatography with PE gave the pur product.

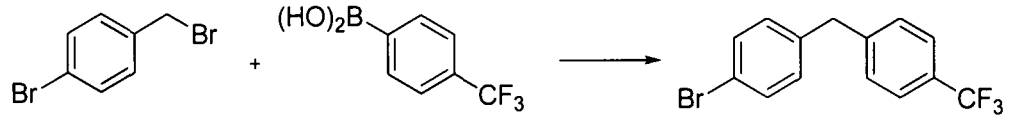
1-Bromo-4-(4'-(trifluoromethoxy)benzyl)benzene.



21 **23** **25**

4-(Trifluoromethoxy)phenylboronic acid (2.50 g, 12.75 mmol) reacted with 1-Bromo-4-(bromomethyl)benzene according to the **general procedure 1** to give a colorless oil (1.22 g, 30 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40 (2H, d, *J*= 8.5 Hz, CH-CBr), 7.14 (4H, m), 7.02 (2H, d, J= 8.2 Hz, CH-CO) and 3.90 (2H, s, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 148.2, 139.8, 139.6, 132.4, 131.1, 130.5, 122.2, 121.5, 120.7 and 41.0 ppm; MS (CI), $[M\text{-}Br]^+$ (100) 251; HRMS calcd for $C_{14}H_{10}BrF_3O$ $[M]^+$ 329.9867, found 329.9863.

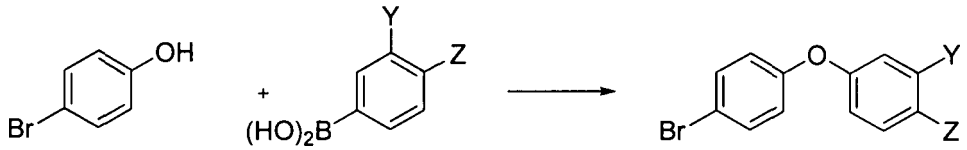
1-Bromo-4-(4'-(trifluoromethyl)benzyl)benzene.



21 **24** **26**

4-(Trifluoromethyl)phenylboronic acid (2.00 g, 10.53 mmol) reacted with 1-bromo-4-(bromomethyl)benzene according to the **general procedure 1** to give a colorless oil (518 mg, 16 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.53 (2H, d, *J*= 8.2 Hz, CH-CBr), 7.41 (2H, d, *J*= 8.4 Hz, CH-), 7.25 (2H, d, *J*= 8.2 Hz, CH-), 7.03 (2H, d, *J*= 8.5 Hz, CH-CCF_3) and 3.96 (2H, s, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 144.9, 139.4, 132.2, 131.1, 129.6, 126.0, 125.9, 123.3, 120.8 and 41.5 ppm; MS (CI), $[M\text{-}Br\text{-}H]^+$ (100) 235; HRMS calcd for $C_{14}H_{10}BrF_3$ $[M]^+$ 313.9918, found 313.9913.

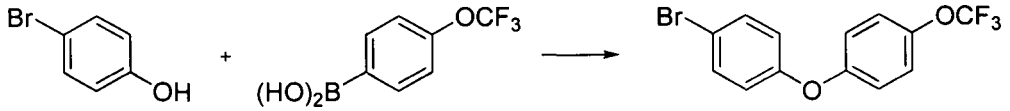
General procedure 2.[40]



A 50 mL round bottom flask was charged with 4-bromophenol (1.00 eq.), $Cu(OAc)_2$ (1.00 eq.), arylboronic acid (2.00 eq.) and powdered 4 Å molecular sieves. The

reaction mixture was diluted in DCM to yield a solution approximately 0.1 M in phenol, and triethylamine (5.00 eq.) was added. After stirring the colored heterogeneous reaction mixture for 18 hrs at 25 °C under ambient atmosphere, the resulting slurry was filtered and the product was isolated from the organic filtrate by flash chromatography (1/9: EtOAc/PE) to give the product.

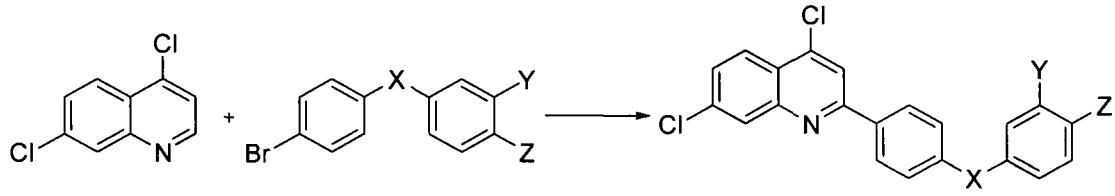
1-Bromo-4-(4'-(trifluoromethoxy)phenoxy)benzene.



22 23 27

4-(Trifluoromethoxy)phenylboronic acid (714 mg, 3.47 mmol) reacted with 4-bromophenol according to the **general procedure 2** to give a colorless oil (409 mg, 71 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.53 (2H, d, J= 9.0 Hz, CH-CBr), 7.18 (2H, d, J= 9.1 Hz, CH), 6.99 (2H, d, J= 9.2 Hz, CH) and 6.89 (2H, d, J= 8.9 Hz, CH-$COCF_3$) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.5, 155.8, 142.1, 133.4, 131.1, 121.5, 117.0, 116.5 and 111.9 ppm.

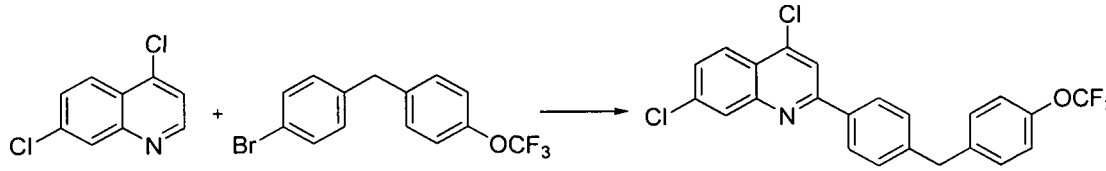
General procedure 3. [43]



The bromide reagent (1.00 eq.) was dissolved in THF (13 mL) in a 2-necked 100 mL round bottom flask and flushed with argon. The solution was cooled at -78°C and *n*-BuLi (1.6 M, 1.10 eq.) was added dropwise and left to stir for 45 mins. 4,7-dichloroquinoline (0.80 eq.) in solution in THF (7 mL) was added to the organolithium dropwise and left at -78 °C for 4 hrs. The reaction was quenched with 10% NH_2OH (15 mL), the reaction mixture was then extracted with DCM (3×10 mL) and washed with water. The organic phase was dried over $MgSO_4$ and the solvents were evaporated. The residue was dissolved in acetone (3.5 mL) and reacted with CAN in water (2.00 g in 10 mL) for 30 mins. The mixture was extracted with DCM (3×30 mL), washed with water (3×10 mL) and dried over $MgSO_4$. Purification

with column chromatography in 1 % EtOAc/*n*Hexane gave the product as a white solid.

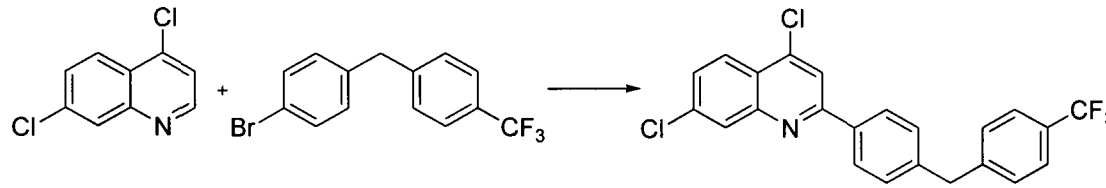
4,7-Dichloro-2-(4'-(4''-(trifluoromethoxy)benzyl)phenyl)quinoline.



25 **28**

1-Bromo-4-(4'-(trifluoromethoxy)benzyl)benzene (646 mg, 1.95 mmol) reacted with 4,7-dichloroquinoline (309 mg, 1.56 mmol) according to the **general procedure 3** to give white crystals (300 mg, 42 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.16 (1H, s, H8), 8.15 (1H, d, *J*= 7.8 Hz, H5), 8.07 (2H, d, *J*= 8.3 Hz, H12), 7.93 (1H, s, H3), 7.53 (2H, dd, *J*= 8.9 and 2.1 Hz, H6), 7.34 (2H, d, *J*= 8.5 Hz, H13), 7.23 (2H, d, *J*= 8.9 Hz, H15), 7.15 (2H, d, *J*= 8.7 Hz, H16) and 4.07 (2H, s, H14) ppm; MS (ES+), $[M+H]^+$ (100) 448; HRMS calcd for $C_{23}H_{15}NOF_3Cl$ $[M+H]^+$ 448.0483, found 448.0491.

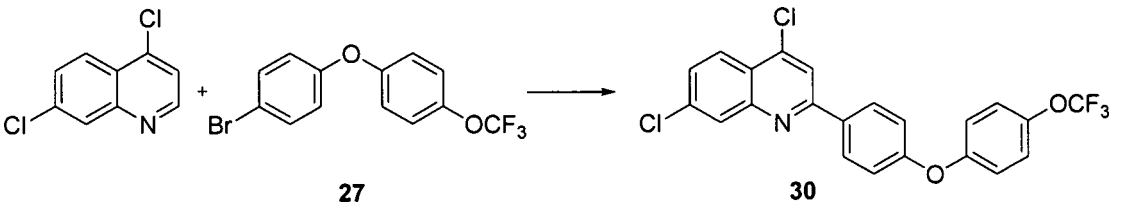
4,7-Dichloro-2-(4'-(4''-(trifluoromethyl)benzyl)phenyl)quinoline.



26 **29**

1-Bromo-4-(4'-(trifluoromethyl)benzyl)benzene (438 mg, 1.40 mmol) reacted with 4,7-dichloroquinoline (222 mg, 1.12 mmol) according to the **general procedure 3** to give white crystals (83 mg, 17 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.17 (1H, s, H8), 8.15 (1H, d, *J*= 7.4 Hz, H5), 8.07 (2H, d, *J*= 8.2 Hz, H12), 7.93 (1H, s, H3), 7.56 (2H, d, *J*= 8.5 Hz, H16), 7.56 (1H, d, *J*= 7.4 Hz, H6), 7.33 (4H, m, H13-H15) and 4.12 (2H, s, H14) ppm; MS (ES+), $[M+H]^+$ (100) 432; HRMS calcd for $C_{23}H_{15}NF_3Cl$ $[M+H^+]$ 432.0534, found 432.0535.

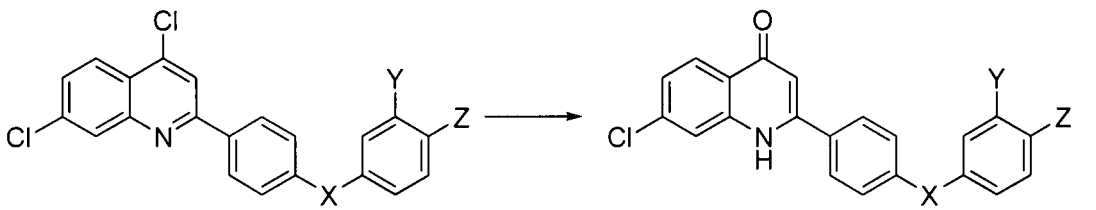
4,7-Dichloro-2-(4'-(4''-(trifluoromethoxy)phenoxy)phenyl)quinoline.



27 30

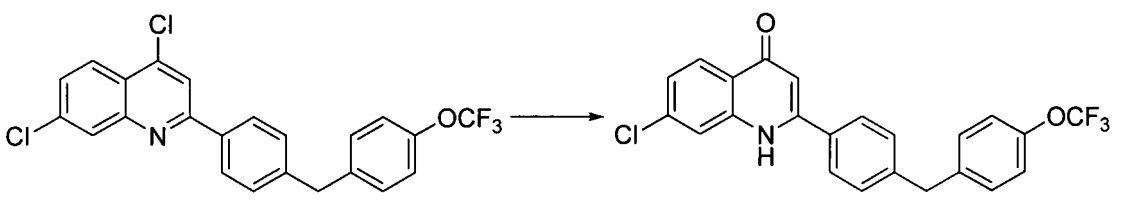
1-Bromo-4-(4-(trifluoromethoxy)phenoxy)benzene (200 mg, 0.6 mmol) reacted with 4,7-dichloroquinoline (95 mg, 0.5 mmol) following **general procedure 3** to give a colourless oil (32 mg, 15%); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.17-8.14 (2H, m, H5-H8), 8.14 (2H, d, *J*= 8.9 Hz, H12), 7.93 (1H, s, H2), 7.56 (1H, dd, *J*= 2.1 and 9.0 Hz, H6), 7.22 (2H, d, *J*= 9.1 Hz, H15), 7.15 (2H, d, *J*= 8.8 Hz, H13) and 7.08 (2H, d, *J*= 9.11 Hz, H16) ppm; MS (ES+), $[M+H]^+$ (100) 450; HRMS calcd for $C_{22}H_{13}NO_2F_3Cl_2$ $[M+H]^+$ 450.0275, found 450.0260; Anal. Calcd for $C_{22}H_{12}NO_2F_3Cl_2$: C, 58.69%; H, 2.69%; N, 3.11%; Found C, 58.61%; H, 2.72%; N, 3.07%.

General procedure 4.



To 4,7-dichloro-2-arylquinoline in DMF (15 mL) was added a solution of 85% formic acid in water (5 mL). The mixture was refluxed for 24 hrs. After cooling down, some precipitate appeared, and the mixture was poured into water (100mL) and filtered to afford the product

7-Chloro-2-(4'-(4''-(trifluoromethoxy)benzyl)phenyl)quinolin-4(1H)-one.

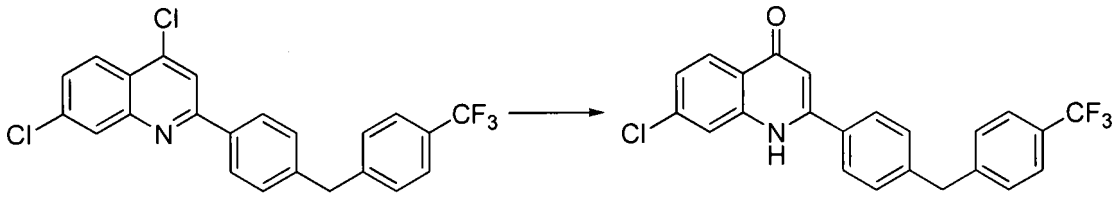


28 31

4,7-Dichloro-2-(4-(4-(trifluoromethoxy)benzyl)phenyl)quinoline (259 mg, 0.58 mmol) was treated according the **general procedure 4** to give the corresponding

quinolone as white crystals (164 mg, 66 %): mp= 278 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.08 (1H, d, J= 8.7 Hz, H5), 7.77 (2H, d, J= 8.3 Hz, H12), 7.77 (1H, d, J= 2.1 Hz, H8), 7.47 (2H, d, J= 8.2 Hz, H15), 7.41 (2H, d, J= 8.6 Hz, H13), 7.35 (1H, dd, J= 8.7 and 2.1 Hz, H6), 7.97 (2H, d, J= 8.0 Hz, H16), 6.37 (1H, s, H3) and 4.09 (2H, s, H14) ppm; IR υ_{max} = 3963, 3926, 3739, 3625, 3269, 2976, 2898, 2364, 2337, 1917, 1631, 1593, 1575, 1543, 1500, 1458, 1400, 1356, 1257, 1230, 1159, 1099, 1080, 1053, 1018, 902, 879, 808 and 735 cm^{-1} ; MS (ES+), $[M+H]^+$ (100) 430; HRMS calcd for $C_{23}H_{16}NO_2F_3Cl$ $[M+H]^+$ 430.0822, found 430.0831.

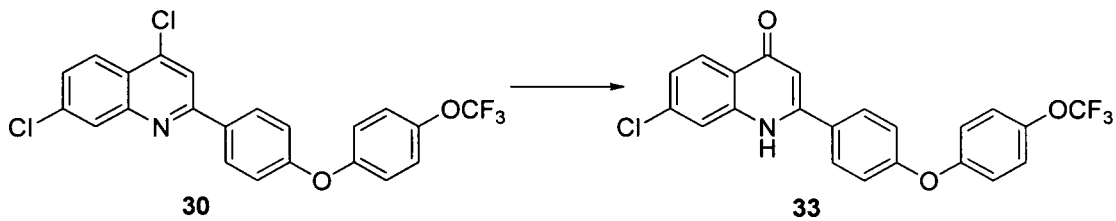
7-Chloro-2-(4'-(4''-(trifluoromethyl)benzyl)phenyl)quinolin-4(1H)-one.



29 **32**

4,7-Dichloro-2-(4-(4-(trifluoromethyl)benzyl)phenyl)quinoline (75 mg, 0.17 mmol) was treated according the **general procedure 4** to give the corresponding quinolone as white crystals (30 mg, 43 %); mp= 280 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.08 (1H, d, J= 8.5 Hz, H5), 7.79 (2H, d, J= 7.9 Hz, H16), 7.79 (1H, d, J= 2.0 Hz, H8), 7.68 (2H, d, J= 8.0 Hz, H15), 7.51 (2H, d, J= 8.2 Hz, H12), 7.48 (2H, d, J= 8.2 Hz, H13), 7.35 (1H, dd, J= 8.6 and 1.7 Hz, H6), 6.37 (1H, s, H3) and 4.16 (2H, s, H14) ppm; MS (ES-), $[M-H]^-$ (100) 412; HRMS calcd for $C_{23}H_{14}NOF_3Cl$ $[M-H]^-$ 412.0716, found 412.0728.

7-Chloro-2-(4'-(4''-(trifluoromethoxy)phenoxy)phenyl)quinolin-4(1H)-one.

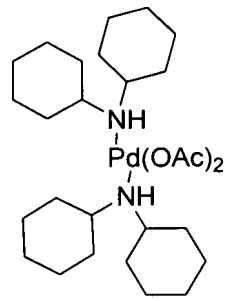


30 **33**

To 4,7-dichloro-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)quinoline (30 mg, 0.06 mmol) was treated according the **general procedure 4** to give the corresponding quinolone as white crystals (20 mg, 77 %); mp= 296 °C; IR υ_{max} = 3880, 3355, 3074,

2974, 2333, 1907,1633, 1595, 1539, 1498, 1415, 1358, 1303, 1251, 1216, 1163, 1099, 1080, 1014, 929, 906, 875, 816, 750 and 665 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 432; HRMS calcd for $C_{22}H_{14}NO_3F_3Cl$ $[M+H^+]$ 432.0614, found 432.0596; Anal. Calcd for $C_{22}H_{13}NO_3F_3Cl$: C, 61.20%; H, 3.03%; N, 3.24%; Found C, 60.99%; H, 3.07%; N, 3.20%.

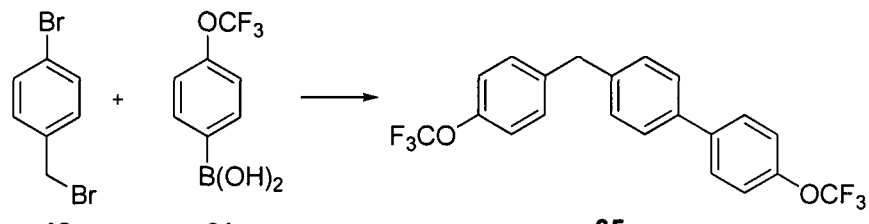
Synthesis of *trans*-$(Cy_2NH)_2Pd(OAc)_2$ (DAPCy).[37]



36

Under a nitrogen atmosphere, Cy_2NH (0.73 g, 4.0 mmol) was added dropwise into a solution of $Pd(OAc)_2$ (0.45 g, 2.0 mmol) in dioxane (20 mL) at r.t.. The mixture was stirred at r.t. for 3 hrs, during which a yellow precipitate occurred. The solvent was removed under reduced pressure. The resulting solid was crystallized from DCM/hexane to give the product as brown crystal (0.98 g, 84 %); mp=140 °C; ^1H NMR ($CDCl_3$) δ_H 6.93 (2H, bs, NH), 2.82 (4H, d, J= 9.9 Hz, CH), 2.44 (4H, m), 1.93-1.67 (30H, m), 1.23 (12H, m); ^{13}C NMR ($CDCl_3$) δ_C 181.0, 67.5, 55.4, 32.5, 32.4, 26.5, 26.3, 26.1 and 24.6 ppm; MS (ES+), $[M+H]^+$ (100) 587; HRMS calcd for $C_{28}H_{53}N_2O_4Pd$ $[M+H]^+$ 585.3046, found 585.2991; Anal. Calcd for $C_{28}H_{53}N_2O_4Pd$: C, 57.28%; H, 8.93%; N, 4.77%; Found C, 57.67%; H, 9.05%; N, 4.59%.

4-(trifluoromethoxy)-4'-(4''-(trifluoromethoxy)benzyl)biphenyl.



19 **21** **35**

4-Bromobenzylbromide (243 mg, 0.97 mmol), potassium phosphate tribasic (824 mg, 3.88 mmol) and Pd(II)Cl_2 (35 mg, 0.05 mmol) were placed in a 2-necked 100 mL round bottom flask. Anhydrous dioxane was added to the mixture. Vacuum and

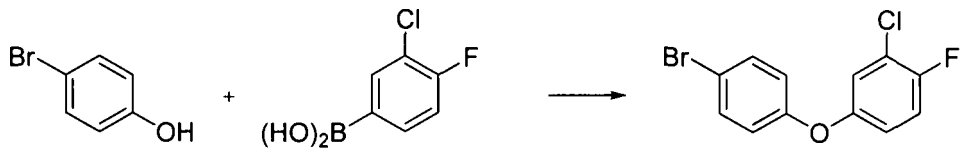
argon cycles were applied 3 times. The mixture was stirred for 10 mins at r.t., then 4-(Trifluoromethoxy)phenylboronic acid was added to the reaction mixture. Vacuum and argon cycles were applied 10 times. The mixture was heated at 100°C for 4 hrs (followed by t.l.c.) finally cooled to r.t., diluted with *n*-hexane and filtered through $MgSO_4$-silica gel. The filtrate was washed with 15% EtOAc/Hex, solvents were evaporated and the crude product was purified via column chromatography with PE to give the product as colorless oil (68 mg, 21 %): ^{1}H NMR ($CDCl_3$) δ_H 7.52 (2H, d, J= 8.7 Hz, CH-), 7.41 (2H, d, J= 8.4 Hz, CH-), 7.28 (2H, d, J= 8.7 Hz, CH-), 7.13 (4H, m, -CH), 7.01 (2H, d, J= 8.4 Hz, CH-C-OCF_3) and 3.90 (2H, s, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 149.8, 148.7, 139.8, 139.6, 139.0, 137.2, 132.4, 132.2, 131.8, 131.1, 129.9, 127.4, 122.9, 122.2 and 41.0 ppm; MS (CI), $[M]^+$ (100) 412; HRMS calcd for $C_{21}H_{14}F_6O_2Pd$ $[M]^+$ 412.0898, found 412.0884.

Tetrakis(triphenylphosphine)palladium(0).[39]

$$2\ PdCl_2 + 8\ PPh_3 + 5\ NH_2NH_2.H_2O \longrightarrow 2\ Pd(PPh_3)_4 + 4\ NH_2NH_2.HCl + N_2 + 5\ H_2O$$

Palladium dichloride (1.36 g, 7.7 mmol), triphenylphosphine (10.21 g, 38.3 mmol) and DMSO (90 mL) are mixed placed in a two-necked flask under argon. The mixture is heated at 140°C until complete dissolution occurs (~30 mins). Hydrazine hydrate (1.5 mL, 30.7 mmol) is then rapidly added over 1 min. A vigorous reaction takes place with evolution of nitrogen. The dark solution is then immediately cooled, crystallisation begins to occur at 125°C. Once at r.t. the mixture is filtered under argon, the yellow solid is washed successively with dry ethanol and diethyl ether. The product dried overnight under high vacuum is yellow crystal (8.06 g, 92%); mp= 116°C.[39]

4-(4'-Bromophenoxy)-2-chloro-1-fluorobenzene.

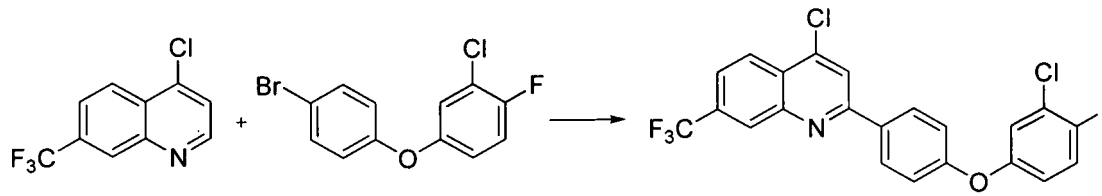


22 **39** **40**

3-Chloro-4-fluorophenylboronic acid (401 mg, 2.29 mmol) reacted with 4-bromophenol (198 mg, 1.15 mmol) following the **general procedure 2** to give a colorless oil (303 mg, 88 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.45 (2H, d, J= 9.0 Hz,

CH-CBr), 7.03 (1H, dd, *J*= 6.1 and 2.9 Hz, CH), 6.87 (2H, d, *J*= 9.0 Hz, CH) and 6.86 (2H, m) ppm.

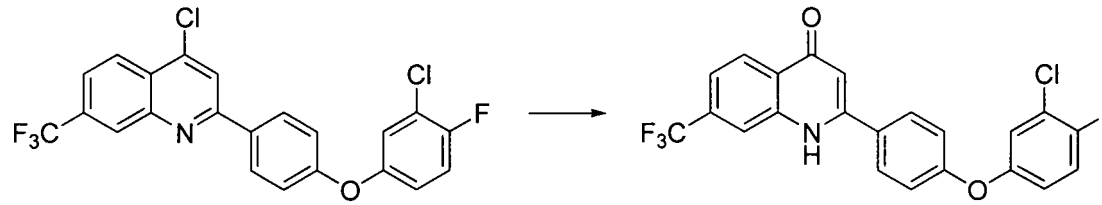
4 -Chloro - 2- (4'- (3''-chloro- 4''-fluorophenoxy) phenyl)- 7 -(trifluoromethyl) quinoline.



42 **40** **43**

According to the **general procedure 3** 1-Bromo-4-(3-chloro-4-fluorophenoxy)benzene (300 mg, 1.0 mmol) reacted with 4-chloro-7-trifluoromethylquinoline (184 mg, 0.80 mmol) to yield the desired compound as a colourless oily solid (28 mg, 6 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.29 (1H, d, *J*=8.4 Hz, H5), 8.15 (1H, s, H8), 7.93 (2H, d, *J*=8.6 Hz, H12), 7.62 (1H, d, *J*=8.5 Hz, H6), 7.51 (1H, dd, *J*= 9.0 and 9.1 Hz, H16'), 7.45 (1H, m, H15), 7.23 (2H, d, *J*= 8.79 Hz, H13), 7.17 (1H, m, H15') and 6.52 (1H, s, H3) ppm; IR υ_{max} = 3687, 3645, 3400, 2976, 2925, 2513, 2362, 2333, 1917, 1581, 1548, 1487, 1428, 1400, 1302, 1270, 1248, 1221, 1169, 1124, 1059, 975, 920, 902, 877, 841, 823, 769, 731 and 688 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 452; HRMS calcd for $C_{22}H_{12}NOF_4Cl_2$ $[M+H]^+$ 452.0232, found 452.0238.

2-(4'-(3''-Chloro-4''-fluorophenoxy)phenyl)-7-(trifluoromethyl)quinolin-4(1H)-one.

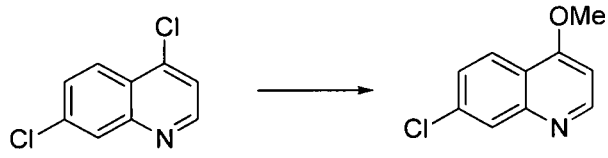


43 **44**

4-Chloro-2-(4-(4-(trifluoromethoxy)phenoxy)phenyl)-7-trifluoromethylquinoline (27 mg, 0.06 mmol) is hydrolysed following the **general procedure 4** to give white crystals (13 mg, 49 %); mp= 289 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.73 (1H, d, *J*= 8.7 Hz, H5), 8.15 (1H, s, H8), 7.93 (1H, d, *J*=8.6 Hz, H12), 7.62 (1H, d, *J*= 8.5 Hz, H6), 7.51 (1H, dd, *J*= 9.0 and 9.1 Hz, H16'), 7.44 (1H, m, H15), 7.23 (2H, d, *J*=8.7 Hz, H13), 7.16 (1H, m, H15') and 6.52 (1H, s, H3) ppm; IR υ_{max} = 3832, 3340,

2967, 2316, 1643, 1602, 1547, 1489, 1413, 1371, 1298, 1224, 1190, 1122, 1057, 879, 819, 735 and 686 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 456; HRMS calcd for $C_{22}H_{12}NO_2F_4ClNa$ $[M+Na]^+$ 456.0390, found 456.0388.

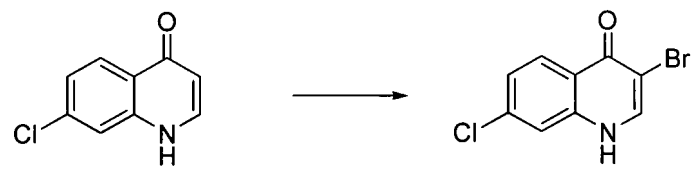
7-Chloro-4-methoxyquinoline.[46]



45 **46**

In a 100 mL three-necked flask, equipped with a thermometer, a reflux condenser and a septum, sodium methoxide (409 mg, 7.60 mmol) and 4,7-dichloroquinoline (1 g, 5.10 mmol) were dissolved in MeOH (20 mL) under heating and magnetic stirring. The reaction mixture was left under reflux for 2 days and quenched with water (20 mL). The crude product was extracted with DCM (3×30 mL) and the combined organic phase were washed with water and dried with $MgSO_4$. After evaporation of the solvent, 7-chloro-4-methoxyquinoline was collected as a white solid (988 mg, 100%): exp mp= 132 °C; lit mp= 129-135°C; R_f= 0.47 (5% MeOH/ DCM); ^1H NMR (400 MHz, $CDCl_3$) δ_H 8.75 (1H, d, J= 5.1 Hz, H2), 8.13 (1H, d, J= 8.7 Hz, H5), 8.02 (1H, d, J=1.9 Hz, H8), 7.44 (1H, dd, J= 8.9 and 2.1 Hz, H6), 6.73 (1H, d, J= 5.3 Hz, H3) and 4.05 (3H, s, -OMe) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 162.7, 152.9, 150.4, 136.8, 128.3, 126.9, 123.8, 120.5, 100.7 and 56.2 ppm; IR υ_{max} = 3895, 3735, 3685, 36560, 3625, 3512, 2987, 2366, 1913, 1616, 1572, 1504, 1450, 1425, 1379, 1311, 1163, 1122, 1070, 981, 887, 843 and 818 cm-1; MS (CI), $[M+H]^+$ (100) 194; HRMS calcd for $C_{10}H_9NOCl$ $[M+H]^+$ 194.0373, found 194.0372.

3-Bromo-7-chloroquinolin-4(1H)-one.[47]

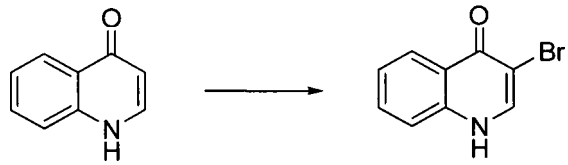


48 **49**

To a solution of 7-chloroquinolin-4(1H)-one (1 g, 5.60 mmol) in acetic acid (25 mL) containing a crystal of iodine, bromine (0.30 mL, 5.60 mmol) was added dropwise. The reaction was left for 2 hours with good stirring. A crystalline yellow solid was

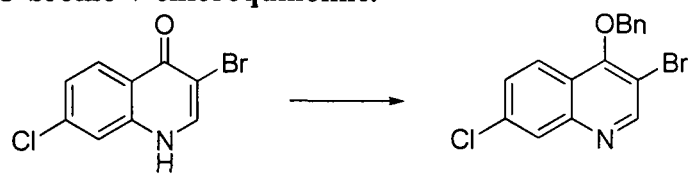
collected, and this solid was stirred with concentrated ammonium hydroxide (15 mL). The crude bromide compound was collected and washed with MeOH (30 mL) to give a white solid: (1.2 g, 85 %); mp= 354°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.52 (1H, s, H2), 8.13 (1H, d, *J*= 8.7 Hz, H5), 7.65 (1H, d, *J*=2.0 Hz, H8) and 7.42 (1H, dd, J=8.7 and 2.0 Hz, H6 ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.3, 141.2, 140.5, 136.9, 128.0, 124.8, 123.2, 118.1 and 105.1 ppm; IR υ_{max} = 3878, 3741, 3687, 3595, 3355, 3070, 2974, 2887, 2567, 2360, 2333, 1844, 1583, 1562, 1506, 1457, 1400, 1350, 1184, 1137, 1093, 1066, 875, 827, 758 and 683 cm^{-1}; MS (CI), $[M-Br]^+$ (100) 180; HRMS calcd for $C_9H_6NOClBr$ $[M+H]^+$ 257.9321, found 257.9315.

3-Bromoquinolin-4-ol.



To a solution of quinolin-4(1H)-one (1 g, 6.88 mmol) in acetic acid (20 mL) containing a crystal of iodine, bromine (0.4 mL, 5.6 mmol) was added dropwise. The reaction was left for 2 hours with good stirring. A crystalline yellow solid was collected, and this solid was stirred with concentrated ammonium hydroxide (15 mL). The crude bromide compound was filtered to give a white solid (1.37 g, 89 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.47 (1H, s, H2), 8.14 (1H, dd, *J*= 8.2 and 0.9 Hz, H5), 8.09 (1H, td, *J*=8.5 and 1.5 Hz, H7), 7.77 (1H, d, *J*= 8.4 Hz, H8) and 7.65 (1H, td, *J*= 8.2 and 1.1 Hz, H6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 140.5, 139.6, 132.3, 125.6, 124.6, 124.3, 118.9 and 104.5 ppm; MS (CI), $[M-Br]^+$ (100) 146; HRMS calcd for C_9H_7NOBr $[M+H]^+$ 223.9711, found 223.9712.

4-(Benzyloxy)-3-bromo-7-chloroquinoline.[48]

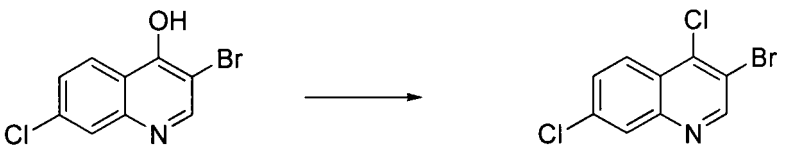


49 **51**

To a stirred solution of 3-bromo-7-chloroquinolin-4(1H)-one (200 mg, 0.8 mmol) and CsF-celite (1.16 mmol) in DMF (20 mL), the benzyl bromide (265 mg, 1.6 mmol) was added. Then the mixture was continued for stirring at r.t. up to

completion of the reaction, indicated by t.l.c. monitoring. The reaction mixture was filtered, the solvent evaporated and the residue dissolved in EtOAc (50 mL). Precipitates were filtered off, washed with ethyl acetate (20 mL) and the filtrate evaporated under reduced pressure. The product was purified by column chromatography on silica gel using 5% MeOH/DCM to give a white solid (162 mg, 60 %): mp= 202 °C; Rf=0.85 (30 % EtOAc/PE; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.44 (1H, d, *J*= 9.1 Hz, H5), 8.03 (1H, s, H2), 7.42-7.32 (5H, m, H6, H8, *meta* and *para* H of Bn), 7.16 (2H, m, *ortho* H of Bn) and 5.29 (2H, s, O-CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 172.1, 143.6, 140.2, 139.0, 129.6, 129.5, 128.9, 126.2, 125.3, 124.1, 115.8, 106.6 and 56.6 ppm; IR υ_{max} = 3882, 3733, 3350, 2974, 2885, 2331, 1946, 1579, 1458, 1379, 1331, 1225, 1090, 1053, 883, 833, 773, 739 and 694 cm^{-1}; MS (ES+), $[M+Na]^+$ (100) 370; HRMS calcd for $C_{16}H_{11}NOClBrNa$ $[M+Na]^+$ 369.9610, found 369.9601.

3-Bromo-4,7-dichloroquinoline.[49]

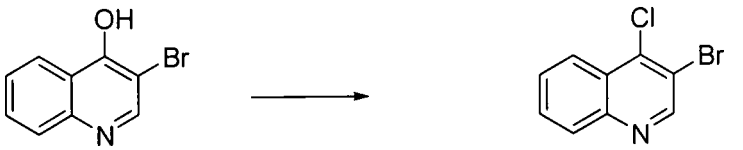


49 **53**

To phosphorus oxychloride (2.0 mL, 21.45 mol), was added 3-bromoquinolin-4-ol (290 mg, 1.13 mmol) with stirring. The mixture was refluxed for 30 min. After cooling the solvent was evaporated in vacuum and the resulting syrup was stirred with some crushed ice (200 mL). After 1 hr, the solid formed was filtered off, washed with cold water (50 mL), and dissolved in DCM (50 mL). The solution was washed once with ice-cold NaOH (1M, 30 mL) and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated to afford the product as a white solid (263 mg, 85%): exp mp= 76 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.95 (1H, s, H2), 8.19 (1H, dd, *J*= 9.1 and 0.6 Hz, H5), 8.11 (1H, d, *J*=1.9 Hz, H8) and 7.62 (1H, dd, *J*= 8.9 and 2.1 Hz, H6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 152.9 (C-N), 151.4, 141.6, 137.2, 129.6, 128.9, 126.0, 125.9 and 118.3 (C-Br) ppm; IR υ_{max} = 3664, 3396, 3049, 2989, 2331, 1909, 1604, 1550, 1473, 1433, 1354, 1331, 1290, 1246, 1149, 1126, 1082, 985, 924, 885, 848, 812, 764 and 696 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 276; HRMS calcd for $C_9H_5NCl_2Br$ $[M+H]^+$ 275.8982, found 275.8993; Anal.

Calcd for $C_9H_4NCl_2Br$: C, 39.03 %; H, 1.46 %; N, 5.06 %; Found C, 39.03 %; H, 1.48 %; N, 5.02 %.

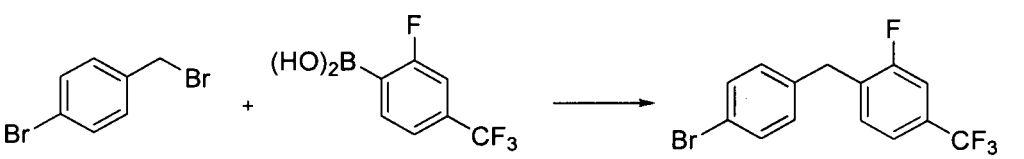
3-Bromo-4-chloroquinoline.[49]



52 **54**

To phosphorus oxychloride (2.0 mL, 21.45 mmol), was added 3-bromoquinolin-4-ol (252 mg, 1.13 mmol), the mixture was refluxed for 30 min. After cooling the solvent was evaporated in vacuum and the resulting syrup was stirred with some crushed ice (200 mL). After 1 hr, the solid formed was filtered off, washed with cold water (50 mL), and dissolved in DCM (50 mL). The solution was washed once with ice-cold NaOH (1M, 30 mL) and dried over $MgSO_4$. The solution was filtered and the solvent was evaporated to afford the product as a white solid (200 mg, 77 %): exp mp= 58°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.93 (1H, s, H2), 8.22 (1H, ddd, J= 8.4, 1.4 and 0.6 Hz, H5), 8.09 (1H, ddd, J=8.4, 1.2 and 0.5 Hz, H8), 7.77 (1H, td, J= 8.4 and 1.4 Hz, H7) and 7.65 (1H, td, J= 8.4 and 1.2 Hz, H6) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 152.1, 147.6, 141.9, 130.7, 130.3, 129.0, 127.8, 124.8 and 118.4 ppm; IR υ_{max} = 3961, 3880, 3782, 33989, 3033, 1953, 1614, 1558, 1483, 1348, 1246, 1167, 1111, 981, 937, 852, 823, 752 and 677 cm^{-1}; MS (CI), $[M\text{-}Br]^+$ (100) 164; HRMS calcd for C_9H_6NClBr $[M+H]^+$ 241.9372, found 241.9377; Anal. Calcd for C_9H_5NClBr: C, 44.58 %; H, 2.08 %; N, 5.78 %; Found C, 44.58 %; H, 2.11 %; N, 5.76 %.

1-(4'-Bromobenzyl)-2-fluoro-4-(trifluoromethyl)benzene.

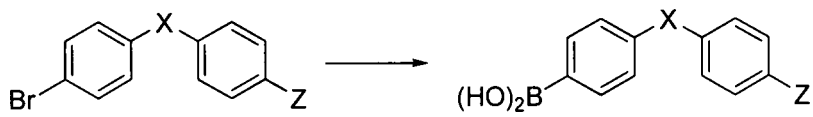


21 **55** **56**

1-Bromo-4-(bromomethyl)benzene (652 mg, 2.60 mmol) and 2-fluoro-4-(trifluoromethyl)phenylboronic acid (516 mg, 2.48 mmol) reacting following the **general procedure 2** to give white crystals (271 mg, 33 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.40 (2H, d, J= 8.5 Hz, CH-CBr), 7.33-7.20 (4H, m), 7.02 (2H, d, J= 8.2

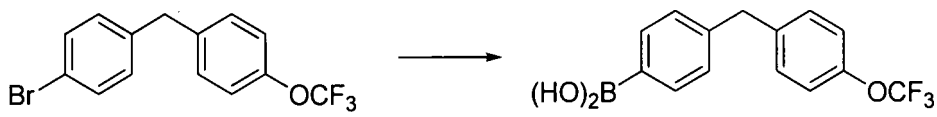
Hz, CH-CO) and 3.97 (2H, s, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 162.1, 159.6, 138.0, 132.2, 131.9, 130.6, 127.8, 125.1, 122.4, 121.6, 121.0, 113.2 and 34.6 ppm; MS (CI), $[M]^+$ (100) 332; HRMS calcd for $C_{14}H_9BrF_4$ $[M]^+$ 331.9824, found 331.9828.

General procedure 5.



To a solution of bromide reagent (1.00 eq.) in anhydrous THF (15 mL) under N_2 at -78°C, solution of *n*-BuLi in hexane (1.6 M, 1.60 eq.) was added. After 1 hr stirring, the clear solution was treated with triisopropylborate (0.86 mL, 3.74 mmol) and stirred for a further hour, allowed to warm at r.t. over 3 hrs. The reaction was left over night and then quenched with HCl (1M, 15 mL). The mixture was extracted with Et_2O (3×20 mL) and washed with water (3×20 mL). Purification of the crude product by flash chromatography with 20 % EtOAC:PE gave the product.

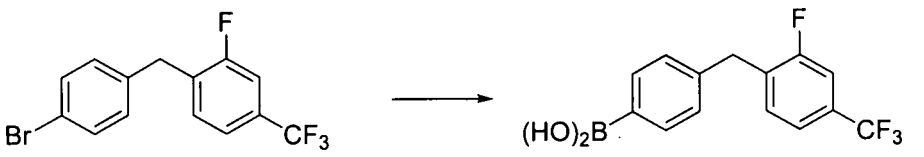
4-(4'-(Trifluoromethoxy)benzyl)phenylboronic acid.



26 **57**

1-Bromo-4-(4-(trifluoromethoxy)benzyl)benzene (619 mg, 1.87 mmol) treated with the **general procedure 5** gave the product as white crystals (550 mg, 100%): mp= 158°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.54 (2H, d, *J*= 8.0 Hz, CH-CB), 7.27 (2H, d, *J*= 8.5 Hz, CH), 7.19 (2H, d, *J*= 8.0 Hz, -CH), 7.15 (2H, d, *J*= 8.0 Hz, CH-CBr) and 3.99 (2H, s, -CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 135.3, 131.8, 129.6, 122.4 and 42.4 ppm; IR υ_{max} = 3849, 3818, 3369, 2976, 2902, 2480, 2335, 1911, 1608, 1560, 1510, 1409, 1340, 1305, 1251, 1221, 1151, 1107, 1020, 921, 862, 816, 752, 727 and 694 cm^{-1}; MS (EI), $[M\text{-}B(OH)]^+$ (100) 268; HRMS calcd for $C_{14}H_{11}BF_3O_3$ $[M\text{-}H]^+$ 295.0753, found 295.1202.

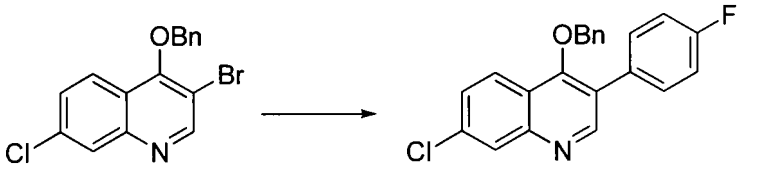
4-(2'-Fluoro-4'-(trifluoromethyl)benzyl)phenylboronic acid.



56 58

1-(4-Bromobenzyl)-2-fluoro-4-(trifluoromethyl)benzene (850 mg, 2.56 mmol) treated with the **general procedure 5** gave the product as white crystals (260 mg, 34 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 7.67 (2H, d, *J*= 8.0 Hz, CH-CB), 7.40 (2H, m), 7.21 (2H, d, J= 7.6 Hz, CH), 7.17 (1H, m, CH) and 4.07 (2H, s, -CH_2) ppm.

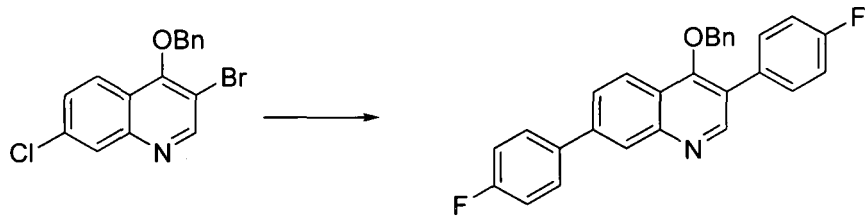
4-(Benzyloxy)-7-chloro-3-(4'-fluorophenyl)quinoline.[52]



51 60

4-(Benzyloxy)-3-bromo-7-chloroquinoline (200 mg, 0.57 mmol) and 4-fluorophenylboronic acid (40 mg, 0.29 mmol) were dissolved in DME (10 mL) and aqueous Na_2CO_3 (1M, 2 mL) was added, the resulting mixture was deoxygenated with a stream of argon. After 20 min, $Pd(PPh_3)_4$ (0.05 equiv) was added, and mixture was brought to reflux, allowed to stir under argon for 12 h and cooled to r.t. . The solution was filtered through celite, washed with Et_2O (10 mL). The aqueous layer was extracted with Et_2O (3×20 mL), and the organic phases were combined and washed with NaOH (1 M, 10 mL) followed by brine. The ethereal solution was dried over $MgSO_4$ and evaporated. Purification of each crude product by flash chromathography using 10 % EtOAc/PE yielded the corresponding product as white crystals (85 mg, 80 %): R_f= 0.63 (50 % PE/EtOAc; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.48 (1H, d, *J*= 8.4 Hz, H5), 7.76 (1H, s, H2), 7.64 (2H, dd, *J*= 8.9 and 5.5, CH of Bn), 7.39-7.29 (5H, m, H6, H8, H of Bn and PhF), 7.18 (2H, d, *J*= 8.2 Hz, CH of Bn), 7.09 (4H, t, *J*= 8.7 Hz, H of PhF) and 5.33 (2H, s, O-CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.7, 163.9, 161.4, 142.6, 140.7, 139.0, 134.9, 131.2, 131.2, 130.8, 130.7, 129.8, 129.0, 126.5, 126.2, 125.0, 122.5, 115.9, 115.7 and 115.5 ppm; MS (ES+), $[M+Na]^+$ (100) 386; HRMS calcd for $C_{22}H_{15}NOFNaCl$ $[M+Na]^+$ 386.0724, found 386.0716.

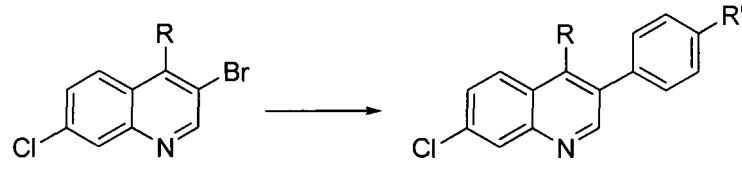
4-(Benzyloxy)-3,7-bis(4'-fluorophenyl)quinoline.[52]



51

4-(Benzyloxy)-3-bromo-7-chloroquinoline (600 mg, 1.73 mmol) and 4-fluorophenylboronic acid (339 mg, 2.42 mmol) were dissolved in DME (20 mL) and aqueous Na_2CO_3 (1M, 4 mL) was added, the resulting mixture was deoxygenated with a stream of argon. After 20 min, $Pd(PPh_3)_4$ (0.05 equiv) was added, and mixture was brought to reflux, allowed to stir under argon for 12 hrs and cooled to r.t.. The solution was filtered through celite, washed with Et_2O (15 mL). The aqueous layer was washed with Et_2O (3×20 mL), and the organic phases were combined and washed with NaOH (1 M, 10 mL) followed by brine. The ethereal solution was dried over $MgSO_4$ and evaporated. Purification of each crude product by flash chromathography using 10 % EtOAc/PE yielded the corresponding phenol as white crystals (403 mg, 41 %): mp= 201°C; Rf= 0.58 (50 % PE/EtOAc; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.60 (1H, d, *J*= 8.3 Hz, H5), 7.83 (1H, s, H2), 7.69 (2H, dd, *J*= 8.9 and 5.5, CH of Bn), 7.53 (1H, dd, *J*= 8.5 and 1.7, H6), 7.44 (1H, s, H8), 7.43-7.33 (5H, m, H of Bn and PhF), 7.23 (2H, d, J= 8.2 Hz, CH of Bn), 7.11 (4H, t, *J*= 8.5 Hz, H of PhF), 7.11 (4H, t, *J*= 8.5 Hz, H of PhF) and 5.44 (2H, s, O-CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.7, 164.3, 163.4, 161.8, 144.0, 142.3, 139.9, 136.2, 135.1, 131.2, 130.4, 129.4, 129.1, 129.0, 128.6, 128.4, 126.3, 126.2, 123.1, 121.7, 116.1, 115.9, 115.3, 115.1 and 114.1 ppm; MS (ES+), $[M+Na]^+$ (100) 446; HRMS calcd for $C_{28}H_{19}NOF_2Na$ $[M+Na]^+$ 446.1332, found 446.1317.

General procedure 6.

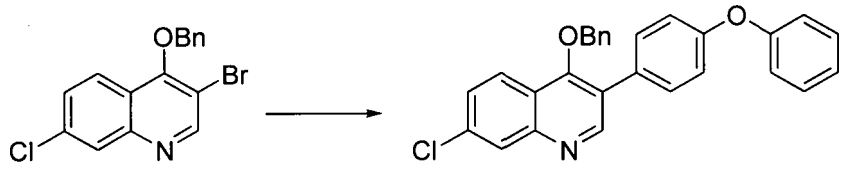


R= OBn or Cl

Quinoline (1.05 eq.) and 4-phenoxyphenylboronic acid (1.00 eq.) were dissolved in DME (10 mL) and aqueous Na_2CO_3 (1M, 4 mL) was added, the resulting mixture

was deoxygenated with a stream of argon. After 20 min, $Pd(PPh_3)_4$ (0.05 equiv) was added, and mixture was brought to reflux, allowed to stir under argon for 12 hrs and cooled to r.t. . The solution was filtered through celite, washed with Et_2O (20 mL). The aqueous layer was extracted with Et_2O (3×20 mL), and the organic phases were combined and washed with NaOH (1M, 10 mL) followed by brine. The ethereal solution was dried over $MgSO_4$ and evaporated. Purification of each crude product by flash chromathography using 10 % EtOAc/PE gave the expected product.

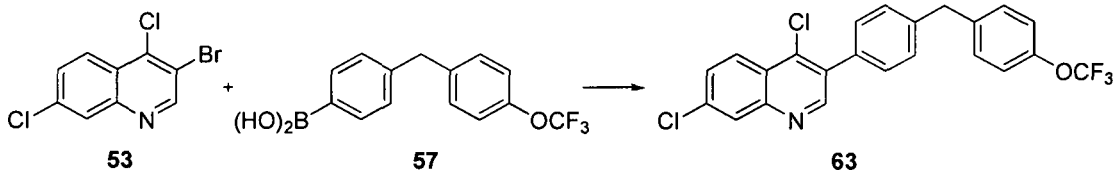
4-(Benzyloxy)-7-chloro-3-(4'-phenoxyphenyl)quinoline.



51 **62**

4-(Benzyloxy)-3-bromo-7-chloroquinoline (430 mg, 1.24 mmol) and 4-phenoxyphenylboronic acid (265 mg, 1.24 mmol) reacted following the **general procedure 6** to yield some white crystals (364 mg, 67 %): Rf= 0.81 (10 % EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.50 (1H, d, J= 8.5 Hz, H5), 7.79 (1H, s, H2), 7.64 (2H, d, J= 8.9 Hz, CH of Bn), 7.39-7.30 (7H, m), 7.19 (2H, d, J= 8.2 Hz, CH of Bn), 7.07-7.03 (4H, m) and 5.34 (2H, s, O-CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 175.8, 157.6, 157.1, 142.5, 140.7, 138.9, 134.9, 130.5, 130.2, 130.1, 129.9, 129.8, 129.0, 126.5, 126.2, 124.9, 123.6, 122.8, 119.3, 119.2, 115.9 and 57.0 ppm; MS (ES+), $[M+Na]^+$ (100) 460; HRMS calcd for $C_{28}H_{20}NO_2NaCl$ $[M+Na]^+$ 460.1080, found 460.1070.

4,7-Dichloro-3-(4'-(4''-(trifluoromethoxy)benzyl)phenyl)quinoline.

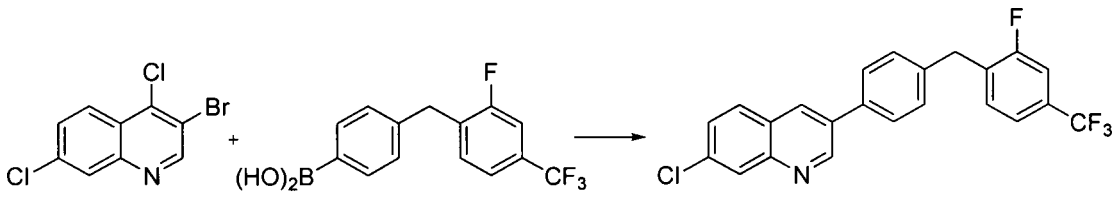


53 **57** **63**

3-Bromo-4,7-dichloroquinoline (263 mg, 0.95 mmol) and 4-(4-(trifluoromethoxy)benzyl)phenyl boronic acid (300 mg, 0.90 mmol) reacted following the **general procedure 6** to yield white crystals (316 mg, 71 %): mp= 74°C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.84 (1H, s, H2), 8.28 (1H, dd, J= 9.1 and 0.4 Hz, H5), 8.14 (1H, d, J= 2.1 Hz, H8), 7.63 (1H, dd, J= 9.1 and 2.1 Hz, H6), 7.48

(2H, d, *J*= 8.2 Hz, H12), 7.33 (2H, d, *J*= 8.6 Hz, H13), 7.27 (2H, d, *J*= 9.7 Hz, H15), 7.18 (2H, d, *J*= 8.9 Hz, H16) and 4.08 (2H, s, H14) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 153.0, 148.6, 146.9, 142.5, 141.4, 140.2, 139.6, 136.5, 134.3, 133.5, 130.7, 130.5, 129.5, 129.4, 129.0, 126.6, 121.6 and 41.4 ppm; IR υ = 3928, 3866, 3737, 3681, 3338, 2974, 2894, 2366, 2339, 1913, 1610, 1552, 1510, 1450, 1338, 1251, 1224, 1155, 1093, 1051, 983, 898, 856, 814, 771, 717 and 677 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 448; HRMS calcd for $C_{23}H_{15}NOF_3Cl_2$ $[M+H]^+$ 448.0483, found 448.0464.

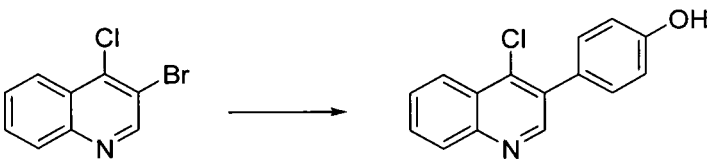
7-Chloro-3-(4'-(2''-fluoro-4''-(trifluoromethyl)benzyl)phenyl)quinoline.



53 **58** **64**

3-Bromo-4,7-dichloroquinoline (176 mg, 0.63 mmol) and 4-(2-fluoro-4-(trifluoromethyl)benzyl)phenylboronic acid (280 mg, 0.60 mmol) reacted following the **general procedure 6** to yield white crystals (113 mg, 45 %); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 9.15 (1H, d, *J*= 2.3 Hz, H2), 8.25 (1H, d, *J*= 2.3 Hz, H4), 8.13 (1H, d, *J*= 1.9 Hz, H8), 7.81 (1H, d, *J*= 8.7 Hz, H5), 7.64 (2H, d, *J*= 8.3 Hz, H12), 7.53 (1H, dd, *J*= 2.1 and 8.7 Hz, H6), 7.37 (2H, d, *J*= 8.2 Hz, H13), 7.33 (2H, m, CH), 7.06 (1H, m, CH) and 4.12 (2H, s, H14) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 151.2, 148.0, 139.4, 136.3, 135.6, 134.1, 133.3, 132.0, 131.9, 130.4, 130.1, 129.6, 128.6, 128.6, 128.1, 126.8, 121.6, 116.0, 113.5, 113.3 and 34.9 ppm; MS (ES+), $[M+H]^+$ (100) 416; HRMS calcd for $C_{23}H_{15}NF_4Cl$ $[M+H]^+$ 416.0829, found 416.0813.

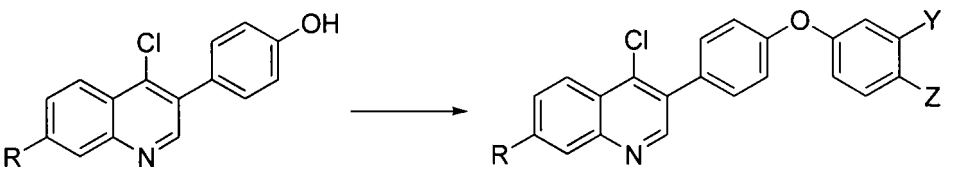
4-(4'-Chloroquinolin-3-yl)phenol.[61]



54 **67**

3-Bromo-4-chloroquinoline (1.05 equiv) and 4-hydroxyphenylboronic acid (1.00 equiv) reacted following the **general procedure 6** to yield white crystals (480 mg, 45%): mp= 192 °C; Rf= 0.26 (30% EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.86 (1H, s, H2), 8.31 (1H, ddd, *J*= 8.3, 1.6 and 0.5 Hz, H5), 8.13 (1H, ddd, *J*=8.4, 1.2 and 0.5 Hz, H8), 7.88 (1H, td, J=8.4 and 1.5 Hz, H7), 7.63 (1H, td, *J*=8.3 and 1.3 Hz, H6), 7.45 (2H, d, *J*= 8.6 Hz, H12) and 6.94 (2H, d, *J*=8.8 Hz, H13) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.1, 152.1, 147.5, 138.3, 133.1, 131.6, 130.5, 129.7, 128.8, 126.4, 126.0, 124.5 and 116.0 ppm; IR υ_{max} = 3670, 3348, 2974, 2885, 2333, 1917, 1608, 1583, 1552, 1516, 1471, 1439, 1375, 1338, 1280, 1228, 1178, 1149, 1091, 1055, 881, 816, 771 and 725 cm^{-1}; MS (CI), $[M+H]^+$ (100) 256; HRMS calcd for $C_{15}H_{11}NOCl$ $[M+H]^+$ 256.0529, found 256.0522.

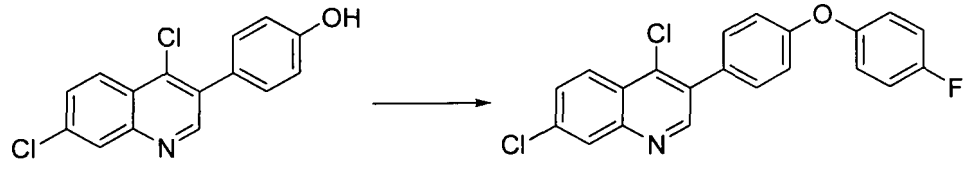
General procedure 7.[40]



R= Cl or H

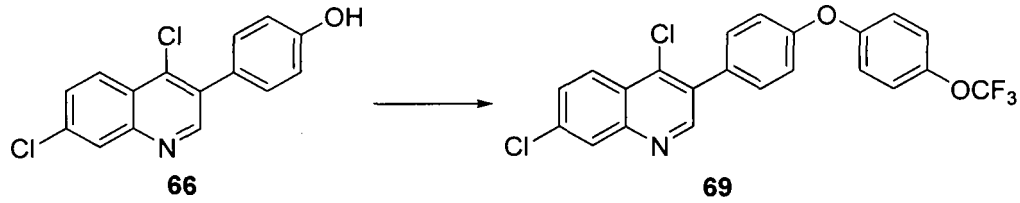
A flask is charged with 4-(4-chloroquinolin-3-yl)phenol (1.0 equiv), $Cu(OAc)_2$ (1.0 equiv), arylboronic acid (2.0 equiv) and powdered 4 Å molecular sieves. The reaction mixture is diluted with DCM to yield a solution approximately 0.1 M in phenol, and triethylamine (5.0 equiv) is added. After stirring the colored heterogeneous reaction mixture for 18 hrs at 25 °C under ambient atmosphere, the resulting slurry is filtered and the diaryl ether is isolated from the organic filtrate by flash chromatography (10% EtOAc/PE).

4,7-Dichloro-3-(4'-(4''-fluorophenoxy)phenyl)quinoline.



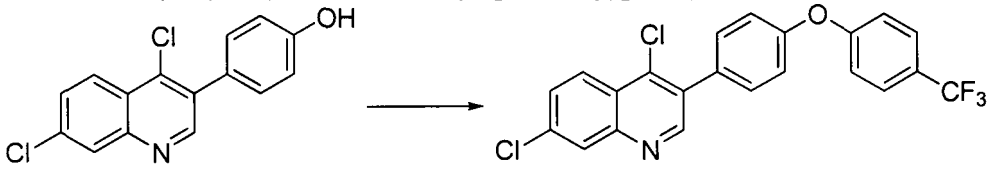
See general procedure 7. White solid (85 mg, 63%); Rf=0.91 in 1/1: EtOAc/PE; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.86 (1H, s, H2), 8.27 (1H, d, *J*= 9.0 Hz, H5), 8.14 (1H, d, *J*=2.1 Hz, H8), 7.64 (2H, d, J=8.4 Hz, H12), 7.63 (1H, dd, *J*=2.2 and 9.0 Hz, H6), 7.56 (2H, d, *J*= 8.7 Hz, H15), 7.19 (2H, m, H16) and 7.16 (2H, d, *J*=7.2 Hz, H13) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 160.1, 156.8, 152.8, 148.7, 140.2, 136.7, 132.9, 132.1 (2C), 132.0 (2C), 129.0, 127.7, 126.5, 119.8 (2C) and 119.1 (2C) ppm; MS (ES+), $[M+H]^+$ (100) 384; HRMS calcd for $C_{21}H_{13}NOFCl_2$ $[M+H]^+$ 384.0358, found 384.0341; Anal. Calcd for $C_{21}H_{12}NOFCl_2$: C, 65.64%; H, 3.15%; N, 3.65%; Found C, 65.50%; H, 3.24%; N, 3.55%.

4,7-Dichloro-3-(4'-(4''-(trifluoromethoxy)phenoxy)phenyl)quinoline.



See general procedure 7. White solid (104 mg, 68%); R_f=0.81 (50% EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.85 (1H, s, H2), 8.27 (1H, d, *J*= 9.0 Hz, H5), 8.14 (1H, d, *J*=2.0 Hz, H8), 7.63 (1H, dd, *J*=2.1 and 9.0 Hz, H6), 7.52 (2H, d, J=8.8 Hz, H12), 7.24 (2H, d, *J*= 9.0 Hz, H15), 7.15 (2H, m, H13) and 7.12 (2H, m, H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 157.8, 155.4, 152.9, 148.7, 145.4, 140.2, 136.6, 133.0, 131.8, 131.4, 129.4, 129.0, 126.5, 125.3, 123.2, 120.8, 118.9 and 100.0 ppm; MS (ES+), $[M+H]^+$ (100) 450; HRMS calcd for $C_{22}H_{13}NO_2F_3Cl_2$ $[M+H]^+$ 450.0275, found 450.0266; Anal. Calcd for $C_{22}H_{12}NO_2F_3Cl_2$: C, 58.69%; H, 2.69%; N, 3.11%; Found C, 58.40%; H, 2.73%; N, 2.98%.

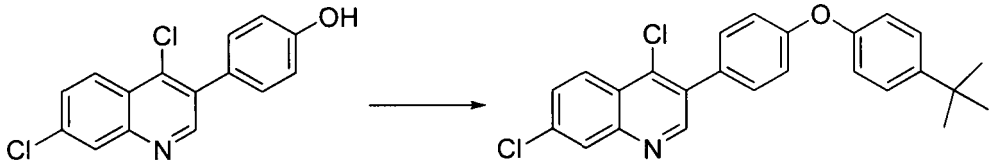
4,7-Dichloro-3-(4'-(4''-(trifluoromethyl)phenoxy)phenyl)quinoline.



66 **70**

See general procedure 7. White solid (152 mg, 90%); R_f=0.91 (50% EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.84 (1H, s, H2), 8.28 (1H, d, *J*= 9.0 Hz, H5), 8.14 (1H, d, *J*=2.0 Hz, H8), 7.63 (1H, dd, *J*=2.1 and 9.0 Hz, H6), 7.50 (2H, d, J=8.8 Hz, H12) and 7.09 (6H, m, H13-H15-H16) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.7, 153.0, 152.5, 148.7, 140.2, 136.5, 133.1, 131.7, 130.7, 129.4, 129.0, 126.6, 125.4, 121.8, 121.7, 118.1, 117.1 and 116.8 ppm; MS (ES+), $[M+H]^+$ (100) 434; HRMS calcd for $C_{22}H_{13}NOF_3Cl_2$ $[M+H]^+$ 434.0326, found 434.0306; Anal. Calcd for $C_{22}H_{12}NOF_3Cl_2$: C, 60.85%; H, 2.79%; N, 3.23%; Found C, 60.36%; H, 2.85%; N, 2.97%.

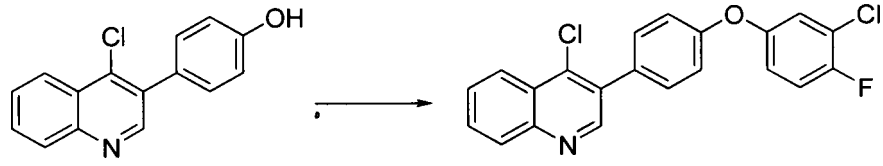
4,7-Dichloro-3-(4'-(4''-(*tert*-butyl)phenoxy)phenyl)quinoline.



66 **71**

See general procedure 7. White solid (140 mg, 80%); R_f=0.79 (50% EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.85 (1H, s, H2), 8.28 (1H, d, *J*= 9.0 Hz, H5), 8.14 (1H, d, *J*=2.1 Hz, H8), 7.63 (1H, dd, *J*=2.1 and 9.0 Hz, H6), 7.49 (2H, d, J=8.7 Hz, H12), 7.40 (2H, d, *J*= 8.8 Hz, H15), 7.12 (2H, d, *J*= 8.7 Hz, H13), 7.05 (2H, d, *J*=8.7 Hz, H16) and 1.35 (9H, s, CH_3 of *tert*-butyl) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.8, 154.2, 153.1, 148.6, 147.4, 140.1, 136.5, 133.3, 131.6 (2C), 130.4, 129.3, 129.0, 127.2 (2C), 126.7, 126.6, 125.4, 121.2, 119.6 (2C), 118.4 (2C) and 31.9 (3C) ppm; MS (ES+), $[M+H]^+$ (100) 422; HRMS calcd for $C_{22}H_{22}NOCl_2$ $[M+H]^+$ 422.1078, found 422.1074.

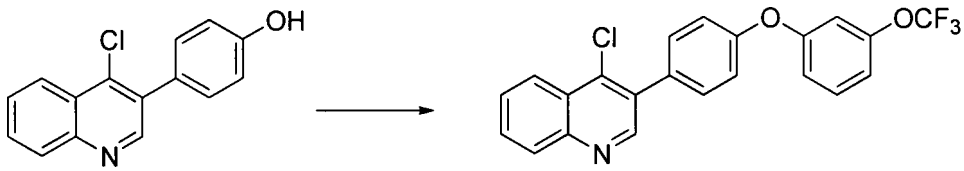
4 -Chloro-3-(4'-(3''-chloro-4''-fluorophenoxy)phenyl)quinoline.



67 **73**

See general procedure 7. White solid (91 mg, 50%); R_f=0.70 (50% EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.86 (1H, s, H2), 8.36 (1H, ddd, *J*= 0.6, 1.4 and 9.0 Hz, H5), 8.15 (1H, ddd, *J*=0.7, 1.3 and 8.4 Hz, H8), 7.80 (1H, dt, *J*=1.4 and 8.4 Hz, H6), 7.49 (2H, dt, *J*=1.4 and 8.4 Hz, H7), 7.54 (2H, d, *J*= 8.7 Hz, H12), 7.19-7.16 (2H, m, H15-H16'), 7.12 (2H, d, *J*=8.8 Hz, H13) and 6.99 (1H, m, H15') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 158.3, 157.7, 151.7, 150.6, 140.3, 132.8, 132.0, 131.9, 130.6, 128.5, 126.8, 125.1, 122.0, 119.5, 118.6, 117.8 and 117.5 ppm; MS (ES+), $[M+H]^+$ (100) 384; HRMS calcd for $C_{21}H_{13}NOFCl_2$ $[M+H]^+$ 384.0358, found 384.0376.

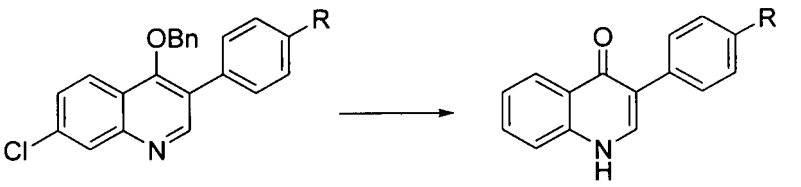
4-Chloro-3-(4'-(3''-(trifluoromethoxy)phenoxy)phenyl)quinoline.



67 **72**

See general procedure 7. White solid (184 mg, 75%); Rf=0.59 (30% EtOAc/PE); ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.87 (1H, s, H2), 8.34 (1H, ddd, *J*= 0.7, 1.4 and 8.4 Hz, H5), 8.15 (1H, dd, *J*=0.7 and 8.4 Hz, H8), 7.78 (1H, dt, *J*=1.4 and 8.4 Hz, H6), 7.68 (2H, dt, *J*=1.1 and 8.3 Hz, H7), 7.55 (2H, d, *J*= 8.7 Hz, H12), 7.38 (1H, t, *J*=8.1 Hz, H15), 7.17 (2H, d, *J*=8.7 Hz, H13) and 7.04-6.98 (3H, m, H15'-H16' and H17') ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 171.5, 158.3, 157.1, 151.8, 150.6, 148.4, 140.2, 132.8, 132.3, 132.2, 132.0, 131.0, 130.5, 128.4, 126.8, 125.1, 119.3, 117.6, 116.1 and 112.5 ppm; MS (ES+), $[M+H]^+$ (100) 416; HRMS calcd for $C_{22}H_{14}NO_2F_3Cl$ $[M+H]^+$ 416.0665, found 416.0670.

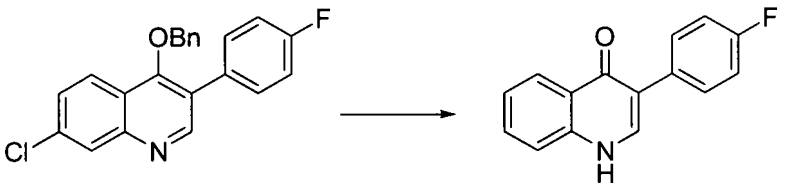
General procedure 8. [62]



R= F or *p*F-Ar

Quinoline reagent was dissolved in MeOH (10 mL), and 10% Pd-C was added, the mixture was then hydrogenated for 30 mins and filtered on scintered funnel, the resulting solid was washed with DCM (10 mL) to afford the product.

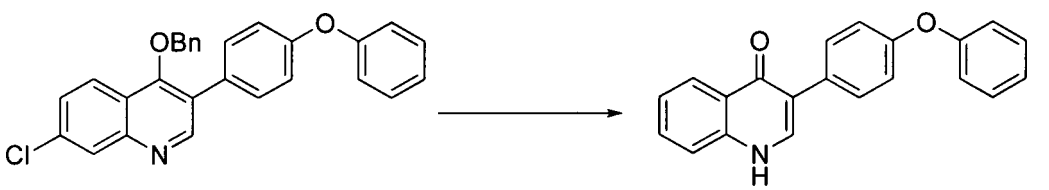
3-(4'-Fluorophenyl)quinolin-4(1H)-one.



60 **79**

4-(Benzyloxy)-7-chloro-3-(4-fluorophenyl)quinoline (175 mg, 0.48 mmol) was treated following **general procedure 8** to give a white crystal (57 mg, 49 %): mp= 282 °C; ^{1}H NMR (400 MHz, MeOD-d$_4$) δ_H 8.37 (1H, dd, *J*= 8.1 and 0.8 Hz, H5), 8.11 (1H, s, H2), 7.75-7.66 (3H, m, CH of PhF and H6), 7.61 (1H, d, *J*= 8.0 Hz, CH of Bn), 7.44 (2H, t, *J*= 8.16 Hz, H7) and 7.16 (2H, t, *J*= 8.16 Hz, CH of PhF) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 175.0, 162.5, 139.6, 138.5, 132.8, 132.0, 130.4, 126.1, 125.9, 123.7, 119.1, 118.6 and 115.0 ppm; IR υ_{max} = 3901, 3816, 3354, 3068, 2974, 2362, 2333, 1861, 1624, 1554, 1504, 1348, 1296, 1228, 1153, 1095, 1055, 1018, 895, 808, 777, 750, 694, 663 cm^{-1}; MS (CI), $[M+H]^+$ (100) 240; HRMS calcd for $C_{15}H_{11}NOF$ $[M+H]^+$ 240.0825, found 240.0825.

3-(4'-Phenoxyphenyl)quinolin-4(1H)-one.

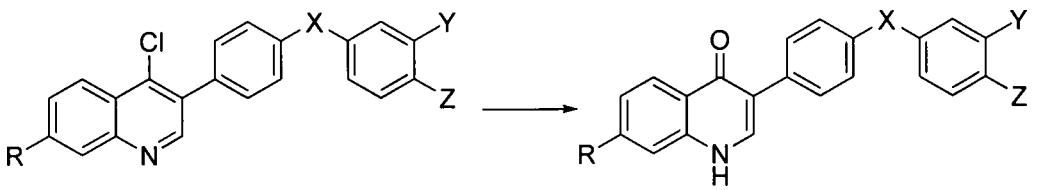


62 **80**

4-(Benzyloxy)-7-chloro-3-(4-phenoxyphenyl)quinoline (200 mg, 0.47 mmol) was treated following **general procedure 8** to give a white crystal (70 mg, 45%): ^{1}H

NMR (400 MHz, DMSO-d_6) δ_H 12.05 (1H, s, -NH), 8.23 (1H, d, J= 9.3 Hz, H5), 7.78 (2H, d, J= 6.6 Hz, OC-C-CH), 7.65 (2H, m, -CH of PhOPh), 7.44-7.31 (4H, m), 7.15 (1H, t, J= 7.02 Hz, H8) and 7.07-7.01 (3H, m) ppm; MS (ES-), $[M-H]^-$ (100) 312; HRMS calcd for $C_{21}H_{14}NO_2$ $[M-H]^-$ 312.1025, found 312.1011.

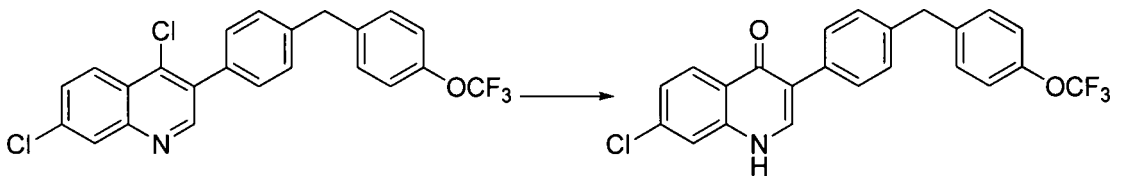
General procedure 9.[63]



R= H or Cl

The 3-substituted-4-chloroquinoline was dissolved in DMF and a solution of 85% formic acid in water (5 mL) was added. The mixture was refluxed for 24 hrs. After cooling down, some precipitate appears, and the mixture was poured into water (100mL) and filtered to afford the product.

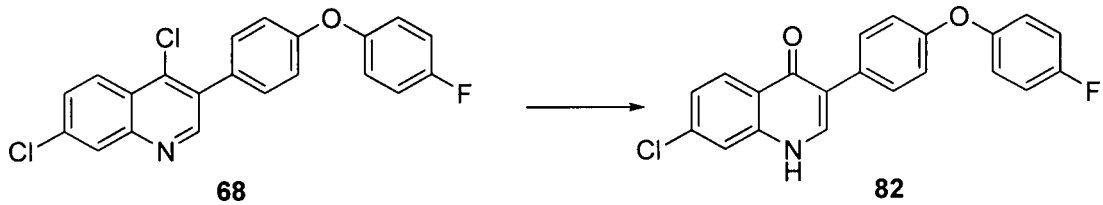
7-Chloro-3-(4'-(4''-(trifluoromethoxy)benzyl)phenyl)quinolin-4(1H)-one.[63]



63 **81**

See general procedure 9. White solid (135 mg, 64 %): mp= 275°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.18 (1H, s, H2), 8.18 (1H, d, J= 8.7 Hz, H5), 7.64 (2H, d, J= 8.2 Hz, H12), 7.62 (1H, d, J= 2.1 Hz, H8), 7.39 (2H, d, J= 8.5 Hz, H13), 7.35 (2H, dd, J= 8.7 and 1.9 Hz, H6), 7.28 (4H, m, H15-H16) and 4.00 (2H, s, H14) ppm; IR υ_{max} = 3903, 3849, 3687, 3354, 2974, 2887, 2478, 2333, 1913, 1625, 1556, 1502, 1462, 1406, 1352, 1317, 1255, 1211, 1167, 1089, 1053, 887, 813, 775, 696 and 671 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 430; HRMS calcd for $C_{23}H_{16}NO_2F_3Cl$ $[M+H]^+$ 430.0822, found 430.0808; Anal. Calcd for $C_{23}H_{15}NO_2F_3Cl$: C, 64.27%; H, 3.52%; N, 3.26%; Found C, 63.88%; H, 3.47%; N, 3.19%.

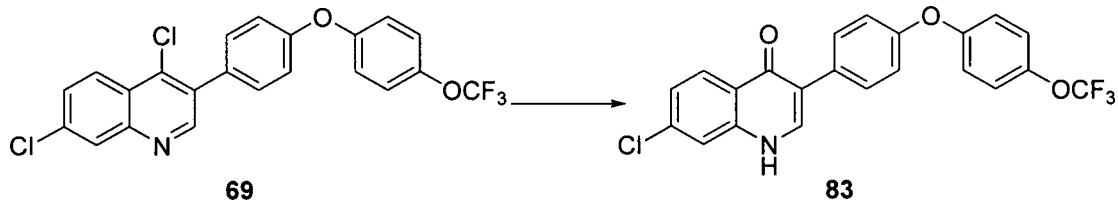
7-Chloro-3-(4'-(4''-fluorophenoxy)phenyl)quinolin-4(1H)-one.



68 **82**

See general procedure 9. White solid (56 mg, 73%): mp= 330 °C; IR υ_{max} = 3955, 3787, 3737, 3650, 3625, 3338, 2974, 2893, 1919, 1628, 1556, 1504, 1458, 1346, 1263, 1213, 1087, 1051, 1010, 883, 829 and 767 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 366; HRMS calcd for $C_{21}H_{14}NO_2FCl$ $[M+H]^+$ 366.0697, found 366.0693.

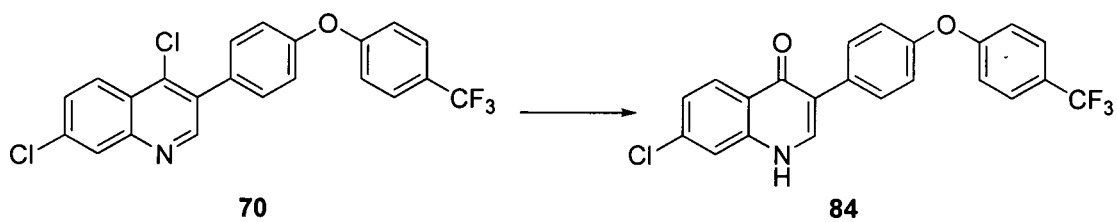
7-Chloro-3-(4'-(4''-(trifluoromethoxy)phenoxy)phenyl)quinolin-4(1H)-one.



69 **83**

See general procedure 9. White solid (59 mg, 61%): mp= 316°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.73 (1H, d, J= 8.7 Hz, H5), 8.07 (1H, s, H2), 8.06 (1H, s, -NH), 7.73 (1H, d, J=8.7 Hz, H12), 7.60 (1H, d, J= 2.0 Hz, H8), 7.28 (1H, dd, J= 2.0 and 8.7 Hz, H6), 7.27 (2H, dd, J= 0.8 and 9.0 Hz, H15), 7.08 (2H, d, J=7.1 Hz, H16) and 7.05 (2H, d, J=8.8 Hz, H13) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.8, 156.2, 155.1, 147.5, 143.7, 140.3, 138.2, 136.8, 132.2, 130.2, 128.0, 123.8, 122.8, 120.29, 119.5, 118.8 and 117.6 ppm; IR υ_{max} = 3880, 3849, 3687, 3357, 3068, 2974, 2887, 2478, 2350, 1915, 1556, 1502, 1462, 1410, 1348, 1304, 1275, 1250, 1211, 1161, 1088, 1053, 1014, 889, 831, 767 and 690 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 432; HRMS calcd for $C_{22}H_{14}NO_3F_3Cl$ $[M+H]^+$ 432.0614, found 432.0611.

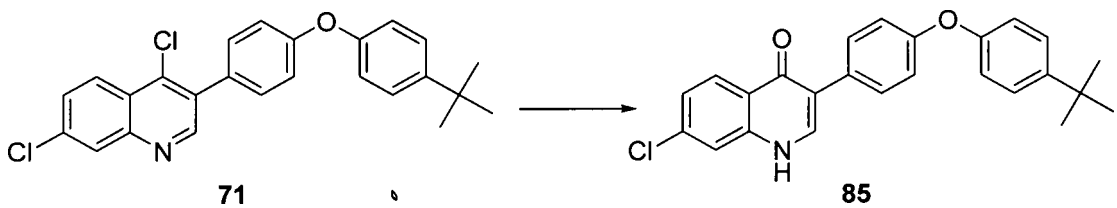
7-Chloro-3-(4'-(4''-(trifluoromethyl)phenoxy)phenyl)quinolin-4(1H)-one.



70 **84**

See general procedure 9. White solid (27 mg, 59%): mp= 318 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.27 (1H, s, H2), 8.21 (1H, d, J= 8.73 Hz, H5), 7.83 (1H, d, J=8.82 Hz, H12), 7.75 (2H, d, J=9.02 Hz, H16), 7.64 (1H, d, J=1.99 Hz, H8), 7.37 (1H, dd, J=2.09 and 8.73 Hz, H6) and 7.17 (4H, m, H13-H15) ppm; IR υ_{max} = 3583, 3001, 2947, 2329, 1916, 1614, 1556, 1506, 1329, 1242, 1169, 1132, 1107, 1066, 833 and 775 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 416; HRMS calcd for $C_{22}H_{14}NO_2F_3Cl$ $[M+H]^+$ 416.0665, found 416.0649.

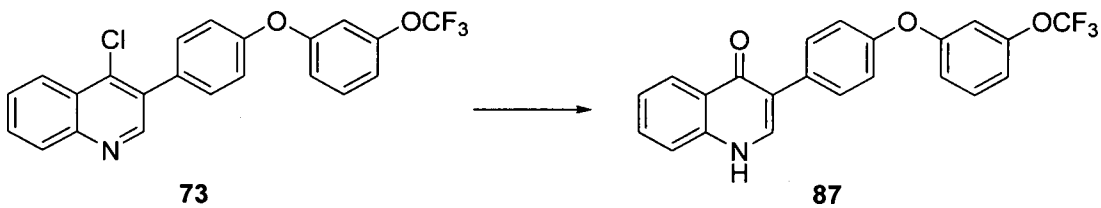
3-(4'-(4''-*tert*-Butylphenoxy)phenyl)-7-chloroquinolin-4(1H)-one.



71 **85**

See general procedure 9. White solid (65 mg, 56%): mp=328°C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.20 (1H, s, H2), 8.19 (1H, d, J= 7.9 Hz, H5), 7.72 (1H, d, J=7.9 Hz, H12), 7.64 (1H, s, H8), 7.41 (2H, d, J= 7.8 Hz, H15), 7.35 (1H, d, J= 7.9 Hz, H6), 7.01 (2H, d, J=7.9 Hz, H16), 6.96 (2H, d, J=7.9 Hz, H13), 2.50 (1H, m, -CH of *t*Bu) and 1.28 (9H, s, -CH_3 of *t*Bu) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 174.5, 156.0, 154.7, 146.1, 140.4, 138.8, 136.4, 131.1, 130.3, 128.3, 127.0, 124.7, 123.9, 120.2, 118.5, 118.3, 117.7, 34.4 and 31.6 ppm; IR υ_{max} = 3880, 3851, 3687, 3398, 3068, 2972, 2322, 1909, 1628, 1556, 1504, 1462, 1408, 1350, 1246, 1174, 1109, 1053, 1014, 889, 829, 769 and 694 cm^{-1} ; MS (ES+), $[M+H]^+$ (100) 404; HRMS calcd for $C_{25}H_{23}NO_2Cl$ $[M+H]^+$ 404.1417, found 404.1432.

3-(4'-(3''-(Trifluoromethoxy)phenoxy)phenyl)quinolin-4(1H)-one.

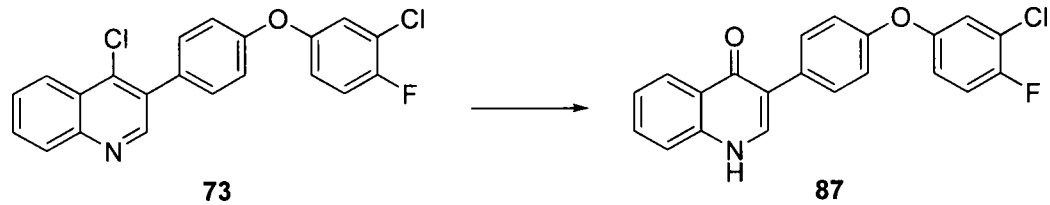


73 **87**

See general procedure 9. White solid (90 mg, 89%): mp= 234 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.22 (1H, dd, J= 1.5 and 8.2 Hz, H5), 8.21 (1H, s, H2), 7.83 (1H, d, J=8.8 Hz, H12), 7.67 (1H, dt, J=1.5 and 8.3 Hz, H7), 7.60 (1H, d, J=8.3 Hz,

H8), 7.52 (1H, t, *J*=8.2 Hz, H15), 7.36 (1H, dt, *J*= 1.1 and 8.1 Hz, H6), 7.14 (2H, d, *J*=8.8 Hz, H13) and 7.07-7.01 (3H, m, H15'-H16' and H17') ppm; ^{13}CNMR (100MHz, DMSO-d_6) δ_C 175.0, 158.9, 149.2, 148.0, 139.7, 138.5, 132.5, 131.9, 131.8, 130.5, 126.0, 125.9, 123.7, 119.3, 119.2, 118.6, 117.1, 115.6 and 111.2 ppm; IR υ_{max} = 3880, 3849, 3687, 3398, 2970, 2322, 1915, 1599, 1556, 1516, 1481, 1352, 1290, 1259, 1209, 1151, 980, 870, 819, 775, 750 and 700 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 398; HRMS calcd for $C_{22}H_{15}NO_3F_3$ $[M+H]^+$ 398.1004, found 398.1001; Anal. Calcd for $C_{22}H_{14}NO_3F_3$: C, 66.50%; H, 3.55%; N, 3.53%; Found C, 66.48%; H, 3.61%; N, 3.44%.

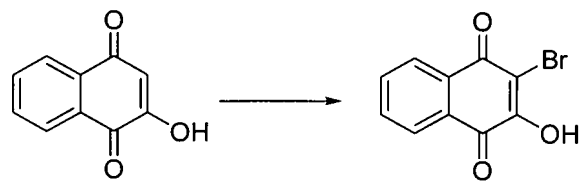
3-(4'-(3''-Chloro-4''-fluorophenoxy)phenyl)quinolin-4(1H)-one.



73 **87**

See general procedure 9. White solid (52 mg, 60%): mp= 250 °C; ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.22 (1H, dd, *J*= 1.3 and 8.3 Hz, H5), 8.20 (1H, s, H2), 7.80 (1H, d, *J*=8.8 Hz, H12), 7.67 (1H, dt, *J*=1.5 and 8.3 Hz, H6), 7.59 (1H, d, *J*=8.2 Hz, H8), 7.46 (1H, t, *J*=9.1 Hz, H16'), 7.35 (1H, dt, *J*= 1.2 and 8.2 Hz, H6), 7.30 (1H, dd, *J*=2.9 and 6.2 Hz, H15), 7.09 (2H, d, *J*=8.8 Hz, H13) and 7.09-7.05 (1H, m, H15') ppm; IR υ_{max} = 3957, 3757, 3676, 3625, 3338, 2974, 2891, 2337, 1612, 1589, 1556, 1491, 1346, 1296, 1265, 1215, 1167, 1091, 1049, 1014, 926, 845, 821, 762, 702 and 669 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 366; HRMS calcd for $C_{21}H_{14}NO_2FCl$ $[M+H]^+$ 366.0697, found 366.0690; Anal. Calcd for $C_{21}H_{12}NO_2FCl$: C, 68.95%; H, 3.58%; N, 3.83%; Found C, 69.20%; H, 3.57%; N, 3.74%.

2-Bromo-3-hydroxynaphthoquinone.[64]

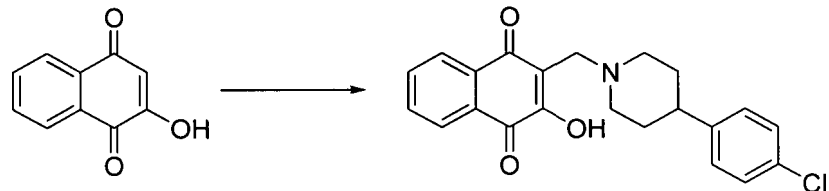


90 **91**

To a solution of 2-hydroxynaphthoquinone (1.00 g, 5.7 mmol) in acetic acid (25 mL) containing a crystal of iodine was slowly added bromine (1 eq). After 2 hrs a

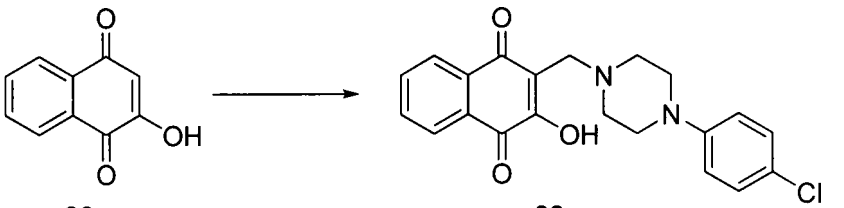
crystalline yellow solid was collected and stirred with NH_4OH (15 mL). An orange crystal was collected from filtration (1.50 g, 100%). R_f =0.54 (10% MeOH/DCM); mp= 196 °C; ^{1}H NMR (200 MHz, DMSO-d_6) δ_H 7.91 (1H, dd, J=7.42, 1.64 Hz), 7.81 (1H, dd, J=7.42, 1.64 Hz), 7.68 (1H, dt, J=7.42, 1.64 Hz), 7.56 (1H, dt, J=7.42, 1.64 Hz) ppm; ^{13}C NMR (100 MHz, DMSO-d_6) δ_C 184.0 (C-OH), 174.2 (C=O), 168.1 (C=O), 135.4, 134.0, 131.1, 130.9, 126.0, 125.9 ppm; MS (CI), $[M+NH_4\text{-}Br]^+$ (100) 192; HRMS calcd for $C_{10}H_9O_3BrN$ $[M+NH_4]^+$ 269.9766, found 269.9758.

2-((4'-(4''-Chlorophenyl)piperidin-1'-yl)methyl)-3-hydroxynaphthoquinone.



To a solution of 2-hydroxynaphthoquinone (67 mg, 0.39 mmol) in absolute ethanol (30 mL) was added formaldehyde (23 mg, 0.77 mmol) and 4-(4-chlorophenyl)piperidine (180 mg, 0.77 mmol). The reaction was quenched after 1 hr with NaOH (10 mL). The mixture was extracted with DCM (3×20 mL) and washed with brine (3×20 mL). Purification of the crude product by flash chromatography (10% MeOH/DCM) gave the product as an orange solid (48 mg, 30%); mp= 148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.01 (2H, d, *J*= 7.60 Hz, CH-C-CO), 7.54 (1H, d, *J*= 7.4 Hz, CH), 7.30 (2H, m, CH-C-Cl), 7.17 (2H, m, CH), 4.22 (2H, s, CH_2), 3.91 (2H, m, CH_2), 2.98 (2H, m, CH_2), 2.77 (1H, m, CH_2), 2.39 (2H, m, CH_2) and 2.04 (2H, m, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 183.2, 181.7, 142.5, 134.0, 133.0, 131.9, 131.4, 129.2, 128.5, 127.1, 126.2, 126.1, 109.0, 54.7, 40.2 and 31.3 ppm; IR υ_{max} = 3986, 3745, 3558, 3014, 2848, 2516, 2353, 1678, 1587, 1527, 1365, 1277, 1234, 1012, 933, 821, 737, 696 and 659 cm^{-1}; MS (ES-), $[M\text{-}H]^-$ (100) 380; HRMS calcd for $C_{22}H_{19}NO_3Cl$ $[M\text{-}H]^-$ 380.1053, found 380.1063.

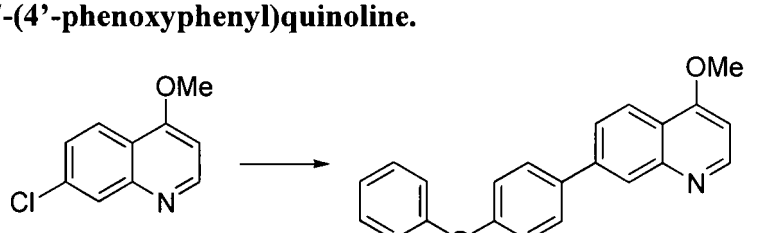
2-((4'-(4''-Chlorophenyl)piperazin-1'-yl)methyl)-3-hydroxynaphthoquinone.



90 **93**

To a solution of 2-hydroxynaphthoquinone (260 mg, 1.48 mmol) in absolute ethanol (30 mL) was added formaldehyde (0.33 mL, 4.74 mmol) and 4-(4-chlorophenyl)piperazine (1.28 g, 4.74 mmol). The reaction was quenched after 1 hr with NaOH (10 mL). The mixture was extracted with DCM (3×20 mL) and washed with brine (3×20 mL). Purification of the crude product by flash chromatography (10 % MeOH/DCM) gave the product as an orange solid: (238 mg, 42 %); mp= 148 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.01 (1H, d, J= 7.8 Hz, CH-C-CO), 7.63 (1H, d, J= 7.6 Hz, CH-C-CO), 7.57 (1H, m, CH), 7.35 (1H, m, CH-C-Cl), 7.23 (2H, m, CH), 6.86 (2H, m, CH), 4.17 (2H, s, CH_2), 3.52 (4H, m, CH_2) and 3.42 (4H, m, CH_2) ppm; ^{13}C NMR (100 MHz, $CDCl_3$) δ_C 183.0, 181.5, 149.0, 134.1, 131.8, 131.7, 129.6, 126.7, 126.3, 126.2, 118.6, 53.2 and 48.0 ppm; IR υ_{max} = 3882, 3803, 3635, 3573, 3064, 2947, 2592, 2316, 1678, 1587, 1529, 1359, 1277, 1226, 1072, 928, 802 and 737 cm^{-1}; MS (ES+), $[M+H]^+$ (100) 383; HRMS calcd for $C_{21}H_{20}N_2O_3Cl$ $[M+H]^+$ 383.1162, found 383.1154.

4-Methoxy-7-(4'-phenoxyphenyl)quinoline.

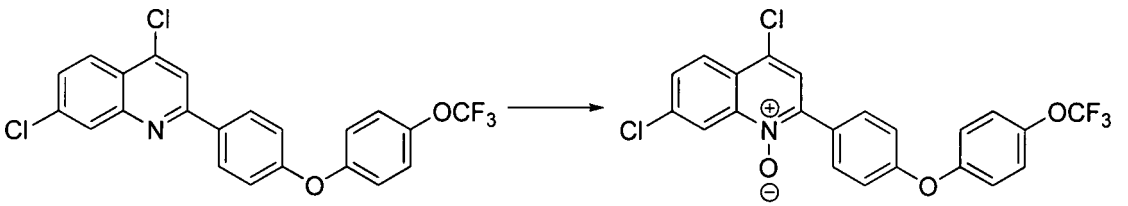


46 **95**

7-chloro-4-methoxyquinoline (100 mg, 0.52 mmol) and 4-(phenoxy)phenylboronic acid (167 mg, 0.78 mmol) were dissolved in DMF (15 mL) and aqueous Na_2CO_3 (1M, 1 mL) was added, the resulting mixture was deoxygenated with a stream of argon. After 20 min, $Pd(PPh_3)_4$ (32 mg, 0.03 mmol) was added, and mixture was brought to reflux, allowed to stir under argon for 12 hrs and cooled to r.t.. The solution was filtered through celite, washed with Et_2O (20 mL). The aqueous layer was washed with Et_2O (3×20 mL), and the organic phases were combined and

washed with NaOH (1M, 10 mL) followed by brine (2×20 mL). The ethereal solution was dried over $MgSO_4$ and evaporated. Purification of the crude product by flash chromatography with pur PE gave the product as a white crystal (100 mg, 58 %): ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.77 (1H, d, *J*= 5.3 Hz, H2), 8.29 (1H, d, *J*=1.8 Hz, H8), 8.23 (1H, d, *J*= 8.7 Hz, H5), 7.75 (1H, dd, *J*= 8.6 and 1.8 Hz, H6), 7.72 (2H, d, *J*= 8.7 Hz, CH), 7.37 (2H, m, CH), 7.15-7.07 (5H, m, CH) and 6.75 (2H, d, *J*= 5.4 Hz, H3) ppm; ^{13}CNMR (100 MHz, $CDCl_3$) δ_C 163.3, 157.9, 157.3, 151.8, 149.0, 142.6, 135.4, 130.2, 129.2, 125.8, 125.6, 124.0, 122.8, 120.5, 119.6, 119.5, 100.4 and 56.3 ppm; MS (CI), $[M+H]^+$ (100) 328; HRMS calcd for $C_{22}H_{18}NO_2$ $[M+H]^+$ 328.1337, found 328.1341.

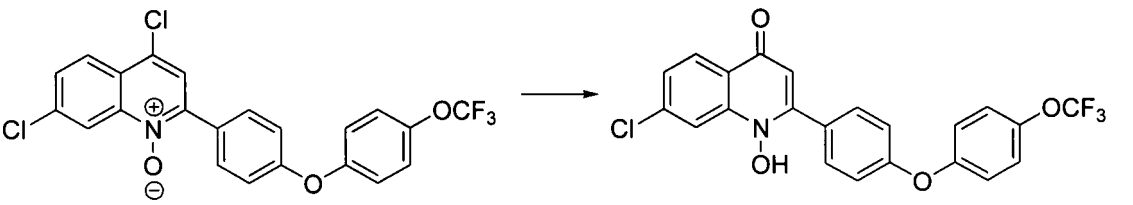
4,7-Dichloro-2-(4'-(4''-(trifluoromethoxy)phenoxy)phenyl)quinoline N-oxide.



31 **97**

4,7-Dichloro-2-(4-trifluorophenoxy)phenyl)quinoline (70 mg, 0.15 mmol) and *m*-CPBA (150 mg, 0.86 mmol) in anhydrous DCM (15 mL) was stirred for 3 hrs at r.t. The solution was washed with a solution of Na_2CO_3 (0.5 M, 2×5 mL) and H_2O (5 mL), dried with $MgSO_4$ and concentrated under reduced pressure. The residue was purified by flash chromatography (EtOAc/ PE: 10/90, *Rf* = 0.20) to give *N*-oxide quinolone product as a white crystal (35 mg, 50 %); mp= 126 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ_H 8.89 (1H, d, *J*= 2.1 Hz, H8), 8.16 (1H, d, *J*=8.9 Hz, H5), 8.01 (2H, d, *J*= 8.9 Hz, CH), 7.70 (1H, dd, *J*= 8.9 and 2.0 Hz, H6), 7.62 (1H, s, H3), 7.24 (2H, d, *J*= 9.1 Hz, CHCCN), 7.13 (2H, d, *J*= 8.9 Hz, CH) and 7.10 (2H, d, *J*= 9.0 Hz, CHCO) ppm; MS (ES+), $[M+H]^+$ (100) 466; HRMS calcd for $C_{22}H_{13}NO_3F_3Cl_2$ $[M+H]^+$ 466.0225, found 466.0234.

7-Chloro-1-hydroxy-2-(4'-(4''-(trifluoromethoxy)phenoxy)phenyl)quinolin-4(1H)-one.



97 **98**

To *N*-oxide quinoline (46 mg, 0.09 mmol) in DMF (10 mL) was added a solution of 85% formic acid in water (3 mL). The mixture was refluxed for 24 hrs. After cooling down, some precipitate appears, and the mixture was poured into water (50 mL) and filtered to afford the product as a white solid (5 mg, 11 %); ^{1}H NMR (400 MHz, DMSO-d_6) δ_H 8.08 (1H, d, J= 8.7 Hz, H5), 7.90 (2H, d, J= 8.5 Hz, Ph), 7.77 (1H, d, J= 2.0 Hz, H8), 7.45 (2H, d, J= 9.0 Hz, Ph), 7.32 (1H, dd, J= 9.0 and 1.6 Hz, H6), 7.22 (4H, m, Ph) and 6.40 (1H, s, H3) ppm; MS (ES-), $[M-H]^-$ (100) 446; HRMS calcd for $C_{22}H_{12}NO_4F_3Cl$ $[M-H]^-$ 446.0407, found 446.0395.

3.7. Literature

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CHAPTER 4

Final Discussion

4. Final Discussion

The aims of this thesis were the synthesis of polar artemisinin derivatives and quinolone analogues and a study of their antimalarial activities. For the novel quinolones prepared, we also investigated their SAR in connection with some molecular modelling.

4.1. Artemisinin

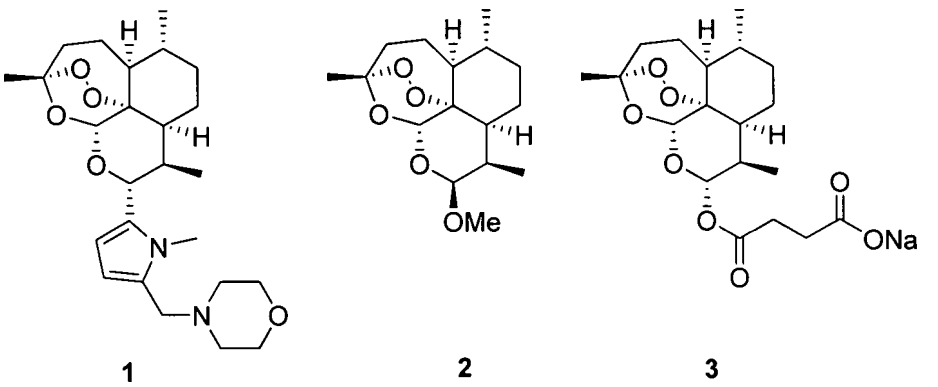
Artemisinin-derived molecules such as artesunate, arteether, artemether, or dihydroartemisinin (DHA) are extremely potent antimalarials that produce very rapid therapeutic response against the parasite's asexual erythrocytic (red blood cell) stage. These compounds also have a strong activity against the parasite blood-stage gametocytes (sexual stage), which can potentially help to reduce the rate of malaria transmission. Artemisinin-derived molecules are being used in recently developed artemisinin combination therapies (ACTs). There is some evidence that use of such combinations can retard the development of resistance to the partner drug and the WHO recommend the use of ACTs to all countries experiencing resistance to conventional monotherapies.[1]

ACTs mix fast-acting and rapidly-cleared artemisinin-derived drugs with other antimalarials with longer half-lives. The co-formulation of artemether in fixed combination with lumefantrine is known as Coartem^{tm} or Riamet^{tm}. Lumefantrine has an elimination half-life of up to 6 days in malaria patients, and is intended to eradicate parasites not killed by the faster acting artemether.[2] In use for many years and the first-line treatment in several parts of SE Asia, the combination of artesunate and mefloquine is sold under the name Artequin^{tm}. Artesunate is also approved in combination with amodiaquine or sulfadoxine/pyrimethamine.

ACTs have been used in Southeast Asia, Africa, and other parts of the world and it is believed that they may slow the spread of drug resistance and reduce the overall malaria transmission rates.

The first aim of this thesis was to develop new classes of semi-synthetic artemisinins. Firstly, we tried to make some sulfone derivatives in order to decrease the lipophilicity of the lipophilic parent. Unfortunately the use of sulfonyl groups at the C-10 anomeric position was not tolerated. In these C-10 sulfonyl we observed elimination that

resulted in the formation of anhydroartemisinin as a by-product. We secondly synthesised a small library of pyrrole analogues. Their antimalarial studies showed some promising results. One of our compounds (**1**) selected was more potent than artemether and sodium artesunate (**Figure 1**) in *in vivo* studies in *Plasmodium Berghei.*



	1	2	3
ED50 mg/kg	1.77	5.88	3.23
ED90 mg/kg	5.20	10.57	>10

Figure 1. Artemisinin derivatives with their *in vivo* activities.

Currently the best semi-synthetic artemisinins in the literature are artemisone[3], Posner's semi-synthetic dimers[4] and some lipophilic derivatives produced by Singh.[5] In studies with Aotus monkeys infected with *P.falciparum* FVO isolate, which is resistant to chloroquine and antifolates, artesunate and artemisone (**4**) were examined at a dose of 10 $mg.kg^{-1}$ for 3 days. The artemisone-treated group cleared parasites within 24 hrs after commencement of treatment, whereas parasites were still present 48 hrs after treatment with artesunate.[3] In three consecutive daily oral doses of 30 mg/kg starting on day 1 after infection, tolyl dimer (**5**) are curative, in sharp contrast to artesunate, which increased survival versus control by less than 1 day.[3] Singh's compound (**6**) is more than twice as active as β-arteether and artesunic acid; The fluorene derivative provided 100% protection at 24 mg/kg for 4 days and 80% at 12 mg/kg for 4 days.[5]

Our morpholine derivative (**1**) is more potent than Singh's compound (**6**) and dimer (**5**). With only a 2-step synthesis from DHA, our compound is the most straightforward to obtain, and less lipophilic than **5** and **6**. Artemisone is still the least lipophilic of these 4 lead compounds and has slightly higher antimalarial activities (**Table 1**).

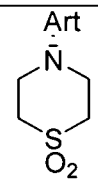
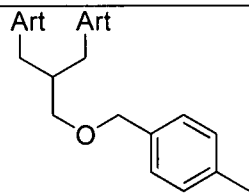
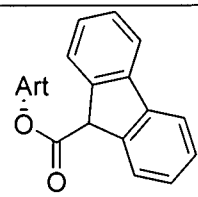
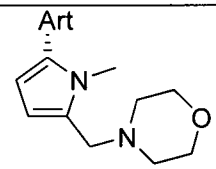
Name	Structure	Number of synthesic steps	Log P	*In vivo* activity Peter's 4-day test Oral dose per day
Artemisone (**4**)		3	2.49	*P.Berghei* ED_{90}= 3.1 $mg.kg^{-1}$
Trioxane dimer (**5**)		4	8.90	*P.Berghei* 100% clearance at 30 $mg.kg^{-1}$
Fluorene derivative (**6**)		3	6.61	P. Yoelii nigeriensis 100% clearance at 24 $mg.kg^{-1}$
Morpholine derivative (**1**)		2	3.40	*P.Berghei* ED_{90}= 5.2 $mg.kg^{-1}$

Table 1. Comparison of *in vivo* activities of our morpholine compound with current semi-synthetic artemisinins.

The peroxide group, present in the form of 1,2,4-trioxane, is essential for the antimalarial activity of these compounds. The disadvantage of all semi-synthetic artemisinin compounds is that their production requires artemisinin as starting material and currently the plant yields of artemisinin remain relatively low. Based on the idea of activation by iron(II), a series of trioxalanes or trioxanes have been made to produce 'bioactive' C-centred radicals.

Vennerstrom and co-workers described a synthetic peroxide antimalarial drug called trioxolanes. After a single 3mg. kg^{-1} oral dose in the murine *P. berghei* model, trioxolanes **7** is clearly more active than artesunate, artemether, chloroquine and mefloquine. Further studies showed the compound, dubbed OZ277, had a half-life significantly longer than dihydroartemisinin, and an overall acceptable toxicity profile.[6]

Several synthetic trioxanes have shown promising antimalarial activity both *in vitro* and *in vivo*.[7-11] O'Neill and co-workers use thiol-olefin co-oxygenation (TOCO) chemistry to afford a series of functionalized 1,2,4-trioxanes, for example **8**, in good yields.[12, 13]

Trioxepanes were also synthesised via TOCO chemistry[14] and keto trioxepane **9** shows 100% and 98% suppression of parasitaemia by intramuscular and oral routes against *P. yoelii* in Swiss mice, respectively, but none of the treated mice survived beyond day 14.[15]

1,2,4,5-Tetraoxanes have been proven to be superior to 1,2,4-trioxolanes in terms of stability and to be superior to trioxane analogues in terms of both stability and activity. Potent tetraoxane antimalarials have been prepared, In vivo, amines **10** and **11** cured all mice at higher doses with a minimum curative dose MCD of ≤ 37.5 (mg/kg)/day (**Figure 2**).[16]

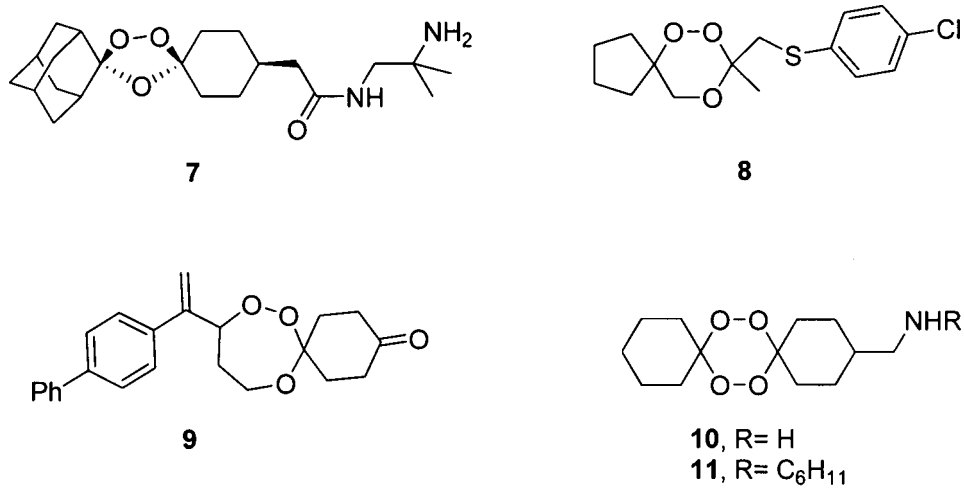
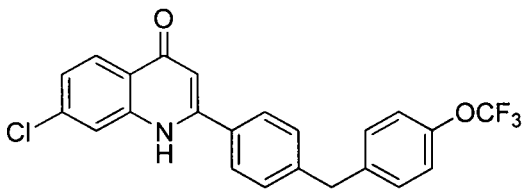


Figure 2. Trioxalane, Trioxane, Trioxepane, Tetraoxane.

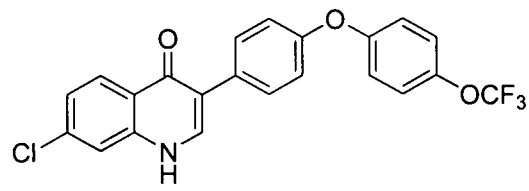
O'Neill and co-workers present evidence that all endoperoxide antimalarial compounds share a common free-iron-dependent mechanism of activation in malaria parasites, regardless of their other structural features; they demonstrate that tagged endoperoxide antimalarial compounds accumulate only in infected erythrocytes within the cytoplasm and the digestive vacuole of the parasite[17, 18] supporting the interaction with specific protein targets such as the SERCA orthologue (PfATP6).[19] Further work is required to investigate the mechanism of action of lead semi-synthetic analogue (**1**) and to compare synthetic analogues such as the tetraoxanes, this forms the basis of future work.

4.2. Quinolone

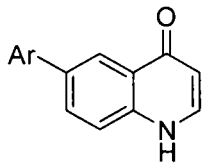
The second aim of this thesis was to investigate the SAR of novel quinolones. The outcome of this study was that 2-substituted quinolones have a better profile than their 3-substituted analogues with IC_{50} values ranging from 30 to 185 nM. It would be interesting to synthesise some 6- and 7-substituted quinolones and evaluate their *in vitro* activities to complete this SAR study (**Figure 3**).



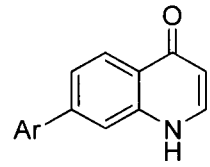
12, 2-substitutedquinolone: IC_{50}= 30 nM



13, 3-substitutedquinolone: IC_{50}= 387 nM



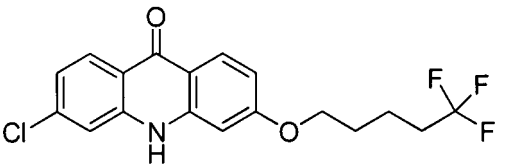
14, 6-substitutedquinolone



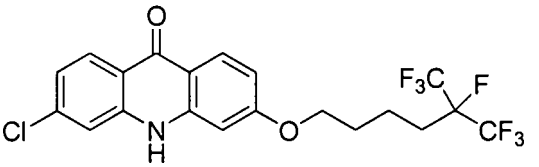
15, 7-substitutedquinolone

Figure 3. 2, 3 and 7-substituted quinolones and their IC_{50}s.

Recently Riscoe et al prepared over 30 acridones and these compounds were tested to elaborate an understanding of the anti-malarial structure-activity relationships. The most potent compounds (**16-17**) are composed of an extended alkyl group terminated by one or more CF_3 groups (**Figure 4**). It seems that the optimal location of CF_3 containing alkyl element is at the 3 position of the tricyclic acridone. Some of these acridones have IC_{50} values in the picomolar range when tested against the mefloquine resistant (D6) strains of *P.falciparum*, which is more potent than any drug in clinical use today (including the quinolines and endoperoxides).[20]



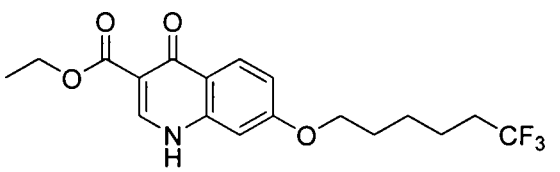
16, 3-(5,5,5-trifluoropentyloxy)-
6-chloroacridone (0.3 nM)



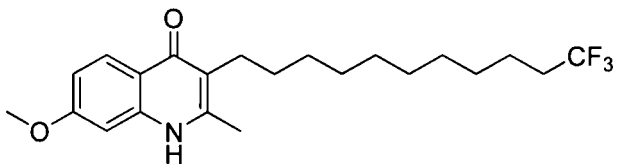
17, 3-(5,6,6,6-tetrafluoro-5-trifluoromethylhexyloxy)-
6-chloro-acridone (~1 pM)

Figure 4. Acridones

Riscoe and co-workers are also developing novel quinolones bearing extended alkyl and alkoxy side chains (**18-19**). Their antiplasmodial activities against the mefloquine resistant (D6) strains of *P.falciparum* reach some IC_{50} values of 1.2 nM (**Figure 5**). Further mechanistic studies lead to the conclusion that these quinolone derivatives target the parasite's cytochrome bc_1 complex as cross-resistance was noted in the atovaquone-resistant clinical isolate Tm90-C2B.



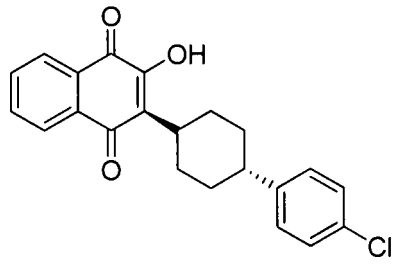
18, IC_{50}= 1.20 nM



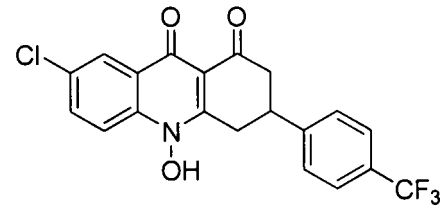
19, IC_{50}= 1.25 nM

Figure 5. 3-Ethoxycarbonyl-7-(6,6,6-trifluorohexyloxy)-4(1*H*)-quinolone (**18**) and 7-Methoxy-2-methyl-3-(11,11,11-trifluoroundecyl)-4(1*H*)-quinolone (**19**).

While their mechanism of action has not been established yet, we can consider a structural resemblance of acridones and quinolones with two well-known antimalarials, atovaquone and floxacrine (**Figure 6**).



20, Atovaquone



21, Floxacrine

Figure 6. Existing antimalarials.

Naphthoquinone and some quinolones target bc_1, Nevertheless fluoroquinolones are antibiotics which do not target bc_1, their targets are DNA gyrases and topoisomerases.[21] Similar molecules, such as aminoquinolines and quinine, accumulate in the acidic food vacuole by an ion-trapping or weak-base mechanism.[22]

The recent, growing failure of atovaquone treatment and increased mortality of patients with malaria or *Pneumocystis* pneumonia has been linked to the appearance of mutations in the cytochrome *b* gene,[23] although other case of Malarone treatment failure have been reported in the absence of these mutations suggests that other mechanisms might also be involved.[24]

It is important to understand the molecular basis of the drug resistance and to develop new drugs that avoid resistance. Trumpower and co-workers have screened a library of 2-hydroxynaphthoquinones (**Figure 7**) and found that compounds with alkyl side-chain effectively inhibit the yeast bc_1 complex. Experimentally measured IC_{50} values showed strong correlations with their molecular modelling into the crystal structure of the yeast cytochrome bc_1 complex, which provides structural and quantitative explanations for their binding efficacy to target the enzyme.[25]

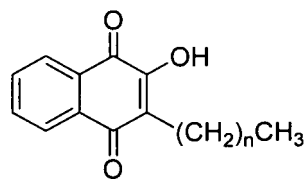


Figure 7. Library of naphthoquinones screened by Trumpower and co-workers.

Chemical synthesis of additional novel quinolones continues in an effort to expand our knowledge of the structure-activity relationships and to optimise the pharmacophore to the fullest of its antimalarial potential. Given the advances in computational approaches to drug discovery, it is likely that novel acridones and quinolones will be designed that are active against atovaquone resistant malaria parasites. The challenge in future work will be to produce inhibitors of the bc_1 complex that have more polar characteristics suitable for oral administration. The 2-arylquinolone series described in this thesis produces a template suitable for further optimisation and studies are underway to incorporate solubilising amine functionality into the quinolone template.

4.3. Literature

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Appendix 1. Crystal data and structure refinement for **86** (Chapter 2).

Identification code	**86**	
Empirical formula	C17 H28 N4 O5	
Formula weight	368.43	
Temperature	100(2) K	
Wavelength	0.71073 Å	
Crystal system	Orthorhombic	
Space group	P 21 21 21	
Unit cell dimensions	a = 10.4618(8) Å	α= 90°.
	b = 17.0721(14) Å	β= 90°.
	c = 20.4445(17) Å	γ = 90°.
Volume	3651.5(5) $Å^3$	
Z	8	
Density (calculated)	1.340 Mg/m^3	
Absorption coefficient	0.099 mm^{-1}	
F(000)	1584	
Crystal size	? x ? x ? mm^3	
Theta range for data collection	1.55 to 28.44°.	
Index ranges	-13<=h<=7, -22<=k<=21, -25<=l<=27	
Reflections collected	20759	
Independent reflections	8291 [R(int) = 0.0717]	
Completeness to theta = 28.44°	93.5 %	
Refinement method	Full-matrix least-squares on F^2	
Data / restraints / parameters	8291 / 0 / 477	
Goodness-of-fit on F^2	0.717	
Final R indices [I>2sigma(I)]	R1 = 0.0427, wR2 = 0.0746	
R indices (all data)	R1 = 0.1084, wR2 = 0.0824	
Absolute structure parameter	-2.5(10)	
Largest diff. peak and hole	0.271 and -0.320 $e.Å^{-3}$	

Table 2. Atomic coordinates (x 10^4) and equivalent isotropic displacement parameters ($Å^2$x 10^3) for **86**. U(eq) is defined as one third of the trace of the orthogonalized U^{ij} tensor.

	x	y	z	U(eq)
C(1)	5783(3)	3114(2)	8524(2)	39(1)
C(2)	4433(3)	4346(2)	8492(2)	29(1)
C(3)	3737(3)	5685(2)	8871(1)	26(1)
C(4)	3276(3)	6001(2)	9528(1)	26(1)
C(5)	2532(3)	5367(2)	9922(2)	40(1)
C(6)	2484(3)	6751(2)	9403(1)	24(1)
C(7)	1178(3)	6569(2)	9091(2)	35(1)
C(8)	425(3)	7315(2)	8936(2)	33(1)
C(9)	1186(3)	7860(2)	8498(1)	28(1)
C(10)	393(3)	8597(2)	8338(2)	47(1)
C(11)	2473(3)	8074(2)	8824(1)	26(1)
C(12)	3268(3)	7335(2)	9002(1)	22(1)
C(13)	3883(3)	6957(2)	8406(1)	23(1)
C(14)	5406(3)	7974(2)	8535(2)	28(1)
C(15)	6770(3)	8070(2)	8309(2)	35(1)
C(16)	4659(3)	8750(2)	8545(2)	34(1)
C(17)	3228(3)	8662(2)	8401(2)	34(1)
C(18)	-1843(3)	5705(2)	9703(2)	49(1)
C(19)	-747(3)	4537(2)	9174(2)	34(1)
C(20)	-72(3)	3131(2)	9048(2)	28(1)
C(21)	696(3)	2584(2)	9483(1)	27(1)
C(22)	1701(3)	3028(2)	9886(2)	38(1)
C(23)	1304(3)	1953(2)	9034(1)	25(1)
C(24)	2422(3)	2262(2)	8624(2)	34(1)
C(25)	2986(3)	1625(2)	8195(2)	33(1)
C(26)	1990(3)	1259(2)	7751(1)	30(1)
C(27)	2631(3)	624(2)	7317(2)	42(1)
C(28)	861(3)	929(2)	8143(1)	25(1)
C(29)	279(3)	1529(2)	8619(1)	21(1)
C(30)	-620(3)	2118(2)	8296(1)	24(1)
C(31)	-2098(3)	1057(2)	8333(1)	23(1)

C(32)	-3549(2)	999(2)	8292(1)	26(1)
C(33)	-1415(3)	358(2)	8015(1)	24(1)
C(34)	-145(3)	566(2)	7702(2)	29(1)
N(1)	5388(2)	3882(1)	8748(1)	30(1)
N(2)	5946(2)	4228(1)	9268(1)	36(1)
N(3)	5406(3)	4879(2)	9371(1)	36(1)
N(4)	4481(2)	4976(1)	8920(1)	27(1)
N(5)	-1337(3)	4893(2)	9686(1)	31(1)
N(6)	-1570(3)	4400(2)	10173(1)	49(1)
N(7)	-1135(3)	3711(2)	10023(1)	40(1)
N(8)	-649(2)	3774(1)	9398(1)	30(1)
O(1)	3750(2)	4233(1)	8031(1)	40(1)
O(2)	4537(2)	6247(1)	8554(1)	26(1)
O(3)	4238(2)	7606(1)	9464(1)	27(1)
O(4)	5513(2)	7620(1)	9158(1)	30(1)
O(5)	4793(2)	7434(1)	8096(1)	26(1)
O(6)	-376(2)	4790(1)	8672(1)	45(1)
O(7)	-1086(2)	2708(1)	8736(1)	26(1)
O(8)	-433(2)	1074(1)	9108(1)	25(1)
O(9)	-1837(2)	1141(1)	9003(1)	24(1)
O(10)	-1720(2)	1781(1)	8013(1)	24(1)

Table 3. Bond lengths [Å] and angles [°] for **86**.

C(1)-N(1)	1.448(3)
C(2)-O(1)	1.199(3)
C(2)-N(1)	1.378(4)
C(2)-N(4)	1.388(3)
C(3)-O(2)	1.429(3)
C(3)-N(4)	1.443(3)
C(3)-C(4)	1.527(4)
C(4)-C(6)	1.547(4)
C(4)-C(5)	1.557(4)
C(6)-C(12)	1.528(4)
C(6)-C(7)	1.540(4)
C(7)-C(8)	1.531(4)
C(8)-C(9)	1.515(4)
C(9)-C(10)	1.542(4)
C(9)-C(11)	1.547(4)
C(11)-C(17)	1.543(4)
C(11)-C(12)	1.555(4)
C(12)-O(3)	1.462(3)
C(12)-C(13)	1.521(4)
C(13)-O(5)	1.405(3)
C(13)-O(2)	1.424(3)
C(14)-O(4)	1.413(3)
C(14)-O(5)	1.438(3)
C(14)-C(15)	1.508(4)
C(14)-C(16)	1.537(4)
C(16)-C(17)	1.533(4)
C(18)-N(5)	1.484(3)
C(19)-O(6)	1.179(3)
C(19)-N(5)	1.358(4)
C(19)-N(8)	1.385(4)
C(20)-O(7)	1.433(3)
C(20)-N(8)	1.443(3)
C(20)-C(21)	1.519(4)
C(21)-C(22)	1.536(4)

C(21)-C(23)	1.551(4)
C(23)-C(24)	1.533(4)
C(23)-C(29)	1.547(4)
C(24)-C(25)	1.518(4)
C(25)-C(26)	1.517(4)
C(26)-C(28)	1.535(4)
C(26)-C(27)	1.553(4)
C(28)-C(34)	1.519(4)
C(28)-C(29)	1.539(4)
C(29)-O(8)	1.469(3)
C(29)-C(30)	1.527(4)
C(30)-O(10)	1.411(3)
C(30)-O(7)	1.436(3)
C(31)-O(9)	1.405(3)
C(31)-O(10)	1.454(3)
C(31)-C(32)	1.523(4)
C(31)-C(33)	1.536(3)
C(33)-C(34)	1.517(4)
N(1)-N(2)	1.349(3)
N(2)-N(3)	1.264(3)
N(3)-N(4)	1.347(3)
N(5)-N(6)	1.327(3)
N(6)-N(7)	1.299(3)
N(7)-N(8)	1.378(3)
O(3)-O(4)	1.473(3)
O(8)-O(9)	1.489(2)

O(1)-C(2)-N(1)	129.7(3)
O(1)-C(2)-N(4)	129.9(3)
N(1)-C(2)-N(4)	100.4(3)
O(2)-C(3)-N(4)	106.2(2)
O(2)-C(3)-C(4)	110.3(2)
N(4)-C(3)-C(4)	113.9(2)
C(3)-C(4)-C(6)	108.4(2)
C(3)-C(4)-C(5)	111.6(2)
C(6)-C(4)-C(5)	113.2(2)

C(12)-C(6)-C(7)	112.7(2)
C(12)-C(6)-C(4)	109.9(2)
C(7)-C(6)-C(4)	112.1(2)
C(8)-C(7)-C(6)	111.9(2)
C(9)-C(8)-C(7)	111.3(3)
C(8)-C(9)-C(10)	110.1(3)
C(8)-C(9)-C(11)	110.3(2)
C(10)-C(9)-C(11)	111.5(2)
C(17)-C(11)-C(9)	111.0(2)
C(17)-C(11)-C(12)	112.6(2)
C(9)-C(11)-C(12)	112.0(2)
O(3)-C(12)-C(13)	111.0(2)
O(3)-C(12)-C(6)	103.5(2)
C(13)-C(12)-C(6)	112.3(2)
O(3)-C(12)-C(11)	105.4(2)
C(13)-C(12)-C(11)	112.5(2)
C(6)-C(12)-C(11)	111.5(2)
O(5)-C(13)-O(2)	105.3(2)
O(5)-C(13)-C(12)	113.7(2)
O(2)-C(13)-C(12)	113.2(2)
O(4)-C(14)-O(5)	108.8(2)
O(4)-C(14)-C(15)	104.4(2)
O(5)-C(14)-C(15)	107.5(2)
O(4)-C(14)-C(16)	113.4(2)
O(5)-C(14)-C(16)	109.5(2)
C(15)-C(14)-C(16)	113.1(3)
C(17)-C(16)-C(14)	114.2(3)
C(16)-C(17)-C(11)	117.1(3)
O(6)-C(19)-N(5)	131.0(3)
O(6)-C(19)-N(8)	127.4(3)
N(5)-C(19)-N(8)	101.5(3)
O(7)-C(20)-N(8)	107.1(2)
O(7)-C(20)-C(21)	110.0(2)
N(8)-C(20)-C(21)	113.5(3)
C(20)-C(21)-C(22)	111.8(2)
C(20)-C(21)-C(23)	107.3(2)

C(22)-C(21)-C(23)	112.3(2)
C(24)-C(23)-C(29)	113.0(2)
C(24)-C(23)-C(21)	113.4(2)
C(29)-C(23)-C(21)	111.4(2)
C(25)-C(24)-C(23)	111.5(3)
C(26)-C(25)-C(24)	112.0(3)
C(25)-C(26)-C(28)	111.5(2)
C(25)-C(26)-C(27)	109.4(3)
C(28)-C(26)-C(27)	112.0(3)
C(34)-C(28)-C(26)	111.8(2)
C(34)-C(28)-C(29)	111.9(2)
C(26)-C(28)-C(29)	113.0(3)
O(8)-C(29)-C(30)	109.3(2)
O(8)-C(29)-C(28)	106.2(2)
C(30)-C(29)-C(28)	114.1(2)
O(8)-C(29)-C(23)	103.0(2)
C(30)-C(29)-C(23)	110.9(2)
C(28)-C(29)-C(23)	112.6(2)
O(10)-C(30)-O(7)	105.5(2)
O(10)-C(30)-C(29)	114.3(2)
O(7)-C(30)-C(29)	113.6(2)
O(9)-C(31)-O(10)	107.5(2)
O(9)-C(31)-C(32)	104.8(2)
O(10)-C(31)-C(32)	107.6(2)
O(9)-C(31)-C(33)	113.7(2)
O(10)-C(31)-C(33)	110.0(2)
C(32)-C(31)-C(33)	112.9(2)
C(34)-C(33)-C(31)	113.8(2)
C(33)-C(34)-C(28)	116.8(2)
N(2)-N(1)-C(2)	111.1(2)
N(2)-N(1)-C(1)	121.5(3)
C(2)-N(1)-C(1)	127.4(3)
N(3)-N(2)-N(1)	108.9(2)
N(2)-N(3)-N(4)	108.3(2)
N(3)-N(4)-C(2)	111.3(2)
N(3)-N(4)-C(3)	122.5(2)

C(2)-N(4)-C(3)	126.0(3)
N(6)-N(5)-C(19)	112.2(3)
N(6)-N(5)-C(18)	120.6(3)
C(19)-N(5)-C(18)	126.7(3)
N(7)-N(6)-N(5)	109.4(3)
N(6)-N(7)-N(8)	106.1(3)
N(7)-N(8)-C(19)	110.6(3)
N(7)-N(8)-C(20)	123.7(3)
C(19)-N(8)-C(20)	125.6(3)
C(13)-O(2)-C(3)	112.7(2)
C(12)-O(3)-O(4)	111.00(18)
C(14)-O(4)-O(3)	108.6(2)
C(13)-O(5)-C(14)	113.1(2)
C(20)-O(7)-C(30)	112.4(2)
C(29)-O(8)-O(9)	111.21(17)
C(31)-O(9)-O(8)	108.95(18)
C(30)-O(10)-C(31)	112.5(2)

Symmetry transformations used to generate equivalent atoms:

Table 4. Anisotropic displacement parameters ($Å^2 x\ 10^3$) for **86**. The anisotropic displacement factor exponent takes the form: $-2\pi^2[\ h^2\ a^{*2}U^{11} + ...\ + 2\ h\ k\ a^*\ b^*\ U^{12}\ ]$

	U^{11}	U^{22}	U^{33}	U^{23}	U^{13}	U^{12}
C(1)	30(2)	29(2)	60(3)	-4(2)	-4(2)	7(2)
C(2)	25(2)	23(2)	38(2)	0(2)	-1(2)	7(2)
C(3)	21(2)	23(2)	36(2)	3(2)	-9(2)	6(2)
C(4)	24(2)	28(2)	27(2)	2(2)	-1(2)	-2(2)
C(5)	36(2)	34(2)	50(2)	7(2)	3(2)	4(2)
C(6)	18(2)	29(2)	24(2)	0(2)	-2(2)	2(2)
C(7)	23(2)	30(2)	51(2)	0(2)	-2(2)	-5(2)
C(8)	17(2)	41(2)	40(2)	-5(2)	-5(2)	3(2)
C(9)	24(2)	33(2)	27(2)	0(2)	-5(2)	9(2)
C(10)	26(2)	44(2)	71(3)	6(2)	0(2)	8(2)
C(11)	24(2)	18(2)	37(2)	-5(2)	-2(2)	3(2)
C(12)	18(2)	25(2)	22(2)	-6(2)	-3(2)	-2(2)
C(13)	21(2)	22(2)	27(2)	3(2)	-3(2)	1(2)
C(14)	22(2)	32(2)	31(2)	-1(2)	-4(2)	-5(2)
C(15)	26(2)	31(2)	47(2)	7(2)	3(2)	-7(2)
C(16)	27(2)	28(2)	45(2)	2(2)	3(2)	-1(2)
C(17)	29(2)	23(2)	51(2)	-1(2)	-2(2)	8(2)
C(18)	39(3)	33(2)	74(3)	-12(2)	4(2)	-6(2)
C(19)	23(2)	49(2)	30(2)	1(2)	3(2)	-13(2)
C(20)	22(2)	28(2)	34(2)	-3(2)	-9(2)	3(2)
C(21)	23(2)	25(2)	33(2)	1(2)	-10(2)	-4(2)
C(22)	33(2)	36(2)	44(2)	-3(2)	-16(2)	-4(2)
C(23)	21(2)	27(2)	26(2)	3(2)	-8(2)	1(2)
C(24)	17(2)	42(2)	44(2)	9(2)	-4(2)	-3(2)
C(25)	15(2)	44(2)	40(2)	14(2)	0(2)	-3(2)
C(26)	25(2)	40(2)	26(2)	6(2)	-1(2)	-2(2)
C(27)	27(2)	59(3)	42(2)	-4(2)	9(2)	2(2)
C(28)	21(2)	32(2)	22(2)	7(2)	0(2)	1(2)
C(29)	16(2)	26(2)	22(2)	11(2)	-1(2)	-2(2)
C(30)	18(2)	29(2)	27(2)	3(2)	-2(2)	-2(2)
C(31)	23(2)	24(2)	21(2)	2(2)	-1(2)	-6(2)

C(32)	17(2)	28(2)	33(2)	1(2)	-2(2)	-3(2)
C(33)	20(2)	26(2)	26(2)	-3(2)	4(2)	1(2)
C(34)	26(2)	34(2)	27(2)	2(2)	4(2)	2(2)
N(1)	23(2)	29(2)	39(2)	-7(1)	-8(1)	4(1)
N(2)	22(2)	32(2)	55(2)	2(2)	-17(2)	6(2)
N(3)	36(2)	26(2)	46(2)	-10(1)	-14(2)	-1(2)
N(4)	21(2)	25(2)	36(2)	0(1)	-11(1)	5(1)
N(5)	27(2)	35(2)	31(2)	-4(1)	7(1)	-10(2)
N(6)	37(2)	71(2)	38(2)	-10(2)	12(2)	-9(2)
N(7)	40(2)	35(2)	45(2)	-9(2)	13(2)	-6(2)
N(8)	26(2)	25(2)	38(2)	4(1)	0(1)	-4(1)
O(1)	37(2)	36(1)	47(2)	-14(1)	-20(1)	12(1)
O(2)	22(1)	19(1)	37(1)	3(1)	5(1)	5(1)
O(3)	20(1)	32(1)	29(1)	-3(1)	1(1)	-3(1)
O(4)	20(1)	38(1)	31(1)	0(1)	-1(1)	-5(1)
O(5)	20(1)	28(1)	28(1)	-1(1)	3(1)	1(1)
O(6)	47(2)	49(2)	40(2)	3(1)	14(1)	9(1)
O(7)	18(1)	25(1)	36(1)	-1(1)	-8(1)	-3(1)
O(8)	16(1)	31(1)	28(1)	8(1)	-7(1)	2(1)
O(9)	16(1)	30(1)	26(1)	2(1)	-3(1)	1(1)
O(10)	18(1)	23(1)	30(1)	7(1)	-7(1)	-4(1)

Table 5. Hydrogen coordinates (x 10^4) and isotropic displacement parameters ($Å^2$x 10^{-3}) for **86**.

	x	y	z	U(eq)
H(1A)	5449	2714	8822	59
H(1B)	6718	3086	8516	59
H(1C)	5448	3022	8083	59
H(3)	2977	5582	8587	32
H(4)	4046	6152	9789	31
H(5A)	3128	4961	10069	60
H(5B)	1877	5131	9641	60
H(5C)	2123	5611	10302	60
H(6)	2315	6998	9838	29
H(7A)	1311	6268	8682	41
H(7B)	673	6238	9393	41
H(8A)	-386	7174	8716	39
H(8B)	213	7589	9348	39
H(9)	1371	7579	8079	34
H(10A)	305	8918	8733	71
H(10B)	-456	8440	8184	71
H(10C)	826	8900	7997	71
H(11)	2266	8346	9244	31
H(13)	3192	6836	8083	28
H(15A)	7216	7567	8344	52
H(15B)	7201	8460	8583	52
H(15C)	6778	8245	7852	52
H(16A)	4762	8995	8981	40
H(16B)	5038	9109	8218	40
H(17A)	3133	8505	7937	41
H(17B)	2825	9183	8449	41
H(18A)	-1256	6040	9950	73
H(18B)	-2684	5706	9914	73
H(18C)	-1923	5904	9255	73
H(20)	506	3348	8703	33

H(21)	95	2318	9792	32
H(22A)	1273	3334	10227	57
H(22B)	2180	3381	9598	57
H(22C)	2289	2653	10088	57
H(23)	1672	1547	9333	30
H(24A)	2121	2699	8345	41
H(24B)	3094	2468	8919	41
H(25A)	3680	1850	7924	40
H(25B)	3364	1213	8476	40
H(26)	1654	1678	7456	36
H(27A)	2003	417	7006	64
H(27B)	2947	198	7594	64
H(27C)	3347	857	7077	64
H(28)	1211	494	8418	30
H(30)	-134	2388	7941	29
H(32A)	-3935	1432	8536	39
H(32B)	-3829	499	8480	39
H(32C)	-3815	1027	7833	39
H(33A)	-1268	-48	8352	29
H(33B)	-1982	130	7677	29
H(34A)	-313	935	7338	35
H(34B)	219	84	7508	35
H(2)	6582	4033	9497	43
H(3A)	5601	5211	9685	43
H(6A)	-1960	4521	10541	58
H(7)	-1147	3288	10270	48

Table 6. Torsion angles [°] for **86**.

O(2)-C(3)-C(4)-C(6)	61.3(3)
N(4)-C(3)-C(4)-C(6)	-179.3(2)
O(2)-C(3)-C(4)-C(5)	-173.5(2)
N(4)-C(3)-C(4)-C(5)	-54.1(3)
C(3)-C(4)-C(6)-C(12)	-54.4(3)
C(5)-C(4)-C(6)-C(12)	-178.7(2)
C(3)-C(4)-C(6)-C(7)	71.8(3)
C(5)-C(4)-C(6)-C(7)	-52.5(3)
C(12)-C(6)-C(7)-C(8)	-52.5(3)
C(4)-C(6)-C(7)-C(8)	-177.1(2)
C(6)-C(7)-C(8)-C(9)	56.2(3)
C(7)-C(8)-C(9)-C(10)	178.8(2)
C(7)-C(8)-C(9)-C(11)	-57.8(3)
C(8)-C(9)-C(11)-C(17)	-177.1(2)
C(10)-C(9)-C(11)-C(17)	-54.4(3)
C(8)-C(9)-C(11)-C(12)	56.1(3)
C(10)-C(9)-C(11)-C(12)	178.7(2)
C(7)-C(6)-C(12)-O(3)	163.0(2)
C(4)-C(6)-C(12)-O(3)	-71.1(3)
C(7)-C(6)-C(12)-C(13)	-77.2(3)
C(4)-C(6)-C(12)-C(13)	48.7(3)
C(7)-C(6)-C(12)-C(11)	50.2(3)
C(4)-C(6)-C(12)-C(11)	176.1(2)
C(17)-C(11)-C(12)-O(3)	70.2(3)
C(9)-C(11)-C(12)-O(3)	-163.9(2)
C(17)-C(11)-C(12)-C(13)	-50.9(3)
C(9)-C(11)-C(12)-C(13)	75.0(3)
C(17)-C(11)-C(12)-C(6)	-178.2(2)
C(9)-C(11)-C(12)-C(6)	-52.2(3)
O(3)-C(12)-C(13)-O(5)	-53.6(3)
C(6)-C(12)-C(13)-O(5)	-168.9(2)
C(11)-C(12)-C(13)-O(5)	64.2(3)
O(3)-C(12)-C(13)-O(2)	66.5(3)
C(6)-C(12)-C(13)-O(2)	-48.8(3)

C(11)-C(12)-C(13)-O(2)	-175.7(2)
O(4)-C(14)-C(16)-C(17)	-93.1(3)
O(5)-C(14)-C(16)-C(17)	28.6(4)
C(15)-C(14)-C(16)-C(17)	148.3(3)
C(14)-C(16)-C(17)-C(11)	53.9(4)
C(9)-C(11)-C(17)-C(16)	-162.6(3)
C(12)-C(11)-C(17)-C(16)	-36.1(4)
O(7)-C(20)-C(21)-C(22)	-173.7(2)
N(8)-C(20)-C(21)-C(22)	-53.7(3)
O(7)-C(20)-C(21)-C(23)	62.7(3)
N(8)-C(20)-C(21)-C(23)	-177.3(2)
C(20)-C(21)-C(23)-C(24)	73.7(3)
C(22)-C(21)-C(23)-C(24)	-49.6(3)
C(20)-C(21)-C(23)-C(29)	-55.1(3)
C(22)-C(21)-C(23)-C(29)	-178.4(2)
C(29)-C(23)-C(24)-C(25)	-52.1(3)
C(21)-C(23)-C(24)-C(25)	179.9(2)
C(23)-C(24)-C(25)-C(26)	56.6(3)
C(24)-C(25)-C(26)-C(28)	-56.6(3)
C(24)-C(25)-C(26)-C(27)	179.0(2)
C(25)-C(26)-C(28)-C(34)	179.6(2)
C(27)-C(26)-C(28)-C(34)	-57.4(3)
C(25)-C(26)-C(28)-C(29)	52.3(3)
C(27)-C(26)-C(28)-C(29)	175.3(2)
C(34)-C(28)-C(29)-O(8)	72.6(3)
C(26)-C(28)-C(29)-O(8)	-160.1(2)
C(34)-C(28)-C(29)-C(30)	-47.9(3)
C(26)-C(28)-C(29)-C(30)	79.4(3)
C(34)-C(28)-C(29)-C(23)	-175.4(2)
C(26)-C(28)-C(29)-C(23)	-48.0(3)
C(24)-C(23)-C(29)-O(8)	162.0(2)
C(21)-C(23)-C(29)-O(8)	-69.0(3)
C(24)-C(23)-C(29)-C(30)	-81.2(3)
C(21)-C(23)-C(29)-C(30)	47.8(3)
C(24)-C(23)-C(29)-C(28)	48.0(3)
C(21)-C(23)-C(29)-C(28)	177.0(2)

O(8)-C(29)-C(30)-O(10)	-55.7(3)
C(28)-C(29)-C(30)-O(10)	63.1(3)
C(23)-C(29)-C(30)-O(10)	-168.6(2)
O(8)-C(29)-C(30)-O(7)	65.5(3)
C(28)-C(29)-C(30)-O(7)	-175.8(2)
C(23)-C(29)-C(30)-O(7)	-47.4(3)
O(9)-C(31)-C(33)-C(34)	-93.8(3)
O(10)-C(31)-C(33)-C(34)	26.8(3)
C(32)-C(31)-C(33)-C(34)	147.0(2)
C(31)-C(33)-C(34)-C(28)	58.4(3)
C(26)-C(28)-C(34)-C(33)	-168.8(3)
C(29)-C(28)-C(34)-C(33)	-40.9(4)
O(1)-C(2)-N(1)-N(2)	179.1(3)
N(4)-C(2)-N(1)-N(2)	-1.2(3)
O(1)-C(2)-N(1)-C(1)	-2.0(5)
N(4)-C(2)-N(1)-C(1)	177.8(3)
C(2)-N(1)-N(2)-N(3)	0.9(3)
C(1)-N(1)-N(2)-N(3)	-178.1(3)
N(1)-N(2)-N(3)-N(4)	-0.1(3)
N(2)-N(3)-N(4)-C(2)	-0.6(3)
N(2)-N(3)-N(4)-C(3)	-175.5(3)
O(1)-C(2)-N(4)-N(3)	-179.1(3)
N(1)-C(2)-N(4)-N(3)	1.1(3)
O(1)-C(2)-N(4)-C(3)	-4.5(5)
N(1)-C(2)-N(4)-C(3)	175.7(3)
O(2)-C(3)-N(4)-N(3)	77.3(3)
C(4)-C(3)-N(4)-N(3)	-44.4(4)
O(2)-C(3)-N(4)-C(2)	-96.8(3)
C(4)-C(3)-N(4)-C(2)	141.5(3)
O(6)-C(19)-N(5)-N(6)	-178.2(4)
N(8)-C(19)-N(5)-N(6)	0.3(3)
O(6)-C(19)-N(5)-C(18)	9.4(6)
N(8)-C(19)-N(5)-C(18)	-172.1(3)
C(19)-N(5)-N(6)-N(7)	1.3(4)
C(18)-N(5)-N(6)-N(7)	174.2(3)
N(5)-N(6)-N(7)-N(8)	-2.3(3)

N(6)-N(7)-N(8)-C(19)	2.6(3)
N(6)-N(7)-N(8)-C(20)	-179.9(3)
O(6)-C(19)-N(8)-N(7)	176.9(3)
N(5)-C(19)-N(8)-N(7)	-1.7(3)
O(6)-C(19)-N(8)-C(20)	-0.6(5)
N(5)-C(19)-N(8)-C(20)	-179.2(3)
O(7)-C(20)-N(8)-N(7)	85.4(3)
C(21)-C(20)-N(8)-N(7)	-36.2(4)
O(7)-C(20)-N(8)-C(19)	-97.5(3)
C(21)-C(20)-N(8)-C(19)	141.0(3)
O(5)-C(13)-O(2)-C(3)	-179.6(2)
C(12)-C(13)-O(2)-C(3)	55.6(3)
N(4)-C(3)-O(2)-C(13)	173.6(2)
C(4)-C(3)-O(2)-C(13)	-62.4(3)
C(13)-C(12)-O(3)-O(4)	14.1(3)
C(6)-C(12)-O(3)-O(4)	134.9(2)
C(11)-C(12)-O(3)-O(4)	-107.9(2)
O(5)-C(14)-O(4)-O(3)	-73.7(3)
C(15)-C(14)-O(4)-O(3)	171.8(2)
C(16)-C(14)-O(4)-O(3)	48.3(3)
C(12)-O(3)-O(4)-C(14)	46.0(3)
O(2)-C(13)-O(5)-C(14)	-96.2(2)
C(12)-C(13)-O(5)-C(14)	28.3(3)
O(4)-C(14)-O(5)-C(13)	33.2(3)
C(15)-C(14)-O(5)-C(13)	145.7(2)
C(16)-C(14)-O(5)-C(13)	-91.2(3)
N(8)-C(20)-O(7)-C(30)	171.8(2)
C(21)-C(20)-O(7)-C(30)	-64.5(3)
O(10)-C(30)-O(7)-C(20)	-177.7(2)
C(29)-C(30)-O(7)-C(20)	56.4(3)
C(30)-C(29)-O(8)-O(9)	17.4(3)
C(28)-C(29)-O(8)-O(9)	-106.2(2)
C(23)-C(29)-O(8)-O(9)	135.3(2)
O(10)-C(31)-O(9)-O(8)	-74.8(2)
C(32)-C(31)-O(9)-O(8)	170.96(19)
C(33)-C(31)-O(9)-O(8)	47.2(3)

C(29)-O(8)-O(9)-C(31)	44.5(3)
O(7)-C(30)-O(10)-C(31)	-97.7(2)
C(29)-C(30)-O(10)-C(31)	27.8(3)
O(9)-C(31)-O(10)-C(30)	35.6(3)
C(32)-C(31)-O(10)-C(30)	147.9(2)
C(33)-C(31)-O(10)-C(30)	-88.7(3)

Symmetry transformations used to generate equivalent atoms:

Table 7. Hydrogen bonds for **86** [Å and °].

D-H...A	d(D-H)	d(H...A)	d(D...A)	<(DHA)
N(2)-H(2)...N(6)#1	0.88	2.46	3.204(4)	143.0
N(2)-H(2)...N(5)#1	0.88	2.65	3.178(4)	119.3
N(2)-H(2)...N(7)#1	0.88	2.68	3.535(4)	165.3

Symmetry transformations used to generate equivalent atoms:

#1 x+1,y,z