BIOACTIVE CHLOROQUINE-BASED LIGANDS AND THEIR GOLD COMPLEXES AS POTENTIAL NOVEL ANTIMALARIAL AGENTS

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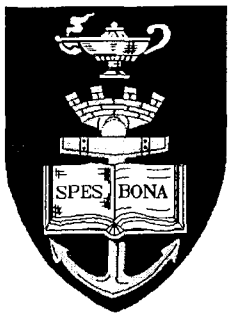

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ABSTRACT

Chloroquine(CQ)-derived 4-aminoquinolines have proven to be the most efficacious antimalarial drugs for both the treatment and prophylaxis of malaria. However, with the advent of drug resistance, their ability to treat the disease has been significantly hindered. Future research into the synthesis of new 4-aminoquinoline derivatives is warranted, since it has been found that the resistance is based on the identity of the side chain and not on the aminoquinoline ring, the functionality by which these compounds derive their activity. Consequently, the synthesis of CQ derivatives with a modified side chain attached to a substituted quinoline ring is a reasonable approach in the search of novel antimalarial compounds that are active against drug-resistant parasite strains.

The work presented in this thesis involved the synthesis of two classes of chloroquine-derived potential antimalarial compounds, namely, (i) acylthioureas, and (ii) 2-hydroxyacetamides. In order to evaluate the enhancing effect on antimalarial activity of gold complexation, corresponding gold complexes of a representative ligand from each class were prepared. All organic ligands and gold complexes were evaluated *in vitro* against strains of the most deadly malaria protozoan, *Plasmodium falciparum*, including the chloroquine-sensitive (CQS) D10 strain, and chloroquine-resistant (CQR) Dd2 and W2 strains.

The synthesised 4-amino-7-chloroquinolinyl acylthioureas were obtained in low yields (due to side reactions and difficulties in purification) of 25 – 65 %, and the aminoquinolinyl 2-hydroxyacetamides were isolated in moderate yields of 21 - 61 % (2 - 3 steps). The acylthiourea chlorogold(I) complex was obtained in 63 %, and the 2-hydroxyacetamide gold(I) dimer complex in 29 % yield.

The biological results revealed that within the acylthiourea class of molecules, compounds **10**, **11**, **12** and **13** (IC_{50} = 0.058 – 0.14 μM) all displayed antimalarial activities comparable to that of CQ (0.033 μM) against the CQS D10 strain, and against the CQR Dd2 strain, **11** (0.228 μM) was found to have an equivalent activity to that of CQ (0.233 μM). All acylthiourea derivatives displayed a marked decrease in activity against the CQR W2 strain, however, indicating cross-resistance with CQ.

The 2-hydroxyacetamides displayed mild activity against both the D10 and W2 strains and were not evaluated further. However, two compounds showed promising activity. The benzylated intermediate **16** displayed high activity against D10 (0.013 μM), Dd2 (0.064 μM) and W2 (0.016 μM) strains, and may provide a good lead compound for further development. Acetamide **18** was found to possess moderate activity against both CQS and CQR strains, however, it displayed the promising property of being more active against the CQR W2 (0.462 μM) strain than the CQS equivalent (0.946 μM), suggesting the potential for overcoming CQ resistance.

Gold complexation of acylthiourea **11** with the gold precursor AuCl(THT) was found to decrease its antimalarial activity, whereas complexation of the 2-hydroxyacetamide **23** with $AuCl(PPh_3)$ resulted in the formation of a dimer complex with a 43-fold increase in activity over the free ligand (IC_{50} = 0.296 μM vs. 12.755 μM, D10 strain). Gold complex structure, including spectator ligand and counter ion, appears to impact on the solubility and therefore the activity of the synthesised compounds. A more extensive study on the effects of these complex properties is required in order to establish the factors necessary for optimum biological activity.

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LIST OF ABBREVIATIONS

δ	Chemical shift
µg	Microgram
µM	Micromolar
Bn	Benzyl
br s	Broad singlet
^{13}C NMR	Carbon Nuclear Magnetic Resonance
CD_3OD	Deuteromethanol
$CDCl_3$	Deuterochloroform
d	Doublet (in ^1H NMR)
D_2O	Deuterowater
DCM	Dichloromethane
dd	Doublet of doublets (in ^1H NMR)
DMF	*N,N*-Dimethylformamide
DMSO	Dimethylsulfoxide
DMSO-d_6	Deuterodimethylsulfoxide
dt	Doublet of triplets (in ^1H NMR)
EI	Electron Impact
eq	Equivalents
Et_3N	Triethylamine
EtOAc	Ethylacetate
EtOH	Ethanol
^1H NMR	Proton Nuclear Magnetic Resonance
h	Hour(s)
hex	Hexane
HRMS	High Resolution Mass Spectrometry
Hz	Hertz
IC_{50}	50 % Inhibitory Concentration
IR	Infrared
J	Coupling constant
m	Multiplet

m.p.	Melting Point
m/z	Mass to charge ratio
MeOH	Methanol
min	Minute(s)
mg	Milligram
mL	Millilitre
mM	Millimolar
mol	Mole
nM	Nanomolar
NMP	*N*-Methyl-2-Pyrrolidinone
P	*Plasmodium*
ppm	Parts per million
q	Quartet
R_f	Retention factor
rt	Room temperature
s	Singlet (in ^{1}H NMR)
t	Triplet (in ^{1}H NMR)
TBAF	Tetrabutylammonium fluoride
THF	Tetrahydrofuran
TLC	Thin Layer Chromatography

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CHAPTER 1

INTRODUCTION

1.1 Background to Malaria

Malaria is one of the most important infectious diseases in the world. Over 300 million cases are reported annually, resulting in more than 1 million deaths.[1] More than 90 % of the infections and deaths occur in low-income countries of sub-Saharan Africa where the economic impact of the disease is severe. The World Health Organisation estimates that the annual GDP loss in Africa as a result of malaria is US$12 billion.[1] In a region already suffering from widespread poverty, the burden of this economic loss has particularly devastating consequences.

Four species of the *Plasmodium* genus are responsible for causing malaria in humans. These include *Plasmodium falciparum*, *P. vivax*, *P. malariae* and *P. ovale*, with most deaths resulting from *P. falciparum* infection. One of the greatest challenges in the fight against malaria has been the emergence of drug resistance, particularly to chloroquine (CQ) (Figure 1.1).[1-4] CQ has been the most widely used drug for the treatment of malaria due to its low cost, low toxicity, activity against all forms of malaria, relative ease of manufacture and chemical stability.[2, 4] However, since 1959, CQ resistance has spread, and now covers most parts of the tropical world.[3, 4] Despite remaining the most frequently used drug for the first-line treatment of malaria infections,[2] resistance in many areas has reached a point where CQ no longer has any clinical efficacy.[5] There is therefore a need for the development of new drugs with different modes of action to circumvent parasite resistance mechanisms.

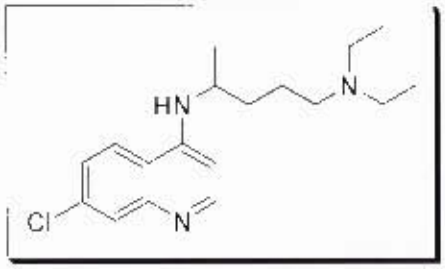


Figure 1.1 Structure of chloroquine (CQ)

1.2 Biology of the Malaria Parasite

1.2.1 Malaria lifecycle

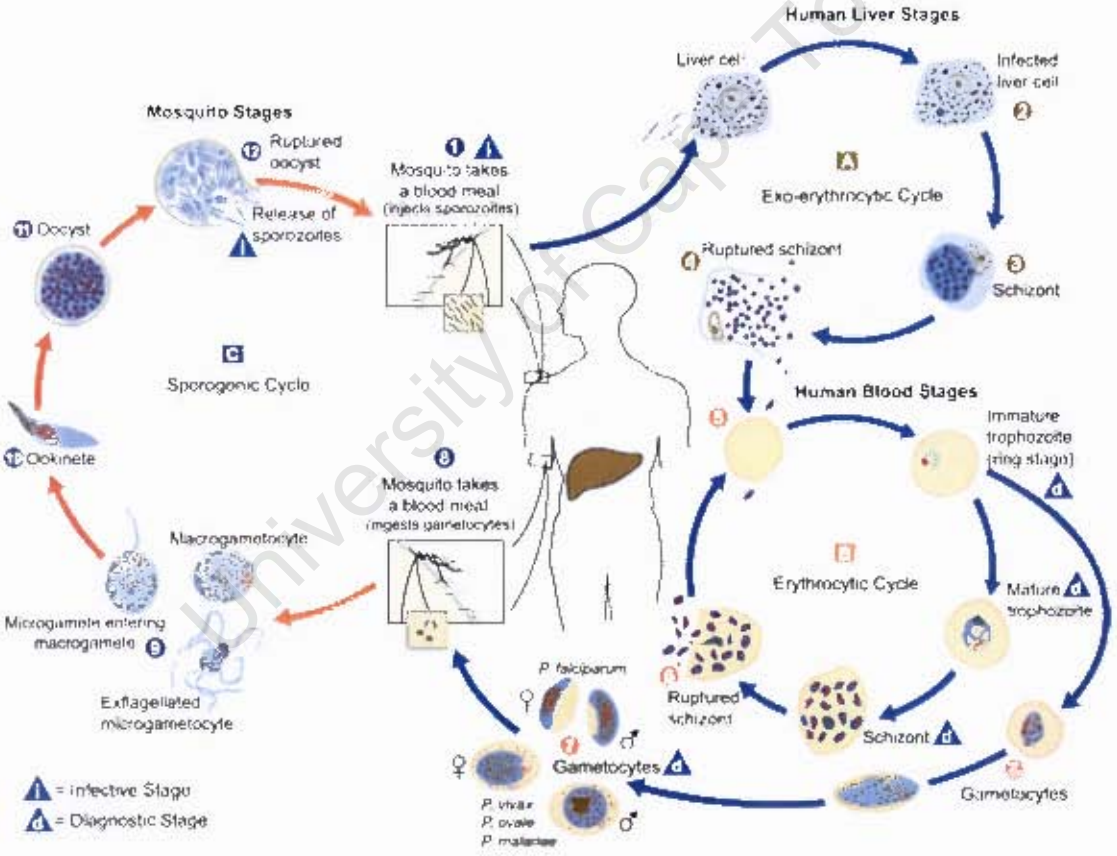


Figure 1.2 Life cycle of the malaria parasite [6]

The life cycle of *Plasmodium* involves two hosts (Figure 1.2). Parasite sporozoites are transmitted to their human host *via* the salivary glands of a biting female anopheles mosquito. The sporozoites migrate to the liver where they invade hepatocytes, multiply and mature into schizonts. In *P. vivax* and

P. ovale, a dormant stage (hypnozoite) can persist in the liver, causing relapses by invading the bloodstream weeks, or even years later.[6] These events comprise the exo-erythrocytic (liver) stage of malaria.[7] Mature schizonts rupture, releasing thousands of merozoites into the bloodstream, where they enter erythrocytes. These mature into trophozoites asexually multiplying into erythrocytic schizonts. This asexual blood stage causes all the symptoms associated with malaria, and has traditionally been the focus of most antimalarial drugs.[6,8,9,10] Each 48 hour asexual reproductive cycle ends in the rupturing of the schizont and the release of merozoites which infect more red blood cells. This cycle results in an exponential growth of the parasite burden within the host.[8,11,12] After several asexual cycles, some merozoites enter erythrocytes and develop into either male or female gametocytes. Gametocyte development is divided into five stages. In the first three, the sexual parasites are sequestered, developing in the capillaries and venules of the human host, and are potentially susceptible to drugs that target the asexual stage of development. In the fourth stage, they re-enter the blood stream, and in the final stage are resistant to all drugs but the 8-aminoquinolines.[9] When both male (microgametocytes) and female (macrogametocytes) are ingested by a feeding anopheles mosquito during a blood meal, the sexual stage can take place. In the mosquito's stomach, microgametes penetrate macrogametes to produce zygotes. These develop into motile ookinetes, which penetrate the midgut wall to become oocysts. After maturation, these rupture to release sporozoites which travel to the mosquito's salivary glands. Inoculation of the sporozoites into a new human host completes the life cycle.[6]

1.2.2 Biochemistry of the food vacuole

1.2.2.1 Haemoglobin Degradation

The malaria parasite has a limited ability to scavenge or synthesise its own amino acids. Instead, these are obtained by the catabolism of host haemoglobin (Hb). This process occurs within the food vacuole (FV) (pH = 5.2-5.6) of the merozoites during the intraerythrocytic stage of the parasite

life-cycle. Hb from the erythrocytic cytosol is transported to the FV by endocytosis.[13] Once there, it is cleaved into smaller fragments by four groups of proteolytic enzymes that are proposed to operate in a semiordered fashion (Figure 1.3).[14] Two aspartic proteases, plasmepsin I and II, initiate the degradative process by cleaving the Phe33-Leu34 bond in the hinge region of the Hb.[15,16] Falcipain-2, a cysteine protease, and falcilysin, a metalloprotease, are involved in further degradation of the haemoglobin into smaller peptide fragments.[14,17,18,19,20] Falcilysin is unable to digest either native haemoglobin or denatured globin, however, it readily hydrolyses smaller polypeptide fragments (up to 20 amino acids) generated by the action of falcipain and plasmepsin. This sequential process eventually leads to the formation of peptides 6-8 amino acids in length.[21]

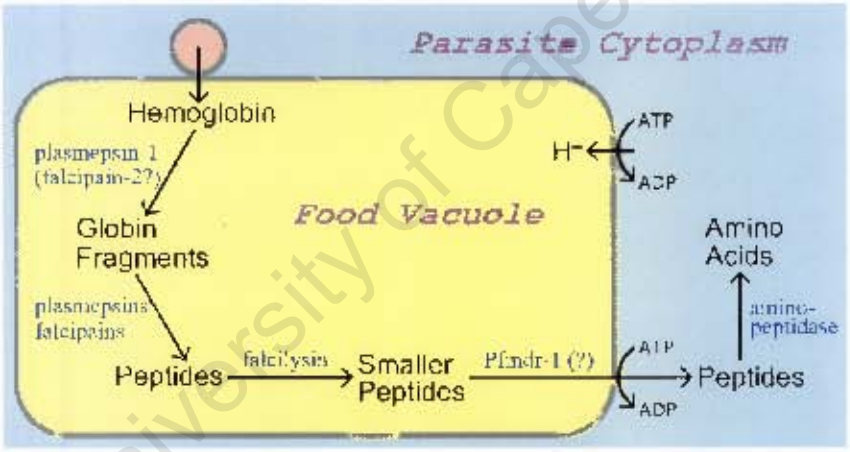


Figure 1.3 Haemoglobin degradation pathway [20]

1.2.2.2 Haem Detoxification and Haemozoin Formation

During the process of Hb catabolism, free haem [ferriprotoporphyrin-IX or Fe(II)PPIX] is produced as a by-product. The iron centre is autoxidised by O_2 producing haematin [aqua/hydroxoferriprotoporphyrin IX or H_2O/HO-Fe(III)PPIX], which is extremely toxic to the parasite.[22] It disrupts cellular metabolism by inhibiting enzymes, peroxidising membranes and producing oxidative free radicals.[23] Detoxification of the haem is necessary for parasite proliferation and growth. It has been proposed to occur *via* dimerization (forming β-haematin), followed by stacking to form haemozoin (Hz) or malaria pigment (see Figure 1.4), which is non-toxic to the parasite.[23,24,25] The

process of dimerization occurs when molecules of ferriprotoporphyrin IX are linked through reciprocal iron-carboxylate bonds to one of the propionic side chains of each porphyrin. These dimers are then bound together through hydrogen bonding to form chains of Hz.

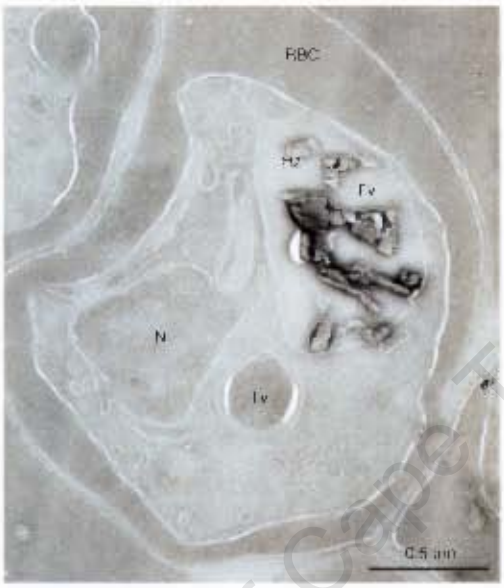


Figure 1.4 Electron micrograph of a *P. falciparum*-infected red blood cell (RBC). Detoxified haem, in the form of haemozoin (Hz), has accumulated inside the food vacuole (Fv). N: Nucleus; Tv: Transport vesicle [23]

Glutathione- (GSH) and hydrogen peroxide-mediated haem degradation have also been suggested as possible detoxification pathways, although these processes are acknowledged to occur in addition to Hz formation.[26,27,28] The presence of Hz crystals in all species of Plasmodium indicates that the processes that lead to their formation are crucial to parasite survival.[23]

1.3 Antimalarial Chemotherapy

1.3.1 Introduction

The desired characteristics of an antimalarial drug for *P. falciparum* malaria include: i) activity against drug resistant strains, ii) provision of a cure within three days (to ensure satisfactory compliance), iii) safety, particularly for small children and pregnant women, iv) formulation of an orally active dosage, and perhaps most importantly, v) affordability.[29,30] It is unfortunate that there exist

trade-offs between desired drug features with all currently available antimalarials. The majority of antimalarial drugs currently in use were not developed following a rational design process to fit identified targets. Rather they have been fortuitous discoveries of natural products with inherent antimalarial activity (quinine and artemisinin), compounds derived from natural products (CQ and artesunate), or compounds active against different infectious diseases (antifolates and tetracyclines).[29] Recent advances in the understanding of the biochemistry of malaria parasites, together with the sequencing of the *P. falciparum* genome, have led to the identification of a number of potential drug targets, and provided insight into the mode of action of older drugs.

1.3.2 Classification of Antimalarial Drugs

The majority of clinically established malaria chemotherapeutic agents can be classified according to i) the stage of the parasite lifecycle upon which they act, and ii) their proposed mechanism of action.[31,32,33]

1.3.2.1 Classification According to the Lifecycle Stage

Depending on which stage of the parasite lifecycle the drug acts, antimalarials can be classified as blood schizonticides, tissue schizonticides, gametocides or sporontocides.[32,34] Blood schizonticides act on the asexual intraerythrocytic forms of the parasite, inhibiting asexual growth within erythrocytes, whereas tissue schizonticides act on the liver stage, inhibiting the development of hepatic schizonts. Since this class of chemotherapeutics act on the developmental stage of the parasite, they commonly serve a prophylactic rather than a curative purpose. Gametocides target the sexual forms of the parasite in the blood, and thereby prevent transmission of infection to the mosquito host. Finally, sporonticides prevent the development of oocysts and sporozoites in the mosquito, thereby restricting further parasite transmission.[34]

1.3.2.2 Classification Based on Mode of Action

Antimalarials can also be classified by the mode of action through which they derive their activity. Most drugs on the market belong to one of the following four subclasses.

1.3.2.2.1 Compounds Acting on Haem Detoxification

This subclass contains many of the most common antimalarials, including the 4-aminoquinolines, chloroquine (**1.1**), amodiaquine (**1.2**) and piperaquine (**1.3**), the quinoline methanols, quinine (**1.4**), quinidine (**1.5**) and mefloquine (**1.6**), as well as the aryl alcohols, halofantrine (**1.7**), lumefantrine (**1.8**) and pyronaridine (**1.9**), all shown in Figure 1.5.

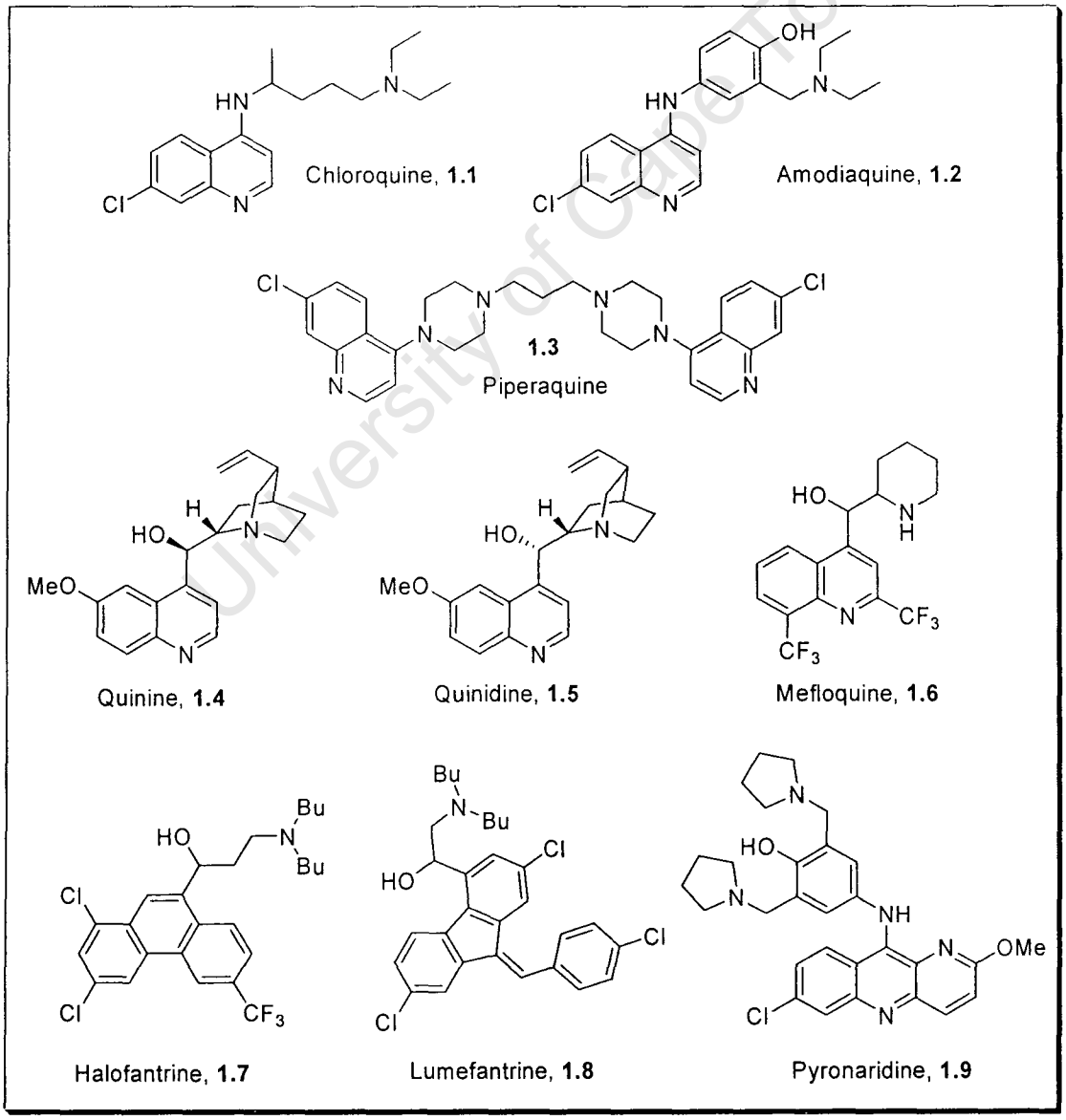


Figure 1.5 Chemical structures of compounds acting on haem detoxification

Developed during the Second World War, chloroquine (**1.1**, Figure 1.5) has been the mainstay of antimalarial chemoprophylaxis for decades. However, since the onset of resistance, new CQ analogues with similar characteristics have been much sought after. One such analogue is amodiaquine (**1.2**).

Although chemically related to CQ, amodiaquine is more effective at treating cases of uncomplicated malaria, and has been shown to be significantly more successful than CQ in areas with low to intermediate CQ-resistance.[3] However, its use has been limited by resistance as well as a high incidence of agranulocytosis and hepatic toxicity.[35] *In vitro* binding to haem and inhibition of haemozoin formation by amodiaquine has been shown to be equivalent to that of CQ.[36] This, together with the existence of cross-resistance with CQ, suggests that both drugs employ a similar mode of action.[37]

Piperaquine (**1.3**, Figure 1.5) is a bisquinoline CQ analogue that was synthesised independently by research groups in France and China in the 1960's.[38] Due to its favourable safety and toxicity profile, and its activity against *P. vivax* and *P. falciparum*, it was widely used in China, even surpassing chloroquine as the recommended antimalarial of choice.[39] Its use, however, began to decline in the 1980's as a result of the emergence of piperaquine-resistant strains of *P. falciparum*. The development of superior artemisinin-based antimalarials also contributed to a decline in its use. More recently, piperaquine has seen a re-emergence as a component of artemisinin combination therapy (ACT) formulations, including Artekin® and Duo-Cotecxin®.[40]

Included in this subclass are some of the first antimalarials to be used, including quinine (**1.4**, Figure 1.6) and its diastereomer at the secondary alcohol position, quinidine (**1.5**). Both are derived from the bark of the cinchona tree, *Cinchona ledgenaria*. Quinine has had the longest period of effective use of all the current antimalarials. Despite evidence of reduced activity against *P. falciparum* in some areas, it remains an important drug for the treatment of severe falciparum malaria.[41,42] Quinine resistance was first reported in the last century, however, resistance has developed slowly and is

still mild.[3] Despite weak binding to haem, quinine inhibition of haemozoin formation has been demonstrated *in vitro*.[41] The mechanism of resistance to quinine is unknown, however, it is postulated to be similar to that of the related quinoline methanol, mefloquine (**1.6**, Figure 1.6).[43]

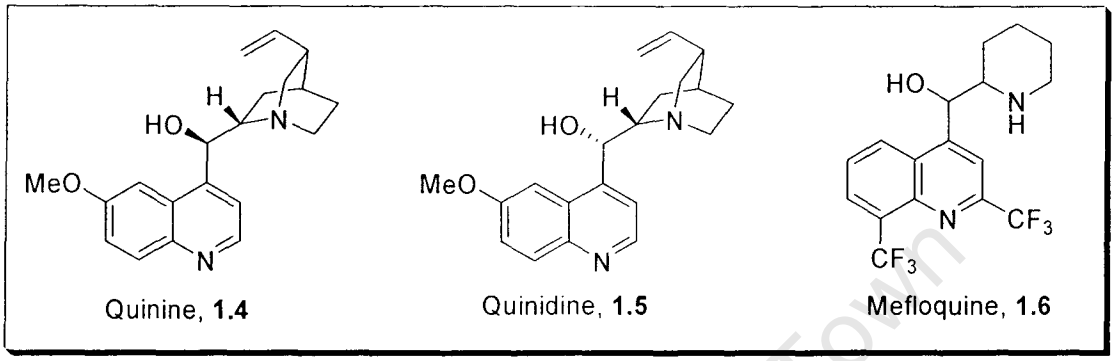


Figure 1.6 Chemical structures of the quinoline methanols, quinine (**1.4**), quinidine (**1.5**) and mefloquine (**1.6**)

Mefloquine is a fluorinated quinoline alcohol that displays good activity against CQ-resistant *P. falciparum*.[3] Its long half-life (14-21 days) has allowed it to be used as a prophylactic, however, this may also have contributed to the rapid development of resistance to the drug, which has been associated with the amplification of the *Pfmdr*1 genes and enhanced drug efflux from resistant parasites.[41,44]

Halofantrine (**1.7**, Figure 1.7) is a phenanthrene alcohol that is metabolised *in vivo* to an active metabolite, desbutylhalofantrine.[3] The drug displays high activity against multi-drug resistant (MDR) malaria,[45] however, due to its cardiotoxicity, administration of the drug is no longer recommended. Halofantrine resistance has also been linked to *Pfmdr*1 gene overexpression, and appears to be related to mefloquine resistance.[41,46]

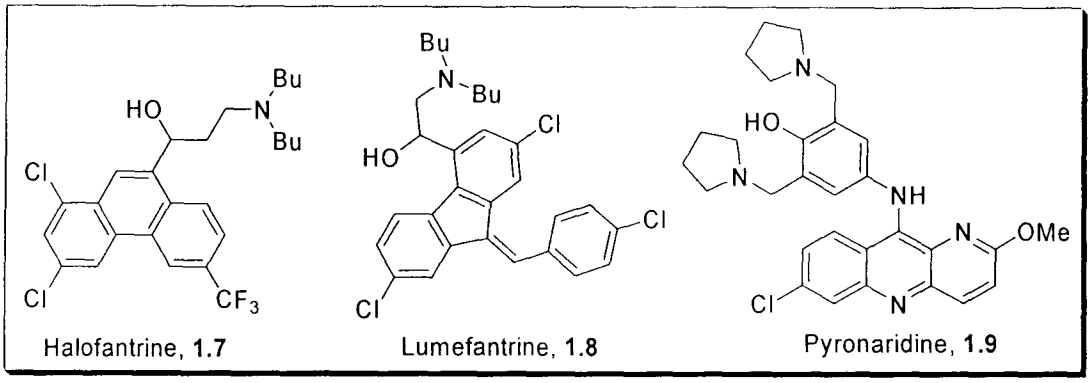


Figure 1.7 Chemical structures of the aryl alcohols, halofantrine (**1.7**), lumefantrine (**1.8**) and pyronaridine (**1.9**)

Lumefantrine (**1.8**) is another recently introduced antimalarial that was developed in China. Its mode of action is similar to the aminoquinolines, and when used in combination with the artemisinin derivative, artemether, displays high activity against MDR *P. falciparum*.[41]

The synthetic acridine-derived compound, pyronaridine (**1.9**), was developed and is widely used in China.[41] Despite displaying low oral bioavailability, and a corresponding high treatment cost due to the large dosage requirements, the drug has been shown to display high *in vitro* activity against MDR falciparum malaria, and has potential to replace CQ as the antimalarial drug of choice in Africa.[47,48]

The exact mode of action of chloroquine and related quinolines remains unresolved. A number of theories have been proposed.[49] These include: (i) direct haem binding, (ii) inhibition of a haem "polymerase" responsible for haemozoin formation, (iii) inhibition of vacuolar phospholipase, (iv) inhibition of protein synthesis, and (v) interaction with DNA. However, the mode of action most widely recognised is related to the accumulation of this basic compound within the acidic food vacuole and its ability to prevent haemozoin formation. Binding of the drug to haem monomers is believed to form an initial drug-haem complex, which attaches to elongation sites on Hz, preventing further stacking of β-haematin.[50,51] The coordination of CQ to haem is also believed to protect the haem from being degraded by reduced

glutathione.[52] These processes result in a build-up of toxic haem, which kills the parasite.

Resistance to CQ was first reported in 1959 in South America. Since then it has spread to most countries where the disease is endemic, resulting in frequent treatment failure and contributing to reported increases in malaria morbidity and mortality.[53] This has spurred extensive studies towards understanding the molecular basis for CQ resistance, in order to circumvent or suppress it. Studies have found that chloroquine-resistant (CQR) parasites accumulate less CQ in their food vacuoles than chloroquine-sensitive (CQS) parasites.[54,55] This decreases the toxicity that would otherwise arise from the interaction of CQ with haem. This reduced accumulation has been attributed to mutations in two food vacuole membrane proteins. Plasmodium falciparum Chloroquine Resistance Transporter (PfCRT) is a 424-amino acid protein consisting of ten helices, and is a member of the drug-metabolite transporter family of proteins (see Figure 1.8).[56] Although its regular physiological function is not known, it is proposed to be involved in peptide transport across the membrane. There are a number of mutations in the gene encoding this protein that have been linked to CQ-resistance. Although these vary according to the geographical origin of the strain, all resistant strains share a common mutation at position K76T (charge loss associated with conversion of lysine to various neutral amino acids).[57] The mechanism by which these mutations are able to reduce CQ accumulation is unknown, however, several leading theories have been suggested. These include i) energy-dependant CQ efflux from CQR but not CQS parasites,[57,58] ii) reduced influx of CQ into CQR parasite food vacuoles,[59] iii) reduced CQ-haem binding due to increased food vacuole pH,[60] and iv) exit of CQ from the food vacuole using PfCRT as a transport channel.[53]

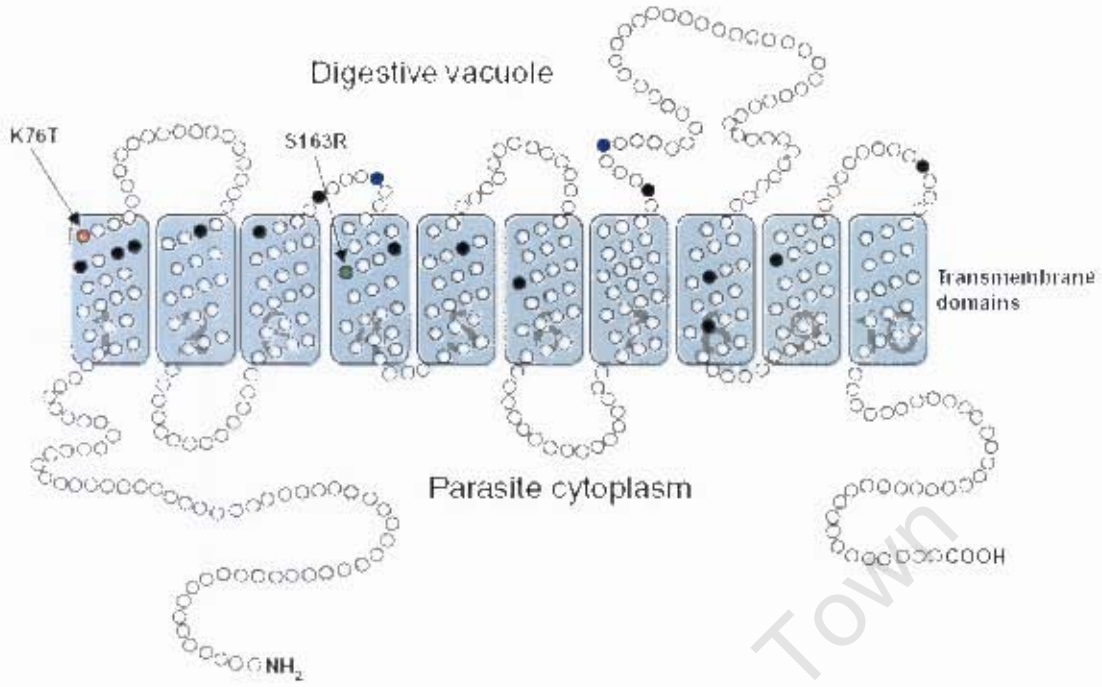


Figure 1.8 The predicted protein structure of PfCRT, which is postulated to possess 10 transmembrane helices, with the N- and C-termini extending into the parasite cytoplasm. Black and red filled circles indicate the positions of mutations. The critical K76T mutation is indicated in red. The green filled circle indicates the position of the S163R mutation in amantadine- and halofantrine-resistant parasites, while blue circles represent additional mutations in drug-pressured parasites[53]

The development of resistance to quinolines has been attributed to mutations in a second gene, pfmdr1, which codes for the food vacuole transmembrane protein, *P. falciparum* P-glycoprotein homolog 1 (Pgh1).[51] This a member of the ATP-binding cassette (ABC) transport protein family, associated with multidrug resistance (MDR) in mammalian cancer cell lines.[61] MDR in cancer cells is believed to originate from an ATP-dependant, transmembrane protein called P-glycoprotein (Pgp), responsible for intracellular drug efflux. The similarities between Pgh1 and Pgp suggest that an analogous energy-dependant drug efflux process by Pgh1 may be the cause of *Plasmodial* resistance to CQ-related quinoline drugs.[61]

Further hypotheses to explain *P. falciparum* resistance to quinolines include: i) elevated levels of glutathione,[62] ii) reduced Na+/H+ exchanger (NHE),[63] iii)

elevated pH levels within the food vacuole,[64,65] and iv) hindered drug access to haematin.[66]

1.3.2.2.2 Inhibitors of Nucleic Acid Synthesis

Within this subclass, two main groups of compounds exist: (i) the dihydrofolate reductase (DHFR) inhibitors, such as pyrimethamine (**1.10**, Figure 1.9) and proguanil (**1.11**), and (ii) the dihydropteroate synthase (DHPS) inhibitors, including sulfones (e.g. dapsone, **1.12**) and sulfonamides (e.g. sulfadoxine, **1.13**).[3,41] The compounds in group (i) derive their activity by the inhibition of DHFR, which causes a depletion of tetrahydrofolate and a consequent reduction in DNA synthesis. The inhibition of DHPS by the compounds in group (ii) results from their ability to mimic *p*-aminobenzoic acid and occupy the active site. This limits the formation of dihydropteroate, which in turn results in a reduction in dihydropyrimidine synthesis, as well as DNA, serine and methionine production.[67]

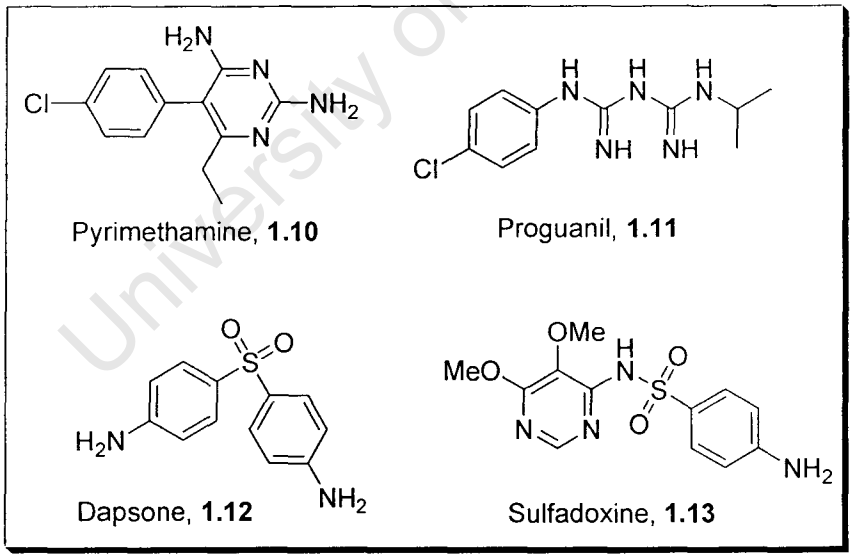


Figure 1.9 Chemical structures of the nucleic acid synthesis inhibitors, pyrimethamine (**1.10**), proguanil (**1.11**), dapsone (**1.12**) and sulfadoxine (**1.13**)

A drug from the first group is usually used in combination with one from the second, because they inhibit sequential stages in the parasite folate biosynthesis pathway, having a combined synergistic effect. However, resistance is now widespread in much of Asia, India and more recently, in

parts of Africa.[68,69] The most widely used combination is sulfadoxine-pyrimethamine (SP), which is favoured due to its low cost, single dosage, and initially high activity. However, it is poorly active against strains with high CQ resistance, and is itself particularly susceptible to the development of resistance, possibly as a result of its long retention time in the body. Resistance occurs as a result of single site mutations in the genes encoding for DHFR and DHPS.[67,70,71]

The combination of proguanil and atovaquone (**1.14**, Figure 1.10) under the trade name, Malarone®, is an additional formulation that has been introduced from the family of folate antagonists. Atovaquone is a derivative of the mitochondrial electron carrier, ubiquinone (Coenzyme Q) (**1.15,** Figure 1.10), a cofactor of dihydroorotate dehydrogenase. Atovaquone activity arises from inhibition of the mitochondrial electron-transport system.[3,72,73] When used alone, atovaquone activity against falciparum malaria is poor and resistance has been shown to develop in one-third of patients.[3] As a result, it is now exclusively sold in combination with proguanil. Malarone® has a greater activity than CQ or mefloquine against MDR *P. falciparum*, and is effective against strains resistant to proguanil alone.[73] The high cost of treatment, however, limits its suitability for malaria treatment in Africa.

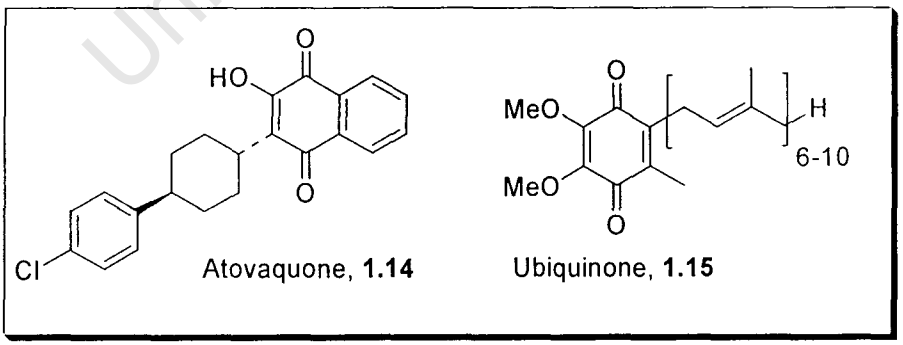


Figure 1.10 Chemical structure of atovaquone (**1.14**), the analogue of Coenzyme Q, ubiquinone (**1.15**)

1.3.2.2.3 Inhibitors of Protein Metabolism

This subclass contains the antibiotics, tetracycline (**1.16**, Figure 1.11) and doxycycline (**1.17**).

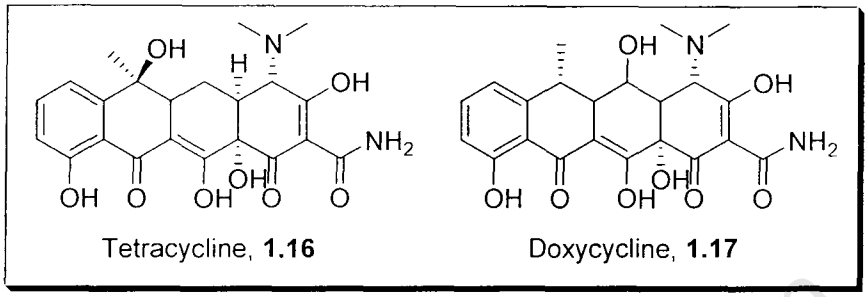


Figure 1.11 Chemical structures of the antibiotics, tetracycline (**1.16**) and doxycycline (**1.17**)

Doxycycline is derived from oxytetracycline, and has an identical spectrum of activity to tetracycline. Its absorption, lipid-solubility, stability and toxicity are, however, superior to that of tetracycline.[74] Doxicycline has a longer plasma half-life than tetracycline, enabling it to be administered once daily, which is a further advantage over tetracycline which must be taken four times daily. Neither drug is considered safe for pregnant women, breastfeeding women or children less than eight years of age, which is a drawback to their use in Africa where many *Plasmodium* infections occur in children under the age of five.[1]

The mechanism of action of these compounds remains uncertain. Doxycycline has been shown to inhibit the expression of plasmodial apicoplast genes, prevent processing of apicoplast-targeted proteins, and interfere with schizogeny, particularly in the progeny of doxycycline-treated parasites.[75] Although the apicoplast is related to plant cell chloroplasts, its exact function is unclear. It has, however, been shown to contain its own genome, encoding ribosomal RNAs, tRNAs, and several proteins [76,77] as well as house enzymes involved in type II fatty acid synthesis.[78] The loss of apicoplast function that these drugs are able to effect in second generation parasites provides them with a slow but potent antimalarial effect. This slow action time, however, necessitates their use in combination with other antimalarials (e.g. quinine) in

order to prevent the possibility of resistance development.[79,80] In fact, co-administration of quinine plus tetracycline has been used for the treatment of uncomplicated *falciparum* malaria since the late 1970's.[74]

1.3.2.2.4 Drugs Generating Oxidative Stress

Cells produce reactive oxygen intermediates (ROI) as by-products of metabolic processes. These ROI are capable of causing damage to many biological components, including lipids, proteins and nucleic acids. [81] Consequently, normal cells have detoxification mechanisms to deal with the oxidative stress imposed by these ROI. Malaria parasites have a high metabolic activity associated with the digestion of host Hb, and consequently produce high levels of ROI. Therefore, drugs that are able to increase levels of oxidative stress in *Plasmodial* cells may overcome cell redox defence mechanisms, which can lead to parasite death. Levels of oxidative stress may either be heightened by direct drug oxidation, or by drug disturbance of natural cell redox cycles.[81]

The two classes of antimalarials that are proposed to act by generating oxidative stress are the artemisinins (**1.18** – **1.23**, Figure 1.12) and the 8-aminoquinolines (**1.24** – **1.25**, Figure 1.13).

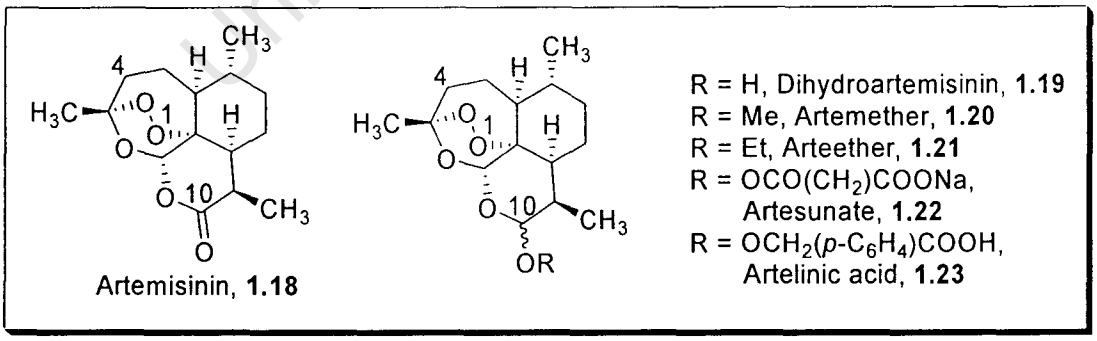
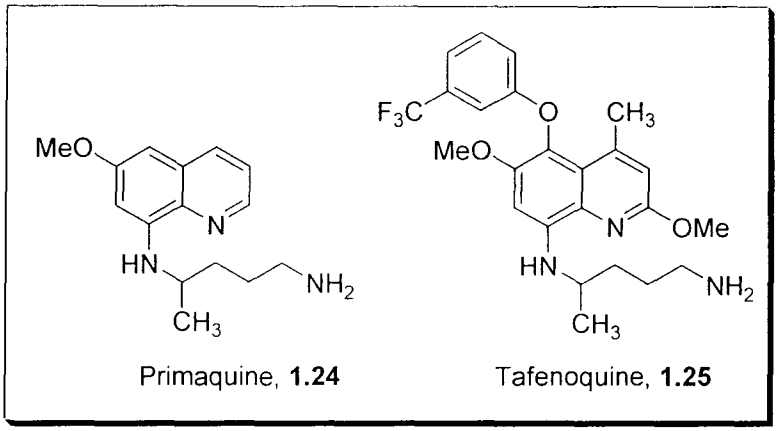


Figure 1.12 Chemical structures of artemisinin (**1.18**) and several of its hemisynthetic derivatives (**1.19 – 1.23**)



Primaquine, **1.24** Tafenoquine, **1.25**

Figure 1.13 Chemical structures of the 8-aminoquinolines, primaquine (**1.24**) and tafenoquine (**1.25**)

Artemisinin (**1.18**) is extracted from the qinghao, or sweet wormwood plant, *Artemisia annua*. There are several derivatives that are more active than artemisinin itself,[82,83] including the two oil-soluble ethers, artemether (**1.20**) and arteether (**1.21**), and the water-soluble artesunate (**1.22**) and artelinic acid (**1.23**). All derivatives are metabolised *in vivo* to the dihydroartemisinin (**1.19**) form. Artemisinins display *in vitro* activity at nanomolar concentrations against both CQ-sensitive and CQ-resistant strains of *P. falciparum*, have no significant toxicity,[84,85,86] even in pregnant women,[87] and are highly successful in treating cases of severe malaria.[88] The artemisinins, and in particular the metabolite dihydroartemisinin, have however been shown to display neurotoxicity in animal studies and *in vitro* studies with neuronal cell cultures,[89,90,91] although it has not yet been established whether it is a definite problem in humans.[92] Further drawbacks associated with the use of artemisinin and its derivatives include their high cost and unreliable supply of the natural parent compound.[41]

The means by which these compounds exert their activity has yet to be fully established, however, it has been suggested that the endoperoxide functionality is a key requirement.[93,94] The mechanism of action is a highly controversial topic and has formed the centre of active debate in the literature.[95,96,97,98,99] One hypothesis suggests iron-mediated cleavage of the peroxide bond to produce a C-centred radical on the C4 carbon (see structure for numbering) that alkylates sensitive biomolecules,[100,101,102] however,

competing arguments refute any correlation between antimalarial efficacy and reactivity towards iron.[97] Artemisinins have also been shown to exert their antimalarial activity by targeting PfATP6, the *Plasmodium falciparum* orthologue of sarco-endoplasmic reticulum Ca^{2+}-ATPase (SERCA).[93] It has been demonstrated that there is a close correlation (R^2>0.9) between inhibitory constants of artemisinins assayed in the PfACT6 model, and artemisinin activity in parasite cultures.

A further proposed mode of action includes inhibition of nuclear factor NF-kB, resulting in the inhibition of nitric oxide synthetase, and a reduction in cellular levels of NO.[103] The lack of certainty in the mode of action of artemisinin-type antimalarials suggests there may yet be undiscovered targets for these drugs.

The 8-aminoquinolines, including primaquine (**1.24**) and tafenoquine (**1.25**) (see Figure 1.13), are the only class of antimalarials active against the sexual (gametocytic) stage of the parasite lifecycle. The mode of action of this class of compounds has yet to be fully established, however, it has been suggested that the activity is derived from drug-induced modifications to parasite DNA.[104] Primaquine and related 8-aminoquinolines have also been shown to target mitochondria,[105] and it has been suggested that their activity is related to electron transport and to the oxido-reduction of parasite ubiquinones.[106]

Primaquine has been widely used to treat hypnozoites (liver reservoirs) that cause relapses of *P. vivax* and *P. ovale* malaria, and has been considered for use as a chemoprophylactic for *P. falciparum* to eliminate the parasite at the hepatic stage, prior to symptomatic malaria.[107,108] Although it has good oral absorption, its short elimination half-life (4 hours) requires that the drug is administered daily. For patients with glucose-6-phosphate dehydrogenase deficiency, primaquine is toxic as it can cause life-threatening haemolysis.

Tafenoquine is an analogue of primaquine that has a longer elimination half-life (14 days) and a larger therapeutic index. It shows potential as a chemoprophylactic of *P. falciparum* and for prevention of *P. vivax* relapses.[41, 109, 110] Compared to primaquine, tafenoquine displays higher

activity against blood and liver stages of malaria,[111, 112] has greater sporontocidal activity,[113] and is more active *in vitro* against MDR asexual blood stages of *P. falciparum*.[108,114,115]

1.4 Gold in Medicine

1.4.1 Background

More than a quarter of the 90 naturally-occurring elements on earth are metals believed to be essential for human life.[116] These metals are key to many biological and biomedical processes in the body, with metal ions, including metalloenzymes, being required for activation or biotransformation of many different organic compounds.[117] There is therefore great scope for the use of inorganic compounds in medicine, as well as a need for research in this field.

Several examples of metals currently used in medicine include platinum in anticancer agents, lithium for manic depression, bismuth in anti-ulcer drugs, silver and mercury in antimicrobial agents, and gold in cancer and rheumatoid arthritis (RA) treatment.[118] It is the medicinal applications of gold, in particular, that are central to this project.

Gold has been used as a cure for many different ailments for centuries.[119] The discovery, in 1890, that gold cyanide could inhibit *Mycobacterium tuberculosis* led to extensive research on the therapeutic properties of gold.[120]

The use of gold(I) compounds for the treatment of RA, termed *chrysotherapy*, was first reported in the 1930's.[121] The discovery of Auranofin (**1.26**, Figure 1.14) in the 1970's,[122] however, provided the first orally administered RA drug, which also had a lower incidence of side effects.[123]

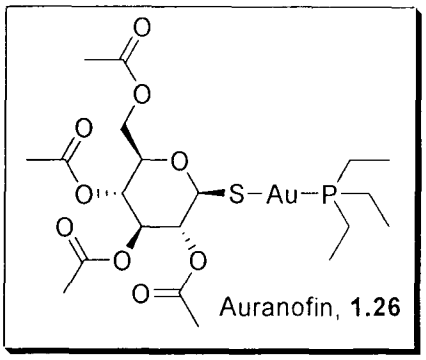


Figure 1.14 Chemical structure of Auranofin (**1.26**)

Studies on the anticancer properties of gold-based drugs were first reported in the 1980's.[124] These studies showed that the most promising anti-tumour compounds were the (phosphine)gold(I) thiolates, i.e. related to the structure of Auranofin.[125] Structure-activity relationship studies revealed active compounds to have the arrangement, P-Au-S. The absence of any one of these components was found to result in loss of activity. The rationale behind using sulphur- and phosphorus-containing ligands is that gold(I) is a 'soft' acid, and as such has a high affinity for these 'soft' bases. Binding to 'hard' nitrogen and oxygen atoms is consequently poor.

In the 1990's, studies were performed that aimed to couple phosphinegold(I) species to biologically active thiols such as 6-mercaptopurine and 6-thioguanine, in order to enhance their activity.[126,127] These thiols are known to be active against human leukaemia. Conclusions from the studies were that the presence of the phosphinegold(I) entity enhanced the potency of the biologically-active thiols, and the overall cytotoxicity of the (phosphine)gold(I) thiolates compared with the free thiols was found to be greatly enhanced in a range of cancer cell lines. In fact, these complexes were shown *in vitro* to exhibit anticancer activity much greater than cisplatin, one of the most active anticancer drugs currently in use. Preliminary *in vivo* studies were also conducted, which showed that this activity was maintained to a certain extent.[128] The most active compound contained both the phosphinegold(I) and 6-mercaptopurine moieties. Structure-activity relationship studies on the phosphine ligands indicated that, in terms of cytotoxicity, cyclohexyl > ethyl > phenyl.[129, 130]

Research has also been reported on gold(III) complexes as anti-tumour agents. The greater charge on the gold(III) atom causes it to coordinate preferentially with 'harder' oxygen and nitrogen ligand donors. The higher charge, however, lowers the chemical stability of gold(III) complexes in solution. Overcoming this instability requires an appropriate choice of ligands, the most successful containing chelating nitrogen donor atoms.[131] The cytotoxicity of gold(III) complexes, thus stabilised, has been shown to be high, and dependant specifically on the presence of the gold(III) centre.[131]

The geometry of Au(III) complexes is four coordinate, square planar, as found for cisplatin. The electronic configuration of the gold(III) metal centre is also the same as cisplatin, i.e. d^8, suggesting it may have a similar mode of biological action (DNA binding and disruption of cellular processes). This was evaluated in a recent study.[131] The results indicated, however, that the DNA interactions of the gold(III) complexes were weak and that DNA was not the primary target for the cytotoxic effects indicated by the complexes.

There are a number of other potential uses of gold as a therapeutic agent. Several gold drugs have been evaluated for anti-HIV activity, with results indicating some inhibition of HIV strains by aurothiomalate and aurothioglucose.[116, 132] The metabolite, dicyanogold(I), produced by all gold drugs, has been proposed for the treatment of AIDS due to its ability to penetrate cells rapidly and its suggested low toxicity.[116] Effectiveness against psoriasis[116] and bronchial asthma[125] was also demonstrated by compounds used in the treatment of RA - a result of their immunosuppressant properties. (Phosphine)gold(I) thiolates have also been evaluated against the parasitic diseases Chagas' disease[133] and malaria[134].

1.4.2 Mode of Action of Gold Drugs

Extensive studies on the pharmacokinetics of gold(I) compounds have been done over the years. Upon entering the body, the gold(I) ligands readily dissociate and exchange with biological thiolates.[119] Most rapid ligand exchange occurs with halide ligands, followed by thiolates, and then

phosphines.[119] The Au(I) metal centre of the drug has a longer residence time in the body than the ligands. It has a half life of approximately 20 days, whereas the ligands tend to be excreted within 24 hours.[135] The gold(I) is distributed throughout the body, however, it accumulates in the lysosomes and the synovium.[136, 137]

Gold(I) drugs are transported in the bloodstream by binding to the free thiol group of cysteine-34 in human serum albumin.[138,139] Once absorbed in the blood, the mechanism of action is not known. A number of pathways have been proposed, and it is likely that therapeutic activity is derived from a combination of biological effects.[119] Several of these are described below.

At the cellular level, gold may act by inhibiting T cell proliferation and modulating the immune system.[140] At the level of transcription, binding of gold(I) drugs to the thiol groups of DNA-binding proteins, such as transcription factors, is strong and may, therefore, allow the gold to influence transcription activity.[141, 142] More specifically, the occupation of the cysteine-rich metal binding site by gold(I) in transcription factors containing the zinc finger motif has also been suggested as a possible mode of action of gold(I) drugs. This would inhibit binding to the specific DNA response elements in promoter and enhancer regions of genes thus disrupting normal cell processes.[143]

Facile ligand exchange of the Au(I), especially with biological thiolates, allows it to inhibit the activity of a variety of different enzymes.[119] The proteolytic enzyme, human neutrophil collagenase is one such example. It is a zinc-dependant protease responsible for collagen breakdown in RA. Au(I) acts by replacing zinc from a zinc-binding site distal to the active site, thereby disrupting its activity. [144] It is noteworthy that Au(I) has the same outer electron configuration as Zn^{2+}, d^{10}, and is therefore able to replace it at these binding sites.

Three widely used gold drugs, Auranofin, aurothiomalate and aurothioglucose, have been shown to inhibit protein kinase C, a metallo-enzyme containing Zn^{2+} bound to cysteine and histidine residues that is crucial to intracellular

signal transduction in proteins. Inhibition of this enzyme has been suggested as a possible mode of action of antirheumatic gold(I) drugs.[145, 146]

Other enzymes that have been shown to be affected by gold(I)-containing compounds include a number of serine-dependant proteases,[147] and the cathepsin family of lysosomal enzymes, which contain an activated cysteine in their active site.[119]

Potential *Plasmodium* enzyme targets of gold complexes include cysteine proteases and oxidoreductases (particularly disulfide reductases). Both these groups contain sulfur functionalities, which aid their affinity for gold(I) centres, as well as provide potential targets for inhibition by S-alkylation/arylation and S-acylation/sulfonylation. In the *Plasmodium* parasite food vacuole, cysteine protease enzymes involved in haemoglobin degradation are of particular importance to parasite survival, and provide an appealing target for gold-based chemotherapy.[148]

Plasmodium disulfide reductases, including glutathione reductase and thioredoxin reductase, are essential to maintaining the redox homeostasis of cells.[149, 150, 151] They are also responsible for removal of free radicals and other xenobiotic particles that are harmful to parasite cells,[149] and have recently been shown to be inhibited by gold(I) complexes.[151, 152]

The lack of enzyme specificity illustrated by these examples indicates that there may be many more possible enzymatic targets of Au(I) drugs. This is an area which would require further study.

Glucose metabolism of cells may be disrupted by binding of gold(I) to the thiol groups of hexose transport protein in the membrane of red blood cells. This may impact the activity and viability of cells.[153]

The aurocyanide metabolite of gold drugs has been shown to exhibit activity as a result of its inhibition of the oxidative burst in various cells (such as polymorphonuclear leukocytes). Oxygen radical production (e.g. O_2^-) by cells

during oxidative burst is essential to their immune response to bacterial infection. Inhibition of this process may, therefore, lead to cell death.[154] Conversion of Au(I) to Au(III) by oxidants, such as ClO^- and H_2O_2, is possible under inflammatory conditions,[155] and may give rise to several disruptive cellular processes responsible for the activity of gold-containing drugs. These include deprotonation of peptide amide groups, causing modification of T cell recognition of peptides, oxidation of disulfide bridges in albumin and insulin,[156] and oxidation of methionine residues of ribonuclease.[116]

1.4.3 Gold Complexes as Antimalarials

Attempts have been made by a number of researchers to extend the use of gold in pharmacy to malaria.[134, 157, 158] These studies aimed to enhance the efficacy of well-known antimalarial drugs by coordinating them to gold.

Singh and Wasi (1987) used Au(III) to form complexes with amodiaquine and primaquine. [157] Their conclusion was that the anti-parasitic activity of the two drugs was independent of their coordination to gold. However, more recently Sánchez-Delgado and co-workers (1997) used Au(I) to coordinate chloroquine (CQ). [134, 158] The results they obtained were very promising, with their complex, $[Au(PPh_3)(CQ)]PF_6$, demonstrating a marked enhancement of the efficacy of chloroquine against the rodent malaria strain, *Plasmodium berghei* both *in vitro* and *in vivo*. [134] Against chloroquine-resistant strains of *P. falciparum*, the complex displayed an enhanced activity of between 4 and 10 times that of chloroquine diphosphate (CQDP). Control cultures indicated that the observed activity was due to the presence of both gold(I) and CQ in the medium.[134] The reversal of CQ-resistance indicated by these studies suggests that the gold-induced activity was derived *via* an alternative mechanism to that of traditional quinoline antimalarials.

A further study by the Sánchez-Delgado group aimed to provide a preliminary structure-activity relationship (SAR) for these gold-chloroquine compounds.[158] Variations in the counter ion, the phosphine substituents, and the charge on the gold atom were made, and the resulting activity against chloroquine-

resistant strains of *P. falciparum* determined. Changing the counter-ion from PF_6^- to NO_3^- gave mixed results with each of the 3 strains of *P. falciparum* used in the study. Changing the substituents on the phosphine ligand between phenyl, ethyl and methyl did not affect the activity except against one strain, where the ethyl substituent produced a four-fold increase in activity. Chloroquine complexes containing a gold(III) metal centre also displayed higher activity than CQDP against both CQ-sensitive and CQ-resistant strains, however, further elaborations of the molecular structure were not attempted. It was therefore not determined whether Au(I) provided a greater enhancement in activity than Au(III).[158]

There are a number of factors affecting the efficacy of these complexes that still need to be determined. These include the counter-ion identity, active ligand characteristics, and spectator ligand characteristics, to name a few. This area of research therefore provides great scope for new studies on chloroquine-derived gold complexes.

1.5 Aims and Objectives

Overall Objective

The main objective of this M.Sc. study is to synthesise novel antimalarial compounds that are able to overcome chloroquine resistance in *Plasmodium falciparum* parasites. This is to be achieved by combining functionalised acylthiourea and glycolic acid amide side chains to an aminoquinoline moiety. The rationale behind this approach is to retain the active functionality of existing antimalarials (the aminoquinoline), while overcoming the resistance recognition pathway by functionalising the side chains. In addition, the side chains may exhibit inherent antimalarial activity that differs from the traditional mode of action of 4-aminoquinoline antimalarials. The proposed target compounds are illustrated in Figure 1.15.

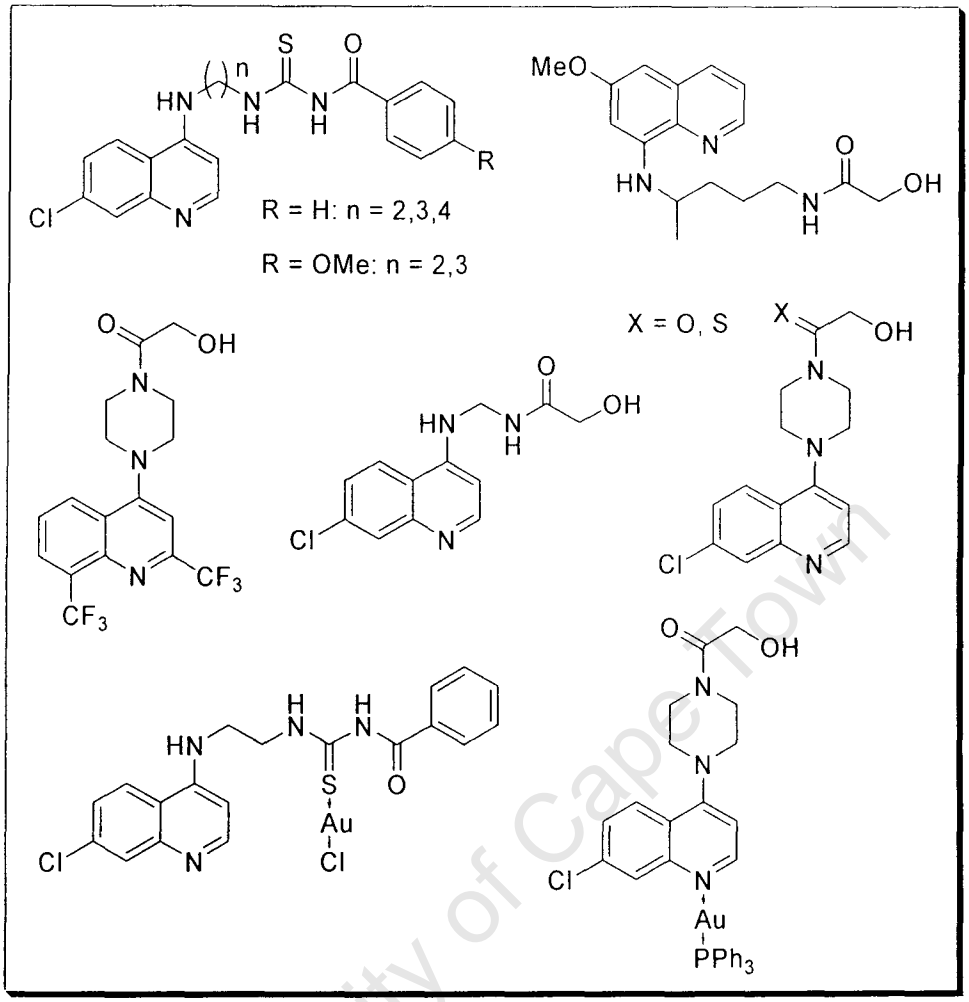


Figure 1.14 Chemical structures of target compounds

Specific Aims

1. To synthesise rationally-designed, novel, aminoquinoline-derived acylthiourea and glycolic acid amide ligands for novel gold complexes
2. To synthesise and characterise novel gold complexes of the two classes of ligands (acylthioureas and glycolic acid amides)
3. To evaluate the antimalarial activity of the prepared compounds *in vitro*, in order to evaluate the effect of gold complexation on antimalarial activity

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CHAPTER 2

SYNTHESIS AND CHARACTERISATION OF 4-AMINOQUINOLINE-BASED ACYLTHIOUREAS

2.1 Introduction

This chapter describes the background, synthesis and characterisation of several novel 4-aminoquinoline-based acylthioureas. The molecules were designed to incorporate the 4-amino-7-chloroquinoline pharmacophore, believed to be the source of activity of chloroquine, with an acylthiourea functionality. All target compounds were designed and synthesised in order to determine their activity against *Plasmodium falciparum* malaria parasites. Compounds were tested against CQ-sensitive and CQ-resistant strains of parasites in order to establish whether they were able to overcome CQ-resistance.

2.1.1 Overcoming CQ Resistance

There are four approaches that have been used to combat the onset of parasite resistance in malaria: drug rotation, combination therapy, redesign of existing drugs to overcome resistance, and resistance reversing agents.[1]

2.1.1.1 Drug Rotation

The concept of drug rotation involves periodically changing the type of chemoprophylactic used in order to circumvent the development drug resistance. In the context of malaria, recent findings have shown that chemosensitivity to CQ has been restored in many areas where use of the drug was stopped due to the prevalence of CQ-resistance.[2,3] This suggests that parasites with the PfCRT K76T mutation are at a survival disadvantage in the absence of CQ drug pressure, and that back-mutation might occur if drug

use is suspended for a sufficient time.[1] However, evidence suggests that recovery of CQ sensitivity is due to an expansion of the wild-type allele rather than any back mutation in *pfcrt*, and therefore drug rotation is unlikely to be a feasible strategy,[4] as resistant PfCRT forms are likely to re-emerge upon the resumption of drug pressure.[5]

2.1.1.2 Combination Therapy

The World Health Organisation (WHO) defines antimalarial combination therapy (CT) as: "The simultaneous use of two or more blood schizontocidal drugs with independent modes of action and different biochemical targets in the parasite."[6]

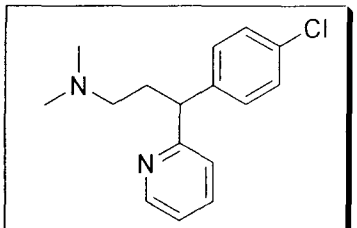
The principle of CT is based on the assumption that the statistical probability of gene mutations simultaneously occurring at two different biochemical targets is infinitely smaller than the probability of such a mutation occurring at any one site. However, this theory requires that the pharmacokinetics of the two drugs is well matched, and that their elimination half-life ($t_{1/2}$) is not too long.[7] If excretion of the drugs occurs sequentially with a sufficient difference between their $t_{1/2}$, resistance may develop. A long $t_{1/2}$ increases parasite drug exposure time, which may also lead to resistance.

Several combinations currently in use include sulfadoxine-pyrimethamine (used for almost two decades for the frontline treatment of malaria, although the recent onset of resistance has caused its usage to decline), chlorproguanil-dapsone,[8] atovaquone-proguanil,[9] and the quinoline combinations, quinine-doxycycline,[10] mefloquine-artesunate,[11] lumefantrine-artemether,[12] and amodiaquine-sulfadoxine-pyrimethamine which has been shown to display good clinical activity.[13] Combination attempts involving CQ, however, have thus far been unsuccessful.[1,13]

2.1.1.3 Resistance Reversal Agents

The fourth strategy that has been proposed to overcome resistance is the use of resistance reversing agents. Since the discovery that co-administration of verapamil with chloroquine is able to reverse CQ resistance,[14] extensive

research has been pursued in this field, with more than forty identified CQ resistance reversing compounds reported.[1,32] Several review articles give an outline of the most promising CQ resistance reversers.[1,32,15,16] It is noted, however, that with the exception of chlorpheniramine (Figure 2.1, **2.1**), resistance reversing agents have yet to be used clinically due to the unacceptably high concentrations required for activity. Chlorpheniramine, traditionally an antihistamine, has been reported to restore CQ sensitivity in highly resistant cases of infection.[17]



Chlorpheniramine, **2.1**

Figure 2.1 Chemical structure of chlorpheniramine

2.1.1.4 Lead-Based Drug Discovery

Drug discovery using available lead compounds is a third viable technique for overcoming resistance, and is perhaps the area receiving the greatest attention in chemical research.[1] This involves structurally modifying an existing active molecule, such that activity is retained and target resistance mechanisms are overcome. Structural modifications to 4-aminoquinolines have produced a multitude of analogues with reported variations in the length of the side chain, in the constituents of the side chain, and in the substituents on the quinoline ring.[18,19,20,21]

2.1.1.4.1 Side Chain and Ring Modified 4-Aminoquinolines

Amodiaquine (AQ) is a CQ analogue with a varied side chain that is currently in use, and has been shown to exhibit activity against many CQR *P. falciparum* strains. However, the development of cross-resistance with several strains has been reported.[22] CQ analogues with shortened (2-3 carbon) and lengthened (10-12 carbon) side chains have been shown to display high activity against CQR parasites,[23,24,25] as have derivatives with

other side chain modifications. These include, among others, ferrocene-containing side chains,[26,27] bulky and branched methyl substituents on the terminal amine,[28] heterocyclic functionalities at the terminal position,[29] and aromatics with H-bond donating substituents.[30] These results suggest that the side chain is of primary importance in the CQ-resistance recognition mechanism, and that resistance is based upon specific binding of the side chain to PfCRT.[31,32] Modifications to the 4-amino-7-chloroquinoline ring such as changing the identity of the 7-chloro- functionality, or further functionalizing different sites on the ring have been reported to play no significant role in enhancing the antimalarial activity of CQ-related derivatives.[23,31] It would appear that this pharmacophore is crucial to the activity of CQ and its analogues.

2.1.1.4.2 The 1,4-bis(3-aminopropyl)-piperazines

The 1,4-bis(3-aminopropyl)-piperazines (Figure 2.1) have activity against both CQS and CQR strains of *Plasmodium falciparum*, with one compound, **2.2**, having activity in animal studies as well.[33] Hydroxamic acid derivatives of a similar series (Figure 2.2, **2.3**), have been shown to target the *P. falciparum* aminopeptidase, PfA-M1, a member of the M1 family of metalloproteases. This protease, active in the parasite cytoplasm, is involved in the latter stages of haemoglobin degradation, responsible for the degradation of small peptides into amino acids, and presents a potentially novel chemotherapeutic target. In addition, the presence of the 4-amino-7-chloroquinoline ring suggests that these compounds may also inhibit the formation of haemozoin.[20]

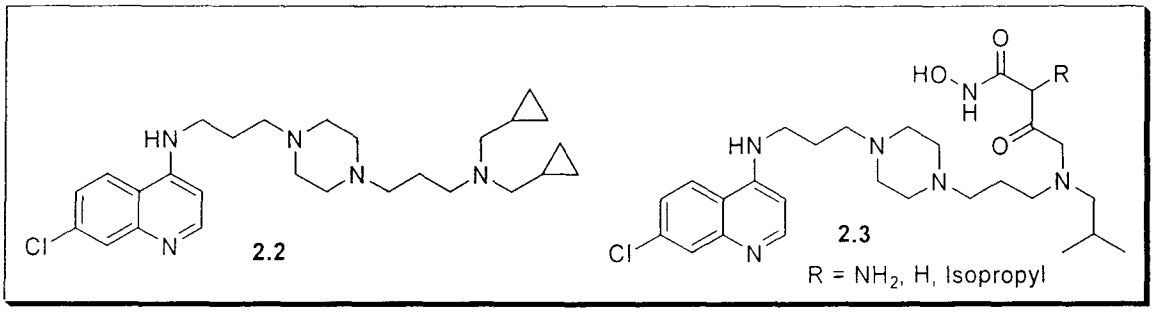


Figure 2.2 Chemical structures of 4-aminoquinoline-based 1,4-bis(3-aminopropyl)piperazines

2.1.1.4.3 Glyoxylylhydrazones

The glyoxylylhydrazones (Figure 2.3, **2.4** and **2.5**) is a second group of 4-aminoquinoline-based molecules with good antimalarial activity. Possible modes of action of these compounds include iron chelation and inhibition of enzymes involved in Hb degradation (cysteine proteinases, falcipains and PfA-M1).[34]

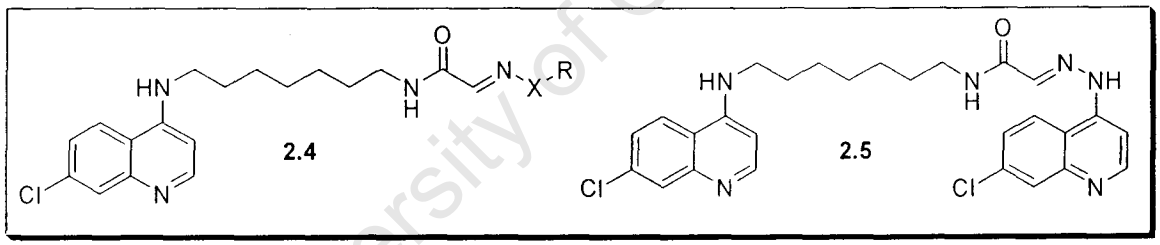


Figure 2.3 Chemical structures of 4-aminoquinoline-based glyoxylylhydrazones

2.1.1.4.4 Isatin-Based 4-Aminoquinolines

4-Aminoquinoline-based isatin thiosemicarbazone hybrids (Figure 2.4, **2.6**) have been reported to exhibit good *in vitro* activity against both CQS (D10) and CQR (K1 and W2) strains of *P. falciparum*. Although the mode of action is undefined, it is postulated to operate either through inhibition of haemozoin formation, inhibition of parasite cysteine proteases, or through metal chelation.[35]

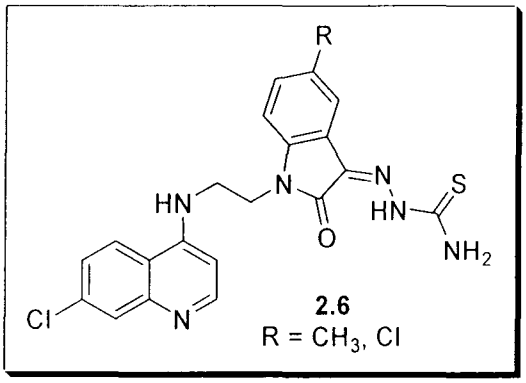


Figure 2.4 Chemical structure of 4-aminoquinoline-based isatin thiosemicarbazones

2.2 Rationale behind using 4-Aminoquinoline-based Acylthioureas

2.2.1 Justification for Research into aminoquinolines

There are several factors that justify the viability of research into aminoquinoline antimalarials.[36] These include: (1) the fact that aminoquinolines, in particular chloroquine, have been the most highly successful class of compounds for both the treatment and prophylaxis of malaria; (2) they are easily synthesised and inexpensive to produce; (3) 4-aminoquinolines are generally well tolerated, and have favourable toxicity profiles for treatment of acute infection; (4) drug resistance is derived from a reduction in drug accumulation, rather than any structural change to the target; (5) the haemoglobin degradation pathway has proven to be a favourable and viable therapeutic target; (6) many 4-aminoquinoline-derived analogues have been shown to be effective against CQ-resistant strains of the parasite, which underlines the potential for further analogue development in this class.

2.2.2 Justification for Research into Acylthioureas

Acylthioureas have found extensive use as antiinfective agents, with biological utility including fungicides and herbicides,[37] antitumour agents,[38] influenza,[39] and platelet-derived growth factor (PDGF) for the treatment of restenosis.[40]
The chemistry of acylthioureas is governed largely by two factors, namely, (i) alkylation, and (ii) metal coordination. Acylthioureas are interesting polydentate molecules with three different nucleophilic centres (S, N, and O) which can react with alkylating agents at different sites.[41] These compounds display high selectivity towards S-alkylation, which is explained by data obtained from semi-empirical and Discrete Fourier Transform (DFT) calculations, which predict the position of the HOMO orbital to be located over the sulfur atom, having predominantly a non-bonding σ-donor character. The LUMO orbitals are essentially of a π-acceptor anti-bonding nature, and are delocalised over the amidic and benzoyl moieties of the molecule.[42]

There are three conformational structures that acylthioureas can adopt, namely, the S, W and U conformers (Figure 2.5), however, it is the S conformation that is preferred, and which leads to S-alkylation. The intramolecular hydrogen bond results in a stable, 6-membered ring, which essentially locks the carbonyl oxygen and limits its accessibility.

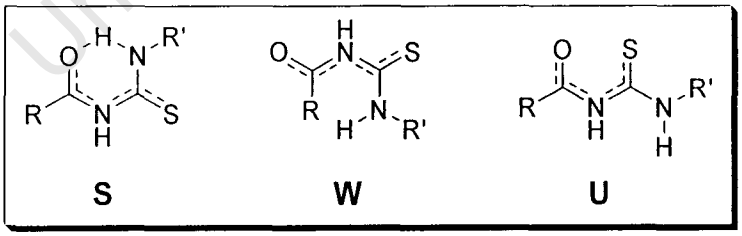


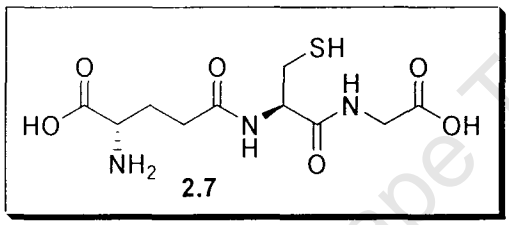
Figure 2.5 Acylthiourea conformations S, W and U [41]

S-alkylation is a particularly attractive property for a molecule to possess in terms of its potential as an antimalarial agent. There are several sulfur-containing *Plasmodium* enzymes, crucial to the survival of the parasite that may be inhibited by the formation of disulfide bonds through S-alkylation. Proteases involved in haemoglobin degradation, in particular, cysteine proteases, contain an active thiol moiety that is a viable target for inhibition

through such a mechanism. The inhibition of these cysteine proteases would impact on the parasite's ability to degrade haemoglobin and obtain essential nutrients, which could lead to parasite death.[43]

The disulfide reductases, glutathione reductase (GR) and thioredoxin reductase (TrxR) are crucial to the redox defence of parasite cells, protecting them from oxidative stress.

Glutathione (GSH) is a γ-L-Glutamyl-L-cysteinylglycine tripeptide (Figure 2.6, **2.7**) that is involved in the removal of destructive xenobiotic particles, particularly free radicals, from the cellular milieu.



2.7

Figure 2.6 Chemical structure of glutathione (GSH)

Intracellular GSH levels in *P. falciparum* depend on a number of factors, including de novo synthesis from the three constituent amino acids Glu, Cys and Gly,[44] glutathione disulfide (GSSG) efflux, and the reduction of GSSG by the homodimeric flavoenzyme, glutathione reductase, as per the equation:[45,46]

$$NADPH + H^+ + GSSG \rightarrow NADP^+ + 2\ GSH$$

CQ resistance has been linked to elevated levels of GSH in parasites, whilst depletion of GSH is postulated to restore CQ sensitivity in CQ-resistant strains of *P. falciparum*.[47] Since intracellular levels of GSH depend largely on the reduction of GSSG by GR, inhibition of this flavoenzyme is a viable means of overcoming the parasite.[48,49] There is a 40% identity in the amino acid sequences of *P. falciparum* GR (PfGR) and human GR (hGR). Significant differences between the enzymes include shape, size and electrostatics of the GSSG binding site (Figure 2.7), which suggests different modes of binding and the potential for selective inhibitors of PfGR.[50] The depletion of GSH in *P. falciparum* trophozoite-infected erythrocytes by GR inhibitors such as 1,3-bis(2-chloroethyl)-1-nitrosourea, 10-arylisoalloxazines, methylene blue, and

ajoene has in fact been demonstrated to impart a significant antiplasmodial effect.[48,51,52,53]

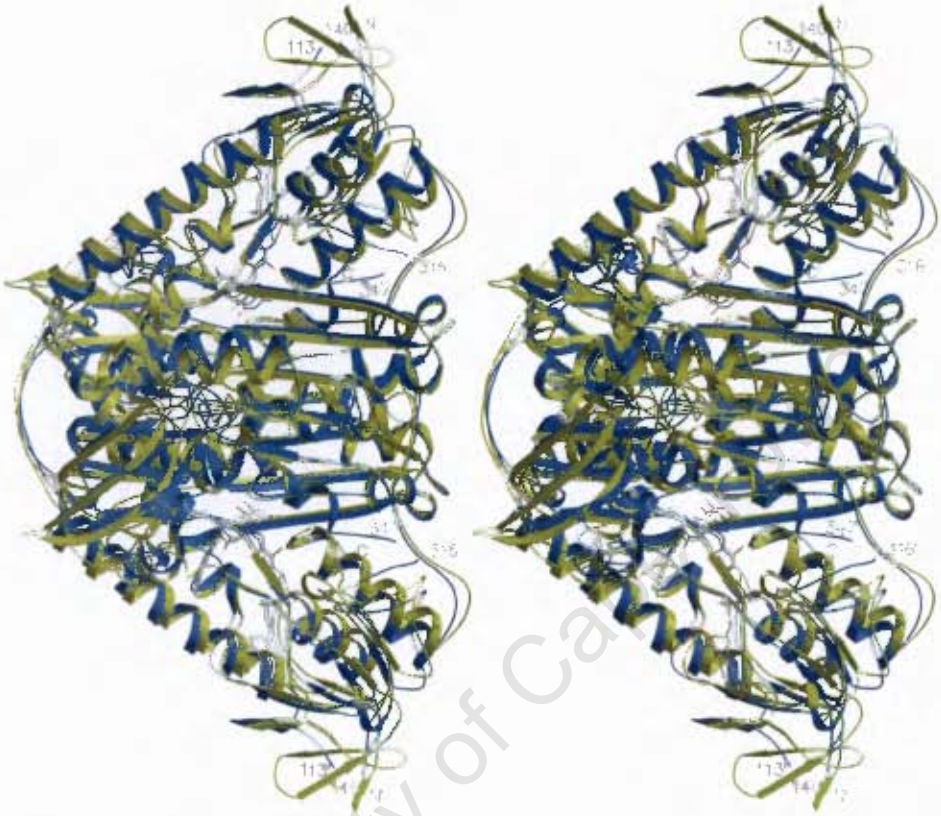


Figure 2.7 Comparison of PfGR and hGR structures. Stereo view of the superimposed backbone ribbons of PfGR (blue) and hGR (yellow). The breaks in the PfGR backbone, occurring where residues were not modeled due to lack of density, are labeled.[52]

Thioredoxin reductase (TrxR) is the second potential disulfide redox enzyme that may be inhibited by acylthiourea S-alkylation. The TrxR substrate, thioredoxin (Trx), is a dithiol protein (~12 kDa in size) that has a number of biological functions including detoxification of reactive oxygen and nitrogen metabolites, ribonucleotide reduction, control of protein folding, and redox regulation of enzymes and transcription factors, and is consequently crucial to maintaining intracellular redox homeostasis.[55,56,57] Trx has an active pair of cysteine amino acids, which cycle between the disulfide ($TrxS_2$) and dithiol [$Trx(SH)_2$] forms. Reduction of Trx is catalysed by the flavin-dependant oxidoreductase, TrxR according to the equation:

$NADPH + TrxS_2 + H^+ \rightarrow NADP^+ + Trx(SH)_2$

Significant differences exist between human TrxR (hTrxR) and *P. falciparum* TrxR (PfTrxR) at the C-terminal redox centre, the site of Trx reduction. In hTrxR, this centre is represented by a cysteine-seleno-cysteine functionality,[58] whereas PfTrxR contains a cysteine pair separated by a four amino acid spacer at this position.[59] This difference in an essential structural and functional motif in the enzyme provides a promising target for the development of selective PfTrxR inhibitors. Indeed, several 6,7-dinitroquinoxaline and nitro-2,1,3-benzothiadiazoles have been identified as highly active and selective inhibitors of PfTrxR *in vitro*.[60]

These enzyme targets offer alternate methods of overcoming the parasite, different to traditional 4-amino-7-chloroquinoline antimalarials, which has the benefit of reducing the likelihood of cross-resistance.

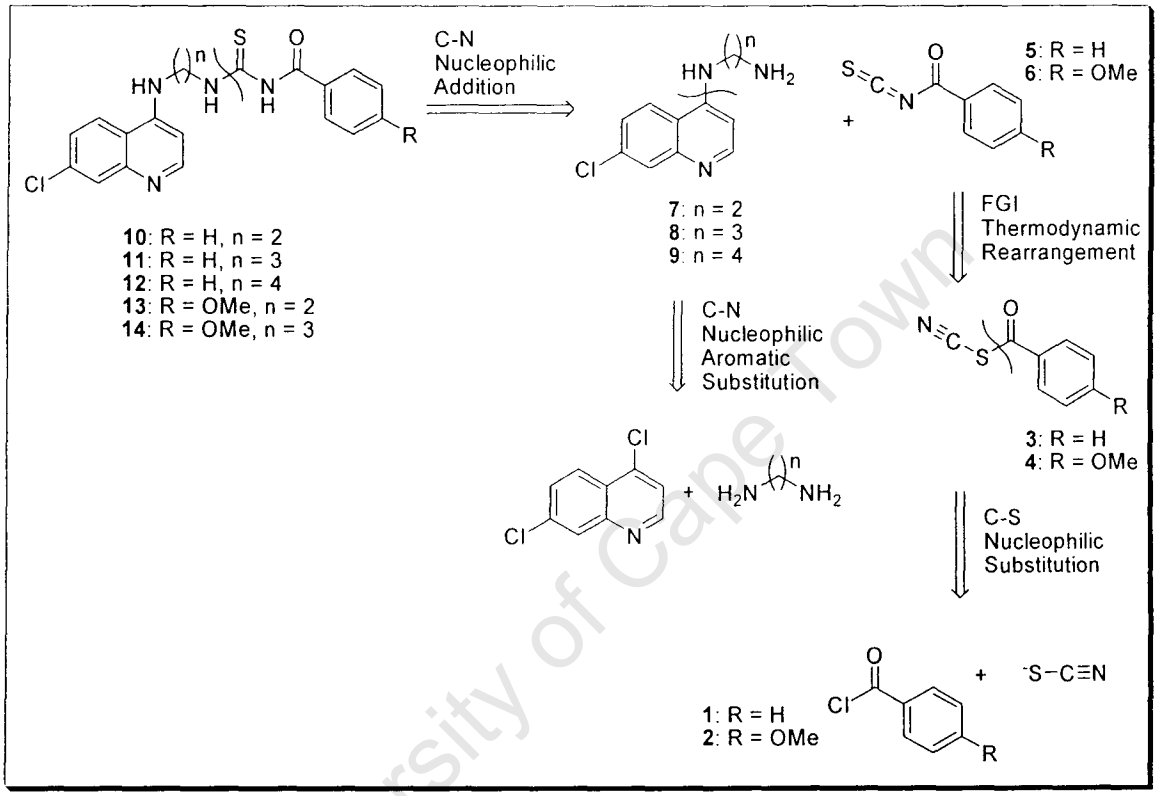
Since one of the goals of this project was to synthesise gold(I) complexes of organic ligands in order to evaluate their potential as antimalarial agents, the gold binding ability of target ligands remained a primary factor in their selection. A variety of acylthioureas have been reported to form metal complexes,[61,62,63] and hence the incorporation of such a functionality in the target ligands was deemed desirable. In particular, the thiocarbonyl was expected to present an ideal site of linear coordination to the thiophilic gold(I) metal centre.

2.3 Synthesis and Characterisation of 4-Aminoquinoline-Derived Acylthioureas

2.3.1 Retrosynthetic Analysis

The retrosynthetic analysis of target compounds **10** – **14** (Scheme 2.1) involves three simple disconnections. A C-N disconnection affords the isothiocyanates (**5**, **6**) and the chloroquinoline amines (**7**, **8**, **9**), which are obtained from the benzoylthiocyanates (**3**, **4**) and 4,7-dichloroquinoline,

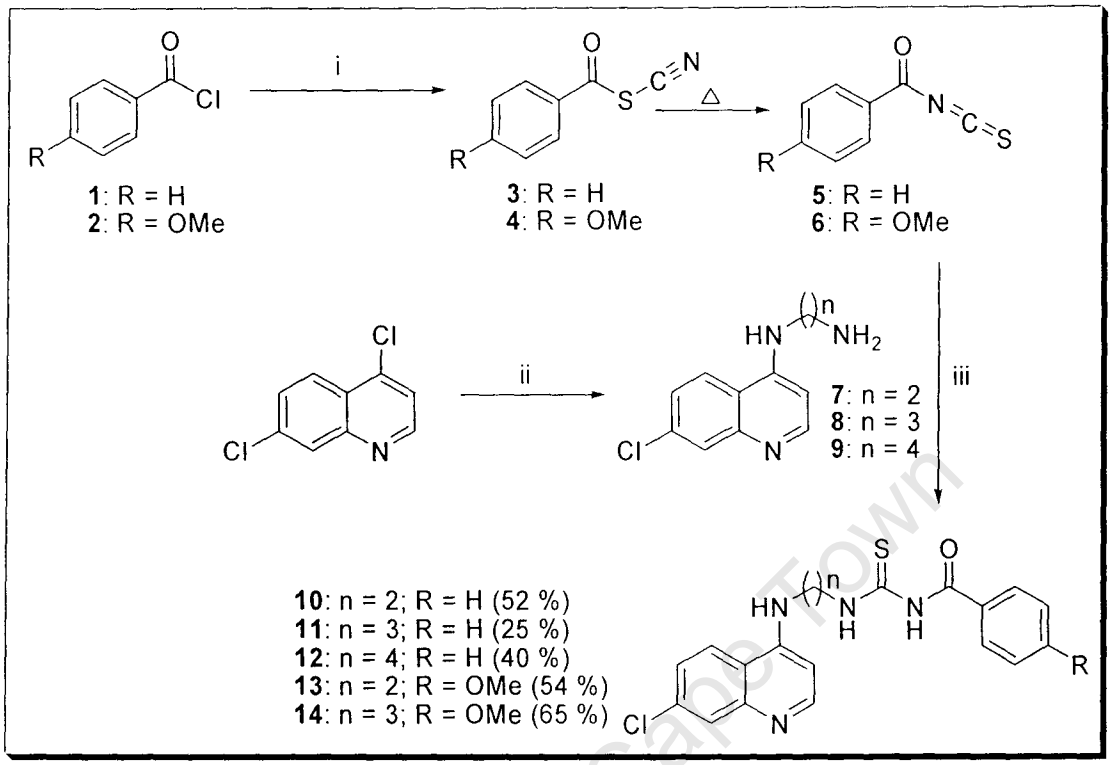
respectively. Conversion of the thiocyanates to the isothiocyanates occurs spontaneously at the elevated temperatures obtained in refluxing acetone. Commercially available benzoyl chloride starting materials **1** and **2** are obtained by the C-S disconnection of **3** and **4**.



Scheme 2.1 Retrosynthetic analysis of 4-aminoquinoline-derived acylthioureas

2.3.2 Synthesis

The synthesis of the acylthiourea target molecules (**10** – **14**, Scheme 2.2) was performed using the procedure reported by Zeng *et al.*[64] This involved reacting benzoylchlorides (**1**, **2**) with thiocyanates (NH_4SCN or KSCN) to afford the benzoylthiocyanates (**3**, **4**), which when heated under reflux, underwent conversion to the isothiocyanates (**5**, **6**). The solution was then filtered to remove the chloride salts formed, before the amines **7** – **9** were added to the solution with subsequent refluxing for 3 hours. Nucleophilic attack at the carbon of the isothiocyanate by the chloroquinoline amines gave the acylthioureas, **10** – **14**, in low to acceptable yields (**10** = 52 %; **11** = 25 %; **12** = 40 %, **13** = 54 %, **14** = 65 %). The chloroquinoline amines were prepared according to the literature procedure described by De *et al.*[65]



Scheme 2.2 *Reagents and conditions* i) KSCN or NH_4SCN, acetone, reflux, 30 min; ii) diaminoalkanes, 80 °C, 1 h; 140 °C, 3 h; iii) For **10**-**12**: 7-chloroquinolinyl alkyldiamines, acetone, reflux, 2 h. For **13**, **14**: acetone or acetone/NMP, r.t, overnight

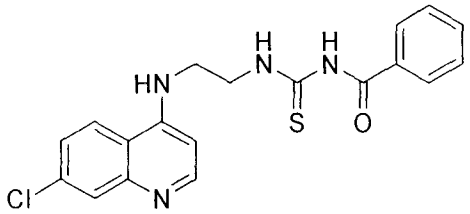
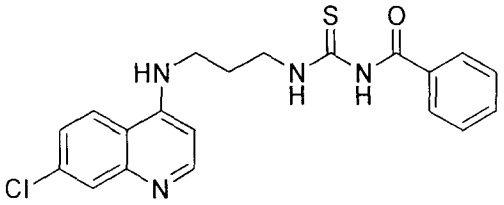
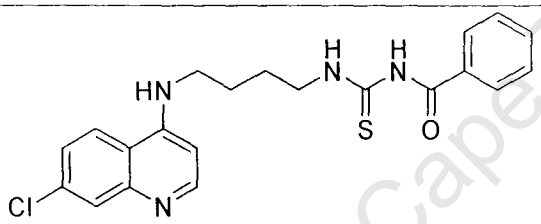
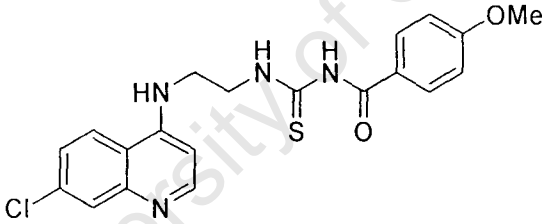
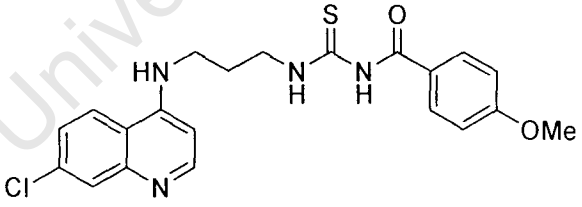
The lower-than-expected product yields were largely due to side reactions in the final step. These may have included amide formation (eliminating thiocyanate), or acylthiourea reaction with a second equivalent of isothiocyanate, or reaction of the isothiocyanate with water (either atmospheric or in the solvent). Purification required, in many cases, multiple columns before the product was obtained in sufficient purity for analysis. This also contributed to the low yields. Because of these difficulties, the protocol was modified for the synthesis of **13** and **14** in an attempt to improve the yield and purity of the products. For **13**, the chloroquine amine was added to the acetone solution of isothiocyanate as a solution of *N*-methyl-2-pyrolidinone (NMP), and the resulting solution stirred at room temperature for 3 days instead of refluxing. It was hoped that the reduced temperature would limit amine attack at the higher energy carbonyl position, leading to exclusive formation of the desired product. This procedure resulted in the formation of a

precipitate of the desired product (25 %). However, despite an improvement in the purity (of this precipitated product), isolation of the remaining product from the NMP proved to be difficult, and resulted in a low overall yield (54 %).

Modifications to the protocol employed for the formation of **14** included the use of NH_4SCN instead of KSCN (for practical reasons – there was no available KSCN at the time of the experiment), omission of the filtration step (to reduce isothiocyananate exposure to atmospheric water), a reduced reaction temperature (room temperature, overnight, to reduce amide formation), and purification of the product from the many reaction impurites by recrystallisation from 95 % EtOH (to increase the yield). These modifications had a minor improvement in the yield (65 %), however, the broad m.p. range (85 – 90 °C) would suggest that further purification of **14** was required.

The structures, isolated yields and respective melting points of the target 4-aminoquinoline-derived acylthiourea compounds are given in Table 2.1 below.

Table 2.1 4-aminoquinoline-derived acylthioureas and their respective yields and melting points

Compound	Chemical Structure	Yield (%)	M.p. (°C)
10		52	191 - 193
11		25	83 - 85
12		40	161 - 163
13		54	206 - 207
14		65	85 - 90

2.3.3 Mechanistic Details

The susceptibility of the 4-position of the quinoline ring to aromatic substitution is based upon the mesomeric effect of the nitrogen, as outlined in Figure 2.8. Resonance structures indicate a greater electrophilicity at the 2- and 4-positions of the ring.

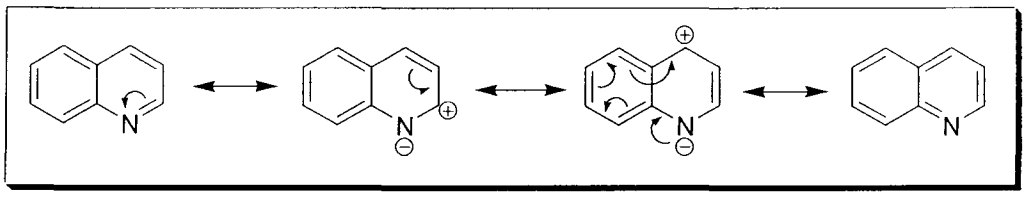
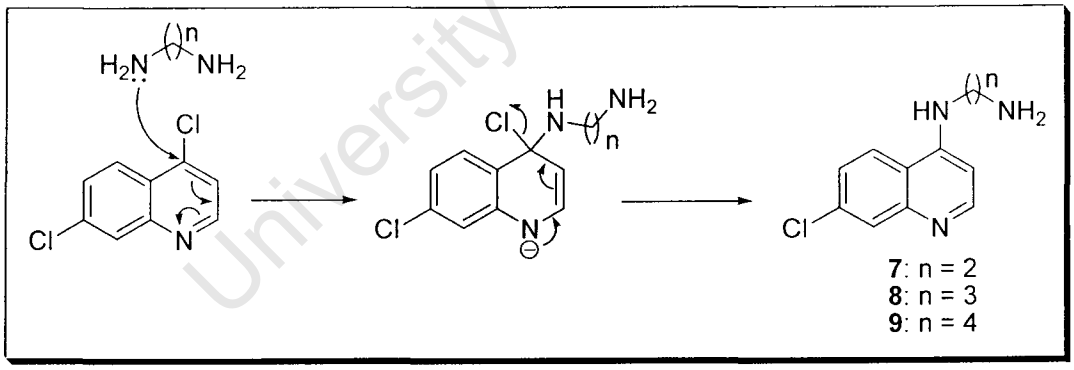


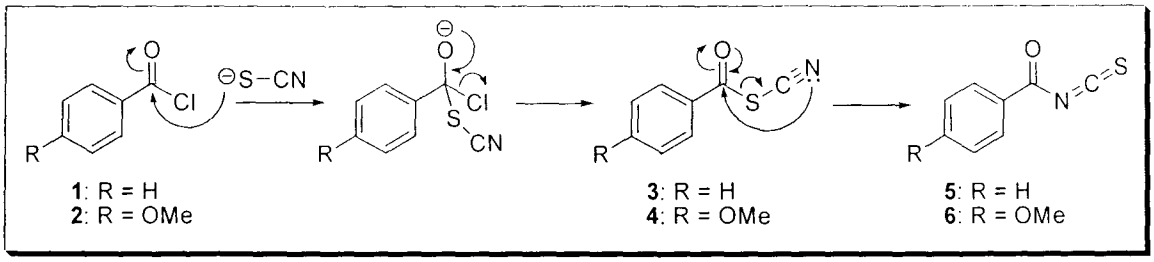
Figure 2.8 Canonical structures depicting mesomeric and resonance effects within the quinoline ring

The chloro- substituent at the 4-position of 4,7-dichloroquinoline ring enhances the electrophilicity of the carbon at that position. The nucleophilic amines of the alkyl diamines therefore attack preferentially at the 4-position resulting in dearomatisation of the ring, leading to the formation of the 4-aminoquinolines (**7** – **9**) *via* an addition-elimination mechanism (Scheme 2.3). Elimination of the chlorine is achieved by the return of the electron pair on the nitrogen to the ring, restoring aromaticity. The driving force behind this regioselective attack is therefore the drive to aromaticity as well as the close proximity of the 4-position to the quinoline nitrogen (7-chloro substitution is unfavoured).



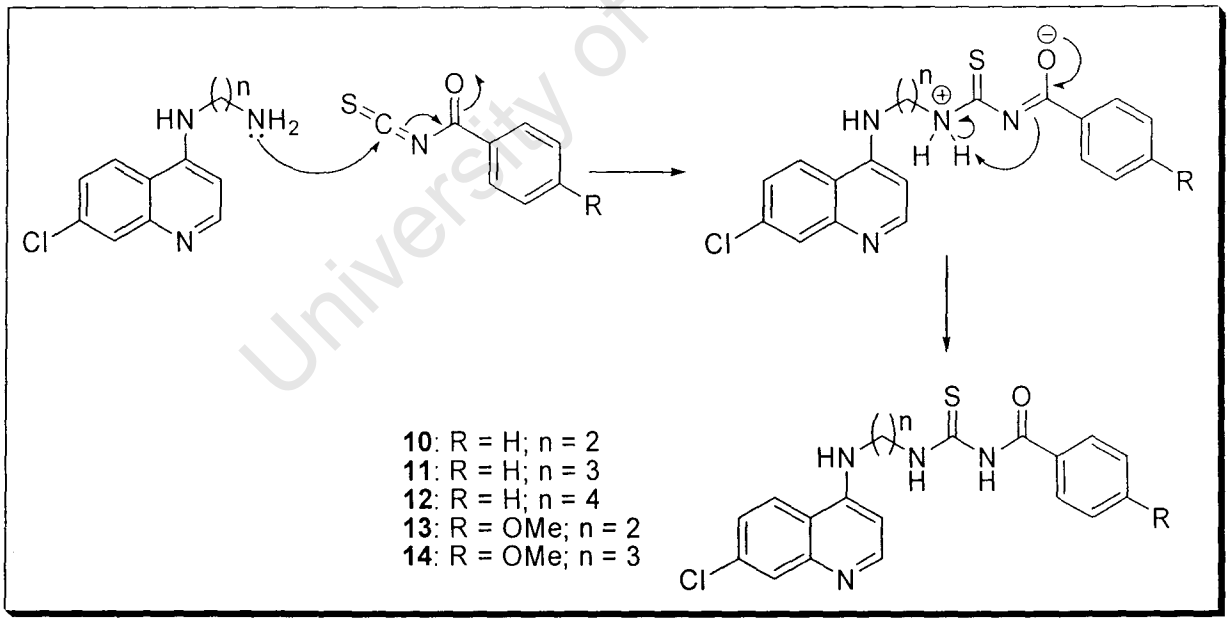
Scheme 2.3 Reaction mechanism for the formation of chloroquine amines **7** - **9**

Isothiocyanate intermediates **5** and **6** were formed from benzoyl chlorides **1** and **2** by substitution of the chloride with NCS^-, followed by conversion of the thiocyanate to the isothiocyanate upon heating. The mechanism is outlined in Scheme 2.4.



Scheme 2.4 Reaction mechanism for the formation of the isothiocyanate intermediates **5** and **6**

Coupling of the isothiocyanates to the chloroquine amines proceeds by nucleophilic attack of the electrophilic isothiocyanate carbon centre by the primary amines of **7** – **9**. This results in a delocalisation of the delivered electrons into the carbonyl, which upon reformation, results in a proton transfer from the primary amine to the amide nitrogen of the isothiocyanate, thus yielding the target acylthioureas (see Scheme 2.5).



Scheme 2.5 Reaction mechanism for the formation of the final acylthiourea target molecules, **10** - **14**

2.3.4 Characterisation of Target Compounds

Characterisation of all compounds was done by means of ^{1}H and ^{13}C NMR, mass spectrometry, and elemental analysis.

The ^{1}H NMR spectra of the products displayed signals for the three NH protons at characteristic positions. The protons at H4 and H5 (Figure 2.9) gave signals at approximately 11 ppm, with the H5 signal further downfield (~11.2 ppm) than H4 (~11.0 ppm) due to its two deshielding carbonyl neighbours. The H1 proton signal was generally found between 7 and 8 ppm. Alkyl proton coupling to the nitrogen-bound protons was generally weak and not seen in the ^{1}H spectra (depending on the NMR solvent used).

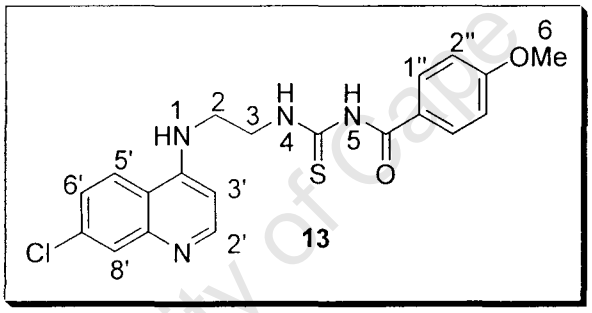


Figure 2.9 Chemical structure and NMR assignment numbering scheme of **13**, a representative target acylthiourea

The aromatic quinoline protons displayed a common pattern in terms of their relative positions and coupling constants, and were generally found between δ 6.5 – 8.5 ppm. The most electrophilic position, due to resonance (as illustrated in Fig. 2.7 above), was the 2-position, and consequently, H2' was consistently the most downfield proton on the quinoline ring. H2' coupled to H3' (the most upfield of the quinoline protons, for similar resonance reasons) with a *J* value of approximately ~5-6 Hz. H5' was usually the second-most downfield quinoline proton, with a large coupling to H6' of ~9 Hz. Occasionally H8' was further downfield than H5', however, in general it was the third-most downfield, with a small ^{4}J coupling to H6' of ~2 Hz. The H6' signal was the fourth-most downfield of the quinoline protons, and gave a doublet of doublets with coupling constants of ~9 and ~2 Hz for coupling to H5' and H8', respectively. Because each quinoline proton gave a doublet with

a characteristic coupling constant, their assignment was relatively straightforward. The non-quinoline aromatic proton signals were generally positioned between δ 7.0 – 7.9 ppm, and were identifiable by their coupling constants of ~7-8 Hz. In cases where the *para*-substituent was H (**10** – **12**), it gave a triplet integrating for 1H, with the *ortho* protons giving a doublet, and the *meta* protons a triplet (both integrating for 2 H). In cases where the *para*-substituent was –OMe, the *ortho* and *meta* protons both gave doublets of the same coupling constant as they coupled to each other.

The most characteristic signals in the ^{13}C NMR spectrum were those of the thiocarbonyl and carbonyl at ~180 ppm and ~165 ppm, respectively. The alkyl (~40 ppm) and methoxy (~55 ppm, when present) carbons also gave easily identifiable signals. Further confirmation of structure obtained from the ^{13}C spectrum was evident in the number and chemical shift of carbon signals.

High resolution mass spectrometry (HRMS) was used to confirm the molecular mass of the product. Product purity was determined by elemental analysis (C, H, N, S).

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CHAPTER 3

AMINOQUINOLINE-BASED 2-HYDROXYACETAMIDES

3.1 Introduction

This chapter describes the background, synthesis and characterisation of several novel aminoquinoline-based 2-hydroxyacetamides (glycolamides). The common feature of these compounds is the α–hydroxy amide functionality, which is derived from the reaction of an amine with the acetonide of glycolic acid (Figure 3.1).

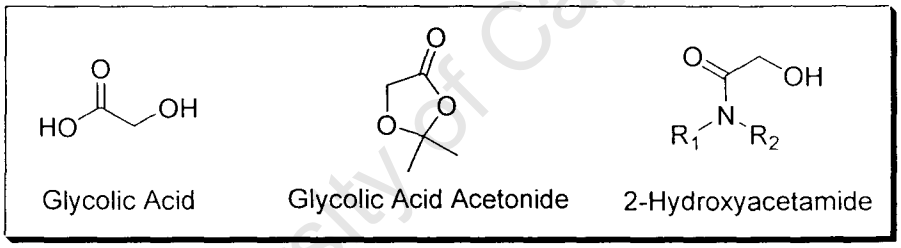


Figure 3.1 Chemical structure of glycolic acid and its corresponding acetonide

Four classes of aminoquinoline molecules were synthesised with this functionality, the differences between the classes lying in the substitution pattern of the quinoline ring and/or the identity of the side chain. The structures of the molecules in each class were based upon known antimalarial drugs currently in use. The four classes of molecules were: i) chloroquine analogues, including *N*-[2-(7-Chloroquinolin-4-ylamino)-ethyl]-2-hydroxyacetamide, its thionated derivative and its benzylated derivative, ii) Piperaquine analogues, including 1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-hydroxyethanone, and its thionated derivative, iii) a piperazinyl mefloquine analogue, 1-(4-(2,8-bis(trifluoromethyl)quinolin-4-yl)piperazin-1-yl)-2-hydroxyethanone, and iv) a primaquine analogue, 2-hydroxy-*N*-{4-(6-methoxyquinolin-8-ylamino)pentyl}acetamide. The target molecules are illustrated in Figure 3.2 below.

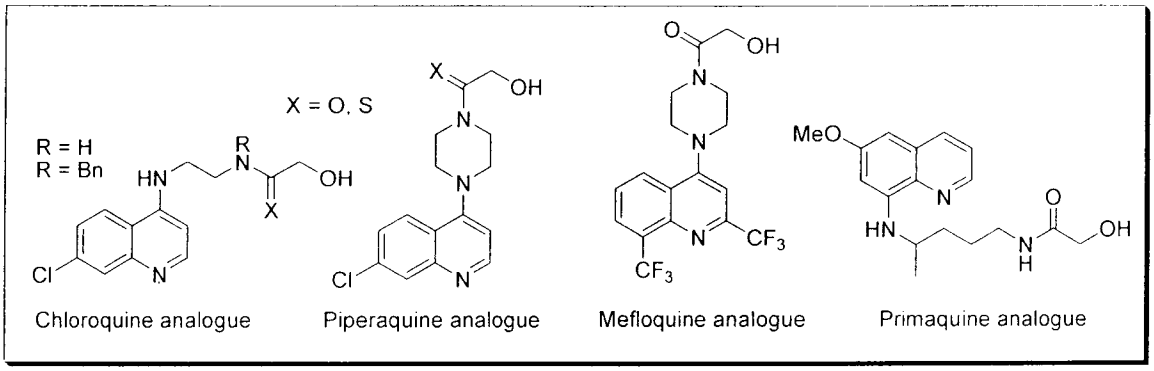


Figure 3.2 Chemical structures of the hydroxyacetamide target molecules

The structures were designed to incorporate the aminoquinoline moiety (by which the known antimalarials are believed to derive their activity), with the 2-hydroxyacetamide functionalised side chain, serving a dual purpose of bypassing parasite recognition mechanisms as well as potentially imparting a novel, inherent, antimalarial activity.

All target compounds were designed and synthesised in order to determine their activity against *Plasmodium falciparum* malaria parasites. Compounds were tested against CQ-sensitive and CQ-resistant strains of parasites in order to establish whether they were able to overcome CQ-resistance.

3.2 Rationale Behind Using Aminoquinoline-based 2-Hydroxyacetamides

The biological activity of the 2-hydroxyacetamide (glycolamide) functionality has been demonstrated in a number of varied targets. These include inhibition of the cell wall biosynthesis of *Mycobacterium tuberculosis*,[1] and inhibition of cyclooxygenase imparting anti-inflammatory activity.[2]

Glycolamides have also been demonstrated to be effective prodrugs, enhancing solubility, lipophilicity and various other pharmacological properties when incorporated into the molecular structure of a number of drugs including

aspirin,[3] Ibuprofen,[4] Naproxen,[5] and the natural product, scutellarin.[6] Such a prodrug property, when applied to an antimalarial compound, could enhance delivery of the active quinoline moiety to parasite targets.

A further potential mode of action of the 2-hydroxyacetamides is iron chelation. Many metabolic processes of the erythrocytic malaria parasite are iron-dependant, and compounds that can chelate, and thereby withhold iron from the parasite, could disrupt parasite growth by inhibiting DNA synthesis, carbohydrate metabolism, proteolysis of host haemoglobin, normal mitochondrial function and electron transport.[7,8,9] Several studies have shown that chelating agents do in fact inhibit parasite growth *in vitro*.[8,10,11] It has also been established that chelators selectively coordinating Fe^{3+} over Fe^{2+} display the greatest antimalarial activity.[7,12] Several of these antimalarial chelators include aroylhydrazones, thiosemicarbazones and glyoxylylhydrazones (see Chapter 2.1.1.4.3).[10,13]

The 2-hydroxyacetamide-iron chelate complex is illustrated in Figure 3.3 below.

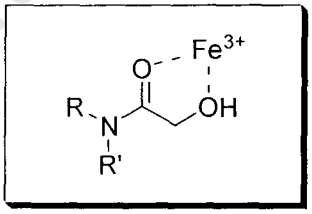


Figure 3.3 Fe-chelate complex with the 2-hydroxyacetamide functionality

The carbonyl oxygen and the α–hydroxy group provide "hard" donor ligands to the "hard" Fe^{3+} providing favourable coordination, according to the HSAB (hard soft acid base) principle. The 5-membered chelate complex provides a stable coordination conformation, with minimal bond distortion, suggesting the potential for antimalarial activity through iron chelation.

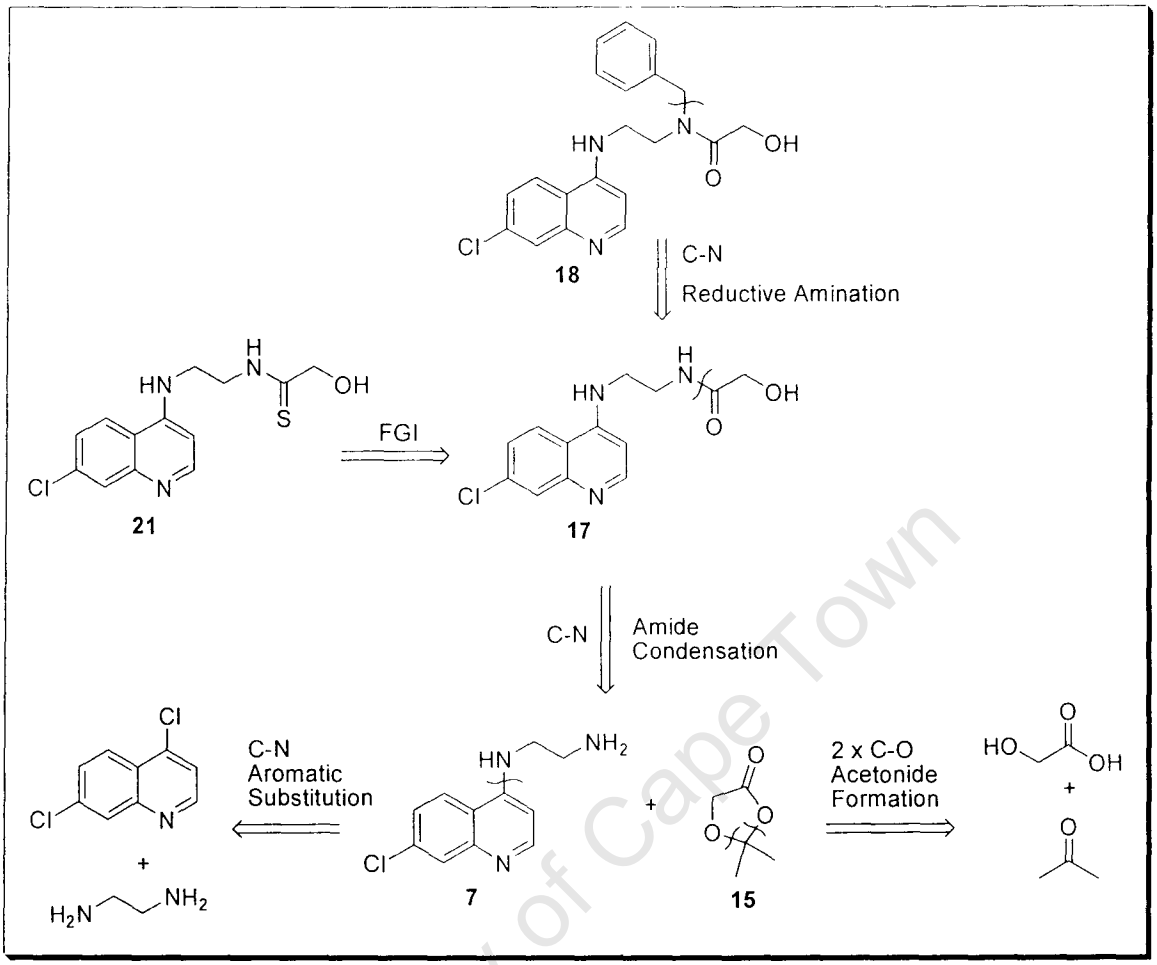
3.3 Synthesis and Characterisation of Aminoquinoline-based 2-Hydroxyacetamides

Four types of molecules were synthesised, as illustrated in Figure 3.2. Since the end design of these molecules was to complex them to gold(I), it seemed desirable to convert the acetamide to a thioacetamide functionality in order to enhance their gold-binding ability [rationale based on the high affinity of gold(I) for sulphur]. Similar 4-amino-7-chloroquinoline molecules have been demonstrated to form complexes with gold(I) by binding through the quinoline nitrogen,[14] consequently it was postulated that conversion to the thioacetamide would facilitate gold-binding through the sulphur of the thiocarbonyl. Thioacetamide conversion was attempted with the two 7-chloroquinoline derivatives.

3.3.1 Chloroquine Analogue

3.3.1.1 Retrosynthetic Analysis

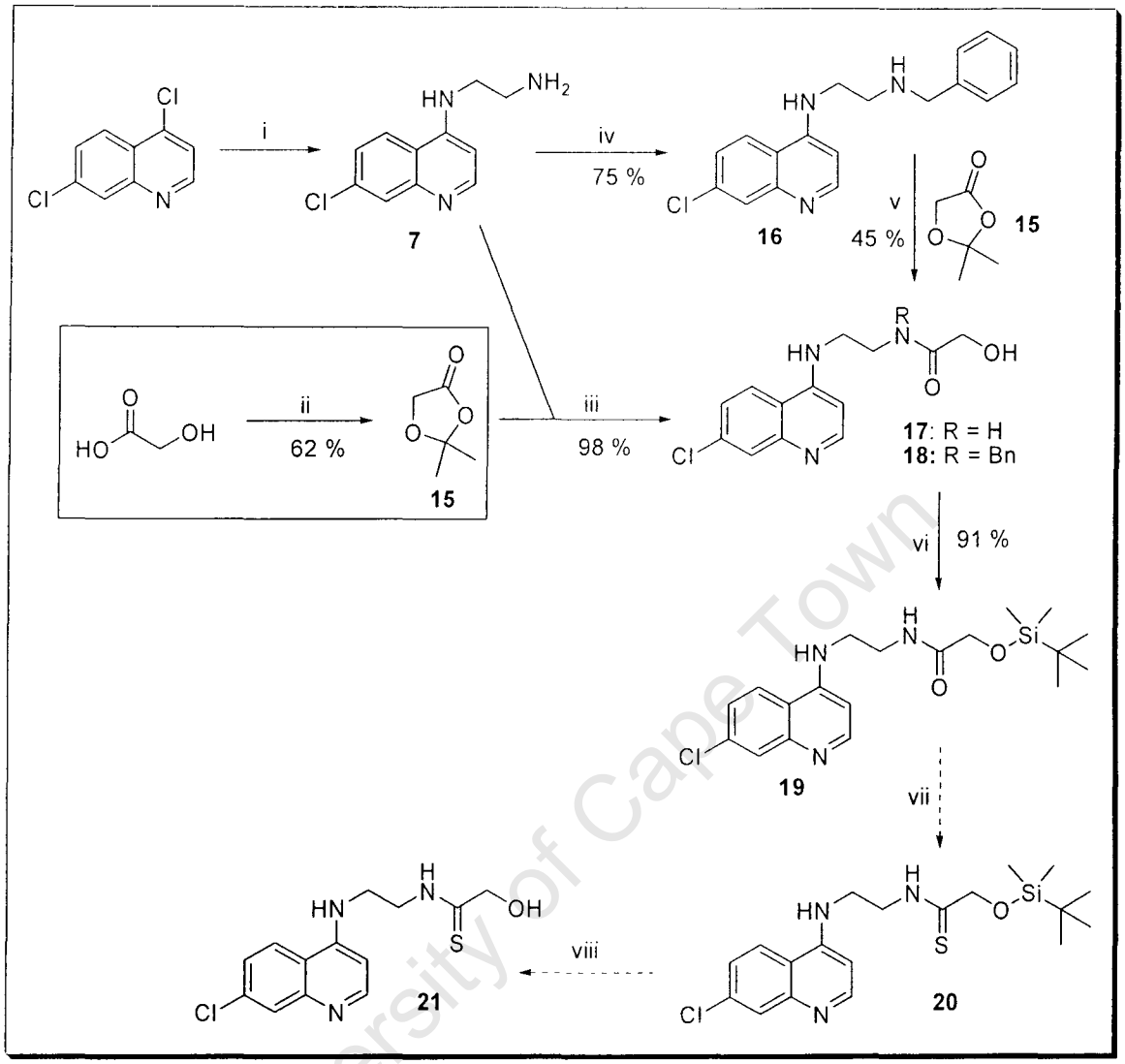
The retrosyntheses of the ethyl 2-hydroxyacetamide derivatives are illustrated in Scheme 3.1 below. C-N disconnection of the benzyl tertiary amide derivative, **18**, leads to the secondary amide, **17**. A functional group inversion (FGI) (thiocarbonyl to carbonyl) of **21** also leads to **17**. C-N amide disconnection of this intermediate leads to the chloroquine diamine, **7**, and the glycolic acid acetonide, **15**. The C-N disconnection of **7** leads to the starting materials 4,7-dichloroquinoline and ethylene diamine. Disconnection of **15** at the two C-O bonds leads to the glycolic acid and acetone starting materials.



Scheme 3.1 Retrosynthetic analysis of the ethyl 2-hydroxyacetamide **16**

3.3.1.2 Synthesis

The synthesis of the ethyl 2-hydroxyacetamide and thioacetamide derivatives is outlined in Scheme 3.2 below.



Scheme 3.2 *Reagents and conditions* i) Ethylene diamine, 80 °C, 1 h; 140 °C, 3 h; ii) Acetone, H_2SO_4 conc., -10 °C, 1 h; iii) Dry CH_2Cl_2, 30 °C, 12 h; iv) Benzaldeyde (1.0 eq.), MeOH/DMF, 25 °C, 5 h; then $NaBH_4$ (2 eq.); v) Dry CH_2Cl_2, 30 °C, 24 h; vi) TBDMSCl (2 eq.), imidazole (2 eq.), dry $CHCl_2$/DMF, 25 °C, 48 h; vii) Lawesson's reagent (1.0 eq.), dry acetontrile, reflux, 1 h; viii) TBAF (3 eq.), dry THF, r.t., 1 h;

The key acetonide intermediate, **15**, was obtained by the acid catalysed cyclisation of glycolic acid with acetone at -10 °C as a transparent oil. Maintenance of the reaction temperature between -15 °C and -10 °C was found to be crucial to obtaining an acceptable yield. In addition to this, it was discovered that the product was volatile under reduced pressure, and attempts to dry the product extract on a high vacuum pump resulted in

significant loss of product. Consequently, the solvent in which the product was extracted after work up was removed under a moderate vacuum by rotary evaporator, and used for reaction without further drying.

The chloroquine amine intermediate N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine, **7**, was synthesised according to the reported literature procedure by De *et al*,[15] in preparation for condensation with the acetonide **15**. It was further derivatised to the benzylated secondary amine **16** by a two step reductive amination procedure. This involved initial formation of the imine by nucleophilic addition of the amine to the benzaldehyde, followed by reduction of the imine double bond with $NaBH_4$ to form N^1-benzyl-N^2-(7-chloroquinolin-4-yl)ethane-1,2-diamine, **16**, in 75 % yield.

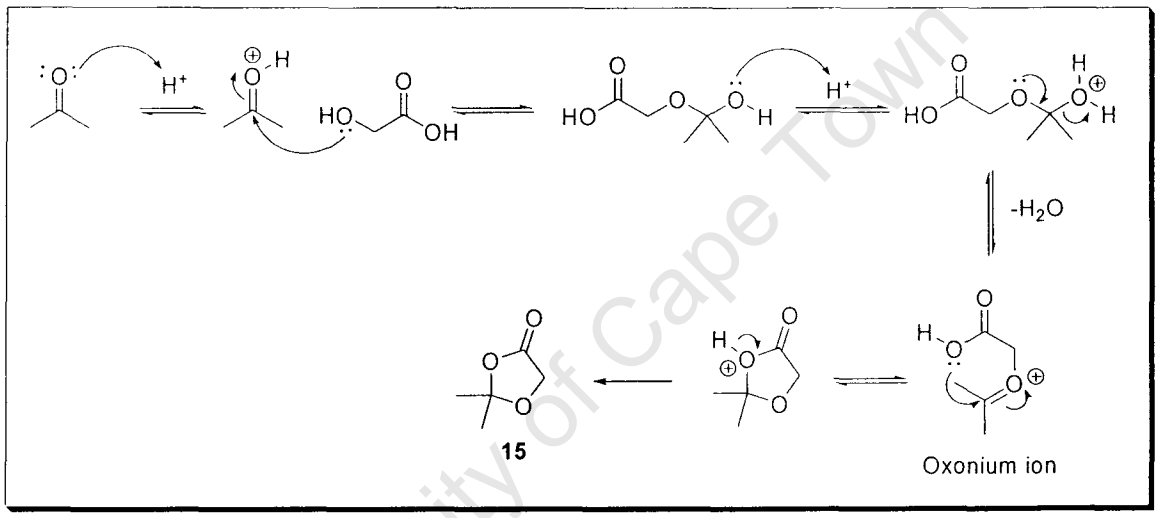
The 2-hydroxyacetamides, **17** and **18**, were synthesised by the condensation of acetonide **15** with amines **7** and **16**, respectively. A yield of 98 % was obtained for **17** and 45 % for **18**. The low yield achieved for **18** was due to incomplete reaction of the starting materials, as well as loss of product during the purification steps. (The product mixture had to be chromatographed twice and recrystallised from EtOH before a sufficiently pure sample was obtained.)

Conversion of **17** to the corresponding 2-hydroxythioacetamide **21** was envisioned to proceed by the synthetic pathway vi – viii (Scheme 3.2). The O – S converting reagent, Lawesson's Reagent (2,4-bis(*p*-methoxyphenyl)-1,3-dithiaphosphetane 2,4-disulfide) (LR) is highly oxophilic. Consequently, protection of the primary alcohol of **17** as a tertiary butyldimethylsilyl ether was required prior to reaction in order to prevent undesired side reactions. Silylation of **17** was achieved after stirring in a CH_2Cl_2/DMF solution of tertiarybutyldimethylsilyl chloride (2 eq.) (TBDMSCl) and imidazole (2 eq.) at room temperature for 48 h. The silyl ether, **19**, was obtained in a good yield of 91 %. Subsequent conversion to the thioacetamide with LR was unfortunately not successful. A single reaction product was obtained after column chromatography, however, ^{1}H NMR analysis did not support the expected molecular structure. It was decided, therefore, to attempt thioacetamide formation with the piperaquine derivative (see Figure 3.2). Step viii was to be

the silyl ether deprotection step, involving Si-O cleavage by tetra-*n*-butylammonium fluoride (TBAF) in dry THF.

3.3.1.3 Mechanistic Details

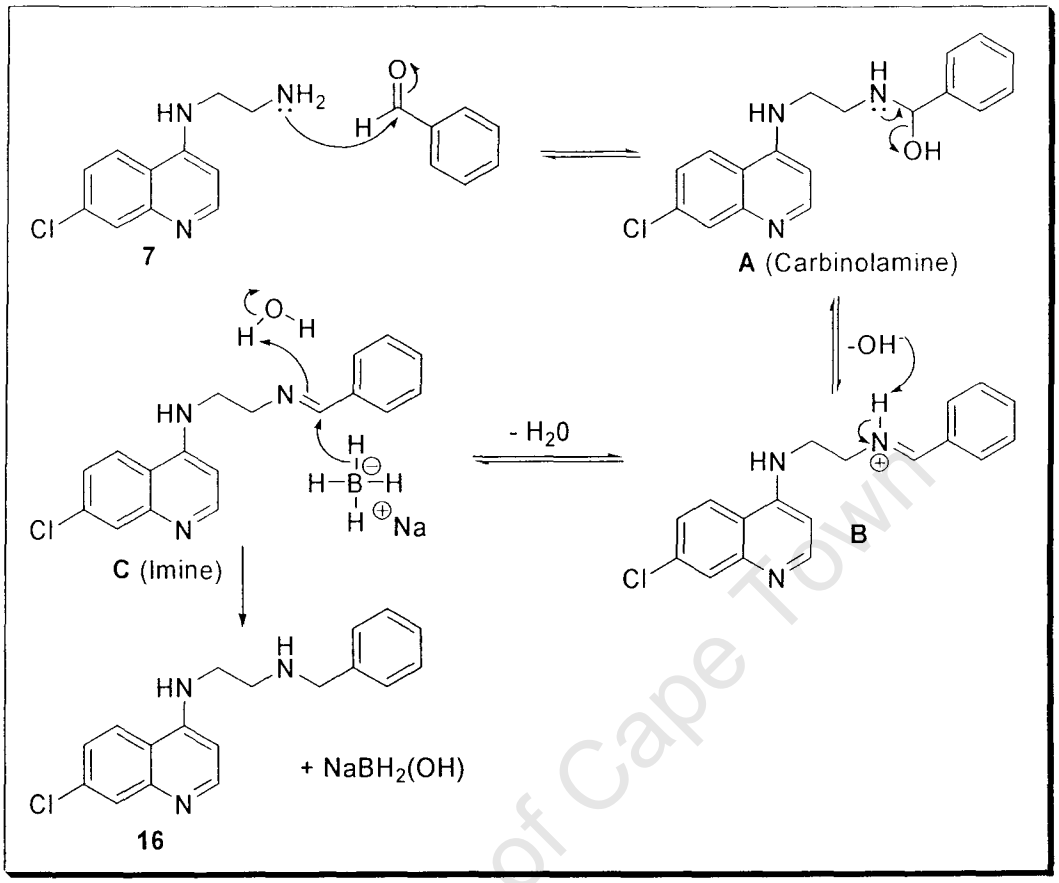
The mechanism of the acid-catalysed glycolic acid acetonide formation is illustrated in Scheme 3.3 below. Each of the steps leading to the formation of the product are in equilibrium. The acidity is provided by ~30 mole % concentrated H_2SO_4.



Scheme 3.3 Mechanism of formation of acetonide **15**

The first step involves protonation of the acetone carbonyl oxygen resulting in a sufficiently electrophilic carbon for attack by the α–hydroxy oxygen of glycolic acid. A second protonation of the acetone oxygen leads to its elimination as water by resonance of the same α-hydroxy oxygen, to form an oxonium ion. This creates a sufficiently electrophilic carbon centre for the weakly nucleophilic carboxylic acid hydroxyl to attack. This step initiates cyclisation, which yields the final product **15** after regeneration of the H^+ catalyst.

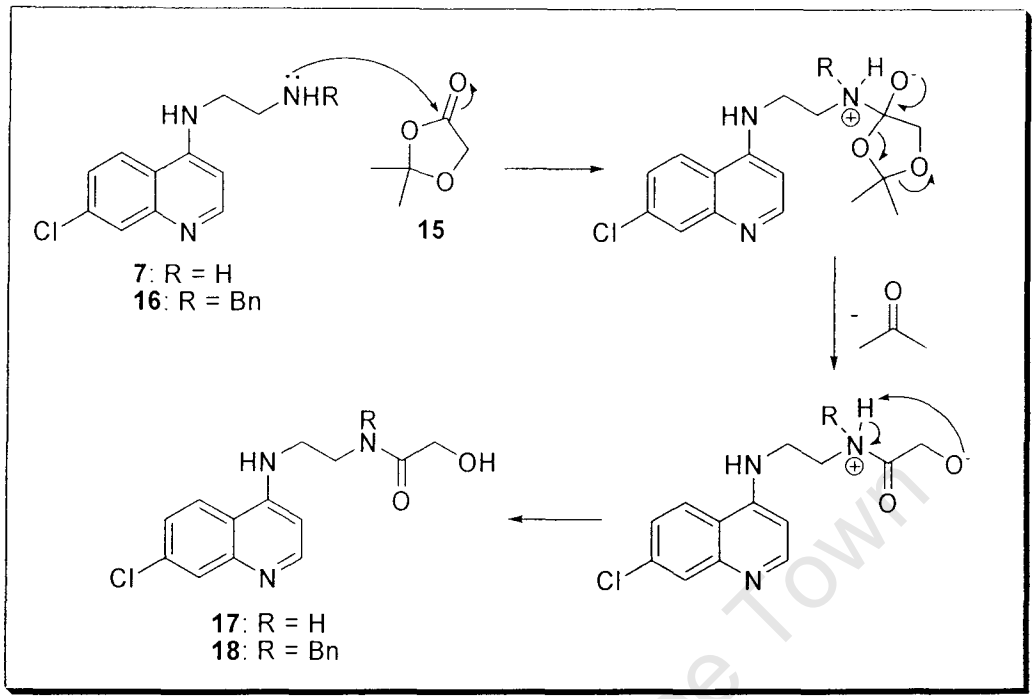
The mechanism of formation of the CQ amine **7** is outlined in Scheme 2.3 (Chapter 2). The mechanistic details of its conversion to the benzylated derivative **16** are illustrated below in Scheme 3.4.



Scheme 3.4 Reaction mechanism for the formation of N^1-benzyl-N^2-(7-chloroquinolin-4-yl)ethane-1,2-diamine, **16**

The alkylimino-de-oxo-bisubstitution reaction leading to the formation of **16** involves the initial formation of an imine, which is then reduced with $NaBH_4$ to the substituted amine. The first step involves nucleophilic addition of the amine to the aldehyde, which occurs in a reversible manner, and is followed by a proton transfer from the amine to the oxygen, to form the carbinolamine **A**. Loss of one water molecule, also in a reversible manner, then results in the formation of the imine **C**. Borohydride reduction of the imine is then accomplished by hydride attack of the double bond, followed by a proton transfer from water to the carbanion, to give the benzylated secondary amine **16**.

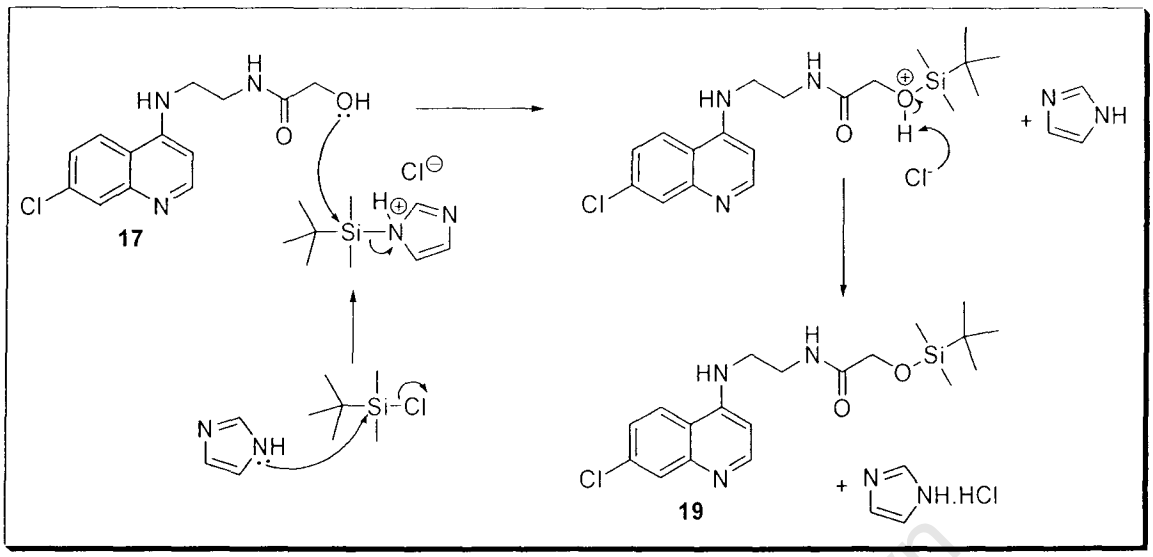
The condensation reactions leading to the formation of the 2-hydroxyacetamides, **17** and **18**, occur *via* the same mechanism, as outlined in Scheme 3.5.



Scheme 3.5 Reaction mechanism for the formation of 2-hydroxyacetamides **17** and **18**

Nucleophilic attack of the acetonide carbonyl by the amine results in formation of the C-N bond, which upon reformation of the carbonyl, results in cleavage of the ester C-O bond, and the elimination of acetone. Proton transfer from the amine to the terminal oxide affords the final 2-hydroxyacetamides **17** and **18**.

The mechanistic details of the formation of the silyl ether, **19**, are outlined in Scheme 3.6 below.



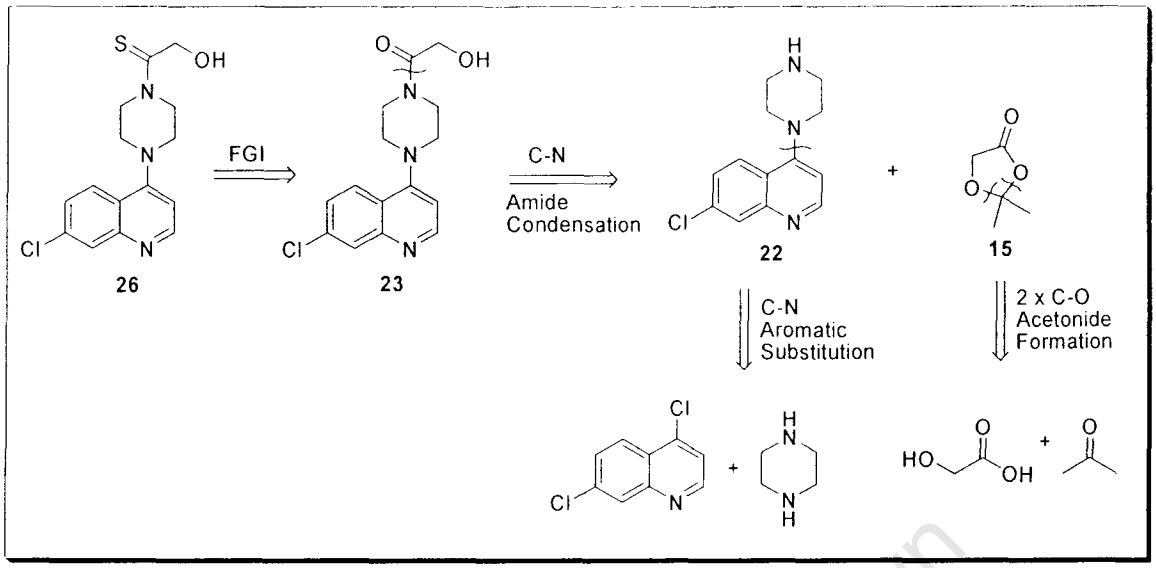
Scheme 3.6 Reaction mechanism for the formation of the silyl ether, **19**

The reaction is initiated by displacement of the silyl chloride by imidazole, resulting in a more reactive imidazolium silyl intermediate. The Si-imidazole bond is then cleaved in an S_N2-type substitution with the hydroxyl of the acetamide. After a proton transfer to form HCl (which is neutralised by the imidazole to imidazolium hydrochloride salt), the silyl ether **19** is obtained.

3.3.2 Piperaquine Analogue

3.3.2.1 Retrosynthetic Analysis

The retrosynthetic analysis of the piperazinyl 2-hydroxyacetamide is similar to that of the ethyl derivative, and is given in Scheme 3.7 below. The FGI (carbonyl to thiocarbonyl) of **26** leads to the α–hydroxyacetamide, **23**. C-N disconnection of **23** gives the piperazinyl chloroquinoline, **22**, and acetonide, **15**. C-N disconnection of **22** yields the aromatic substitution starting materials 4,7-dichloroquinoline and piperazine. Disconnection of **15** is described above.

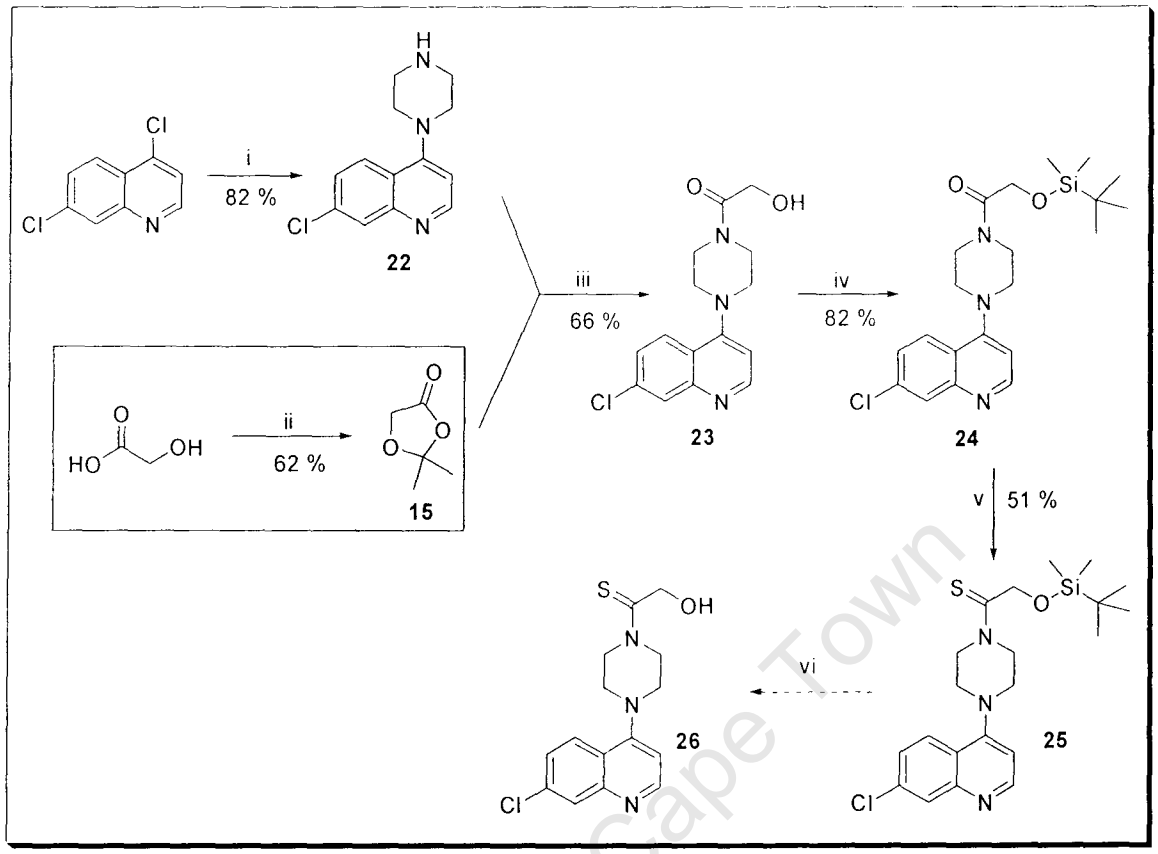


Scheme 3.7 Retrosynthetic analysis of the piperazinyl 2-hydroxyacetamide

3.3.2.2 Synthesis

The synthesis of the piperazinyl 2-hydroxyacetamide is outlined in Scheme 3.8 below. Once again, the acetonide, **15**, is a key intermediate.

The secondary amine, **22**, with which the acetonide was reacted to form the 2-hydroxyacetamide target, was synthesised from 4,7-dichloroquinoline and a 5 equivalent excess of piperazine. The excess was required to limit dimerisation, i.e. both piperazine amines reacting with 4,7-dichloroquinoline. Product **22** was obtained in 82 % yield, with side reactions such as dimerisation contributing to a reduction in product formation. Synthesis of the 2-hydroxyacetamide, **23**, was performed in a similar manner to that of **17** and **18**, by condensation with the acetonide **15** under anhydrous conditions. Product **23** was obtained in a moderate yield of 66 %. This lower yield is possibly due to steric hindrance around the secondary amine limiting reaction with the acetonide. A similar low yield (48 %) was obtained for the benzylated secondary amine **18**, which would experience a greater degree of steric repulsion around the amine as a result of the bulky benzyl group.



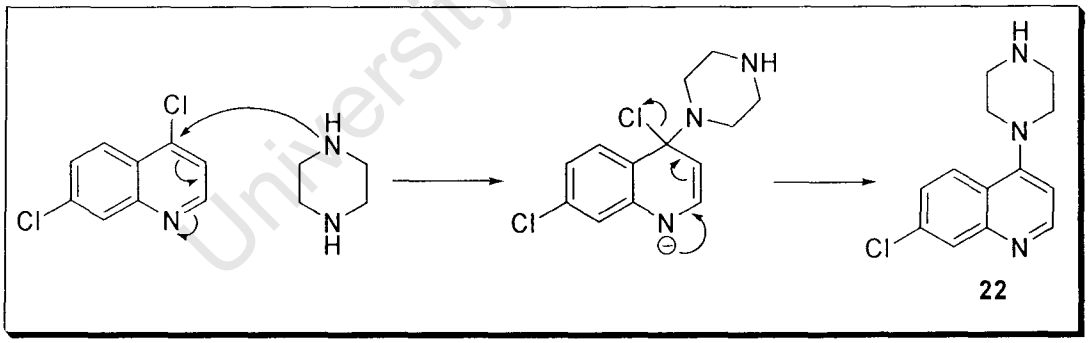
Scheme 3.8 *Reagents and conditions* i) Piperazine (5 eq.), K_2CO_3 (0.3 eq.), Et_3N (1.5 eq.), NMP, 135 °C, 2 h; ii) Acetone, H_2SO_4 conc., -10 °C, 1 h; iii) Dry CH_2Cl_2, 30 °C, 40 h; iv) TBDMSCl (2.2 eq.), imidazole (2.6 eq.), dry CH_2Cl_2, 30 °C, 192 h; v) Lawesson's reagent (0.6 eq.), dry THF, 40 °C, 72 h; vi) TBAF, dry THF;

Following the lack of success in obtaining the thio-derivative of the chloroquine analogue, an attempt was made to convert the piperazinyl 2-hydroxyacetamide (piperaquine analogue) to the 2-hydroxythioacetamide. The synthetic pathway (steps iv – vi) is indicated in Scheme 3.8 above. In order to prevent side reactions of the terminal hydroxyl with Lawesson's Reagent (L.R.), its protection as a silyl ether was required. This was accomplished by stirring a CH_2Cl_2 solution of **23**, TBDMSCl and imidazole at 30 °C for 8 days. An excess of approximately 2 equivalents of TBDMSCl and imidazole was required for the reaction to proceed to completion, possibly as a result of hydrolysis of the TBDMSCl by residual moisture in the solvent. The silyl ether, **24**, was obtained in good yield (82 %). Carbonyl to thiocarbonyl conversion (step v) was then accomplished with LR by stirring in dry THF at 40 °C for 3 days. The thioacetamide product of the thiation reaction, **25**, was

obtained in a moderate yield of 51 %. The final step to produce the 2-hydroxythioacetamide product, **26**, required removal of the silyl protecting group with tetra-*n*-butylammonium fluoride (TBAF). This step, however, proved unsuccessful, and resulted in decomposition of the silyl ether substrate. Attempts were made to modify the protocol (lowering the temperature; lowering the concentration of TBAF; using dilute KOH solution instead of TBAF), but these all proved unsuccessful. It is possible that the F^- delivered by the TBAF cleaved the amide C-N bond instead of the Si-O bond, however, confirmation of this possibility was not able to be established within the time frame of this project. This step, therefore, remains an item for future work.

3.3.2.3 Mechanistic Details

The mechanism for the formation of the 7-chloro-4-piperazinylquinoline, **22**, is similar to that by which the chloroquine amine, **7**, was formed. The mechanism involves an aromatic addition elimination step, as outlined in Scheme 3.9.

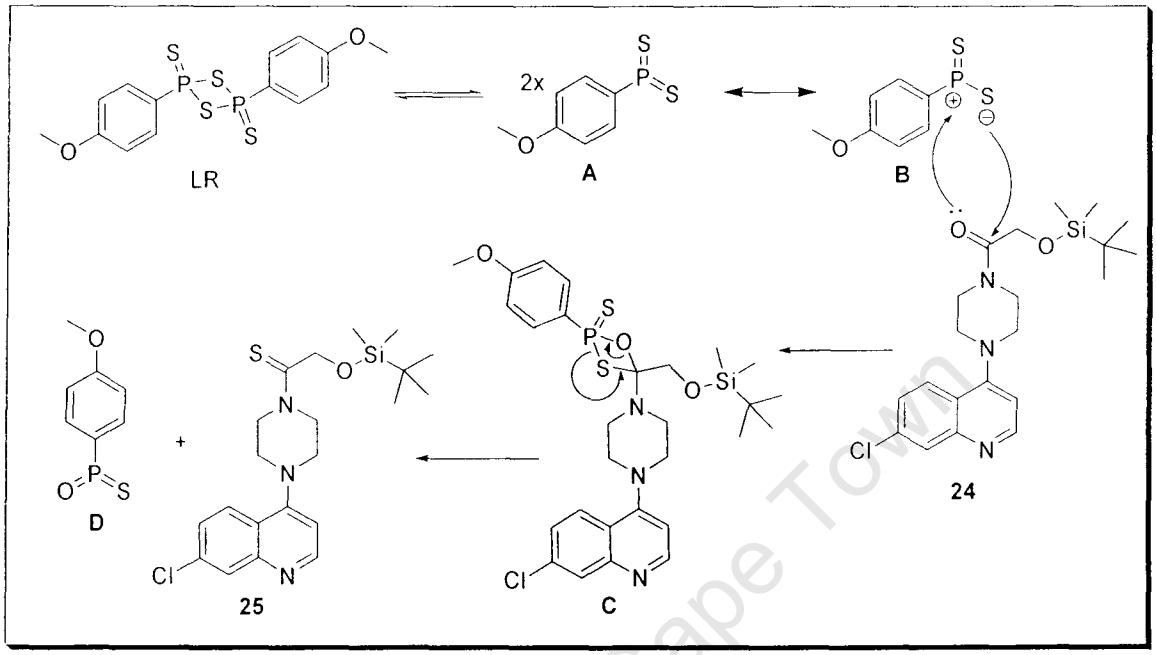


Scheme 3.9 Reaction mechanism for the formation of piperazinyl quinoline **22**

The reaction mechanisms for acetamide formation and silylation of the piperazinyl 2-hydroxyacetamide derivatives proceed in the same manner as for the ethyl derivatives, outlined in Scheme 3.5 and Scheme 3.6, respectively.

The mechanism of thionation of **24** is illustrated in Scheme 3.10. The first step involves opening of the Lawesson's Reagent phosphetane ring to form

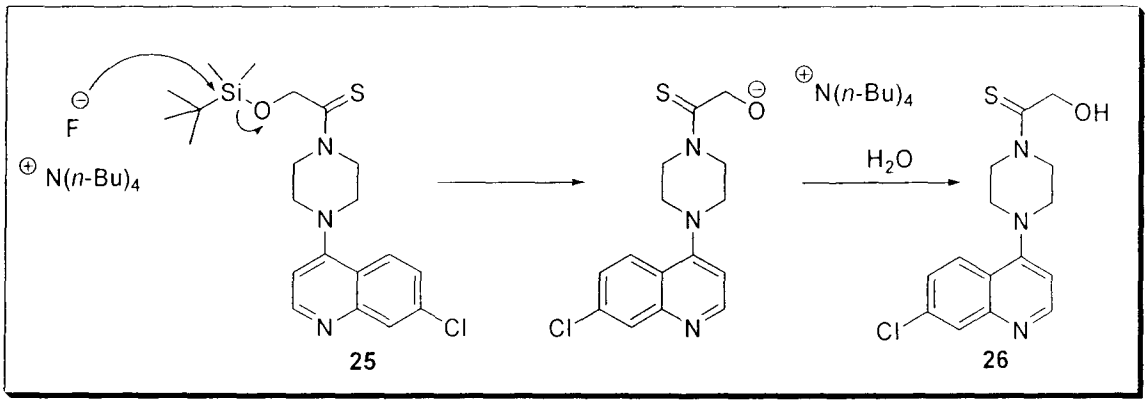
two equivalents of the reactive dithiophosphine ylide, which has two resonance forms (**A** and **B**, Scheme 3.10).



Scheme 3.10 Reaction mechanism of thionation of **24** with Lawesson's reagent (LR)

Nucleophilic attack of the carbonyl by the ylide results in the formation of a 4-membered ring (**C**) which breaks down in a Wittig-type concerted process to yield the thioacetamide **25** and the phosphine oxide side product.

The mechanism by which the thioacetamide is deprotected by TBAF is indicated in Scheme 3.11. The reaction involves a single S_N2 substitution of the oxygen on the silicon centre by an incoming fluoride ion delivered by the TBAF reagent. The released oxide, present in the reaction mixture as an ammonium salt, is protonated to the desired alcoholic product by aqueous work up.

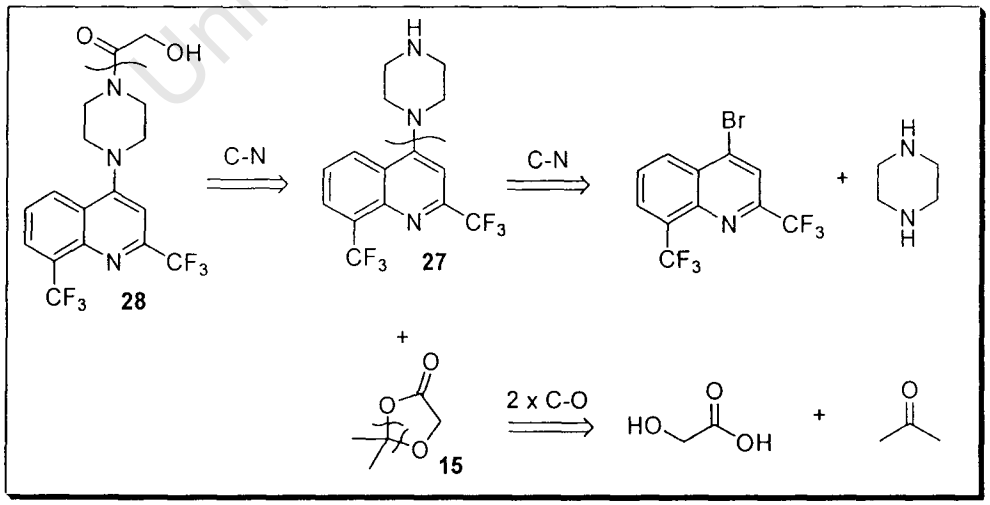


Scheme 3.11 Reaction mechanism for the deprotection of the silyl ether, **25**, to the 2-hydroxy thioacetamide, **26**

3.3.3 Mefloquine Analogue

3.3.3.1 Retrosynthetic Analysis

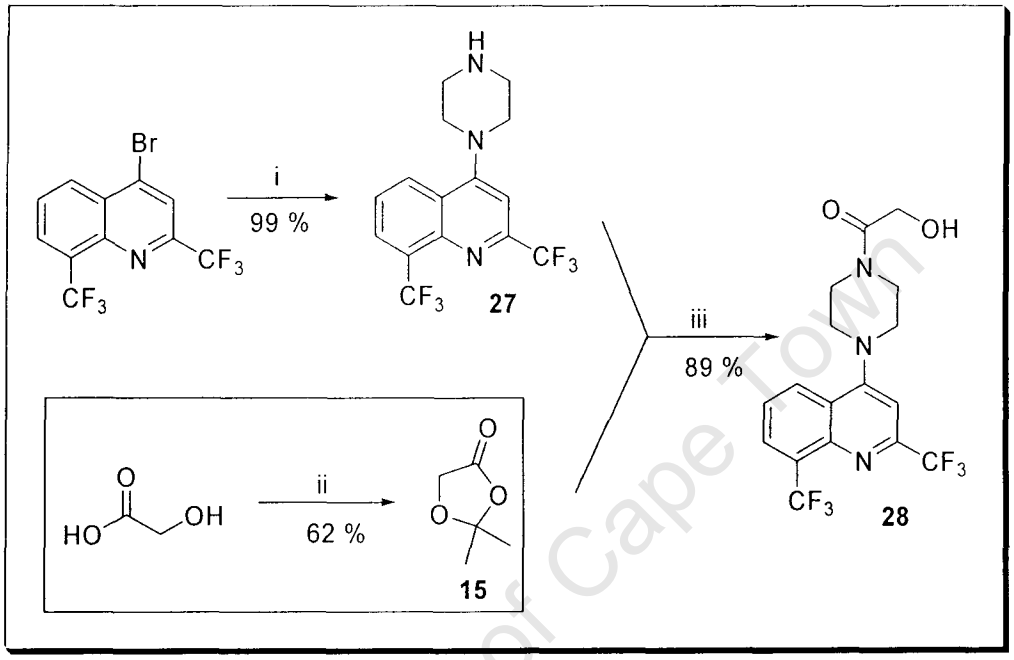
The retrosynthesis of the mefloquine analogue (Figure 3.2), **28**, is illustrated in Scheme 3.12 below. The first disconnection (C-N) gives the acetonide, **15**, and the piperazinyl 2,8-bis(trifluoromethyl)quinoline intermediate, **27**. C-N disconnection of **27** yields the starting materials 4-bromo-2,8-bis(trifluoromethyl)quinoline and piperazine.



Scheme 3.12 Retrosynthetic analysis of the mefloquine analogue, **28**

3.3.3.2 Synthesis

The synthesis of this 2-hydroxyacetamide is outlined in Scheme 3.13 below, and follows a similar pathway to that used in the synthesis of **23** (see Scheme 3.8).



Scheme 3.13 *Reagents and conditions* i) Piperazine (5 eq.), K_2CO_3 (0.3 eq.), Et_3N (1 eq.), NMP, 135 °C, 2 h; ii) Acetone, H_2SO_4 conc., -10 °C, 1 h; iii) Dry CH_2Cl_2, 30 °C, 40 h;

Synthesis of the piperazinyl intermediate **27** was accomplished in excellent yield (99 %) under the same reaction conditions as for **22**, i.e. with 5 equivalents of piperazine, base (Et_3N, K_2CO_3), NMP as solvent, and heating at 135 °C for 2 hours. The subsequent amide condensation was carried out under nitrogen in dry CH_2Cl_2 at 40 °C to afford the 2-hydroxyacetamide product, **28**, in a yield of 89 %.

3.3.3.3 Mechanistic Details

The reaction mechanism leading to the formation of the piperazinyl quinoline intermediate, **27**, follows an addition elimination pathway, similar to that outlined in Scheme 3.9. The only differences between the mechanisms of **22** and **27** lie in the identity and position of the quinoline 'spectator' substituents, and in the acid that is produced by the elimination of the halide at the 4-

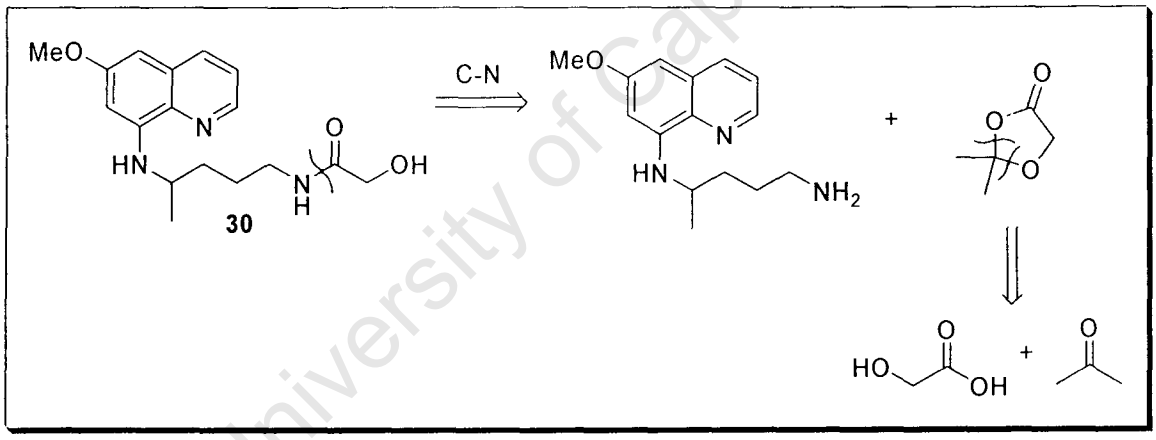
position. Formation of **27** leads to the production of HBr, whereas **22** produces HCl.

The mechanism of acetamide formation is outlined above in Scheme 3.5.

3.3.4 Primaquine Analogue

3.3.4.1 Retrosynthetic Analysis

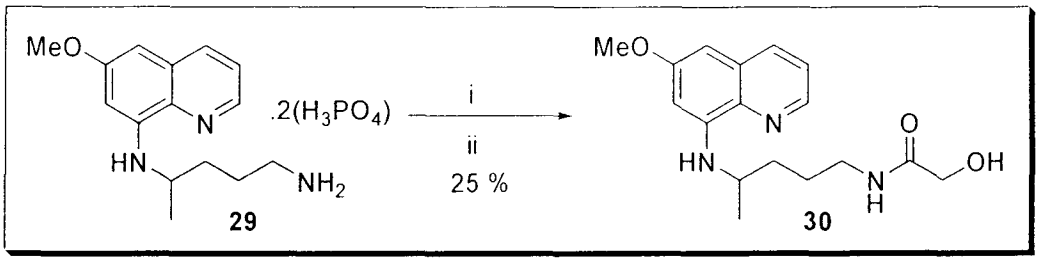
The retrosynthetic analysis of this 2-hydroxyacetamide is given below in Scheme 3.14 and comprises a C-N disconnection which gives primaquine and the glycolic acid acetonide, **15**. The acetonide is disconnected to glycolic acid and acetone as seen before.



Scheme 3.14 Retrosynthetic analysis of the primaquine analogue, **30**

3.3.4.2 Synthesis

The coupling of primaquine to the acetonide, **15**, was preceded by neutralisation of primaquine diphosphate. This was performed by stirring a CH_2Cl_2 solution of the salt with 6 equivalents of Et_3N for 30 minutes at room temperature. The acetonide was then added to the solution *in situ* and the reaction allowed to proceed. The product was obtained in a low yield of 25 %, possibly as a result of incomplete neutralisation. The details of the synthesis are outlined in Scheme 3.15 below.



Scheme 3.15 *Reagents and conditions* i) Et_3N (6 eq.), CH_2Cl_2, r.t., 30 min; ii) Acetonide **15** (1.7 eq.);

3.3.4.3 Mechanistic Details

The mechanism of the amide condensation reaction between the primaquine free-base and the acetonide is the same as that illustrated in Scheme 3.5 (Section 3.3.1.4), above.

3.4 Characterisation of Target Compounds

Characterisation of all compounds was done by means of ^1H and ^{13}C NMR, infra-red spectroscopy, mass spectrometry, melting point and elemental analysis.

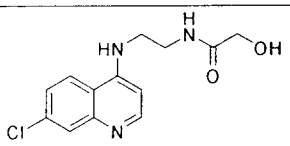
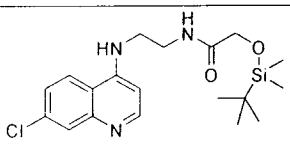
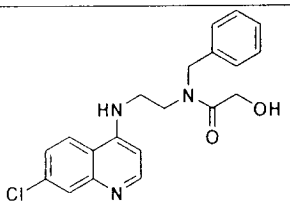
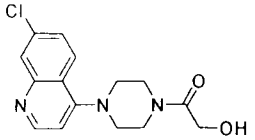
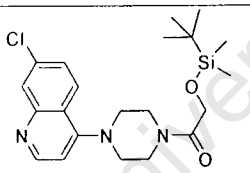
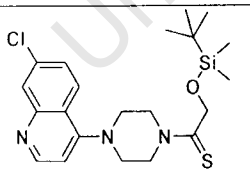
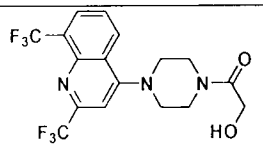
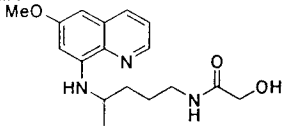
The characteristic signal in the ^1H NMR spectra of the 2-hydroxyacetamides was the methylene singlet, integrating for two protons, at approximately δ 4 ppm (see Table 3.1 below). Splitting of the singlet into a doublet by coupling to the hydroxyl proton was only occasionally seen (only for compounds **23** and **28**), and only when the NMR solvent was $CDCl_3$.

The assignment of the quinoline protons was carried out in a similar manner, as described in Section 2.3.4, Chapter 2. In general, the proton at the H8' position gave a signal that was further downfield than that of the H5' position, for the compounds in this chapter. The rest of the quinoline protons gave signals that followed the pattern (chemical shift, coupling constant, relative position) as described in Chapter 2.

The presence of the acetamide carbonyl functionality was confirmed by ^{13}C NMR, with C=O signals ranging between 169.46-174.51 ppm. Infra-red

spectroscopy was used to confirm this, and C=O stretching frequencies were found in the range 1641-1663 cm^{-1}. These characteristic values are given in Table 3.1 below:

Table 3.1 Characterisation data of carbonyl and methylene signals used in the analysis of the target 2-hydroxyacetamides, with their yields and melting points

Code	Chemical Structure	^{1}H NMR: δ Methylene Signal (ppm)	^{13}C NMR: δ C=O signal	I.R.: C=O Stretching Frequency /cm^{-1}	Yield (%)	M.p. (°C)
17		3.81	173.31	1641	98	213-215
19		4.15	174.33	1649	91	193-196
18		4.42	174.51	1655	47	181-183
23		4.23	170.43	1660	66	151-152
24		4.25	169.46	1654	82	oil
25		4.70	200.09 (C=S)	986 (C=S)	51	oil
28		4.26	170.56	1663	89	173-175
30		3.91	171.50	1646	25	127-130

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CHAPTER 4

GOLD(I) COMPLEXES

4.1 Introduction

This chapter describes the background, synthesis and characterisation of two gold(I) complexes of the synthesised organic ligands in Chapter 2 and Chapter 3. One ligand was selected from each class to provide a representative complex. Two different gold precursors were used in the syntheses, due to the different properties of the ligands. The acylthiourea ligand **11** was complexed using the gold salt, Au(THT)Cl,[1] because it was postulated that facile gold binding would occur through the thiourea sulfur atom due to the high affinity of gold(I) for sulfur. The 2-hydroxyacetamide ligand **23**, however, possessed no such sulfur moiety, and consequently binding was postulated to occur through the quinoline nitrogen, in a similar manner to several published examples in literature.[1,2,3,4] The gold precursor employed in this case was $Au(PPh_3)Cl$. The proposed gold(I) complexes are illustrated in Figure 4.1 below.

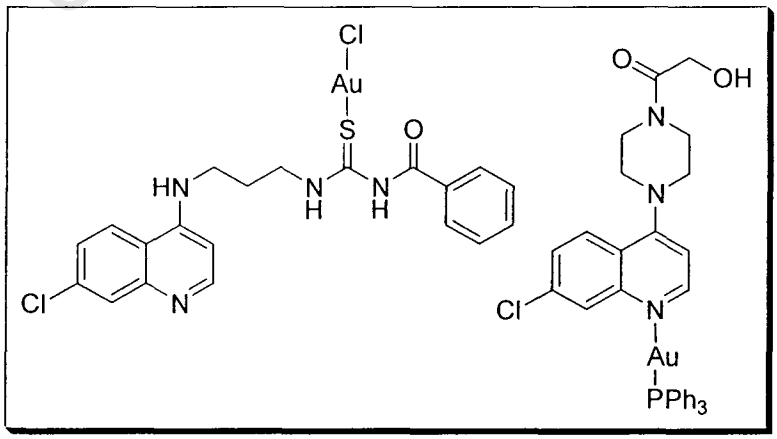


Figure 4.1 Chemical structures of the proposed gold(I) complexes

[1] THT = tetrahydrothiophene

4.2 Rationale Behind Using Gold(I) Complexes in Antimalarial Chemotherapy

Despite the initially reported lack of activity imparted to antimalarial compounds by gold complexation, as described by Singh and Wasi (1987),[5] subsequent studies have shown that gold complexation of chloroquine and substituted 4-aminoquinolines, in fact has a significant enhancing effect on both the *in vitro* and *in vivo* activities of these compounds. [1,2,3,4] In an attempt to expand these findings to include the quinoline-derived acylthioureas and 2-hydroxyacetamides described in this project, gold complexes of each class were prepared.

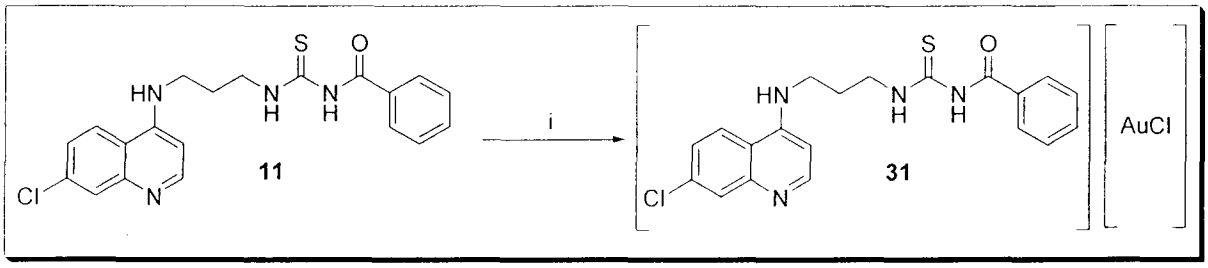
4.3 Synthesis and Characterisation of the Gold(I) Complexes

4.3.1 Acylthiourea Gold(I) Complex

The gold precursor selected for this complexation reaction was Au(THT)Cl. The selection was based upon the presence of the thiourea functionality in the ligand, which was predicted to eliminate THT from the precursor and result in *S*-coordination. The quinoline nitrogen remained a possible alternate site of coordination.

4.3.1.1 Synthesis

The reaction between the acylthiourea ligand **11** and Au(THT)Cl was performed under a nitrogen atmosphere in anhydrous tetrahydrofuran (THF) (standard THF caused a drop in the yield). The product **31** precipitated out of solution, and after washing with THF was dried and analysed. The synthesis is outlined below in Scheme 4.1.



Scheme 4.1 *Reagents and conditions*: i) Au(THT)Cl, anhydrous THF, 0 °C – r.t., 3 h

4.3.1.2 Characterisation

The synthesised complex **31** was characterised by spectroscopy (^{1}H and ^{13}C NMR, infra-red), elemental analysis, and melting point.

In order to establish the site of gold coordination, the NH proton signals neighbouring the thiourea had to be assigned in the ^{1}H NMR spectrum. These are the closest protons to the sulfur through which gold binding was postulated to occur, hence significant changes in the chemical shifts (downfield movement, specifically) of these protons would suggest *S*-coordination. The proton signal of the NH at the 4-position of the quinoline ring was also assigned, in order to establish whether *N*-coordination occurred. This proton would also be expected to shift downfield as *N*-coordination would draw electron density away from the amine nitrogen, according to the mesomeric effect as illustrated below in Figure 4.2.

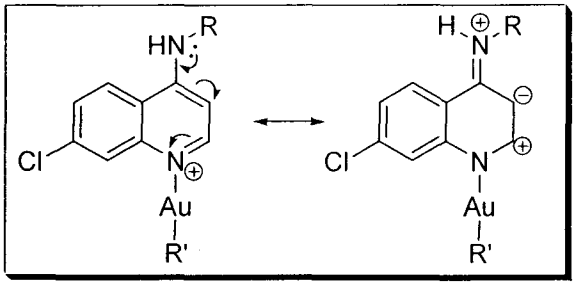


Figure 4.2 Two resonance forms indicating the mesomeric effect, induced by quinoline *N*-coordination, on the NH at the 4-postion of the quinoline ring

The NH proton signal at the quinoline 4-position was assigned from the ^{1}H NMR spectrum of CQ amine **7** at ~7.2 ppm. This signal is centred at ~7.4 ppm in the spectrum of the acylthiourea **11**. The two NH signals at 10.9 and 11.2 ppm were therefore assigned as the thiourea NH signals, with the broad singlet at 11.2 ppm assigned to NH6 and the faintly split multiplet at 10.9 assigned to NH5. The ^{1}H NMR spectra of **11** and the corresponding chlorogold(I) complex **31** are provided in Figure 4.3, with characteristic NH signals indicated.

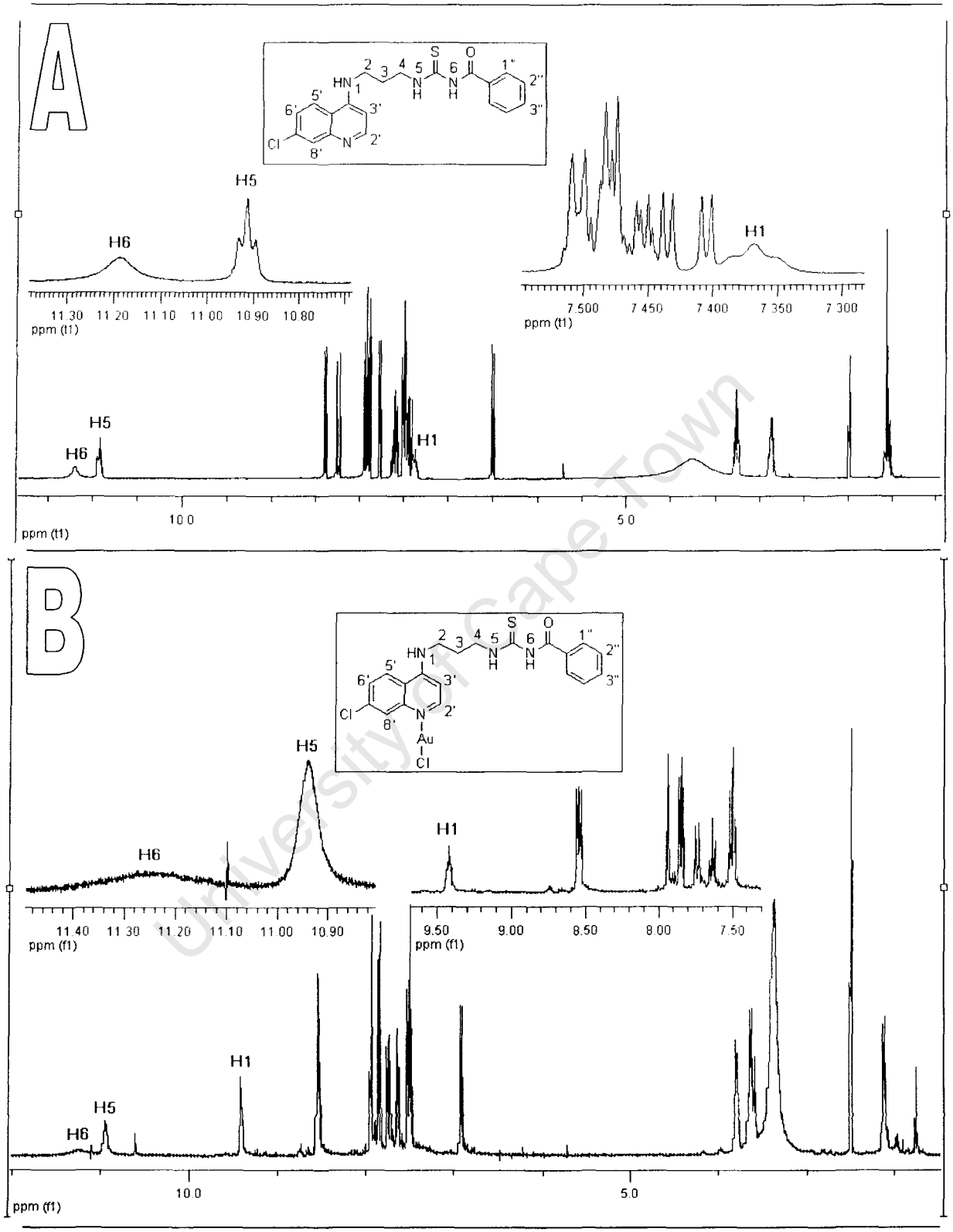


Figure 4.3 ^{1}H NMR spectra of **11** (A) and the corresponding gold complex **31** (B)

The ^{1}H NMR spectrum of **31** clearly indicates the unchanged chemical shift of the thiourea NH protons. However, the signal for the NH proton at the quinoline 4-position shifted dramatically to 9.4 ppm (a 2.0 ppm difference!). The aromatic proton shifts of **11** and **31** are given in Table 4.1 below. The differences in the chemical shifts of the quinoline protons are clearly greater than those of the benzene ring protons, which would be relatively unaffected by *N*-coordination to the quinoline ring.

Table 4.1 Comparison between the ^{1}H NMR chemical shifts of the aromatic and NH protons of ligand **11** and corresponding gold complex **31**

	NH1	NH5	NH6	H2'	H3'	H5'	H6'	H8'	H1''	H2''	H3''
11	7.37	10.92	11.18	8.39	6.50	8.24	7.42	7.77	7.91	7.59	7.48
31	9.37	10.94	11.25	8.55	6.92	8.52	7.74	7.91	7.85	7.50	7.63
Difference	-2.00	-0.02	-0.07	-0.16	-0.42	-0.28	-0.32	-0.14	+0.06	+0.09	-0.15

Based on the ^{1}H NMR evidence, coordination of the gold appears to be through the quinoline nitrogen, and NOT through the thiourea sulfur, as initially predicted. The revised gold complex product structure is illustrated in Figure 4.4 below.

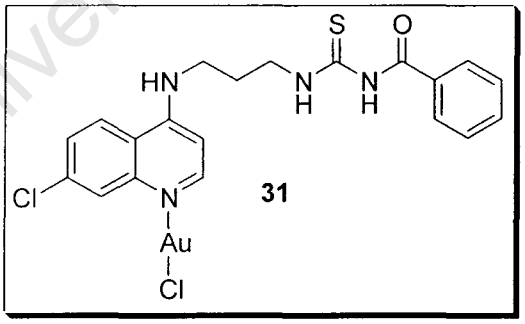


Figure 4.4 Chemical structure of gold(I) complex, **31**

Confirmation of this result was evidenced in the ^{13}C NMR spectrum, where contrary to the predicted downfield shift of the thiocarbonyl carbon, the signal remains relatively unchanged at 179.59 ppm (C=S signal of free ligand **11** = 182.23 ppm).

The infra-red spectrum of **31** indicated relatively little shift in the carbonyl stretching frequency with a value of 1688 cm^{-1} (C=O stretching frequency of **11** = 1676 cm^{-1}), adding further evidence for *N*-coordination.

Although spectroscopic data suggest the site of coordination to be through the quinoline nitrogen, and indicate the presence of the ligand in the final compound, further evidence to confirm the complete structure of **31** could not be obtained. Elemental analysis produced values that exceeded the bounds of experimental error for any of the predicted molecular structures. The differing melting points (**11** = 83 - 85 °C; **31** = 185 °C) and precipitation of the product from the solution of THF (**11** is highly soluble in THF) did indicate the formation of a new compound, however, whether the product was obtained as a dimer (**11**-Au-Au-**11**; or **11**-Au-**11**), or a monomer with a chloro spectator ligand, could not be established. Mass spectrometry data would be helpful in supporting structural assignment; however, indisputable assignment of coordination site as well as complex structure can only be obtained by crystal structure analysis. The difficulty in obtaining such data, however, lies in the poor solubility of **31** in all laboratory organic solvents used, except DMSO, in which the complex decomposes within a few hours. Consequently, crystals could not be grown within the timeframe of this project. Complete analysis and characterisation (including mass spectrometry) remains an item for future work.

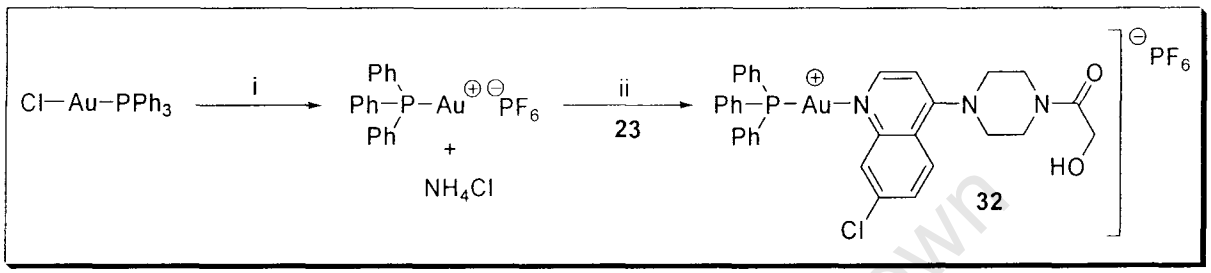
4.3.2 2-Hydroxyacetamide Complex

The gold precursor selected for this complexation reaction was $Au(PPh_3)Cl$. The selection was based upon the similarity of the ligand **23** to reported quinoline-containing gold(I)-complexed ligands. The protocol employed for the reaction was that reported by Sánchez-Delgado and co-workers (1996).[2]

4.3.2.1 Synthesis

In order to increase the electrophilicity of the gold(I) centre, and facilitate *N*-coordination of the quinoline nitrogen, NH_4PF_6 was added to the solution of

$Au(PPh_3)Cl$ prior to addition of the ligand. The resulting NH_4Cl salt precipitated from solution leaving the more reactive $(PPh_3)Au^+PF_6^-$ intermediate. Addition of **23** to the solution, followed by refluxing for 48h, resulted in the formation of the gold complex. The details of the synthesis, and the predicted structure of the complex, are given in Scheme 4.2 below.



Scheme 4.2 *Reagents and conditions*: i) Anhydrous CH_3CN, NH_4PF_6 (2eq.), reflux, 30 min; ii) **23** (2 eq.), reflux, 48 h;

The product was purified by filtration (to remove the NH_4Cl) and washing with THF (to remove unreacted starting material), to yield the product as a yellow powder (characteristic of gold complexes).[4]

4.3.2.2 Characterisation

The synthesised complex **32** was characterised by spectroscopy (^1H and ^{13}C NMR, infra-red), elemental analysis, and melting point.

Confirmation of the production of a novel compound, which contained the complete ligand **23**, was provided by melting point and NMR spectroscopy, respectively. The poor solubility of the product in all laboratory solvents used except for the polar solvents, CH_3CN and DMSO further indicated the formation of a novel gold complex.

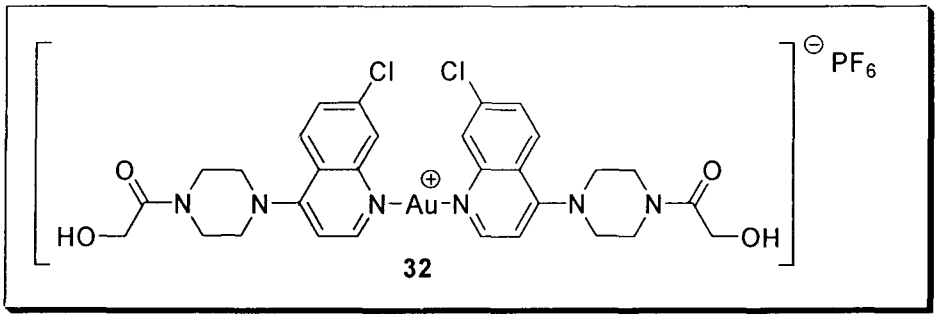
Evidence from the ^1H NMR spectrum indicated *N*-coordination of the gold(I) centre to the quinoline nitrogen. The chemical shifts of the methylene protons alpha to the amide had the same value in both the spectrum of the ligand **23** and complex **32** (4.15 ppm), thus negating the likelihood of *O*-coordination

through either the carbonyl or hydroxyl of the glycolamide group [formation of a 5-membered chelate complex is unlikely due to the preference of gold(I) for linear coordination]. The quinoline protons, however, indicated significant differences in their chemical shifts. These are given in Table 4.2 below.

Table 4.2 Comparison between the ^{1}H NMR chemical shifts of the quinoline and α-methylene protons of ligand **23** and corresponding gold complex **32**

	H2'	H3'	H5'	H6'	H8'	α-CH_2
23	8.70	7.00	8.08	7.54	7.97	4.15
32	8.65	7.16	8.23	7.68	7.98	4.15
Difference	-0.05	-0.16	-0.15	-0.14	-0.01	0

Further analysis of the ^{1}H NMR spectrum revealed the surprising absence of any triphenylphosphine signals, suggesting elimination of the triphenylphosphine ligand by the quinoline functionality. This was unexpected since gold(I) has a greater affinity for phosphorus than for nitrogen. The absence of the triphenylphosphine was confirmed by ^{31}P NMR spectroscopic analysis which revealed a single signal at -143 ppm (a heptet, corresponding to the PF_6 counter-ion). Since Au(I) forms two-coordinate complexes, both positions would have to be occupied, suggesting the formation of a dimer complex, i.e. **[23**-Au-**23]**PF_6. It is likely that the high temperature of the reaction (82 °C), and the long reaction time (48 h), resulted in dissociation of the triphenylphosphine from the gold centre, and allowed two quinoline ligands to coordinate. This would have been aided by the 2 equivalents excess of ligand to gold precursor.



32

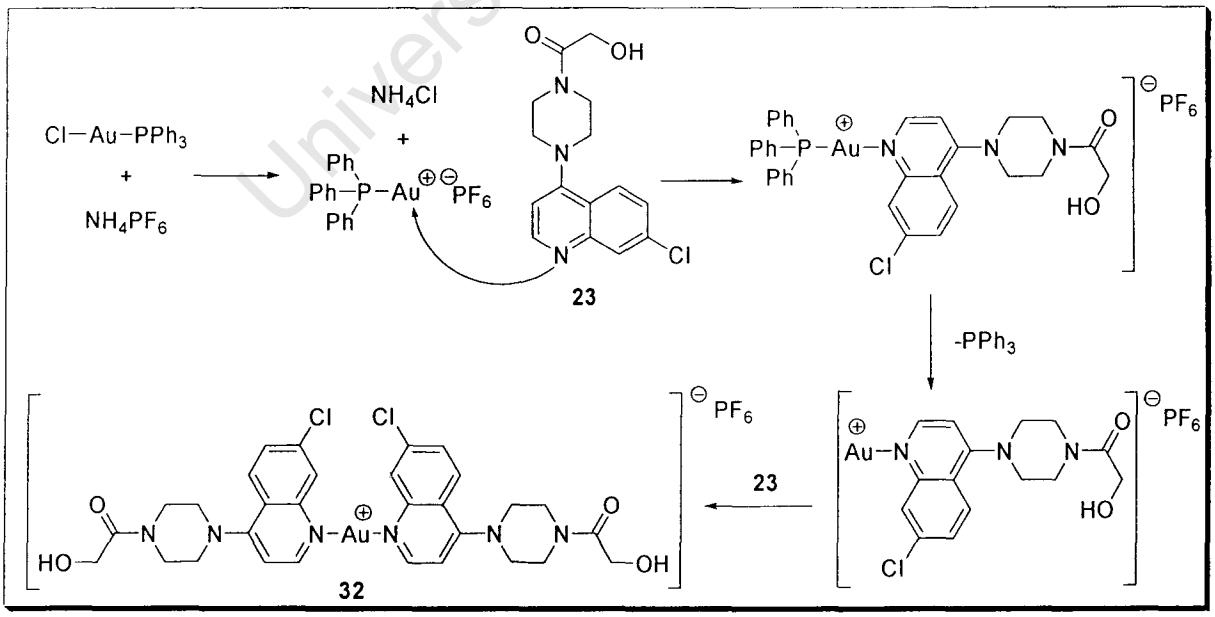
Figure 4.5 Proposed chemical structure of the 2-hydroxyacetamide gold complex

This proposed dimer structure, as illustrated in Figure 4.5, is supported by the elemental analysis data, which gives percentage C, H and N values close to the calculated values (Found: C, 40.06; H, 4.30; N, 8.95. $C_{30}H_{32}Au^{35}Cl_2F_6N_6O_4P.(Et_2O)$ requires C, 39.74; H, 4.12; N, 8.18 %). Analytical data from mass spectrometry, although not obtained within the timeframe of the project, would help to confirm this proposed structure, and remains an item for future work.

Once again, it should be mentioned that true confirmation of complex structure can only be obtained unambiguously from a crystal structure. The limited solubility of the complex in most laboratory solvents used, however, precluded crystallisation and prevented such an analysis from being undertaken during the course of this project.

4.3.2.3 Mechanistic Details

The mechanism leading to the formation of **32** is outlined in Scheme 4.3 below.



Scheme 4.3 Reaction mechanism for the formation of the 2-hydroxyacetamide gold complex **32**

Coordination of the first equivalence of **23** to the gold(I) precursor occurs in an S_N1-type mechanism, after the chloride ligand has been removed by the NH_4^+. Elimination of the PPh_3, according to the proposed mechanism, may either occur before or after this coordination. Departure of the PPh_3 is followed by coordination of the second equivalence of **23** to form the final complex **32**.

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CHAPTER 5

BIOLOGICAL RESULTS AND DISCUSSION

5.1 Introduction

The following chapter describes the results of the *in vitro* biological evaluation of the synthesised compounds against strains of *P. falciparum*. Assays were carried out against both chloroquine-sensitive (CQS) and chloroquine-resistant (CQR) strains, in order to establish whether the synthesised compounds were able to overcome CQ resistance. The results are reported as IC_{50} values, that is, the inhibitory concentration of the compound necessary to produce a therapeutic effect in 50 % of the test sample. The lower the IC_{50} value of a compound, the greater is its efficacy. The descriptions of the assays and experimental details used in biological evaluation of the target compounds are given in the experimental section.

The antimalarial activities of the synthesised target compounds were determined in three different *in vitro* assays in the laboratories of Associate Professor P.J. Smith (University of Cape Town, Medical School) (D10 and Dd2 strains) and Professor P.J. Rosenthal (University of California at San Francisco, USA) (W2 strain), in all cases using chloroquine as the control drug.

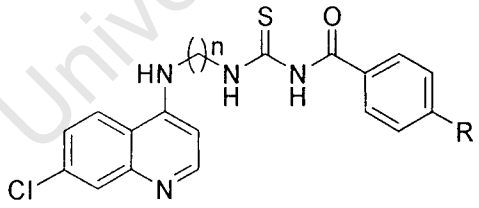
5.2 *In Vitro* Antimalarial activity of the 4-Aminoquinoline-based Acylthioureas

5.2.1 Results and Discussion

The antimalarial activities of the 4-aminoquinoline-based acylthioureas **10** - **14** as determined in the three different *in vitro* assays are presented in Table 5.1.

Table 5.1 *In vitro* antimalarial activity of 4-aminoquinoline acylthioureas

Compound Code	D10		Dd2		W2	
	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)
10	0.054	0.140	ND	-	-	0.502
11	0.034	0.085	0.091	0.228	-	1.105
12	0.024	0.058	ND	-	-	1.492
13	0.026	0.063	ND	-	-	0.257
14	0.110	0.256	NTF	-	-	1.274
CQ	0.011	0.034	0.078	0.244	-	0.091



10: n = 2; R = H
11: n = 3; R = H
12: n = 4; R = H
13: n = 2; R = OMe
14: n = 3; R = OMe

ND = Not Determined; NTF = Not Tested Further

Against the CQS strain, D10, compounds **10** – **14** displayed good to moderate activity, with **11** (0.085 µM), **12** (0.058 µM) and **13** (0.063 µM) showing a high activity that is comparable to chloroquine (0.034 µM). Compound **10** (0.140 µM) was found to be four times less active than CQ, and **14** (0.256 µM) had the lowest activity of the tested compounds (eight times less active than CQ) and was not tested further against the CQR strain. These preliminary results suggest that increasing the alkyl chain length (from n = 2 to n = 4) causes an

increase in activity against this strain, except where the acyl benzene ring contains a para-methoxy substituent, in which case it causes a marked decrease in activity.

When tested against the CQR strain, Dd2, three of the compounds (**10**, **12**, **13**) gave results that were too varied for an IC_{50} value to be determined. The only successfully-tested compound, **11**, showed moderate activity (0.228 μM) against the strain.

Against the CQR W2 strain, none of the compounds exhibited any significant antimalarial activity, relative to CQ. The most active compounds were **10** (0.502 μM) and **13** (0.257 μM), and the least active was **12**. This may suggest that an alkyl chain length of n = 2 is important for activity against this strain, since both active compounds contain that functionality. The data also appear to indicate that increasing the chain length causes a sequential decrease in the antimalarial activity as n increases. This is in direct contrast to the results against the D10 strain. The importance of the *p*-methoxy substituent on the benzene ring appears to be minor, since compound **13** exhibited the second-poorest activity of the group, whereas **12** was the most active. Notwithstanding the aforementioned, the number of compounds is not large enough to allow any meaningful structure-activity relationship to be delineated.

Quantification of the degree of cross resistance between two parasite strains is given in the form of a resistance index: a ratio of the IC_{50} value against the resistant strain to that against the sensitive strain. These indices are given in Table 5.2.

Table 5.2 Resistance indices of 4-aminoquinoline acylthioureas

Compound	Resistance Index (Dd2)	Resistance Index (W2)
10	-	3.59
11	2.68	13.00
12	-	25.72
13	-	4.08
14	-	4.98
CQ	7.18	2.68

The resistance index against the Dd2 strain of compound **11** is significantly lower than the corresponding index for chloroquine, possibly indicating that the parasite resistance pathway in this strain does not recognise the compound as effectively as it does CQ. However, despite this lower index and the superior activity to CQ against Dd2, the compound's activity fell almost three-fold from the D10 results.

The resistance indices of **10** – **14** against the W2 strain are all greater than that of CQ. This indicates significantly high levels of cross-resistance, which would require further modifications to the molecular structures to be overcome.

5.2.2 Conclusion

Conclusions that can be drawn from these results about the structural requirements for activity are minimal, since no trends are evident throughout the three assays and the number of compounds evaluated is too small. Cross-resistance is evident with these molecules, and further derivatisations will be required before CQ resistance is overcome.

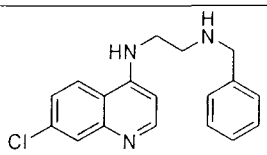
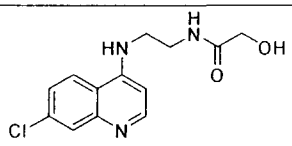
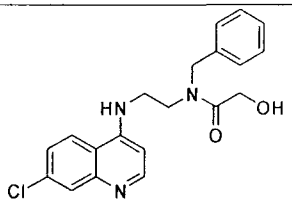
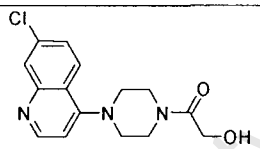
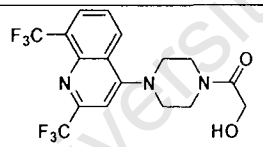
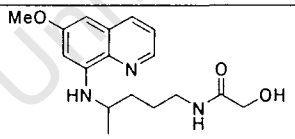
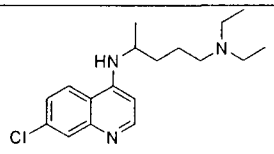
5.3 *In Vitro* Antimalarial Activity of the Aminoquinoline-derived 2-Hydroxyacetamides

5.3.1 Results and Discussion

The antimalarial activities of the aminoquinoline-based 2-hydroxyacetamides **17**, **18**, **23**, **28** and **30**, as well as the intermediate **16**, as determined in the three different *in vitro* assays are presented in Table 5.3.

The results of the assay against the CQS D10 strain indicated than none of the five target molecules were significantly active relative to CQ. The most active 2-hydroxyacetamide, **18**, had an IC_{50} value of 0.946 μM, which when compared to the IC_{50} value of CQ (IC_{50} = 0.034 μM), was 28 times less active. Consequently, none of the 2-hydroxyacetamides were considered for testing against the CQR strain Dd2. Biological evaluation against the D10 strain was also done on all the synthetic intermediates, with poor activity being exhibited by most, except for **16** which displayed exceptional activity (IC_{50} = 0.013 μM), approximately 3 times more active than CQ against this CQS strain. The structure of **16**, as presented in Table 5.3, contains a benzyl-substituted terminal secondary amine attached to the chloroquinoline amine substructure, present in many of the synthesised compounds. It appears that this benzyl-substituted amine functionality is an important determinant of activity, since the benzylated tertiary amide, **18**, possesses the greatest activity of the synthesised 2-hydroxyacetamide target molecules. Removal of the benzyl group causes a significant drop in activity, as seen by the 100 % decrease in activity from **18** to **17** (0.946 μM to 2.038 μM). The presence of the 2-hydroxyacetamide functionality appears to not impart any antimalarial activity, but rather to greatly reduce the activity of a compound, as seen by the reduction in activity when **16** is converted to **18** (0.013 μM to 0.946 μM). This is confirmed by the very poor activity of the primaquine-derived 2-hydroxyacetamide **30**. Consequently, the only compound in this class selected for further testing against the CQR Dd2 strain was the active intermediate, **16**.

Table 5.3 *In vitro* antimalarial activity of the aminoquinoline-derived 2-hydroxyacetamides

Compound	Chemical Structure	D10		Dd2		W2	
		IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)	IC_{50} (µg/ml)	IC_{50} (µM)
16		0.004	0.013	0.020	0.064	-	0.016
17		0.57	2.038	-	-	-	5.518
18		0.35	0.946	-	-	-	0.462
23		3.90	12.755	-	-	-	>20
28		7.36	18.070	-	-	-	>20
30		9.40	29.617	-	-	-	>20
CQ		0.011	0.034	0.078	0.244	-	0.091

Against the Dd2 strain **16** once again displayed good activity with an IC_{50} value of 0.064 µM (approximately four times more active than CQ), however, this value was five times higher than the activity against the CQS strain (resistance index = 4.92, see Table 5.4), indicating a susceptibility to cross-resistance with CQ against this strain.

The activities of the 2-hydroxyacetamides against the CQR W2 strain confirmed the results obtained against the D10 strain, with poor activities exhibited by most compounds. The most active compound, once again, was the benzylated intermediate **16** with an activity of 0.016 μM [600 % more active than CQ (IC_{50} = 0.091 μM)]. The most active 2-hydroxyacetamide was the benzylated tertiary acetamide **18** with an IC_{50} = 0.462 μM.

The resistance indices of the compounds in this class are presented in Table 5.4 below. The resistance index of the active intermediate **16** in the W2 strain is 1.23, which is significantly lower than its resistance index in the Dd2 strain (4.92). This result is in line with the values obtained for the CQ control, which indicate a greater degree of resistance in the Dd2 strain than that obtained with the W2 parasites. Compound **17** has a resistance index of 2.71 in the W2 strain, which is equivalent to the CQ value (2.68), indicating the existence of cross-resistance. An important finding of this study was the resistance index of **18** = 0.49, which indicates that the compound has a greater activity against the CQR strain than the CQS equivalent, suggesting it is able to overcome CQ resistance. There is therefore potential for further development of **18** as an antimalarial agent effective against CQ-resistant parasites.

Table 5.4 Resistance indices of the aminoquinoline-derived 2-hydroxyacetamides

Compound	Resistance Index (Dd2)	Resistance Index (W2)
16	4.92	1.23
17	-	2.71
18	-	0.49
23	-	ND
28	-	ND
30	-	ND
CQ	7.18	2.68

ND = Not Determined

5.3.2 Conclusion

In general, the 2-hydroxyacetamide compounds synthesised displayed poor activity against *P. falciparum* parasites, however, two compounds showed good promise for potential further development. The intermediate **16** exhibited high activity against both the CQS (IC_{50} = 0.013 μM) and CQR (Dd2: IC_{50} = 0.064 μM; W2: IC_{50} = 0.016 μM) strains tested. Despite this high activity, the results indicated the presence of cross-resistance with CQ (resistance indices: Dd2 - 4.92; W2 - 1.23), which would need to be addressed before the compound could be progressed for further development as an antimalarial drug. The benzyl-substituted tertiary acetamide **18** was the most active of the 2-hydroxyacetamide targets synthesised, and displayed a greater activity against the CQR strain W2 (IC_{50} = 0.462 μM) than the sensitive D10 strain (IC_{50} = 0.946 μM). The possibility that this compound is able to overcome CQ resistance mechanisms in the parasite makes it a potential candidate for future drug development.

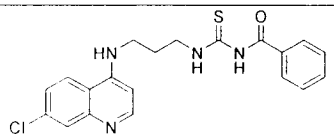
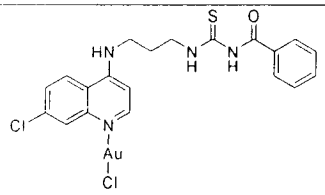
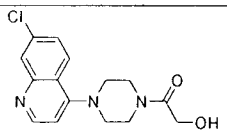
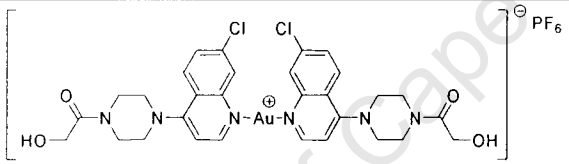
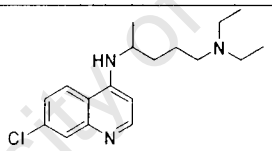
5.4 *In Vitro* Antimalarial Activity of the Gold(I) Complexes

Biological evaluation of the two synthesised gold complexes was performed exclusively against the CQS D10 strain. Our collaborators involved in the *in vitro* assays experienced difficulties with CQR parasite strains producing variable results. Consequently, results against the Dd2 and W2 strains were not available for these compounds.

5.4.1 Results and Discussion

The antimalarial activities of the synthesised gold(I) complexes, and their free ligands, as determined in D10 *in vitro* assays are presented in Table 5.5.

Table 5.5 *In vitro* antimalarial activities of the gold(I) complexes and their ligands

Compound	Chemical Structure	D10	
		IC_{50} (µg/ml)	IC_{50} (µM)
11		0.034	0.085
31		>10	>16
23		3.90	12.755
32		0.282	0.296
CQ		0.011	0.034

The *in vitro* antiplasmodial activity of the chlorogold complex **31** was found to be low, and beyond the range of analysis (IC_{50} >16 µM). Comparison of the IC_{50} value of the complex to the free ligand (IC_{50} = 0.085 µM) indicates a resultant decrease in antimalarial activity as a result of gold complex formation. The low activity obtained for the complex, however, may largely be as a result of poor solubility in the cell culture medium.

In contrast, complex **32** (IC_{50} = 0.296 µM) displayed a marked increase in activity over its free ligand **23** (IC_{50} = 12.755 µM), with a 43-fold increase in antiplasmodial activity. This suggests great potential for improving the activity of many of the other 2-hydroxyacetamide ligands, particularly the active compounds **16** and **18**.

5.4.2 Conclusion

These preliminary results may illustrate the impact of gold complex structure on the antimalarial activity. It would appear that the spectator ligand (e.g. Cl^-; PPh_3) on the gold metal centre plays a key role in the solubility as well as the activity of the resultant complex. The presence or absence of a counter ion (e.g. $^-PF_6$) may also contribute to the activity. These results provide an initial indication of the effect of complex formation on the antimalarial activity of ligands, however, complexation of the remaining acylthioureas and 2-hydroxyacetamides with both precursors would be required before a conclusive understanding could be established.

CHAPTER 6

CONCLUSIONS

Successful synthesis of five 4-aminoquinoline-based acylthioureas, five aminoquinoline-derived 2-hydroxyacetamides, and two gold(I) complexes was achieved.

The acylthioureas were obtained in low yields (25 – 65 %) as a result of side reactions and difficulties in purification. The antimalarial activities of these compounds against the CQS D10 strain, although varied, were generally good (IC_{50} = 0.058 μM – 0.256 μM), the most active derivatives being **11**, **12** and **13**. However, against the CQR strains, the activities decreased significantly for all compounds [IC_{50}(W2) = 0.257 μM – 1.492 μM]. No conclusive structural requirements for activity could be established from the biological results. Cross-resistance with CQ was observed for most compounds, with the exception of **18** and **32**.

The aminoquinoline-derived 2-hydroxyacetamides were obtained in varied yields (21 - 61 %; 2 - 3 steps). Thioacetamide conversion of the 2-hydroxyacetamide functionality was attempted with Lawesson's Reagent unsuccessfully. The biological results of these compounds indicated that in general, the 2-hydroxyacetamide compounds had poor activity against *P. falciparum* parasites. Two compounds, however, showed good promise for further development. The benzylated intermediate **16** displayed high activity against both CQS and CQR strains, and may provide a good lead compound for further development. Despite the moderate activity of acetamide **18** against both the CQS and CQR strains, it displayed the promising property of being more active against the CQR strain than the CQS equivalent. The potential of this compound for overcoming CQ resistance should be further

explored *via* a more comprehensive structure-activity relationship study, since it remains a potential candidate for future drug development.

Gold complexation of the ligands **11** (acylthiourea) and **23** (2-hydroxyacetamide) was successfully accomplished with two different gold(I) precursors. The results of the *in vitro* assays were significantly different for each complex. The chlorogold(I) acylthiourea complex **31** displayed poor antiplasmodial activity, whereas the dimer complex **32** resulted in a marked enhancement in the activity of the ligand. The importance of gold precursor selection on activity was thereby at least partially illustrated. Gold complex structure, including spectator ligand and counter ion, appears to impact on the solubility and therefore the activity of the synthesised compounds. A more extensive study on the effects of these complex properties is required in order to establish the factors necessary for optimum biological activity.

CHAPTER 7

PROPOSED FUTURE WORK

The potential for further development of the two lead compounds, **16** and **18**, is proposed to be evaluated *via* a comprehensive structure-activity relationship (SAR) study. This SAR study should focus on assessing the effects of modifications to the side chain, since it has been demonstrated that the activity of related aminoquinoline antimalarials is based upon the 7-chloroquinoline ring, whereas resistance is based upon parasite recognition of the alkyl side chain (see Section 2.1.1.4.1).

Suggested variations to **16** include, (i) evaluating the importance of the methylene spacer length, and (ii) evaluating the effect of substitutions on the benzene ring (see Figure 7.1). Common aromatic substituents in antimalarial chemotherapy include chlorine, bromine, hydroxy, methoxy and trifluoromethyl groups among others, and the antimalarial effect imparted by substitutions with these groups would be of particular interest. The effect on activity of different substitution patterns, (mono-, di- or tri-substituted *ortho*, *meta*, *para*) would also require evaluation.

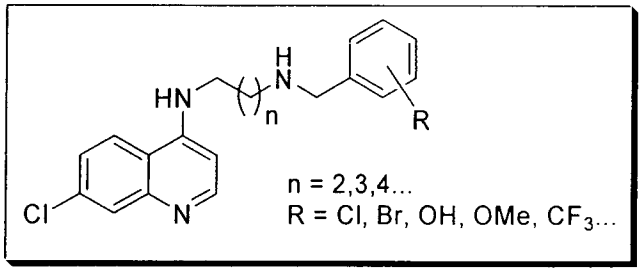


Figure 7.1 Suggested variations to the molecular structure of **16**

Potential variations of **18** are indicated in Figure 7.2 below. Due to the similarity in structure between **16** and **18**, these include the modifications

given in Figure 7.1, as well as possible changes to the tertiary amide 2-hydroxyacetamide substituent (see Figure 7.2). Since **18** displayed such a favourable resistance index, it would be of interest to determine an R_2 substituent that could impart an antimalarial activity equivalent to **16** while retaining a resistance index equivalent to **18**.

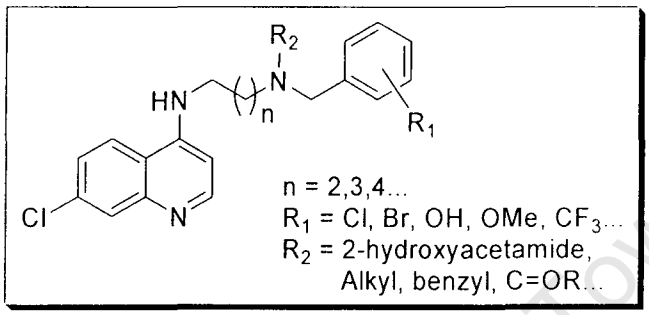


Figure 7.2 Suggested variations to the molecular structure of **18**

Within the context of gold complexation, the impact on the antimalarial activity of the gold complex structure has yet to be fully established. While it would appear that complexation with the chlorogold(THT) precursor results in a decrease in activity, reaction with different ligands would need to be performed for this result to be confirmed. Equally, the activity enhancement resulting from gold complex formation with the PPh_3AuCl precursor would also need to be confirmed by reaction with a variety of ligands.

Preliminary SAR studies on the effect of the spectator ligand (e.g. PMe_3, PEt_3, PPh_3) and the counter ion (e.g. PF_6^-, NO_3^-) have been performed, with inconclusive results.[1] Consequently, verification of the structural components necessary for optimum biological activity is still required, and remains an item for future work. This may involve replacing the phosphine spectator ligand with amine or thiol derivatives, and/or replacing the PF_6^- counter ion with appropriate alternatives.

It would also be of interest to test the lead compounds in appropriate animal models, since the *in vitro* activity does not necessarily translate into *in vivo* activity.[2]

The mode of action of the gold complexes could be evaluated to establish whether activity is derived from DNA interactions, such as intercalation. It has been reported that gold(III) complexes are able to intercalate DNA, and it may be though this process that their activity is derived.[3,4] Such an investigation could involve spectroscopic titration of increasing amounts of DNA (e.g. calf thymus DNA) to a fixed concentration of the complex, to determine any shifts in the maxima of the peaks. A shift would indicate an altered DNA structure. Viscosity experiments could also be done to identify any changes to the DNA structure as a result of exposure to the gold complex. Finally, gel electrophoresis studies on whole plasmids incubated at varying concentrations of the complex could also indicate DNA interaction through changes in the proportion of supercoiled:circular plasmid bands.[5] Such assays could be performed on the complexes and the free ligands as a means of comparison.

The lipophilicity/hydrophobicity of the ligands and complexes could be measured in order to provide a quantitative value for the change in solubility arising from gold complexation. Lipophilicity is assessed through the log*P* parameter, *P* being the octanol-water partition coefficient, a ratio of solute solubility in octanol *versus* water.[6,7,8] The higher the log*P*, the greater the lipophilicity, the converse being true for hydrophobicity. Such a study could be done by dissolving a known quantity of ligand or complex in a mixture of octanol/water, and measuring the concentration in each layer by UV/vis spectroscopy. The log*P* could also be determined using HPLC, by correlating the retention times of ligands and complexes with those of related compounds with known log*P* values.[9]

References

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CHAPTER 8

EXPERIMENTAL

8.1 General

All commercial chemicals were purchased from Merck (South Africa) or Sigma-Aldrich. All solvents were freshly distilled and dried by appropriate techniques with the exception of analytical grade absolute ethanol (EtOH), methylene chloride and acetone. Reactions were monitored by thin layer chromatography (TLC) using Merck F254 silica gel plates and were visualised by ultraviolet light or staining with iodine vapour or anisaldehyde. Silica gel chromatography was performed using Merck kieselgel 60.

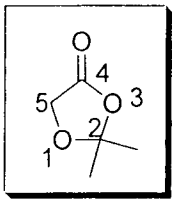
Melting points were determined on a Reichert-Jung Thermovar hot-stage microscope and are uncorrected. Infrared spectra were recorded on a Thermo Nicolete FTIR instrument in the range 4000 – 600 cm^{-1} as KBr disks. Microanalyses were determined using a Fisons EA 1108 CHNO-S instrument.

^{1}H NMR spectra were recorded on a Varian Mercury Spectrometer at 300 MHz or a Varian Unity Spectrometer at 400 MHz. All spectra were recorded in deuterochloroform, deuterodimethylsulfoxide, deuteroacetone or deuteromethanol using tetramethylsilane as an internal standard. All chemical shifts were recorded in ppm.

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

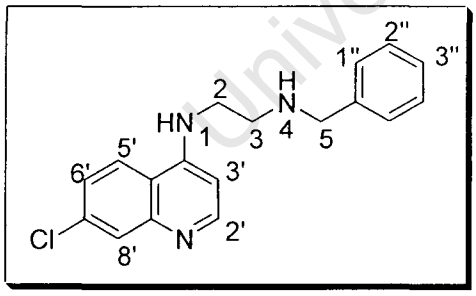
8.2 Chemical Synthesis and Characterisation

2,2-Dimethyl-(1,3)-dioxolan-4-one, 15



Glycolic acid (5.00 g, 65.7 mmol) was dissolved in acetone (55 ml) and stirred at -12 °C. Conc. H_2SO_4 (1 ml) was added drop-wise to the reaction mixture, such that the temperature did not rise above -10 °C. The solution was stirred for 45 minutes at -10 °C, before being poured onto crushed ice and neutralised to pH 7 with powdered $NaHCO_3$. The inorganic precipitate was filtered and washed with EtOAc (3 x 30 ml), and the filtrate extracted with additional EtOAc (50 ml). After drying (Na_2SO_4) and the removal of solvent under reduced pressure (rotary evaporator), the product was obtained as a colourless oil (4.754 g, 62 %); R_f(EtOAc:Hexane, 30:70) 0.41; δ_H (400 MHz, $CDCl_3$) 4.30 (2H, s, H5), 1.55 (6H, s, 2 x CH_3); δ_C (100 MHz, $CDCl_3$) 171.35, 112.63, 63.51, 25.74 (2 C)

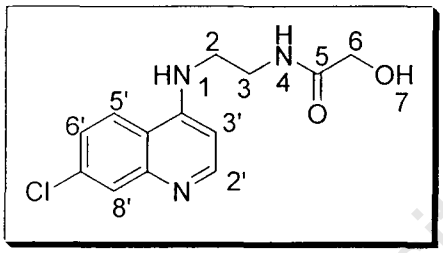
N^1-benzyl-N^2-(7-chloroquinolin-4-yl)ethane-1,2-diamine, 16



Benzaldehyde (0.301 ml, 3.00 mmol) was added dropwise to a solution of N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine, **7**, (0.663 g, 3.0 mmol) in MeOH (20 ml) and *N*,*N*-dimethylformamide (DMF) (10 ml), and the resulting mixture stirred at 25 °C for 5 hours. $NaBH_4$ (0.232 g, 6.00 mmol) was then added to the reaction mixture which was stirred for a further 1 hour. Excess $NaBH_4$ was quenched by dropwise addition of water to the reaction vessel until effervescence ceased. The solution was then evaporated under reduced pressure and the residue purified by column chromatography (MeOH-CH_2Cl_2, 5:95 - 10:90) to give the benzyl chloroquinoline amine as a white powder (0.699 g, 75 %), m.p. 142 – 144 °C; R_f(MeOH-CH_2Cl_2, 10:90) 0.14; IR ν_{max} (Nujol)/cm^{-1} 3211 (N-H),

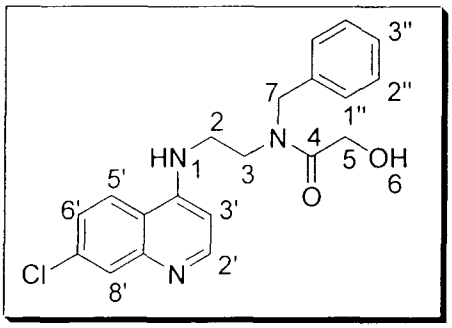
2926 (C-H), 1579 (C=N), 1545 (Ar C=C); δ_H (400 MHz, DMSO-d_6) 8.34 (1H, d, *J* = 5.6 Hz, H2'), 8.20 (1H, d, *J* = 9.2 Hz, H5'), 7.77 (1H, d, *J* = 2.0 Hz, H8'), 7.39 (1H, dd, *J* = 2.0, 9.2 Hz, H6'), 7.31 (2H, d, *J* = 7.2 Hz, H1''), 7.25 (2H, t, *J* = 7.2 Hz, H2''), 7.18 (1H, t, *J* = 7.2 Hz, H3''), 6.45 (1H, d, *J* = 5.6 Hz, H3'), 3.72 (2H, s, H5), 3.35 (2H, t, *J* = 6.4 Hz, H2), 2.78 (2H, t, *J* = 6.4 Hz, H3); δ_C (100 MHz, DMSO-d_6) 152.47, 151.00, 149.48, 141.00, 134.23, 128.80 (2C), 128.68 (2C), 127.89, 127.34, 124.81, 124.66, 118.08, 99.41, 53.28, 47.19, 42.97; HRMS (EI) *m/z* 311.11804 (M^+ $C_{18}H_{18}N_3Cl$ requires 311.11893); (Found: C, 68.25; H, 5.89; N, 13.32. $C_{18}H_{18}{}^{35}Cl\,N_3$ requires C, 69.34; H, 5.82; N, 13.48 %).

N-[2-(7-Chloroquinolin-4-ylamino)-ethyl]-2-hydroxyacetamide, 17



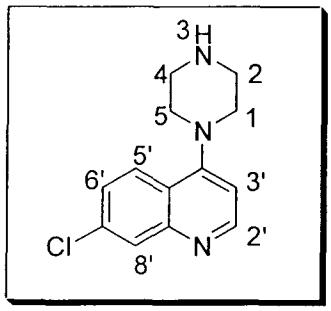
Glycolic acid acetonide **15** (115 mg, 0.99 mmol) and N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine, **7**, (220 mg, 0.99 mmol) were stirred in anhydrous CH_2Cl_2 under N_2 at 30 °C for 12 hours. The solution was then evaporated under reduced pressure, and the residue purified by column chromatography (MeOH-CH_2Cl_2, 1:1) to give the glycolic acid amide as colourless needles (271 mg, 98 %), m.p. 213 -215 °C; R_f(MeOH-CH_2Cl_2, 1:1) 0.27; I.R. ν_{max}(KBr)/cm^{-1} 3340 (O-H), 3066 (N-H), 2777 (C-H), 1641 (C=O), 1613 (Ar C=C),1593 (C=N); δ_H (400 MHz, DMSO-d_6-D_2O) 8.33 (1H, d, *J* = 5.6 Hz, H2'), 8.09 (1H, d, *J* = 9.2 Hz, H5'), 7.76 (1H, d, *J* = 2.0 Hz, H8'), 7.40 (1H, dd, *J* = 2.0, 8.8 Hz, H6'), 6.53 (1H, d, *J* = 6.0 Hz, H3'), 3.81 (2H, s, H6), 3.41 (2H, t, *J* = 6.2 Hz, H3), 3.32 (2H, t, *J* = 6.2 Hz, H2); δ_C (100 MHz, DMSO-d_6) 173.31 (C=O), 152.62, 150.76, 149.71, 134.10, 128.22, 124.84, 124.54, 118.11, 99.37, 62.17, 43.21, 37.45; HRMS (EI) *m/z* 279.0769 (M^+ $C_{13}H_{14}{}^{35}ClN_3O_2$ requires 279.0775); (Found: C, 55.71; H, 5.25; N, 15.54. $C_{13}H_{14}{}^{35}ClN_3O_2$ requires C, 55.82; H, 5.04; N, 15.02 %).

N-benzyl-N-(2-(7-chloroquinolin-4-ylamino)ethyl)-2-hydroxyacetamide, 18



Glycolic acid acetonide **15** (133 mg, 1.15 mmol) and N^1-benzyl-N^2-(7-chloroquinolin-4-yl)ethane-1,2-diamine **16** (357 mg, 1.15 mmol) were stirred in anhydrous CH_2Cl_2 under N_2 for 24 hours at 30 °C. The solvent was then evaporated under reduced pressure and the residue chromatographed (MeOH-EtOAc, 1:5) twice and recrystallised from 95 % EtOH to yield the product as pale yellow cubes (201 mg, 47 %), m.p. 181 – 183 °C; R_f(MeOH-CH_2Cl_2, 10:90) 0.37; I.R. ν_{max}(KBr)/cm^{-1} 3314 (O-H), 3062 (N-H), 1655 (C=O), 1602 (C=C), 1579 (C=N); δ_H (400 MHz, $CDCl_3$/CD_3OD) 8.33 (1H, d, *J* = 5.6 Hz, H2'), 7.83 (1H, s, H8'), 7.69 (1H, d, *J* = 8.4 Hz, H5'), 7.30 (4H, m, *H1''*H2''H3''H6'), 7.12 (2H, d, *J* = 7.2 Hz, H1''*H2''*), 6.16 (1H, d, *J* = 5.2 Hz, H3'), 4.42 (2H, s, H5), 4.26 (2H, s, H7), 3.78 (2H, t, *J* = 5.6 Hz, H3), 3.34 (2H, t, *J* = 5.6 Hz, H2); δ_C (100 MHz, $CDCl_3$/CD_3OD) 174.51 (C=O), 151.32, 150.26, 148.34, 135.34, 134.75, 129.25 (2C), 128.39, 127.93, 127.50, 126.57 (2C), 125.64, 121.87, 98.07, 60.16, 50.73, 45.82, 42.45; HRMS (EI) *m/z* 369.12385 (M^+ $C_{20}H_{20}{}^{35}ClN_3O_2$ requires 369.12440); (Found: C, 64.70; H, 5.74; N, 10.65. $C_{20}H_{20}{}^{35}ClN_3O_2$ requires C, 64.95; H, 5.45; N, 11.36 %).

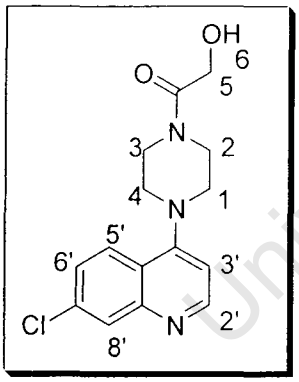
7-chloro-4-(piperazin-1-yl)quinoline, 22



Prepared according to the procedure reported in literature.[1] A solution of 4,7-dichloroquinoline (2.00 g, 9.80 mmol), piperazine (4.228 g, 49.08 mmol), K_2CO_3 (4.08 mg, 2.96 mmol) and Et_3N (2.04 ml, 14.72 mmol) in *N*-methyl-2-pyrrolidinone (12 ml) was stirred under N_2 for 2 hours at 135 °C. After cooling to room temperature, the mixture was diluted with CH_2Cl_2, washed with brine

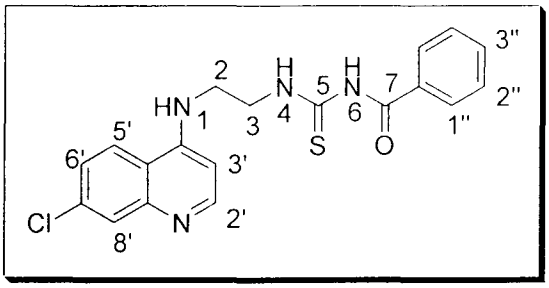
(2 x 50 ml), dried ($MgSO_4$), and concentrated under reduced pressure. The resulting oil was purified by column chromatography (MeOH-CH_2Cl_2, 1:4) to afford the amine as a yellow powder (1.980 g, 82 %), m.p. 112 – 114 °C (lit. 113-115 °C); R_f(MeOH-CH_2Cl_2, 10:90) 0.15; I.R. ν_{max}(KBr)/cm^{-1} 3256 (N-H), 1606 (C=C), 1569 (C=N); δ_H (300 MHz, DMSO-d_6): 8.75 (1H, d, *J* = 5.1 Hz, H2'), 8.10 (1H, d, *J* = 8.7 Hz, H5'), 8.01 (1H, d, *J* = 2.1 Hz, H8'), 7.58 (1H, dd, *J* = 2.1, 8.7 Hz, H6'), 7.10 (1H, d, *J* = 4.8 Hz, H3'), 3.17 (4H. t, *J* = 4.6 Hz, H1H2*H4H5*), 3.08 (4H, t, *J* = 4.8 Hz, *H1H2*H4H5); δ_C (75 MHz, DMSO-d_6): 155.39, 125.20, 149.54, 133.75, 128.05, 126.03, 125.98, 121.16, 109.80, 48.77 (2C), 42.87 (2C); HRMS (EI) *m/z* 247.0871 (M^+ $C_{13}H_{14}{}^{35}ClN_3$ requires 247.0876); (Found: C, 62.50; H, 5.64; N, 17.54. $C_{13}H_{14}{}^{35}ClN_3$ requires C, 63.03; H, 5.70; N, 16.96 %).

1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-hydroxyethanone, 23



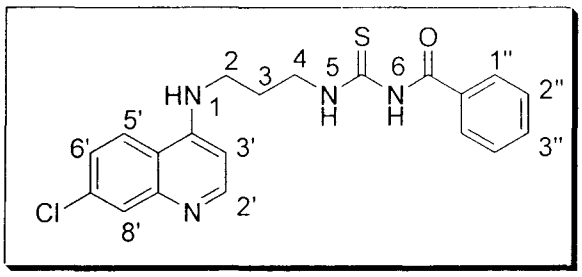
Glycolic acid acetonide **1** (352 mg, 3.03 mmol) and 7-chloro-4-(piperazin-1-yl)quinoline, **22**, (751 mg, 3.03 mmol) were dissolved in anhydrous CH_2Cl_2 (6 ml) and stirred at 30 °C for 40 hours. After evaporation under reduced pressure, the residue was purified by column chromatography (EtOAc, 100% - MeOH-EtOAc, 5:95) to yield the crystalline amide as light green cubes (615 mg, 66 %), m.p. 151 -152 °C; R_f(MeOH-EtOAc, 5:95) 0.18; I.R. ν_{max}(KBr)/cm^{-1} 3373 (O-H), 2908 (C-H), 2845 (C-H), 1660 (C=O), 1607 (C=C), 1571 (C=N); δ_H (300 MHz, DMSO-d_6) 8.70 (1H, d, *J* = 5.1 Hz, H2'), 8.08 (1H, d, *J* = 9.0 Hz, H5'), 7.97 (1H, d, *J* = 2.1 Hz, H8'), 7.54 (1H, dd, *J* = 9.0, 2.1 Hz, H6'), 7.00 (1H, d, *J* = 5.1 Hz, H3'), 4.55 (1H, bs, H6), 4.15 (2H, s, H5), 3.69 (4H, m, H2H3*H1H4*), 3.21 (4H, m, *H2H3*H1H4); δ_C (75 MHz, $CDCl_3$) 170.43 (C=O), 156.08, 151.93, 150.17, 135.23, 129.13, 126.73, 124.50, 121.75, 109.39, 59.85, 51.90 (2C), 43.49, 42.26; HRMS (EI) *m/z* 305.0921 (M^+ $C_{15}H_{14}{}^{35}ClN_3$ requires 305.0931); (Found: C, 58.89; H, 5.24; N, 13.72. $C_{13}H_{16}{}^{35}ClN_3O_2$ requires C, 58.92; H, 5.27; N, 13.74 %).

***N-(2-(7-chloroquinolin-4-ylamino)ethylcarbamothioyl)benzamide*, 10**



Benzoyl chloride (0.22 ml, 1.88 ml) was added drop-wise to a solution of KSCN (224 mg, 2.30 mmol) in acetone (10 ml). The mixture was stirred under reflux for 30 minutes, and then cooled to room temperature. The inorganic precipitate was removed by filtration leaving benzoylisothiocyanate in solution. Chloroquinoline amine **7** (500 mg, 2.26 mmol) was then added to this solution, which was refluxed for 2 hours. After cooling to room temperature, the acetone was removed under reduced pressure, and cold water added to the residue. After stirring for 30 minutes, the water was filtered off, and the residue purified by column chromatography (CH_2Cl_2, 100 % - MeOH-CH_2Cl_2, 5:95) to yield the product as a white powder (380 mg, 52 %), m.p. = 191 – 193 °C; R_f(MeOH-CH_2Cl_2, 10:90) 0.41; I.R. ν_{max}(KBr)/cm^{-1} 3351 (N-H), 2941 (C-H), 2875 (C-H), 1681 (C=O), 1612 (C=C), 1582 (C=N), 1166 (C=S); δ_H (400 MHz, DMSO-d_6): 11.29 (1H, broad s, H4), 11.02 (1H, t, *J* = 5.6 Hz, H1), 8.40 (1H, d, *J* = 5.2 Hz, H2'), 8.24 (1H, d, *J* = 9.2 Hz, H5'), 7.89 (2H, d, *J* = 7.2 Hz, H1''), 7.78 (1H, d, *J* = 2.0 Hz, H8'), 7.61 (1H, t, *J* = 7.4 Hz, H3''), 7.49 (2H, t, *J* = 7.8 Hz, H2''), 7.45 (1H, dd, *J* = 2.2, 9.2 Hz, H6'), 6.69 (1H, d, *J* = 5.4 Hz, H3'), 3.96 (2H, quartet, *J* = 6.0 Hz, H3), 3.59 (2H, quartet, *J* = 6.0 Hz, H2); δ_C (100 MHz, DMSO-d_6) 181.42, 168.47, 152.59, 150.85, 149.70, 134.14, 133.61, 132.94, 129.17 (2C), 129.08 (2C), 128.19, 124.90, 124.73, 118.16, 99.71, 43.82, 41.40; HRMS (EI) *m/z* 384.0813 (M^+ $C_{19}H_{17}{}^{35}ClN_4OS$ requires 384.0812); (Found: C, 58.55; H, 4.66; N, 13.32; S, 7.97. $C_{19}H_{17}{}^{35}ClN_4OS$ requires C, 59.29; H, 4.45; N, 14.56; S, 8.33 %).

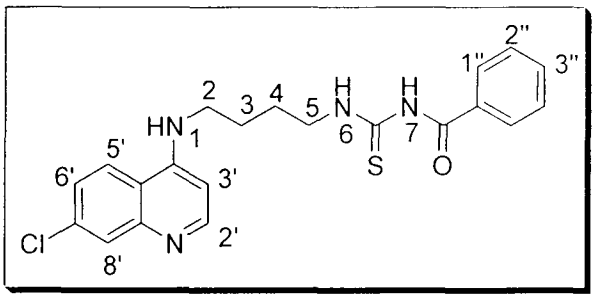
N-(3-(7-chloroquinolin-4-ylamino)propylcarbamothioyl)benzamide, 11



Benzoyl chloride (0.49 ml, 4.21 mmol) was added drop-wise to a solution of KSCN (500 mg, 5.15 mmol) in acetone (25 ml) and the mixture stirred under reflux for 30 minutes. After cooling to room temperature, the inorganic salt was filtered off, to yield benzoylisothiocyanate in solution. Chloroquinoline amine **8** (992 mg, 4.21 mmol) was added to this solution and the mixture refluxed for 2 hours. After cooling to room temperature, the acetone was removed under reduced pressure, and cold water added to the flask. After stirring for 30 minutes, the water was filtered off, and the residue purified by column chromatography (MeOH-CH_2Cl_2, 5:95 increased to 10:90) to afford the product as yellow cubes (418 mg, 25 %), m.p. = 83 – 85 °C;

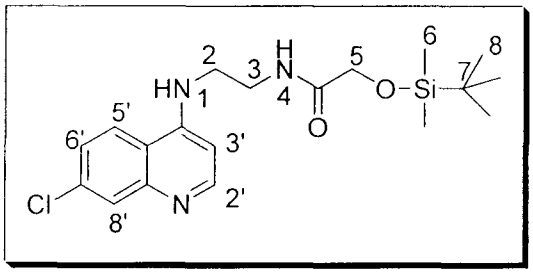
R_f(MeOH-CH_2Cl_2, 15:85) 0.33; I.R. ν_{max}(KBr)/cm^{-1} 3244 (N-H), 2943 (C-H), 1676 (C=O), 1609 (C=C), 1579 (C=N), 1167 (C=S); δ_H (300 MHz, DMSO-d_6) 11.18 (1H, bs, H6), 10.92 (1H, t, *J* = 5.3 Hz, H5), 8.39 (1H, d, *J* = 5.4 Hz, H2'), 8.24 (1H, d, *J* = 9.0 Hz, H5'), 7.91 (2H, m, H1''), 7.77 (1H, d, *J* = 1.8 Hz, H8'), 7.59 (2H, m, H2''), 7.48 (1H, m, H3''), 7.42 (1H, dd, *J* = 9.0, 2.1 Hz, H6'), 7.37 (1H, bs, H1), 6.50 (1H, d, *J* = 6.0 Hz, H3'), 3.76 (2H, q, *J* = 6.5 Hz, H4), 3.37 (2H, q, *J* = 6.0 Hz, H2), 2.05 (2H, quin, *J* = 6.8 Hz, H3); δ_C (75 MHz, CD_3OD) 182.23, 169.35, 152.61, 152.29, 149.48, 136.37, 134.19, 133.69, 129.77 (2C), 129.06 (2C), 127.47, 126.03, 124.35, 118.80, 99.71, 44.15, 41.63, 28.00; HRMS (EI) *m/z* 398.09620 (M^+ $C_{20}H_{19}{}^{35}ClN_4OS$ requires 398.09681); (Found: C, 59.50; H, 5.34; N, 12.17; S, 7.67. $C_{20}H_{19}{}^{35}ClN_4OS$ requires C, 60.22; H, 4.80; N, 14.05; S, 8.04 %).

N-(4-(7-chloroquinolin-4-ylamino)butylcarbamothioyl)benzamide, 12



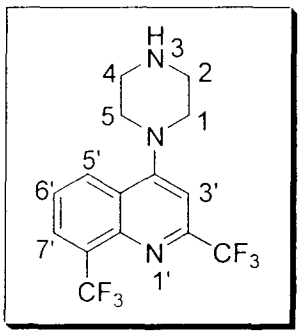
Benzoyl chloride (0.20 ml, 1.72 mmol) was added dropwise to a solution of KSCN (205 mg, 2.11 mmol) in acetone (10 ml), and the mixture refluxed for 30 minutes. After cooling to room temperature, the inorganic salt formed was filtered off leaving benzoylisothiocyanate in solution. Chloroquinoline amine **9** (473 mg, 1.90 mmol) was then added to this solution, and the mixture refluxed for 2 hours. After cooling to room temperature, the acetone was removed under reduced pressure, and cold water added to the flask. After stirring for 30 minutes, the water was filtered off, and the residue purified by column chromatography (CH_2Cl_2, then CH_2Cl_2-MeOH, 5:95) to afford the product as a yellow powder (283 mg, 40 %), m.p. = 161 – 163 °C; R_f(MeOH-CH_2Cl_2, 10:90) 0.40; I.R. ν_{max}(KBr)/cm^{-1} 3234 (N-H), 2939 (C-H), 2866 (C-H), 1668 (C=O), 1614 (C=C), 1582 (C=N), 1163 (C=S); δ_H (400 MHz, CD_3OD) 8.33 (1H, d, *J* = 5.6 Hz, H2'), 8.12 (1H, d, *J* = 8.8 Hz, H5'), 7.75 (2H, dd, *J* = 7.2 Hz, 1.2 Hz, H1''), 7.75 (1H, *J* = 2.0 Hz, H8'), 7.62 (1H, tt, *J* = 7.6 Hz, 1.4 Hz, H3''), 7.50 (2H, t, *J* = 7.6 Hz, H2''), 7.40 (1H, dd, *J* = 8.0 Hz, 2.2 Hz, H6'), 6.57 (1H, d, *J* = 6.0 Hz, H3'), 3.79 (2H, t, *J* = 6.4 Hz, H5), 3.46 (2H, t, *J* = 6.8 Hz, H2), 1.87 (4H, m, H3H4); δ_C (100 MHz, CD_3OD) 180.89 (C=S), 168.29 (C=O), 151.99, 150.41, 147.58, 135.55, 133.04, 132.60, 128.64 (2C), 127.89 (2C), 125.71, 125.03, 123.30, 117.46, 98.56, 44.65, 42.46, 25.61, 25.45; HRMS (EI) *m/z* 412.1131 (M^+ $C_{21}H_{21}{}^{35}ClN_4SO$ requires 412.1125); (Found: C, 59.09; H, 5.20; N, 12.23; S, 7.08. $C_{21}H_{21}{}^{35}ClN_4SO$ requires C, 61.08; H, 5.13; N, 13.57; S, 7.77 %).

2-(tert-butyldimethylsilyloxy)-N-(2-(7-chloroquinolin-4-ylamino)ethyl)acetamide, 19



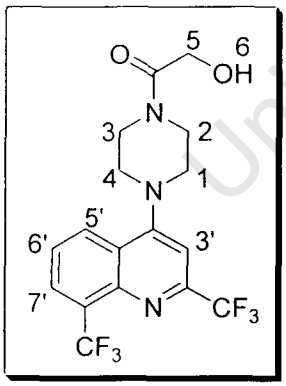
A solution of **17** (300 mg, 1.07 mmol) in dry CH_2Cl_2 (5 ml) and dry DMF (5 ml) was stirred at 25 °C before t-butyldimethylsilyl chloride (323 mg, 2.14 mmol) and imidazole (160 mg, 2.35 mmol) were added. The mixture was stirred at the same temperature under N_2 atmosphere for 48h. After completion of the reaction (confirmed by TLC) the CH_2Cl_2 was removed under reduced pressure. The residue was diluted with EtOAc (100ml), and washed with water (7 x 100 ml) to remove the DMF. After drying ($MgSO_4$) and removal of the solvent under reduced pressure, the product was obtained as colourless needles (383 mg, 91 %), m.p. 193 – 196 °C; R_f (MeOH/CH_2Cl_2, 10:90) 0.28; I.R. ν_{max}(KBr)/cm^{-1} 3381 (N-H), 2952 (C-H), 2928 (C-H), 1649 (C=O), 1612 (C=C), 1577 (C=N); δ_H (400 MHz; $CDCl_3$) 8.49 (1H, d, *J* = 5.2 Hz, H2'), 7.95 (1H, d, *J* = 2.4 Hz, H8'), 7.79 (1H, d, *J* = 8.8 Hz, H5'), 7.37 (1H, dd, *J* = 8.8, 2.0 Hz, H6'), 7.19 (1H, bs, H4), 6.83 (1H, bs, H1), 6.29 (1H, d, *J* = 5.2 Hz, H3'), 4.15 (2H, s, H5), 3.77 (2H, m, H2*H3*), 3.43 (2H, m, *H2*H3), 0.90 (9H, s, H8), 0.09 (6H, s, H6); δ_C (100 MHz; $CDCl_3$) 174.33 (C=O), 151.35, 150.40, 148.46, 135.20, 127.96, 125.65, 122.05, 117.17, 98.12, 63.19, 45.83, 38.51, 25.77 (3C), 18.18, -5.56 (2C); HRMS (EI) *m/z* 393.16331 (M^+ $C_{19}H_{28}{}^{35}ClN_3O_2Si$ requires 393.16393); (Found: C, 57.91; H, 6.62; N, 10.33. $C_{19}H_{28}{}^{35}ClN_3O_2Si$ requires C, 57.92; H, 7.16; N, 10.67 %).

4-(piperazin-1-yl)-2,8-bis(trifluoromethyl)quinoline, 27



The reaction was carried out according to the protocol of Molyneaux and co-workers [2] to yield the product as a light yellow powder (1.506 g, 99 %), m.p. 132 – 134 °C (lit.[2] 128 – 131 °C); R_f(MeOH/CH_2Cl_2, 10:90) 0.26; I.R ν_{max}(KBr)/cm^{-1} 3423 (N-H), 2952 (C-H), 2836 (C-H), 1593 (C=N), 1518 (C=C); δ_H (300 MHz; $CDCl_3$) 8.24 (1H, d, *J* = 8.4 Hz, H7'), 8.07 (1H, d, *J* = 7.2 Hz, H5'), 7.60 (1H, t, *J* = 7.8 Hz, H6'), 7.20 (1H, s, H3'), 3.28 – 3.25 (4H, m, H1H5), 3.20 – 3.17 (4H, m, H2H4); δ_C (75 Mhz; $CDCl_3$) 159.27,149.25, 148.80, 145.30, 128.60, 128.12, 125.40, 124.33, 123.20, 121.88, 105.20, 53.67 (2C), 45.90 (2C); HRMS (EI) *m/z* 349.1008 (M^+ $C_{15}H_{13}F_6N_3$ requires 349.1014); (Found: C, 50.22; H, 4.16; N, 12.12. $C_{15}H_{13}F_6N_3$ requires C, 51.58; H, 3.75; N, 12.03 %).

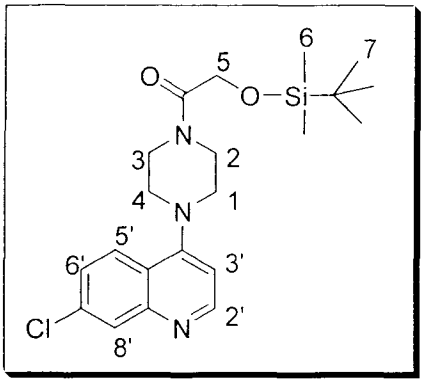
1-(4-(2,8-bis(trifluoromethyl)quinolin-4-yl)piperazin-1-yl)-2-hydroxyethanone, 28



27 (300 mg, 0.86 mmol) was dissolved in dry CH_2Cl_2 (10 ml) and stirred at 40 °C under N_2. Glycolic acid acetonide (150 mg, 1.29 mmol) was added drop-wise and the solution stirred for 2 days (completion of the reaction was confirmed by TLC). Crystalline product was obtained after purification by column chromatography (Hexane/EtOAc, 90:10 – 40:60) as yellow plates (312 mg; 89 %), m.p. 173 – 175 °C; R_f(Hexane/EtOAc, 10:90) 0.33; I.R. ν_{max}(KBr)/cm^{-1} 3446 (O-H), 2915 (C-H), 2850 (C-H), 1663 (C=O), 1592 (C=N), 1519 (C=C); δ_H (300 MHz; $CDCl_3$) 8.24 (1H, d, *J* = 8.7 Hz, H7'), 8.13 (1H, d, *J* = 7.2 Hz, H5'), 7.68 (1H, t, *J* = 7.8 Hz, H6'), 7.24 (1H, s, H3'), 4.26 (2H, d, *J* = 4.5 Hz, H5), 4.00 (2H, bs, H2H3 *ax*/eq), 3.62 (2H, bs, H2H3 ax/*eq*), 3.54 (1H, t, *J* = 4.5 Hz, H6), 3.31 (4H, t, *J* = 5.0 Hz, H1H4); δ_C (75 Mhz; $CDCl_3$) 170.56 (C=O), 158.16, 149.09, 145.24, 128.97, 127.42, 126.23,

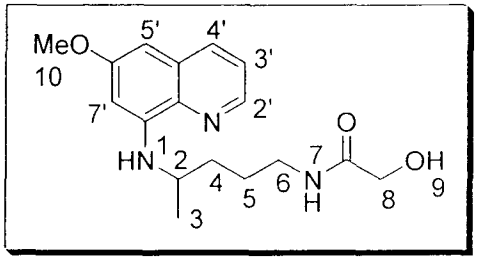
125.35, 124.23, 122.99, 121.71, 105.85, 59.86, 52.21, 51.92, 43.35, 42.15; HRMS (EI) *m/z* 407.1072 (M^+ $C_{17}H_{15}F_6N_3O_2$ requires 406.1069); (Found: C, 50.63; H, 4.14; N, 9.70. $C_{17}H_{15}F_6N_3O_2$ requires C, 50.13; H, 3.71; N, 10.32 %).

2-(tert-butyldimethylsilyloxy)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone, 24



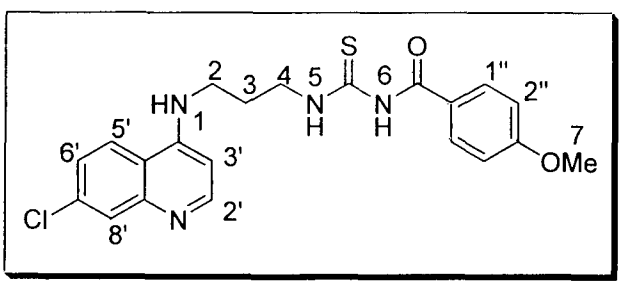
A dry CH_2Cl_2 (20 ml) solution of **23** (190 mg, 0.62 mmol), TBDMSCl (112 mg, 0.75 mmol) and imidazole (51 mg, 0.75 mmol) was stirred at 30 °C for 5 days. At this point the reaction was incomplete (confirmed by TLC), therefore additional TBDMSCl (91 mg, 0.60 mmol) and imidazole (84 mg, 1.23 mmol) were added to the reaction mixture and stirring continued for a further 3 days. The purified silyl ether product was recovered following purification by column chromatography (EtOAc/Hexane, 0:100 – 50:50) as a light orange oil (213 mg, 0.51 mmol, 82 %); R_f (EtOAc/Hexane, 50:50) 0.12; I.R. ν_{max}(NaCl)/cm^{-1} 2955 (C-H), 2928 (C-H), 1654 (C=O), 1608 (C=C), 1578 (C=N); δ_H (400 MHz; $CDCl_3$) 8.64 (1H, d, *J* = 4.8 Hz, H2'), 7.96 (1H, d, *J* = 2.0 Hz, H8'), 7.85 (1H, d, *J* = 9.2 Hz, H5'), 7.36 (1H, dd, *J* = 2.0, 9.2 Hz, H6'), 6.73 (1H, d, *J* = 5.2 Hz, H3'), 4.25 (2H, s, H5), 3.78 (4H, t, *J* = 5.0 Hz, H2H3), 3.10 (4H, broad m, H1H4), 0.81 (9H, s, H7), 0.02 (6H, s, H6); δ_C (100 MHz; $CDCl_3$) 169.46 (C=O), 156.52, 151.90, 150.11, 135.20, 128.97, 126.61, 124.79, 121.85, 109.30, 64.26, 51.94 (2C), 45.24, 41.84, 25.78 (3C), 18.23, -5.40 (2C); HRMS (EI) *m/z* 419.1789 (M^+ $C_{21}H_{30}{}^{35}ClN_3O_2Si$ requires 419.1796).

2-hydroxy-N-(4-(6-methoxyquinolin-8-ylamino)pentyl)acetamide, 30



A suspension of primaquine diphosphate, **29**, (465 mg, 1.0 mmol) in dry CH_2Cl_2 (10 ml) was stirred at room temperature and Et_3N (0.84 ml, 6.0 mmol) added dropwise. Stirring was continued for a further 30 minutes before the drop-wise addition of glycolic acid acetonide (200 mg, 1.7 mmol). The reaction mixture was left to stir overnight at room temperature under N_2 atmosphere. The resulting product was obtained after purification by column chromatography on silica gel (Hexane/EtOAc, 20:80) as a green powder (78 mg; 25%), m.p. 127 – 130 °C; R_f(Hexane/EtOAc, 20:80) 0.20; I.R. ν_{max}(KBr)/cm^{-1} 3348 (O-H), 3325 (N-H), 2960 (C-H), 2924 (C-H), 1646 (C=O), 1615 (C=C), 1578 (C=C), 1521 (C=N); δ_H (400 MHz; CD_3COCD_3) 8.51 (1H, dd, *J* = 4.0, 1.6 Hz, H2'), 8.03 (1H, dd, *H* = 8.4, 1.6 Hz, H4'), 7.37 (1H, dd, *J* = 8.3, 4.0 Hz, H3'), 7.29 (1H, bs, *H1*H7), 6.46 (1H, d, *J* = 2.4 Hz, H7'), 6.31 (1H, d, *J* = 2.4 Hz, H5'), 6.13 (1H, bd, *J* = 8.8 Hz, H1*H7*), 3.91 (2H, s, H8), 3.86 (3H, s, H10), 3.71 (1H, m, H2), 3.30 (2H, m, H6), 1.70 (4H, m, H4H5), 1.29 (3H, d, *J* = 6.4 Hz, H3); δ_C (75 MHz; $CDCl_3$) 171.50 (C=O), 159.33, 144.92, 144.42, 135.35, 134.93, 129.94, 121.91, 96.91, 91.86, 62.18, 55.26, 47.87, 38.82, 33.57, 26.12, 20.54; HRMS (EI) *m/z* 317.1725 (M^+ $C_{17}H_{23}N_3O_3$ requires 317.1739); (Found: C, 60.27; H, 7.21; N, 11.29. $C_{17}H_{23}N_3O_3$ requires C, 64.33; H, 7.30; N, 13.24 %).

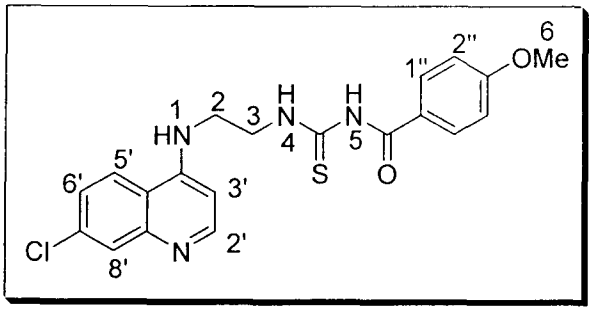
N-(3-(7-chloroquinolin-4-ylamino)propylcarbamothioyl)-4-methoxy benzamide, 14



4-methoxybenzoylchloride (0.31 ml, 2.26 mmol) was added dropwise to a stirring solution of NH_4SCN (206 mg, 2.71 mmol) in dry acetone (80 ml) at room

temperature, and the resulting mixture refluxed for 30 minutes. After cooling to room temperature, N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine, **8**, (500 mg, 2.12 mmol) was added and the solution stirred at room temperature overnight. The solvent was removed under reduced pressure and the residue washed successively with water, ether and hexane. After recrystallisation from 95 % EtOH, product was obtained as off-white plates (0.589 mg; 65 %), m.p. 85 – 90 °C; R_f(MeOH/CH_2Cl_2, 5:95) 0.20; I.R. ν_{max}(KBr)/cm^{-1} 3346 (N-H), 3206 (N-H), 2972 (C-H), 2936 (C-H), 1654 (C=O), 1609 (C=C), 1581 (C=N), 1158 (C=S); δ_H (400 MHz; $CDCl_3/CD_3OD$) 8.37 (1H, d, *J* = 5.6 Hz, H2'), 7.85 (1H, d, *J* = 9.2 Hz, H5'), 7.83 (1H, d, *J* = 2.4 Hz, H8'), 7.80 (2H, d, *J* = 9.2 Hz, H1''), 7.31 (1H, dd, *J* = 9.2 Hz, 2.4 Hz, H6'), 6.94 (2H, d, *J* = 9.2 Hz, H2''), 6.39 (1H, d, *J* = 5.6 Hz, H3'), 3.87 (2H, t, *J* = 6.8 Hz, H4), 3.84 (3H, s, H7), 3.44 (2H, t, *J* = 6.4 Hz, H2), 2.08 (2H, quin, *J* = 6.4 Hz, H3); δ_C (100 MHz; $CDCl_3/CD_3OD$) 180.91 (C=O), 166.92, 164.02, 150.78, 147.89, 135.52, 129.86 (2C), 127.05, 125.52, 125.41, 123.43, 122.09, 117.18, 114.30 (2C), 98.50, 55.53, 42.56, 39.84, 27.15; HRMS (EI) *m/z* 428.10691 (M^+ $C_{21}H_{21}{}^{35}ClN_4O_2S$ requires 428.10737); (Found: C, 54.48; H, 4.93; N, 5.23; S, 5.38. $C_{21}H_{21}{}^{35}ClN_4O_2S$ requires C, 58.80; H, 4.93; N, 13.06; S, 7.48 %).

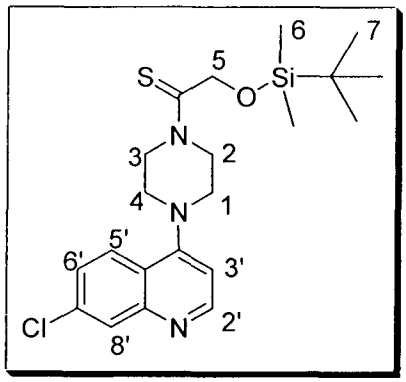
N-(2-(7-chloroquinolin-4-ylamino)ethylcarbamothioyl)-4-methoxybenzamide, 13



4-methoxybenzoylchloride (0.124 ml, 0.90 mmol) was added dropwise to a stirring solution of KSCN (87.5 mg, 0.90 mmol) in dry acetone (20 ml) at room temperature and the resulting mixture refluxed for 30 minutes. After cooling to room temperature, the inorganic salt was filtered off to yield 4-methoxybenzoylisothiocyanate. An *N*-methyl-2-pyrolidinone (3 ml) solution of N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine, **7**, (200 mg, 0.90 mmol) was added dropwise to this solution under

N_2, and the resultant mixture stirred at room temperature for 3 days. The precipitate thus formed was filtered, washed with EtOAc (2 x 30 ml), and dried under reduced pressure to yield the pure product (94 mg, 25 %). The filtrate was then evaporated to remove the acetone, and subsequently redissolved in EtOAc (150 ml), washed with water (5 x 50 ml) and dried ($MgSO_4$). The product in the filtrate (106 mg, 28 %) was obtained as a white powder after purification by column chromatography ($MeOH/CH_2Cl_2$, 5:95). Overall yield: 200 mg, 54 %, m.p. 206 – 207 °C; R_f($MeOH/CH_2Cl_2$, 10:90) 0.50; I.R. ν_{max}(KBr)/cm^{-1} 3302 (N-H), 3249 (N-H), 2939 (C-H), 2837 (C-H), 1652 (C=O), 1606 (C=C), 1584 (C=N), 1176 (C=S); δ_H (400 MHz; DMSO-d_6) 11.12 (1H, s, H5), 11.07 (1H, bt, *J* = 5.8 Hz, H4), 8.43 (1H, d, *J* = 5.6 Hz, H2'), 8.33 (1H, d, *J* = 9.2 Hz, H5'), 7.94 (2H, d, *J* = 8.8 Hz, H1''), 7.81 (1H, d, *J* = 2.4 Hz, H8'), 7.75 (1H, bt, *J* = 5.0 Hz, H1), 7.50 (1H, dd, *J* = 2.4, 9.2 Hz, H6'), 7.02 (2H, d, *J* = 8.8 Hz, H2''), 6.75 (1H, d, *J* = 5.6 Hz, H3'), 3.97 (2H, q, *J* = 6.1 Hz, H3), 3.83 (3H, s, H6), 3.62 (2H, q, *J* = 5.9 Hz, H2); δ_C (100 MHz; DMSO-d_6) 180.81 (C=S), 166.88 (C=O), 163.01, 150.89, 150.54, 147.45, 134.00, 130.68 (2C), 126.24, 124.50, 124.20, 123.91, 117.12, 113.68 (2C), 98.92, 55.49, 42.94, 40.79; HRMS (EI) *m/z* 414.09122 (M^+ $C_{20}H_{19}{}^{35}ClN_4O_2S$ requires 414.09172); (Found: C, 57.01; H, 4.72; N, 12.94; S, 7.46; $C_{20}H_{19}{}^{35}ClN_4O_2S$ requires C, 57.90; H, 4.62; N, 13.50; S, 7.73 %).

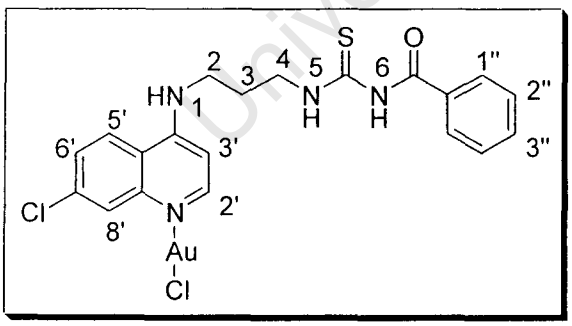
2-(tert-butyldimethylsilyloxy)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanethione, 25



A solution of 2-(tert-butyldimethylsilyloxy)-1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)ethanone, **24**, (111 mg, 0.26 mmol) in dry THF (15 ml) was added dropwise to a stirring solution of Lawesson's Reagent, 2,4-bis(*p*-methoxyphenyl)-1,3-dithiaphosphetane 2,4-disulfide, (64 mg, 0.16 mmol) in dry THF (15 ml) at room temperature. The mixture was stirred under nitrogen at 40 °C for 3

days to allow complete conversion to the thioamide. The solvent was removed under reduced pressure and the residue dissolved in EtOAc (50 ml), washed with water (3 x 50 ml) and dried ($MgSO_4$). After purification by column chromatography (Hexane/EtOAc, 60:40), the product was obtained as a pale green oil (58 mg, 51 %); R_f(Hexane/EtOAc, 60:40) 0.30; I.R. ν_{max}(NaCl)/cm^{-1} 2928 (C-H), 2856 (C-H), 1607 (C=C), 1578 (C=N), 986 (C=S); δ_H (400 MHz; $CDCl_3$): 8.72 (1H, d, *J* = 4.8 Hz, H2'), 8.03 (1H, d, *J* = 2.0 Hz, H8'), 7.93 (1H, d, *J* = 8.8 Hz, H5'), 7.43 (1H, dd, *J* = 9.2, 2.0 Hz, H6'), 6.80 (1H, d, *J* = 4.8 Hz, H3'), 4.70 (2H, s, H5), 4.64 (2H, bt, *J* = 5.0 Hz, H2H3 *ax*/eq), 4.19 (2H, bt, *J* = 4.8 Hz, H2H3 ax/*eq*), 3.27 (4H, m, H1H4), 0.87 (9H, s, H7), 0.11 (6H, s, H6); δ_C (100 MHz; $CDCl_3$) 200.09 (C=S), 155.93, 151.93, 150.17, 135.29, 129.11, 126.75, 124.68, 121.77, 109.33, 71.65, 52.44, 51.37, 50.38, 49.80, 25.80 (3C), 18.13, -5.03 (2C); HRMS (EI) *m/z* 435.15617 ($C_{21}H_{30}{}^{35}ClN_3OSSi$ requires 435.15674); (Found: C, 57.24; H, 6.96; N, 8.24; S, 6.88. $C_{21}H_{30}{}^{35}ClN_3OSSi$ requires C, 57.84; H, 6.93; N, 9.64; S, 7.35 %).

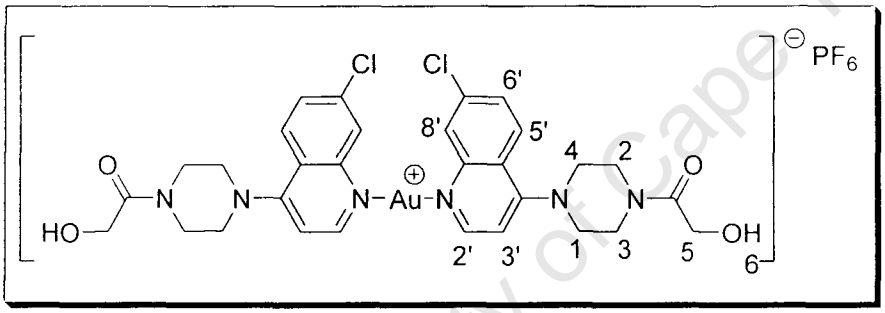
N-{3-[7-chloro-1-(chlorogold)quinolin-4-ylamino]propylcarbamothioyl} benzamide, 31



A solution of Au(THT)Cl (50 mg, 0.16 mmol) was stirred in anhydrous THF under nitrogen at 0 °C prior to the addition of an anhydrous THF solution of **11** (62 mg, 0.16 mmol). The mixture was stirred overnight (0 °C to r.t.), whereafter the precipitated product was filtered, washed with THF, and dried under reduced pressure. The purified product was obtained as a yellow powder (62 mg, 63 %), m.p. 185 °C (melting and decomposition from this temperature); I.R. ν_{max}(KBr)/cm^{-1} 3227 (N-H), 2933 (C-H), 1688 (C=O), 1613 (C=C),1589 (C=N); δ_H (400 MHz, DMSO-d_6) 11.25 (1H, bs, H6), 10.94 (1H, bs, H5), 9.37 (1H, bs, H1), 8.55 (1H, d, *J* = 6.8 Hz, H2'), 8.52 (1h, d, *J* = 9.2 Hz, H5'), 7.91 (1H, d, *J* = 1.6 Hz, H8'), 7.85 (2H, d, *J*

= 7.6 Hz, H1''), 7.74 (1H, dd, *J* = 8.8, 1.6 Hz, H6'), 7.63 (1H, t, *J* = 7.4 Hz, H3''), 7.50 (2H, t, *J* = H2''), 6.92 (1H, d, *J* = 7.2 Hz, H3'), 3.80 (2H, t, *J* = 6.4 Hz, H4), 3.63 (2H, m, H2), 2.12 (2H, quin, *J* = 6.6 Hz, H3); δ_C (100 MHz, DMSO-d_6) 179.59 (C=S), 167.61 (C=O), 155.37, 142.87, 138.33, 137.93, 132.99, 128.37 (2C), 128.33 (2C), 127.95, 126.76, 125.54, 118.96, 115.45, 98.59, 42.78, 41.06, 26.13; (Found: C, 28.83; H, 2.46; N, 5.30; S, 5.11. $C_{20}H_{19}Au^{35}Cl_2N_4OS$ requires C, 38.10; H, 3.04; N, 8.89; S, 5.08 %).

{[Goldtriphenylphosphine]-1-[4-(7-chloroquinolin-4-yl)piperazin-1-yl]-2-hydroxyethanone}hexafluorophosphate, 32



Triphenylphosphine gold(I) chloride (49 mg, 0.10 mmol) and NH_4PF_6 (32 mg, 0.20 mmol) were refluxed in dry acetonitrile (6 ml) under N_2 for 45 min. The ligand, 1-(4-(7-chloroquinolin-4-yl)piperazin-1-yl)-2-hydroxyethanone (60 mg, 0.20 mmol) was then added, and the solution stirred and refluxed for 36 h. The NH_4Cl thus produced was removed by filtration through celite. The filtrate was rotavapped, and the residue washed sequentially with CH_2Cl_2, water and Et_2O, to yield the desired gold complex as a yellow powder (59 mg, 29 %), m.p. 205 – 206 °C; I.R. ν_{max}(KBr)/cm^{-1} 3468 (N-H), 3334 (O-H), 2728 (C-H), 2657 (C-H), 1634 (C=O), 1605 (C=N), 1541 (C=C), 841 (PF_6); δ_H (300 MHz; DMSO-d_6) 8.65 (1H, d, *J* = 6.9 Hz, H2'), 8.23 (1H, d, *J* = 9.3 Hz, H5'), 7.98 (1H, s, H8'), 7.68 (1H, d, *J* = 8.7 Hz, H6'), 7.16 (1H, d, *J* = 6.9 Hz, H3'), 4.15 (2H, s, H5), 3.90 (4H, bs, H2H3), 3.73 (4H, bs, H1H4); δ_C (75 MHz; DMSO-d_6) 170.52 (C=O), 159.85, 142.58, 140.36, 137.78, 128.85, 126.25, 119.51, 117.31, 105.33, 60.22, 50.67, 42.43; δ_P (121 MHz; DMSO-d_6) -143.03 (PF_6); (Found: C, 40.06; H, 4.30; N, 8.95. $C_{30}H_{32}Au^{35}Cl_2F_6N_6O_4P.(Et_2O)$ requires C, 39.74; H, 4.12; N, 8.18 %).

8.3 Procedures for Biological Assays

8.3.1 Methodology

In Vitro* Activities of Compounds Against D10 and Dd2 *P. falciparum

The test compounds were tested in duplicate on one occasion against chloroquine sensitive (CQS) or chloroquine resistant (CQR) strain of *Plasmodium falciparum* (D10 or Dd2). Continuous *in vitro* cultures of asexual erythrocyte stages of *P. falciparum* were maintained using a modified method of Trager and Jensen (1976).[3] Quantitative assessment of antiplasmodial activity *in vitro* was determined via the parasite lactate dehydrogenase assay using a modified method described by Makler (1993).[4]

The samples were prepared to a 2mg/ml stock solution in 10% DMSO and sonicated to enhance solubility. Samples were tested as a suspension if not completely dissolved. Stock solutions were stored at -20°C. Further dilutions were prepared on the day of the experiment. Chloroquine (CQ) was used as the reference drug in all experiments. A full dose-response was performed for all compounds to determine the concentration inhibiting 50% of parasite growth (IC_{50} – value). Test samples were tested at a starting concentration of 1000ng/ml, which was then serially diluted 2-fold in complete medium to give 10 concentrations; with the lowest concentration being 1.95ng/ml. The same dilution technique was used for all samples. CQ was tested at a starting concentration of 100ng/ml. The highest concentration of solvent to which the parasites were exposed to had no measurable effect on the parasite viability (data not shown). The IC_{50}-values were obtained using a non-linear dose-response curve fitting analysis via Graph Pad Prism v.4.0 software.

In Vitro* Activities of Compounds Against W2 *P. falciparum

W2-strain *P. falciparum* parasites (1 % parasitemia, 2 % hematocrit) were cultured in 0.5 ml of medium in 48-well culture dishes.[3] The test inhibitors from 10 mM stocks in DMSO were added to cultured parasites to a final

concentration of 20 μM. From 48-well plates, 125 μM of culture was transferred to two 96-well plates (in duplicate). Serial dilutions (1 %) of inhibitors were made to final concentrations of 10 μM, 2 μM, 0.4 μM, 80 nM, 16 nM and 3.2 nM. Cultures were maintainedat 37 °C for 2 days after which the parasites were washed and fixed with 1 % formaldehyde in PBS. After 2 days, parasitemia was measured by flow cytometry using the DNA stain YOYO-1 as a marker for cell survival.[5]

References

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