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Simplified Reversed Chloroquines to Overcome Malaria Resistance to Quinoline-based Drugs

Bornface Gunsaru
Portland State University

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by

Bornface Gunsaru

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Dissertation Committee:
David H. Peyton, Chair
Albert S. Benight
Jason Podrabsky
Reuben H. Simoyi
Robert M. Strongin

Portland State University

ABSTRACT

Malaria is a major health problem, mainly in developing countries, and causes an estimated 1 million deaths per year. *Plasmodium falciparum* is the major type of human malaria parasite, and causes the most infections and deaths. Malaria drugs, like any other drugs, suffer from possible side effects and the potential for emergence of resistance. Chloroquine, which was a very effective drug, has been used since about 1945, but its use is severely limited by resistance, even though it has mild side effects, and is otherwise very efficacious. Research has shown that there are chloroquine reversal agents, molecules that can reinstate antimalarial activity of chloroquine and chloroquine-like drugs; many such reversal agents are composed of two aromatic groups linked to a hydrogen bond acceptor several bonds away. By linking a chloroquine-like molecule to a reversal agent-like molecule, it was hoped that a hybrid molecule could be made with both antimalarial and reversal agent properties. In the Peyton Lab, such hybrid "Reversed Chloroquine" molecules have been synthesized and shown to have better antimalarial activity than chloroquine against the *P. falciparum* chloroquine-sensitive strain D6, as well as the *P. falciparum* chloroquine-resistant strains Dd2 and 7G8. The work reported in this manuscript involves simplifying the reversal agent head group of the Reversed Chloroquine molecules, to a single aromatic ring instead of the two rings groups described by others; this modification retained, or even enhanced, the antimalarial activity of the parent Reversed Chloroquine molecules. Of note was compound PL154, which had IC_{50} values of 0.3 nM and 0.5 nM against chloroquine-sensitive D6 and chloroquine-

resistant Dd2. Compound PL106 was made to increase water solubility (a requirement for bioavailability) of the simplified Reversed Chloroquine molecules. Molecular modifications inherent to PL106 were not very detrimental to the antimalarial activity, and PL106 was found to be orally available in mice infected with *P. yoelli*, with an ED_{50} value of about 5.5 mg/kg/d.

Varying the linker length between the quinoline ring and the protonatable nitrogen, or between the head group and the protonatable nitrogen, did not have adverse effects on the antimalarial activities of the simplified Reversed Chloroquine molecules, in accord with the trends observed for the original design of Reversed Chloroquine molecules as found from previous studies in the Peyton Lab. The simplified Reversed Chloroquine molecules even tolerated aliphatic head groups (rather than the original design which specified aromatic rings), showing that major modifications could be made on the Reversed Chloroquine molecules without major loss in activity.

A bisquinoline compound, PL192, was made that contained secondary nitrogens at position 4 of the quinoline ring (PL192 is a modification of piperaquine, a known antimalarial drug that contains tertiary nitrogens at position 4 of the quinoline ring); this compound was more potent than piperaquine which had an IC_{50} value of 0.7 nM against CQS D6 and an IC_{50} of 1.5 nM against CQR Dd2, PL192 had IC_{50} values of 0.63 nM against chloroquine sensitive D6 and 0.02 nM against chloroquine resistant Dd2.

Finally, the mechanism of action of these simplified "Reversed Chloroquines" was evaluated; it was found that the simplified "Reversed Chloroquines" behaved like chloroquine in inhibiting β-hematin formation and in heme binding. However, the simplified "Reversed Chloroquines" were found to inhibit chloroquine transport for chloroquine resistant *P. falciparum* chloroquine resistance transporter expressed in *Xenopus* oocytes to a lesser extant than the classical reversal agent verapamil. From these studies it was noted that the simplified "Reversed Chloroquines" may not behave as well as classical reversal agents would in restoring chloroquine efficacy, but they are very potent, and so could be a major step in developing drug candidates against malaria.

To my parents David and Nelia Gunsaru, for believing in me and investing all your resources so that I could have an education.

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LIST OF ABBREVIATIONS

CNS Central nervous system

CQ Chloroquine

CQS Chloroquine-sensitive

CQR Chloroquine-resistant

DNA Deoxyribonucleic acid

dhfr Dihydrofolate reductase

dhfs Dihydrofolate synthase

DMF Dimethylformamide

DMSO Dimethyl sulfoxide

DV Digestive vacuole

FP Ferriprotoporphyrin IX

G6PD Glucose-6-phosphate dehydrogenase

NMR Nuclear magnetic resonance

OTRADI Oregon Translational Research and Drug Development Institute

PfCRT *Plasmodium falciparum* chloroquine resistance transporter

PRBC Parasitized infected red blood cell

RA Reversal agent

RBC Red blood cell

RCQ Reversed chloroquine

SAR Structure activity relationship

THF Tetrahydrofuran

CHAPTER 1

Introduction

Malaria Problem

Malaria is a major health problem, mainly in Sub-Saharan Africa, some parts of Asia, and South America as shown in Figure 1.1.[5;10] Malaria is caused by protozoan parasites belonging to the genus *Plasmodium.*[11;12] There are four major species of the parasite that cause malaria in humans, namely *P. falciparum, P. vivax, P. ovale,* and *P. malaria,* but a fifth parasite, *P. knowlesi,*[13;14] is now being recognized. *P. falciparum* is the most virulent kind of human malaria parsite.[15] *Plasmodia* cause an estimated 300 million clinical infections each year, and perhaps 1 million deaths annually, primarily in sub-Saharan Africa.[16-18]

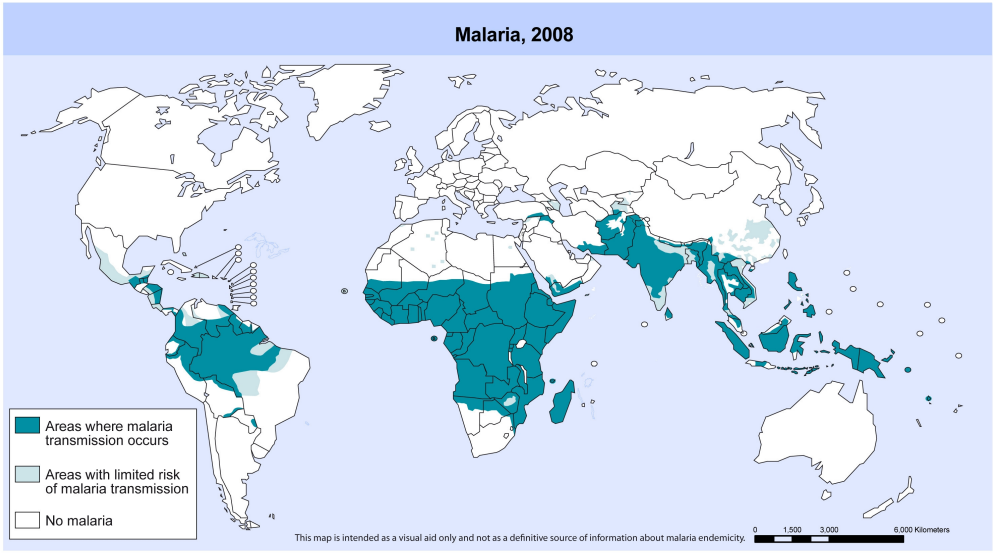


Figure 1.1: World map showing malaria risk areas shaded dark.

About 90% of the deaths occur in sub-Saharan Africa in children under the age of 5.[18] In fact, in every 30 seconds a child dies of malaria in Africa.[18] More than 40% of the world's population is in malaria risk areas.[18] Malaria symptoms include development of a fever, sore joints, vomiting, and headache.[19] Malaria can develop into convulsions, coma, and death if left untreated.[19] Children who suffer from severe cases of malaria can have learning impairments resulting from brain damage even if they survive.[20] Children who have repeated cases of malaria can develop anemia, lethargy, and generally poor development.[20] Pregnant women infected with malaria can develop placental complications, which could lead to children with low birth weights, or even death of the fetus, in addition to the rest of the malaria-related problems.[20]

The Malaria Life Cycle

The *Anopheles* mosquito is the vector that carries the parasite. During a human blood meal, an infected *Anopheles* mosquito passes the sporozoites to the human host (Figure 1.2). The sporozoites infect liver cells, where they mature into schizonts. The infected liver cells then can rupture and release merozoites, which then can infect red blood cells, where they become ring stage trophozoites. CQ is active on the ring stage of the malaria life cycle,[21] and since the simplified RCQs reported in this document are CQ-like, they are expected to act on the ring stage as well. The trophozoites mature into schizonts, which rupture the red blood cells, releasing merozoites. These merozoites infect other red blood cells; the blood stage of the life cycle is responsible for the clinical symptoms of the disease as well as death.[22]

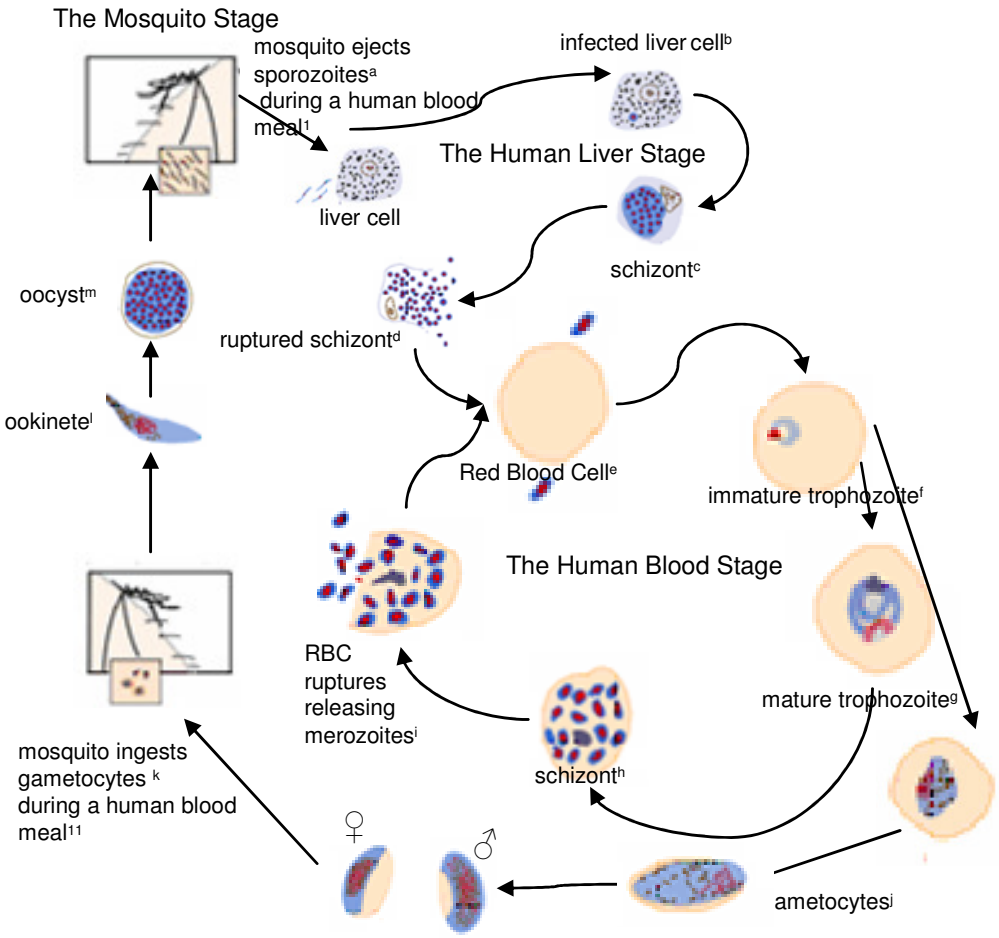


Figure 1.2: The malaria life cycle adapted from the CDC website.[1] During a blood meal, a malaria-infected mosquito injects sporozoites into the human host[a]. Sporozoites infect liver cells and mature into schizonts, which rupture the liver cells and release merozoites[b-d]. After this liver stage, the parasites undergo asexual multiplication in the erythrocytes. The merozoites infect red blood cells (where they degrade hemoglobin as a food source) [e]. Trophozoites in infected erythrocytes, mature into schizonts, the red blood cell ruptures releasing more merozoites into the blood stream[g-i]. Some parasites differentiate into gametocytes. CQ and other quinoline ring system drugs are believed to act on the blood stages of the malaria life cycle.[6] The gametocytes, male (micro gametocytes) and female (macro gametocytes) are ingested by mosquito during a blood meal[k]. In the mosquito's stomach, the micro gametes enter the macro gametes creating zygotes. The zygotes in turn become ookinetes which develop into oocysts[l-m]. The oocysts grow, rupture, and release sporozoites which travel to the mosquito's salivary glands. Injection (during a blood meal) of the sporozoites[a] into a new human host repeats the malaria life cycle[a].

Some parasites of *P. falciparum* may mature from the ring stage to become male and female gametocytes, which may be ingested by an *Anopheles* mosquito during a blood

meal. In the stomach of the mosquito, the male gametocytes penetrate the female gametocytes forming zygotes. The zygotes become elongated and motile ookinetes. The ookinetes invade the midgut wall of the mosquito where they develop into oocysts. The oocysts grow, rupture, and release sporozoites which travel to the mosquito's salivary glands. The sporozoites can be passed on to a human host, thus perpetuating the cycle. In the liver stage, *P. vivax* and *P. ovale* may also develop hypnozoites, which remain dormant and can persist in the liver. Relapses can occur when invasion of the red blood cells occurs, and this can take weeks, months, or even years.[1]

The History of Antimalarial Drugs

Quinine was introduced in 1632 when the bark of Cinchona trees from South America was used to treat fevers.[23] Resistance to quinine was noted in 1910.[24] Quinine is still in use today, although it is not a primary drug. Primary drugs are used as a first line of defense against malaria; usually they are given in combination with another drug to reduce the effects of resistance. During the second world war, the Japanese invaded Java, which was the main source of Cinchona tree plant extract that had supplied quinine for the American soldiers.[25] This resulted in massive shortages in the antimalarial drug quinine, which in turn resulted in increased death rates for soldiers infected with malaria. It became clear that alternative drugs were required, and so increased research on new antimalarial drugs began.[25]

A Malaria Drug Development Program was set up, which lead to the development of antimalarial drugs like chloroquine (CQ), amodiaquine, primaquine, proguanil, and pyrimethamine.[25]

CQ was introduced in about 1945, it was discovered earlier on by the Germans, although they abandoned its development,[23] but resistance to it was reported in 1957.[24] Amodiaquine was first used in 1951, but resistance was first reported in 1971.[24] Both CQ and amodiaquine still are in use today, although sparingly due to resistance. Artemisinin and its derivatives were introduced in the 1970s, but reports of resistance to artemisinin began to surface in 1998.[26] Mefloquine was first used in 1977, but resistance was reported in 1982.[24] However, mefloquine is still in use today. Halofantrine was used from 1988 to 1990, but discontinued due to the sometimes-fatal cardio toxicity associated with its use.[24;27]

Combination drugs were introduced to limit resistance. Combination therapy relies on the premise that the probability of development of resistance to two drugs with independent mechanisms of action is extremely low, of the order of once every 10^{12} treatments.[28] Sulfadoxine/pyrimethamine (Fansidar) was a combination drug introduced in 1967, but resistance was noted in the same year it was introduced.[24] This was rather disappointing because Fansidar is cheap, practicable (only one dose is needed because it eliminates from the body slowly), and widely used in Africa.[29] Fansidar is still in use today although resistance severely limits its usefulness.

Atovaquone/proguanil (Malarone) was first used in 1996, but resistance to it was reported in 2002.[30] Malarone is still in use today. It can be seen that malaria has developed resistance to most, if not all, of the drugs that have been introduced, and so there is a need to reverse this resistance.

From this history it can be seen that several drugs are used against malaria,[31-34] including those derived from the 4-substituted quinoline ring system. Examples of drugs that fall in this class are chloroquine, amodiaquine, piperaquine, mefloquine, and quinine, shown in Figure 1.3.

Drugs of the 4-substituted quinoline class are generally thought to act on the blood stages of the malaria life cycle.[35] 7-chloro-4-aminoquinoline derivatives, including CQ and amodiaquine, are among the most potent antimalarial drugs reported to date.[36-38] CQ has been postulated to prevent the conversion of ferriprotoporphyrin IX (FP) into an insoluble material called hemozoin, as shown in Figure 1.4.[6]

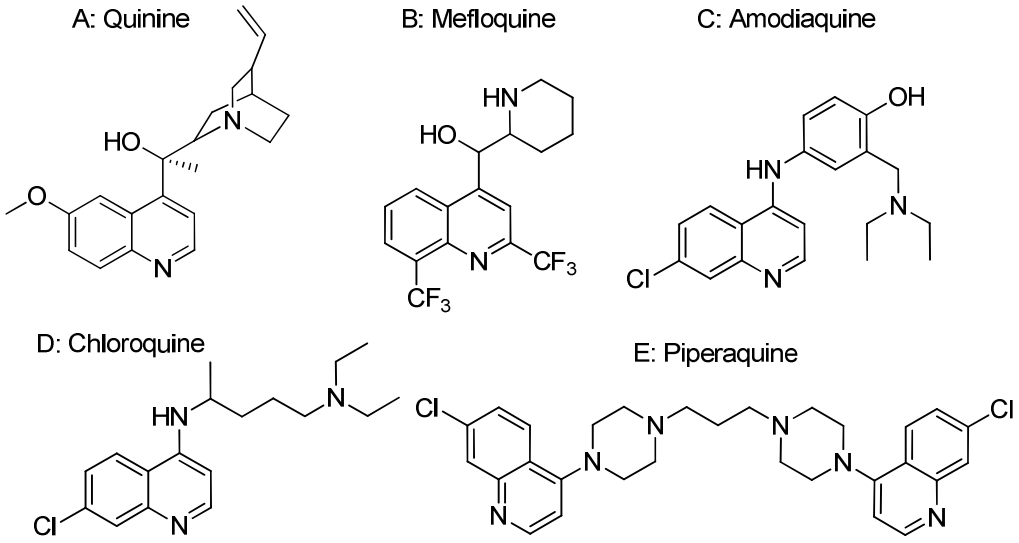


Figure 1.3: Examples of the 4-substituted quinoline ring system drugs. A: quinine, having a 7-methoxy functional group off the quinoline ring; B: mefloquine, which has triflouromethyl functional groups at the 2 and 8 positions; C: amodiaquine, which has a 7-chloro functional group; D: chloroquine, which has the 7-chloro functional group; E: piperaquine, which has two quinoline rings; A, B, C, D, and E have aliphatic chains at the 4 position of the quinoline ring.

The release of soluble FP upon hemoglobin digestion by the parasite reaches a level which would become toxic to the parasite if it were not sequestered into hemozoin.[6] It is also noted that CQ accumulates in the digestive vacuole (DV) of malaria parasites;[39] this accumulation is reduced in chloroquine-resistant (CQR) strains.[40] Out of these quinoline ring system drugs, CQ emerged as the most important. CQ was safe, effective, widely used, and inexpensive. CQ could be used to treat pregnant women and children who account for most of the deaths associated with malaria, but *P. falciparum* developed resistance to it by 1957, as mentioned above, in Southeast Asia

(Thai-Cambodia border) and in South America.[41;42] In 1978 CQ resistance had spread into East Africa.[43] Between 1978 and 1988, reports of resistance to CQ began to surface in all the countries of tropical Africa.[43] This is important because CQR malaria accounts for the majority of malaria associated-deaths.[44]

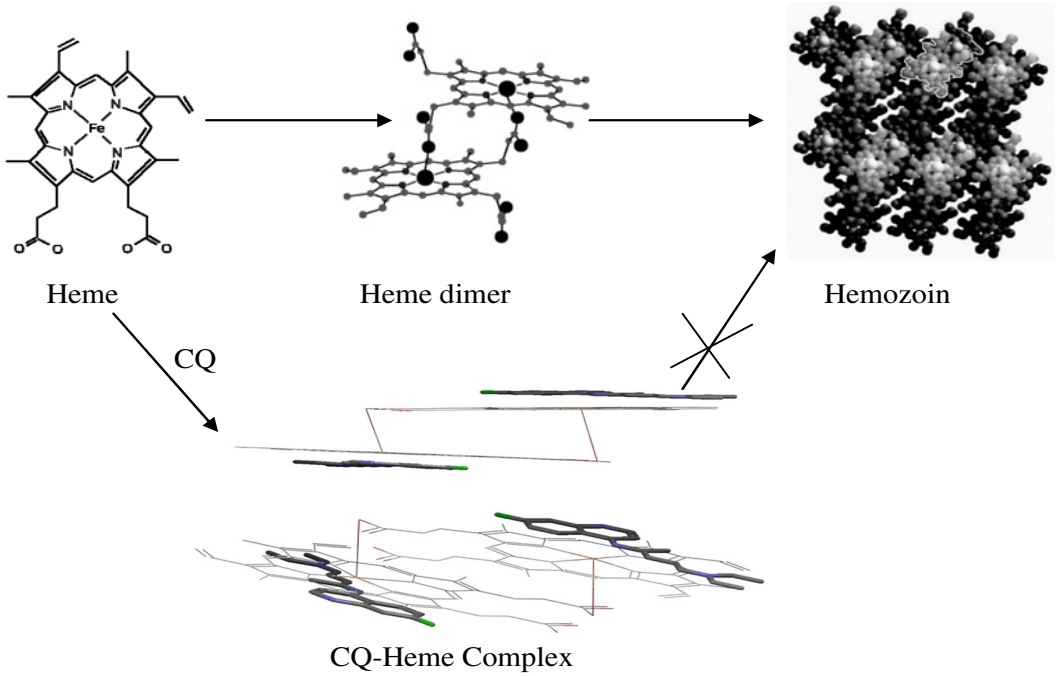


Figure 1.4: Presumed CQ mode of action. Free heme in the parasite forms heme dimers which mineralize into hemozoin. In the presence of CQ hemozoin formation is inhibited. CQ caps the ends of heme dimers to form a sanguage like complex.[6;7]

CQ resistance is linked to a mutation in codon 76 of the *P. falciparum* CQR transporter (PfCRT), resulting in a change of lysine to threonine (K76T).[45] PfCRT is located on the membrane of the DV of *P. falciparum* during the blood stage of the parasite's life cycle.[46] Mutations at other positions, when in the presence of K76T, can give rise to different strains, having different degrees of resistance. *P. falciparum*

strains with the K76T point mutation accumulate less CQ in the DV, presumably because CQ efflux from the DV is enhanced, as shown in Figure 1.5.[8] It has been noted that all clinical isolates that did not respond to CQ treatment have this point mutation.[45]

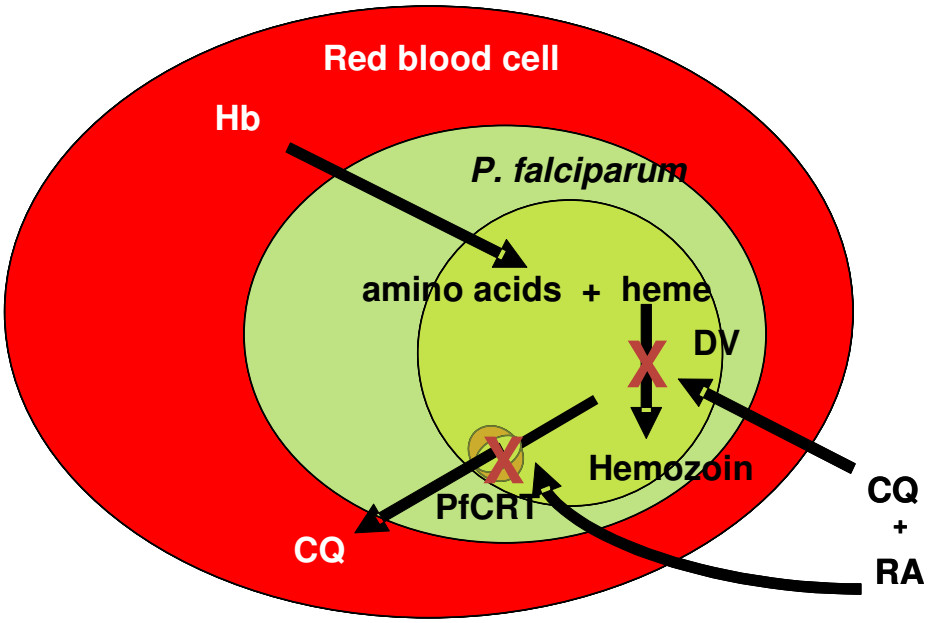


Figure 1.5: CQ resistance and presumed mechanism. CQ enters the parasites DV by passive diffusion through the red blood cell and *P. falciparum* membranes. The pH in the DV is low (~5), hence CQ becomes protonated and trapped in the DV. The malaria parasite digests Hemoglobin into amino acids (used for the parasites metabolism) and free heme which is toxic to the parasite. Heme is mineralized by the parasite to hemozoin which is not toxic to the parasite. CQ prevents heme from mineralizing; this is lethal to the parasite. CQR strains accumulate less CQ in the DV and this has been associated with PfCRT.[8;9] RA are compounds that reverse the CQ resistance.

Mutations in codon 86, resulting in the change of asparagine to tyrosine (N86Y) in the *P. falciparum* multi-drug resistance gene (*pfmdr1*), have also been associated with CQ resistance.[47]

Primaquine (and other 8-aminoquinolines under development) primarily acts on the hepatic stage of the life cycle.[34;35] Examples of 8-aminoquinolines include primaquine, tafenoquine, pamaquine, and pentaquine, and are shown in Figure 1.6. Out of these 8-aminoquinolines, primaquine has been mainly used to treat patients infected with *P. vivax* and *P. ovale* and to prevent relapses from malaria.[48;49] Primaquine cannot be prescribed to patients with glucose-6-phosphate dehydrogense (G6PD) deficiency or pregnant women (because G6PD deficiency cannot be detected in the fetus), because it results in hemolysis.[49] The mechanism of action of primaquine is not clearly understood; two of the modes of action that have been proposed are given below. Primaquine is presumed to be metabolically activated in the liver to generate metabolites which can act on parasite nucleophiles. Also, infected hepatocytes and erythrocytes contain ferrous iron that can facilitate the generation of oxidative radical species which act on the parasite.[50] These 8-aminoquinolines have been demonstrated by Whichard, *et al*. to bind to DNA, which could then inhibit DNA function, and therefore result in their antimalalarial action.[51] Howells, *et al.* suggested that the resistant parasites overcome the damaging effects of 8-aminoquinolines on their mitochondria by synthesizing more mitochondria to compensate for the functional loss.[52]

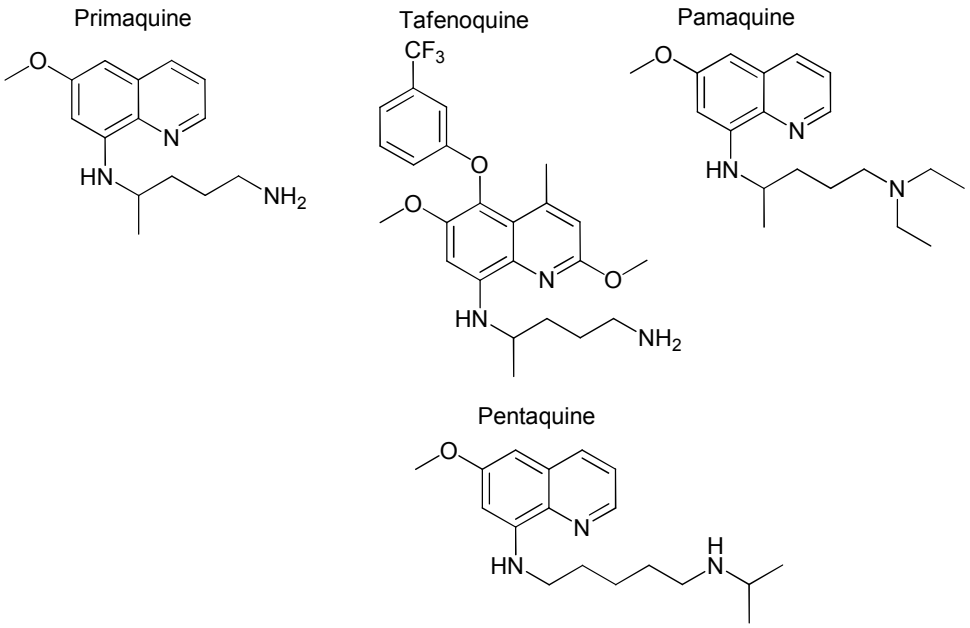


Figure 1.6: Examples of 8-aminoquinolines. Primaquine, tafenoquine, pamaquine, and pentaquine.

Antifolates are another class of compounds that act against malaria. They have been shown to inhibit the synthesis of folate cofactors selectively which are needed for amino acid and nucleotide synthesis.[53] The enzyme inhibited by these drugs is dihydrofolate reductase.[54] Sulfadoxine and pyrimethamine in combination (Fansidar) show synergism, increasing their effectiveness against *P. falciparum*.[22] Examples of antifolates are shown in Figure 1.7.

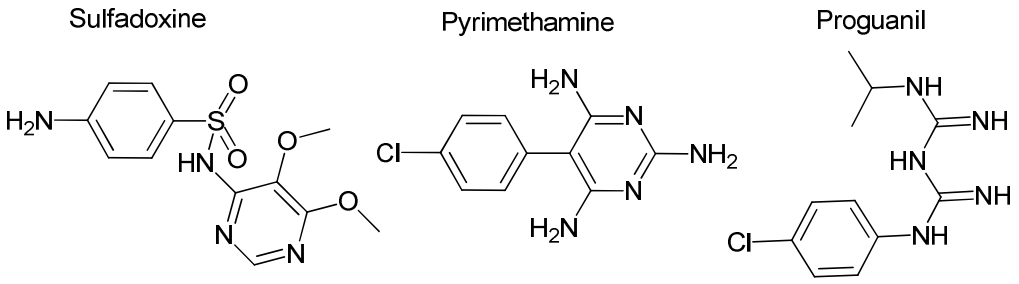


Figure 1.7: Examples of antifolates. Sulfadoxine, pyrimethamine, and proguanil.

Point mutations at the dihydrofolate reductase (*dhfr*) codon 108, which result in a change of serine to asparagine (S108N), are linked to pyrimethamine resistance, while a change of serine to threonine (S108T) along with a change in alanine to valine (A16V), is associated with resistance to cycloproguanil and chloroproguanil.[47] Mutations have also been found which are associated with sulfadoxine resistance in dihydropteroate synthase (*dhps*) genes on codons 436, 437, 540, and 581.[55;56] Mutations in both *dhfr* and *dhps* both affect their catalytic functions in folate biosynthesis.

Artemisinin and two of its most important derivatives are shown in Figure 1.8. Artemisinin is water insoluble, thus not very bioavailable; hence the derivatives artesunate and artemether were synthesized to increase water solubility and bioavailability.[57] These compounds have a wide spectrum of activity, including being effective against the ring stage of the parasite.[58] Artemisinins may also suppress

gametocyte transmission.[58;59] Artemisinins accumulate in the *P. falciparum*-infected red blood cells, and are thought to kill the parasite via the action of free radicals generated by the action of ferrous iron or exogenous free iron.[60]

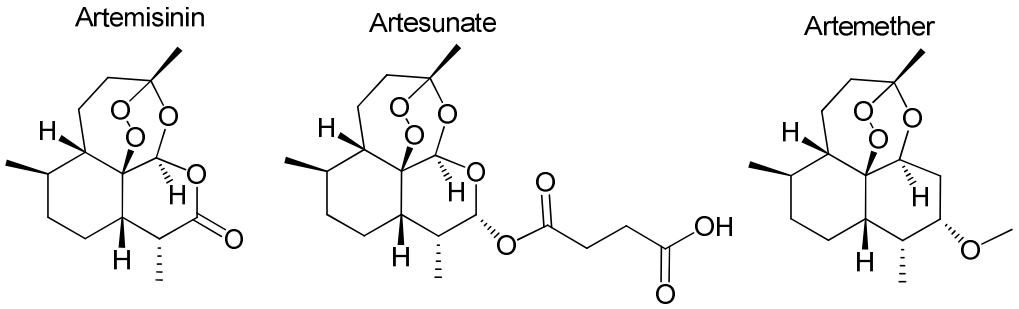


Figure 1.8: Examples of the artemisinin group of compounds. Artemisinin, artesunate, which has a carboxylic acid side chain, and artemether, having a methoxy side chain.

Although artemisinin has been quite effective in the fight against malaria, there are reports on artemisinin resistance due to a change in serine to asparagine (S769N) on the sarco/endoplasmic reticulum calcium-dependant ATPase (SERCA; *PfATPase6*) gene.[61] However, others have shown doubts on artemisinin even inhibiting SERCA; *PfATPase6.*[62]

These are the main groups of drugs in use today, although other drugs like doxycycline are still used in different parts of developing countries.[63;64]

Other Motivations for Continued Development of New Antimalarial Drugs

A number of different side effects have been linked to antimalarial drugs. For example, Malarone is associated with abdominal pain, nausea, vomiting, and headaches. Malarone is not safe for pregnant women, nor children weighing less than 25 pounds.[19] Mefloquine-induced side effects include headaches, nausea, dizziness, CNS-associated vivid dreams, and anxiety.[19] The side effects associated with artemisinin combination therapies include mild gastralgia, vomiting, dizziness, and asthenia.[65] These side effects have, in turn, resulted in the erratic use of antimalarial drugs, which then fuels the progression of antimalarial drug resistance.[66]

The high cost of many of the more effective drugs, relative to the ability of the endemic market's ability to pay, is another factor that leads to erratic antimalarial drug use. Detailed research on malaria drugs has often been limited, and as a result prescribers are not in a position to give a detailed explanation of drug usage as well as side effects.[66] There is a continued need for the development of newer drugs as a result of resistance, side effects, and the cost of many of the current drugs on the market. CQ, with its few side-effects, safety, and low cost is a regrettable loss. In fact, CQ is still being used, although it often fails due to CQ resistance. It would be good if there were a way to re-introduce CQ or something very much like it, as an effective drug.

Reversal Agents

Reversal agents (RAs) are compounds that are known to reverse CQ resistance. RAs in the presence of CQ, can reinstate the antimalarial activity of CQ in CQR strains of *P. falciparum*. RAs help prevent the export of CQ from the DV as shown in Figure 1.5, so that CQ can exert its antimalarial action in the DV.

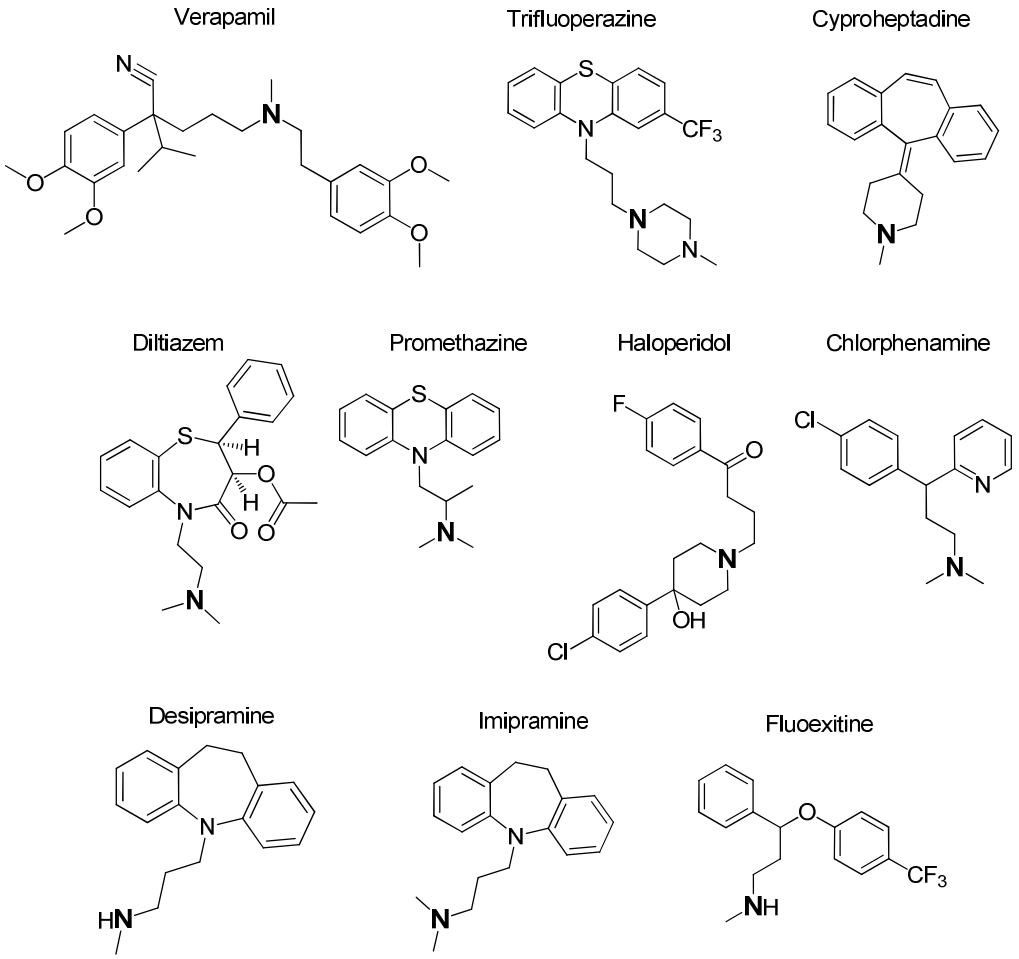


Figure 1.9: Examples of reversal agents. They all have 2 phenyl head groups linked via an aliphatic chain to a hydrogen bond acceptor (generally nitrogen, shown in bold in the drawings).

Verapamil (Figure 1.9) was discovered to reverse CQ resistance,[67] and in due course certain tricyclic antidepressants[68] and antihistamines[69] were also been shown to

reverse CQ resistance; these compounds are termed chemosensitizers, or RAs. Examples of RAs shown to overcome antimalarial resistance are shown in Figure 1.9.

A three-dimensional QSAR pharmacophore model for CQR reversal was developed from imipramine, desipramine, and 15 of their analogues.[2] The pharmacophore was constructed from two aromatic hydrophobic interaction sites linked by an aliphatic chain to a hydrogen bond acceptor site (generally nitrogen)[2] as shown in Figure 1.10.

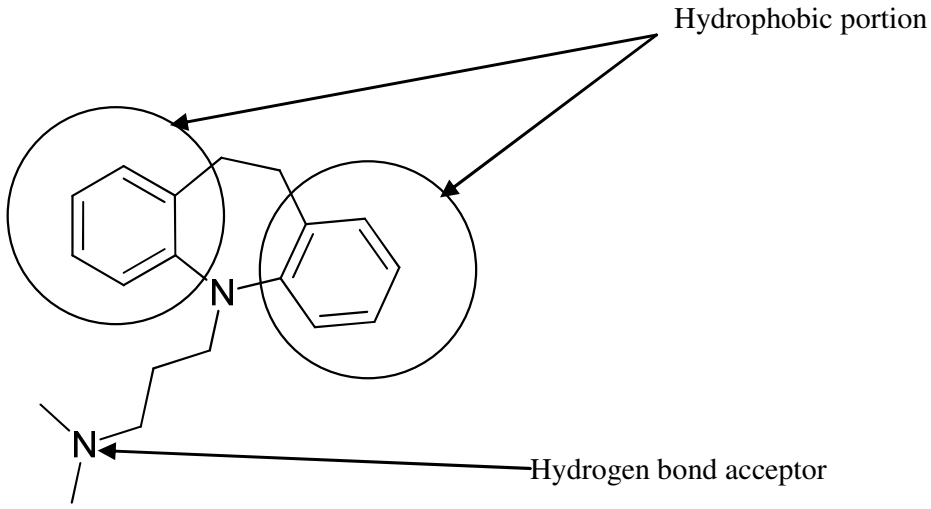


Figure 1.10: The RA pharmacophore as proposed by Bhattacharjee and his group is two aromatic rings linked via an aliphatic chain to a hydrogen bond acceptor (generally nitrogen). Note that the RA shown in this diagram in imipramine

Initial Work on Reversed Chloroquines (RCQs)

In the Peyton Laboratory (Portland, Oregon), it was postulated that by linking a RA-like moiety to a CQ-like moiety, it would be possible to create a class of hybrid molecules that have antimalarial properties and can overcome resistance as well.[4]

Others, have modified CQ and created new compounds with improved activity to CQR strains of *P. falciparum*.[70-74] One advantage of these hybrid molecules is that, in principle, it might be administered at a lower dose than the RA component of a cocktail. Proteins involved in drug efflux in the DV of the malaria *P. falciparum* parasite may also fail even to recognize this hybrid molecule and thus fail to export the drug, rendering the drug active in the DV. Reducing the dose could make the hybrid drug cheaper and reduce the side effects, including toxicity that may be associated with administering two separate drugs. Figure 1.11 shows PL01, the first such molecule, which was synthesized by Steven Burgess in the Peyton Laboratory.[4]

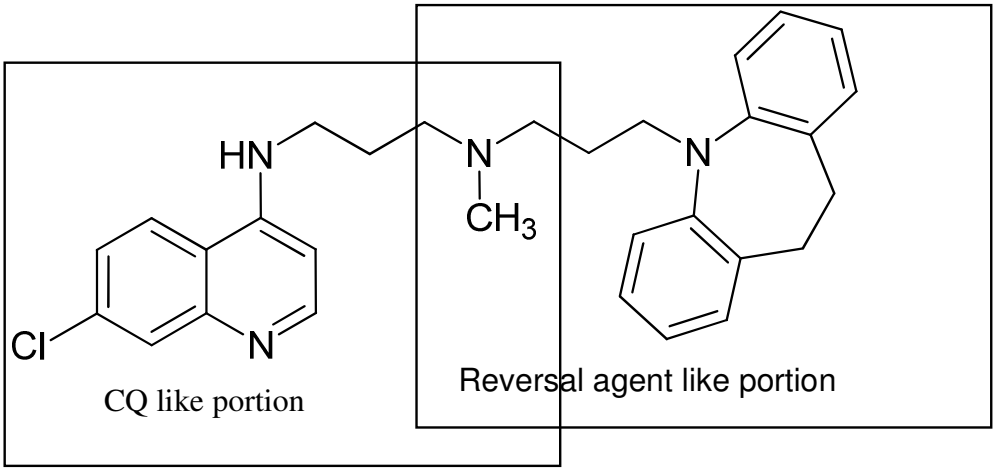
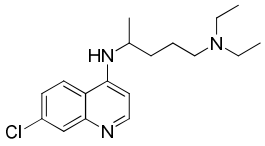
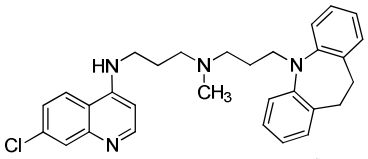
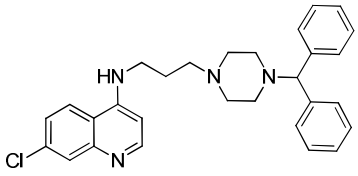


Figure 1.11: The hybrid molecule PL01. The first molecule synthesized in the Peyton lab that is composed of a link between a CQ like portion and a RA like portion (i.e. an imipramine like portion).

The postulated hybrid molecule approach was demonstrated to be viable, as shown by the results in Table 1.1. The hybrid compound PL01 was potent against both CQS and CQR strains of *P. falciparum.* In fact, PL01 had better activity than CQ for both CQS and CQR strains of *P. falciparum*.[4] PL06 (Figure 1.12) was subsequently made by Steven Burgess in the Peyton Lab. PL06 is a modification of PL01 that deleted the center ring of the tricyclic group of the "tricyclic antidepressant" imipramine RA; however it retains the antimalarial activity of PL01.[75]

Table 1.1: IC_{50} values of the previous compounds made in the Peyton Lab.[4] The values of CQ have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50}.

Compound	Structure	IC_{50} value in nM	
		D6	Dd2
CQ		6.9	102
PL01		2.9	5.3
PL06		2.4	3.7

It was thus decided to alter the RA head group and so deviate further from the RA pharmacophore proposed by Bhattacharjee, *et al.,*[2] which stated that there was need for two aromatic rings and a hydrogen bond acceptor several bonds away.

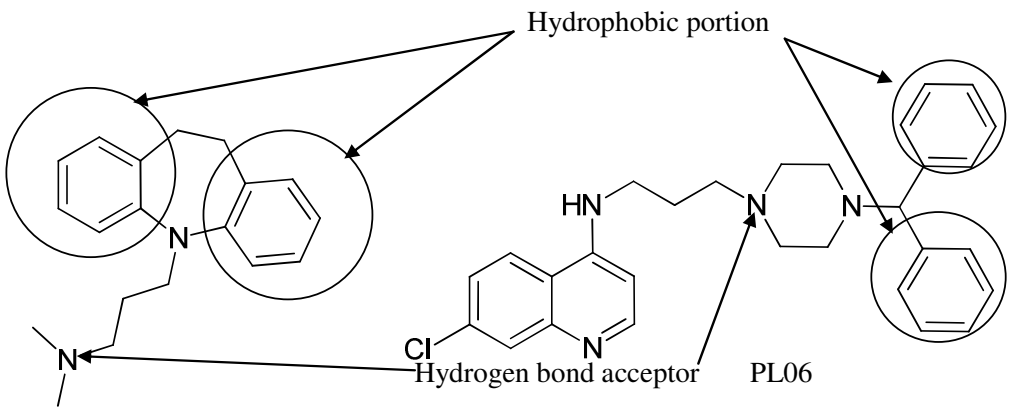


Figure 1.12: PL06 a modification of PL01, this diagram shows how PL06 fits the pharmacophore proposed by Bhattarcharjee, *et al*.[2]

The next Chapter introduces work that was done to further simplify the RCQs.

CHAPTER 2

The Approach

In the first Chapter, the idea of RCQs was introduced. In the work presented here, the RA is more drastically changed from the RA pharmacophore described by Bhattacharjee, *et al*.,[2] in order to obtain an expanded structure activity relationship (SAR) of the RCQs. Initially, PL112 was made which contained a biphenyl ring system shown in Figure 2.1 instead of the branched pair of aromatic rings proposed by Bhatacharjee, *et al*..[2] From PL112 we can see that the hydrogen bond acceptor of the pharmacophore has been maintained but the hydrophobic portions have been significantly altered; instead a biphenyl group is presented in place of the branched aromatic rings that are described in the pharmacophore by Bhattacharjee, *et al*..[2]

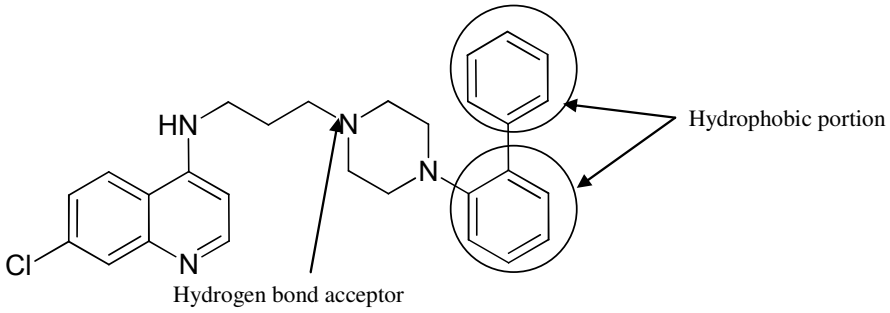
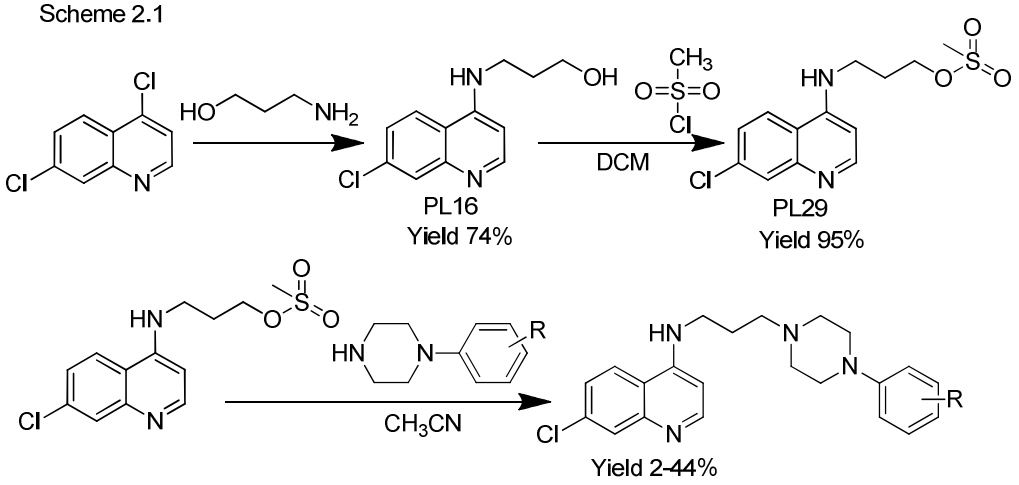


Figure 2.1: PL112 the first compound synthesized with a variation in the RA head group to a biphenyl group.

PL112 and most of the compounds mentioned in this thesis were made via Scheme 2.1. First, 4,7-dichloroquinoline was treated with excess 3-aminopropan-1-ol to make PL16. Next, PL16 was treated with methanesulphonylchloride to make PL29.[4] PL29 was then used as a starting material for addition of the piperazine analogues to make PL112 and most of the compounds mentioned in this thesis.

Scheme 2.1



Despite these alterations to the RA portion of the RCQs, it was noted that PL112 had good antimalarial activity (Table 2.1). This was a surprising improvement to the activity of PL06, and so further variations in the head group were made, specifically altering the arrangement of the RA phenyl groups from meta, to ortho, and then to the para position as shown in Figure 2.2.

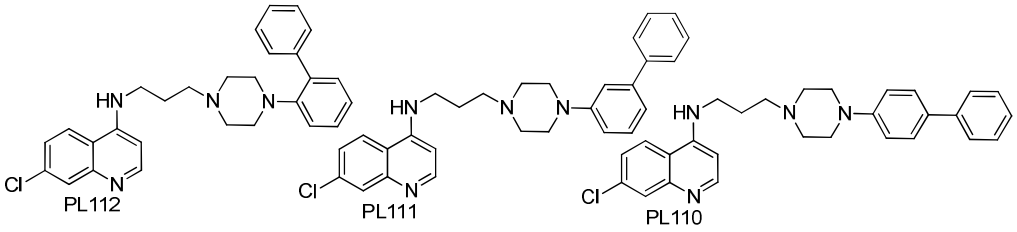
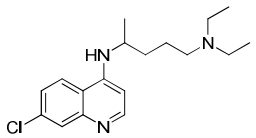
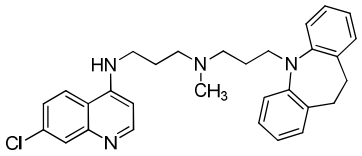
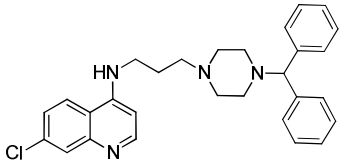
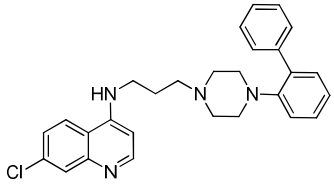
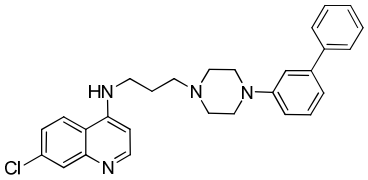
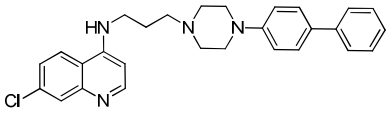


Figure 2.2: Initial compounds synthesized with a biphenyl head group with variations from the ortho, to meta, then to the para position.

This initial group of compounds was designed as an enquiry as to how far the RA head group could be perturbed with retention of antimalarial activity. These results are shown in Table 2.1, and demonstrate that having a biphenyl head group enhances the activity of the compounds slightly. PL110 which has a linear arrangement of the phenyl groups has increasingly lower IC_{50} values than the other compounds in the series, with IC_{50} values of 0.7 nM and 0.6 nM for CQS D6 and CQR Dd2, respectively. These results were surprising because the linear head groups appear to deviate from the branched phenyl groups pharmacophore described by Bhattacharjee, *et al.*.[2]

Table 2.1: Biphenyl ring system compounds. The values of CQ, PL01, and PL06 have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

Compound	Structure	IC_{50} value in nM	
		D6	Dd2
CQ		6.9	102
PL01		2.9	5.3
PL06		2.4	3.7
PL112		1.2	2.6
PL111		0.9	1.8
PL110		0.7	0.6

These results are a clue that a simple model of CQ linked to the RA pharmacophore is not the full explanation of how more potent IC_{50} values may be obtained for the RCQs. Figure 2.3 shows a graphical representation of the activities of the biphenyl ring system compounds.

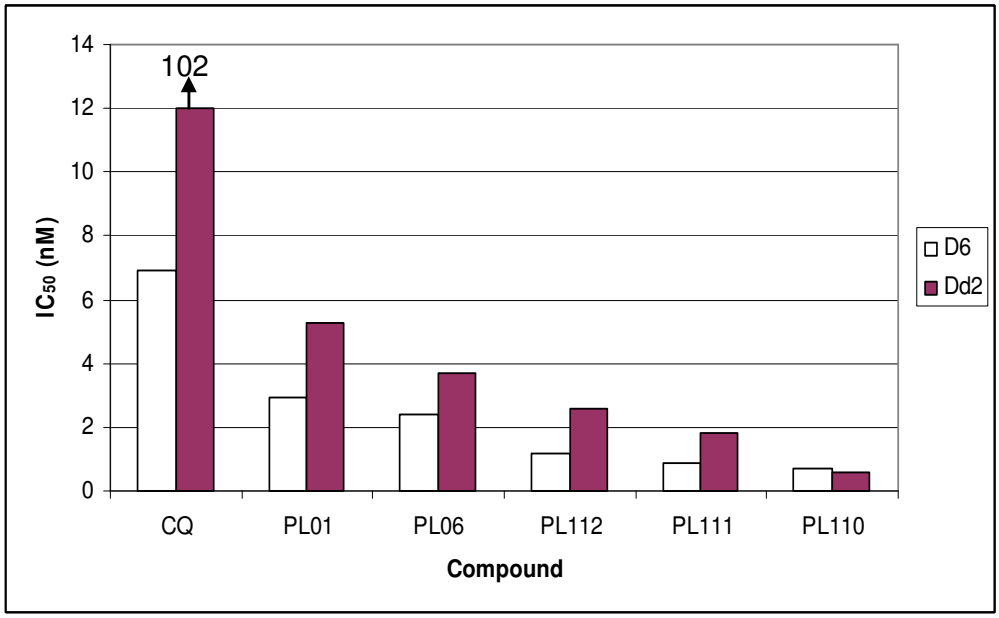


Figure 2.3: Activity of biphenyl ring system compounds PL112, PL111, and PL110. CQ, PL01, and PL06 have been included for comparison. There is a 30% uncertainty in the activities of the compounds that results from weighing and obtaining IC_{50} values. The white bars are for CQS strain D6 and the grey bars are for CQR strain Dd2.

Single Phenyl Head Group

From the results above, it was hypothesized that one of the phenyl groups could be removed without substantial loss of antimalarial activity, as illustrated in Figure 2.4.

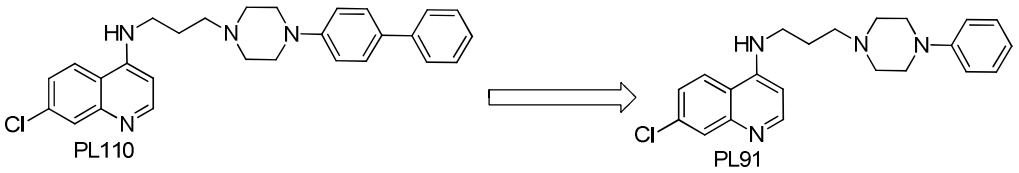
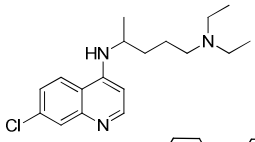
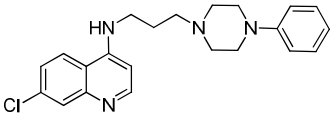
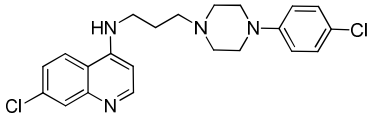
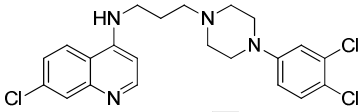
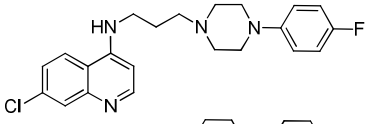
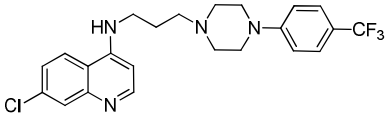
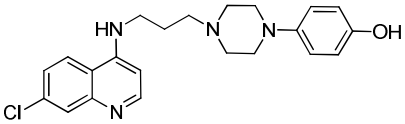
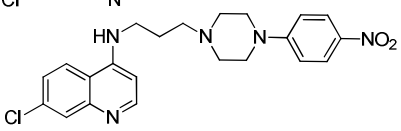


Figure 2.4: Transition from biphenyl head group to single phenyl head group.

A set of compounds with a single phenyl head group (the aromatic portion of the RA) was synthesized as outlined in Scheme 2.1. PL91 had surprisingly low IC_{50} values of 0.5 nM for both CQS D6 and CQR Dd2 of *P. falciparum*. This result was surprising because the head group is just a single aromatic group instead of the branched aromatic head groups defined by the pharmacophore. Due to this success, more compounds were made to further investigate an expanded SAR of the single head group. Initially, compounds with electron withdrawing groups on the phenyl group shown in Table 2.2 were synthesized. PL154 gave quite impressive IC_{50} values of 0.3 nM for CQS D6 and 0.5 nM for CQR Dd2. PL154 and PL156 were made to test the effect of having halogens as electron withdrawing groups. From these two compounds, it can be seen that interchanging the halogens (fluorine and chlorine) does not strongly affect the activity of the compounds. PL155 was made to test the effect of having two electron withdrawing groups on the RA aromatic head group. This change also does not strongly affect the activity of these compounds. PL159 has an additional oxygen group that generally increases water solubility as well as has electron withdrawing

properties. PL159 has hydrogen bonding potential which may have had some detrimental effects on its antimalarial activity.

Table 2.2: Single aromatic head group and electron withdrawing groups. The values of CQ have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

Compound	Structure	IC_{50} value in nM	
		D6	Dd2
CQ		6.9	102
PL91		0.5	0.5
PL154		0.3	0.5
PL155		0.9	0.7
PL156		2.0	0.2
PL158		0.06	0.2
PL159		4.1	4.1
PL257		0.9	0.8

The electron withdrawing groups did not result in large changes in the activities most of the simplified RCQs, although PL159 has a slight loss in antimalarial activity.

PL158 and PL257 had stronger electron withdrawing groups, which seemed not to reduce the activity of the compounds. It was thus noted that these changes in the electron withdrawing groups on the phenyl group were well tolerated. This is an important factor in these antimalarial compounds, because if mutations arise in the parasite, new substitutions can be made easily without loss of activity. Also, if the compounds are found to perturb certain biological functions in the body (i.e., cause toxicity and/or side-effects), alterations in the compounds can be made without major deleterious effects on the antimalarial activity of the compounds. Figure 2.5 shows a graphical representation of the activities of the single aromatic head group compounds.

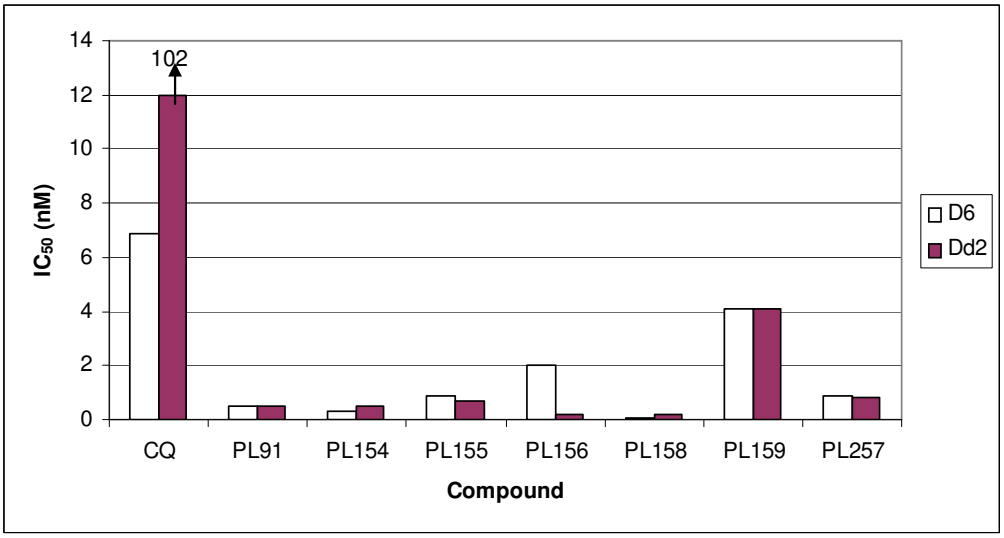
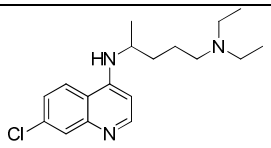
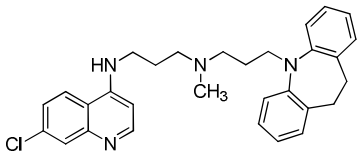
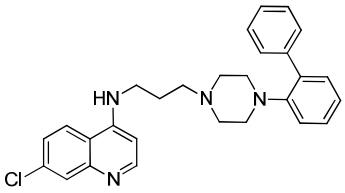
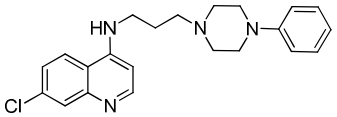
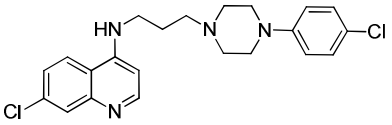
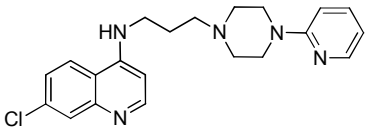
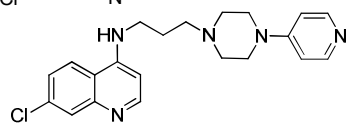
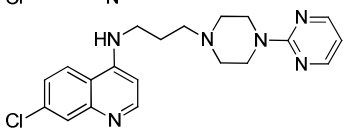


Figure 2.5: Single aromatic head group and electron withdrawing groups. The values of CQ have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50}. The white bars are for CQS strain D6 and the grey bars are for CQR strain Dd2.

Better Solubility

Lipinski's rules suggest that bioavailable small compounds generally have a ClogP value of <5.[76] In this document, ClogP is a calculated value for an organic compound given by Chemdraw Ultra 12.0 that estimates the distribution of a compound between *n*-octanol and water. In essence it is a measure of how water soluble or hydrophilic a compound is. The compounds synthesized this far, had on average, very good activities, but the ClogP values were greater than 5, suggesting that they were rather hydrophobic, and hence may be less bioavailable than desired. The initial set of compounds was found to be insoluble in water; chloride salts of these compounds were made which were equally insoluble. The salts were prepared by dissolving the compound in methanol, and then adding excess methanolic acid at less than 0^{o}C. Finally the solvent was evaporated off to get the salts. The next set of compounds that were synthesized had ClogP values of less than 5. The compounds were synthesized as outlined in Scheme 2.1. The lower ClogP values were achieved by substituting some of the carbon atoms in the compound with nitrogens. These new compounds (Table 2.3 and Figure 2.6), as free bases, were insoluble in water as well; however their chloride salts were soluble in water.

Table 2.3: Compounds with Clog P values <5. The values of CQ and PL01 have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

Compound	Structure	IC_{50} value in nM		ClogP
		D6	Dd2	
CQ		6.5	102	5.1
PL01		2.9	5.3	8.9
PL112		1.2	2.6	7.7
PL91		0.5	0.5	5.8
PL154		0.3	0.5	6.6
PL106		2.7	1.8	4.3
PL109		0.5	1.6	4.3
PL261		1.4	2.3	4.1

Despite these atomic alterations, and resultant change in ClogP value, PL106 was potent to within an order of magnitude as PL91, the analogous compound, with IC_{50} values of 2.7 nM and 1.8 nM for CQS D6 and CQR Dd2. PL109 (Table 2.3) shows that moving the nitrogen in the pyridine ring from the ortho to the para position does not adversely affect the activity of the PL106. PL261 was synthesized to increase solubility further. This compound tolerated the addition of another nitrogen group in that the activity of the compound did not change by a great margin.

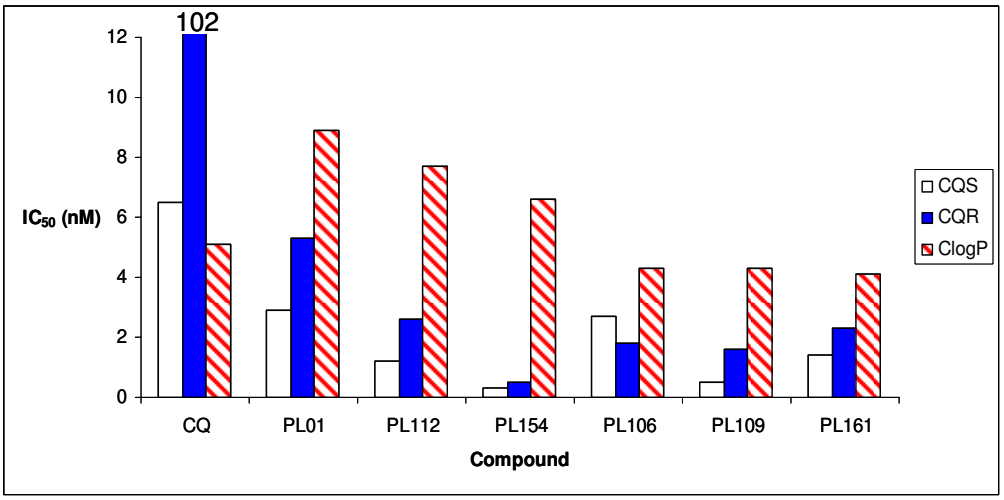


Figure 2.6: Compounds with Clog P values <5. The values of CQ and PL01 have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7). The white bars are for CQS strain D6 and the grey bars are for CQR strain Dd2, the bars with the diagonal

Toxicity is another hurdle that any drug development program needs to overcome. PL106 and PL261 have superior cytotoxicities of 13 300 and 28 000 respectively against human hepatic cancer cells. These compounds (PL106 and PL261) have high antimalarial potency and yet very low toxicity, for a "therapeutic index"

(cytotoxicity/efficacy) of 4 900 for PL106 D6 and 7 300 for PL106 Dd2; for PL261 the "therapeutic index" is 20 000 for D6 and 12 100 for Dd2. These values are far better than our calculated "therapeutic index" values for CQ: 1 700 for D6 and only 120 for Dd2 and thus show that these compounds could be potential drug candidates.

In vivo Studies

In vivo tests were done, to test for bioavailability of the compounds as well as possible signs of toxicity. PL106 was tested at the Swiss Tropical and Public Health Institute (Basel, Switzerland) by Sergio Wittlin and Reto Brun, in mice infected with *P. berghei*, and cured 1 out of 3 mice at a dose of 30 mg/kg/d in a 30-day trial, as shown in Table 2.4. Detection of parasitemia was done on day 4, and it was noted that the control mice had 63 580 parasitized red blood cells (RBCs), while the mice dosed with PL106 had no detectable parasitized RBCs on day 4. It is expected that at a higher dosage, all of the mice may be cured of malaria. Also, PL106 may be administered in combination with another drug that may clear the remaining parasites. These results were very encouraging, and lead us to do more *in vivo* studies as shown below. *P. falciparum* is very specific to the human host, hence mouse models of *P. falciparum* are not generally viable.[77] However, work has been done to express the different stages of the *P. falciparum* life cycle in mice, so that in the future it is hoped that the complete life cycle of *P. falciparum* can be expressed in a single mouse.[77]

Table 2.4: *In vivo* mouse studies with *P. berghei*. PL106 was administered at 30 mg/kg/d for 4 days. Parasetemia was determined on the 4th day. 0 represents undetectable levels of parasetemia.

Test	Parasitized RBC	Mouse survival in days
Control 1	635 80	4
PL106, mouse 1	0	14
PL106, mouse 2	0	16
PL106, mouse 3	0	30

Tests on *P. yoelii* have been done by Marty Smilktein, at the Oregon Translational Research and Drug Development Institute (OTRADI, Portland, Oregon) to test further the mouse efficacy of some selected compounds. Results for these tests are shown in Table 2.5. For these experiments CF-1 out-bred mice were infected with *P. yoelli*. Forty mice were injected intravenously through the tail vein with 5 X 10^5 infected RBCs. The mice were placed in groups of 4 with a water control, in order to evaluate PL154, PL157, PL106, and PL261. The mice were dosed at 1, 4, 16, 64 mg/kg/d with the simplified RCQ salts and weighed daily. Treatments were administered once daily X 3, then 24 hours after the final dose, blood smears were made and parasetemia determination by counting by light microscopy. Compounds PL106 and PL261 were found to be orally available, with ED_{50} values of 5.5 and 6.0 mg/Kg/d respectively.

Table 2.5: *In vivo* mouse tests with *P. yoelii.*

Compound	ED_{50} (mg/Kg/d)
PL154	<<15
PL157	<<15
PL106	5.5
PL261	6.0

Short toxicity studies were also carried out on the mice. One mouse received 500 mg/kg/d X 2 days, and another mouse received 500 mg/kg/d X 1 of PL157. Both mice had previously been infected with *P. yoelii* 2-3 days before dosing. The maximum weight loss was ~10%, and this weight loss leveled off after dosing stopped, suggesting absence of ongoing toxicity. The only visible effect that was noted was minimal lethargy 1 hour after the highest dosing which may have reflected the large volume (0.5 mL) required for this high dose. From these results it was noted that 500 mg/kg of PL157 was well tolerated, hence the compounds were not toxic according to the experiment.

Summary of the Approach

From these results, it is possible to introduce the biphenyl head group to the RCQs and still maintain antimalarial activity. Modifications that result in a single phenyl group were also well tolerated by these compounds, and it was noted that electron withdrawing groups resulted in compounds with increased potency. However, these

compounds were rather water insoluble and so pyrimidine and pyridine head groups were introduced to increase solubility. This change did not adversely affect the antimalarial activities of the simplified RCQ compounds. From the *in vivo* mouse studies, it can be seen that PL106 is a viable drug candidate because it was orally available. Figure 2.7 shows a graph of *in vitro* potencies of all the compounds presented in this chapter. From this graph we see that the compounds all have activities lower than CQ against either CQS or CQR *P. falciparum* strains D6 and Dd2 respectively, showing that modifications on the head group can be made without loss of activity.

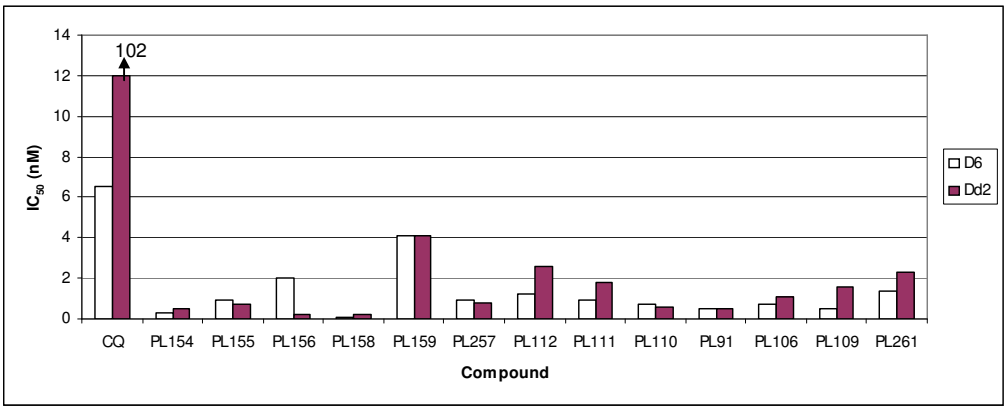


Figure 2.7: IC_{50} values for CQ and synthesized PL compounds. The white bars are for CQS D6 strain and the grey bars are for the CQR Dd2 strain, of *P. falciparum* malaria.

CHAPTER 3

Assessing the Boundaries for the Simplified RCQs Pharmacophore

Aliphatic Head Group

In the previous chapter, simplified RCQs with good bioavalability and high activity were introduced. In the work presented here, compounds were synthesized to assess the boundaries of the RCQ pharmacophore. Initially, compounds were made to test whether having an aliphatic, rather than an aromatic, head group would be detrimental to the antimalarial activity of the compounds. Bhattacharjee, *et al*. proposed that RAs contain aromatic head groups, so having aliphatic head groups is a major deviation from the proposed pharmacophore, as shown in Figure 3.1

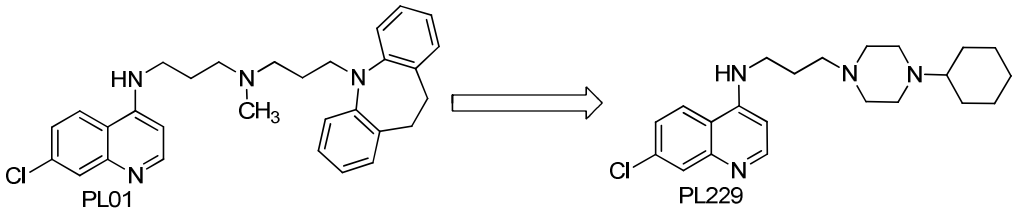
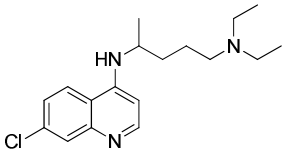
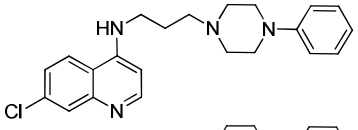
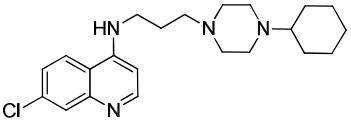
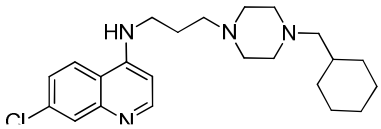


Figure 3.1: PL229: A compound with an aliphatic head group instead of the aromatic head group proposed by Bhattacharjee, *et al.*

In the Peyton Lab an RCQ having an adamantane head group has been synthesized by Steven Burgess and shown to have antimalarial activity of < 2nM for both CQS D6 and CQR Dd2 *P. falciparum* strains of malaria. Table 3.1 shows the antimalarial results for the aliphatic head group compounds that were synthesized for this study.

These compounds were synthesized as outlined in Scheme 2.1. PL228 was synthesized to test the addition of a methylene group between the piperazine ring and the now aliphatic head group. Surprisingly, PL228 and PL229 had IC_{50} values within the same order of magnitude with the other simplified RCQs. This further verified the fact that even major variations in the head group do not negatively affect the activity of these compounds.

Table 3.1: Antimalarial activity of compounds with aliphatic head groups. The values of CQ and PL91 have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

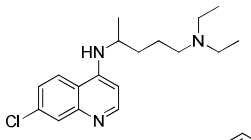
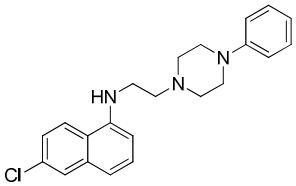
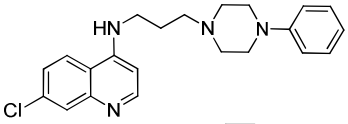
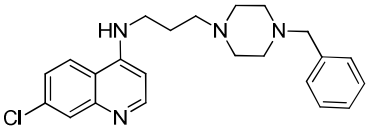
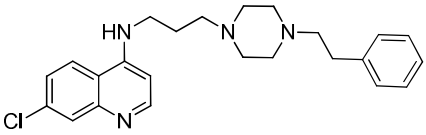
Compound	Structure	IC_{50} value in nM	
		D6	Dd2
CQ		6.9	102
PL91		0.5	0.5
PL229		1.0	2.0
PL228		0.2	0.4

Linker Length

Previous work in the Peyton Lab[78] has shown that varying the linker length between the quinoline ring and the protonatable nitrogen, or between the protonatable nitrogen

and the head group, does not have adverse effects on the antimalarial activities of these compounds. This finding was tested with the simplified RCQs. PL274 was made as outlined in Scheme 2.1, although 3-aminopropan-1-ol was substituted by 2-aminoethan-1-ol. PL227 was synthesized as outlined in Scheme 2.1, and DM1020 was obtained from Steven Burgess of DesignMedix (Portland, OR). Table 3.2 and Figure 3.2 show the results for this antimalarial testing. It can be seen that varying the linker length does not strongly and adversely reduce the antimalarial activity of the compounds, in accord with previous results on other compounds in the Peyton Lab.[78]

Table 3.2: Compounds with variable linker lengths. The values of CQ have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

Compound	Structure	IC_{50} value in nM	
		D6	Dd2
CQ		6.9	102
PL274		0.5	0.5
PL91		0.3	0.5
DM1020		2.4	7.0
PL227		0.5	1.0

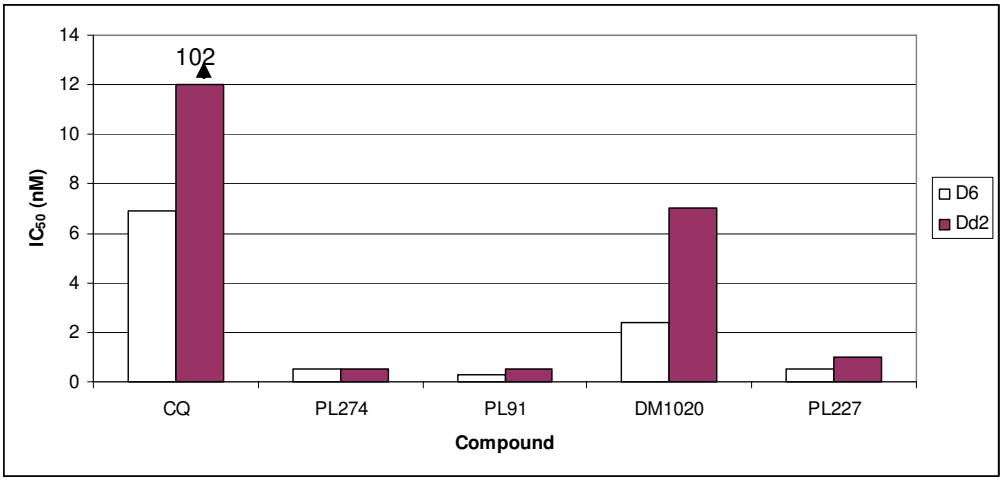


Figure 3.2: Compounds with variable linker lengths. The values of CQ have been included for comparison. There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

PL163 and PL157 (shown in Figure 3.3) were synthesized as outlined in Scheme 2.1, and PL163 has a methyl head group, which has mild electron donating properties, while PL157 has electron donating properties provided by the methoxy functional group. These modifications did not seem to have a major impact on the activity of the compounds. PL163 had IC_{50} values of 0.1 and 1.3 nM for CQS D6 and CQR Dd2 respectively. While PL157 had IC_{50} values of 1.3 and 0.3 nM, for CQS D6 and CQR Dd2 respectively.

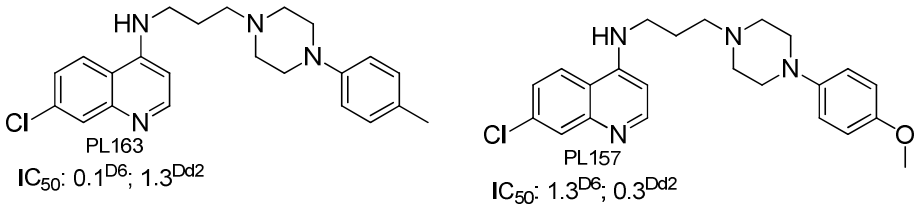


Figure 3.3: PL163 and PL157 with electron donating groups on the head group.

PL223, shown in Figure 3.4, was made with a naphthyl head group. This compound has a head group that somewhat mimics the quinoline ring. Despite this modification, there was no major change in activity from PL91. PL223 had IC_{50} values of 1.1 nM for both CQS D6 and CQR Dd2. Synthesis of this compound was as outlined in Scheme 2.1.

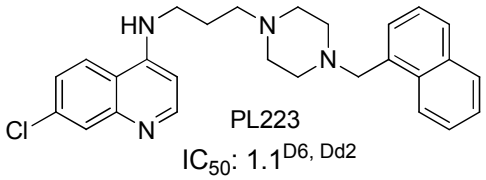


Figure 3.4: PL223 a compound with a naphthyl head group.

Compound 5b and 5c made by Fattorusso, *et al.*,[5] shown in Figure 3.5 are somewhat similar to PL106 and PL109, however, there are differences in the linkers between the pyridine rings and the quinoline rings. These differences seem to result in more potent compounds for PL106 and PL109 though the *Plasmodia* species tested were different. The higher activities of PL106 and PL109 maybe because these compounds resemble CQ, and also the head group and the quinoline ring of these compounds are behaving

like a RA. This is different in 5b and 5c which have a shorter chain towards the protonatable nitrogen which is shown in bold. Bhattacharjee, *et al*. proposed that the protonatable nitrogen is a few bonds away from the hydrophobic groups.[2]

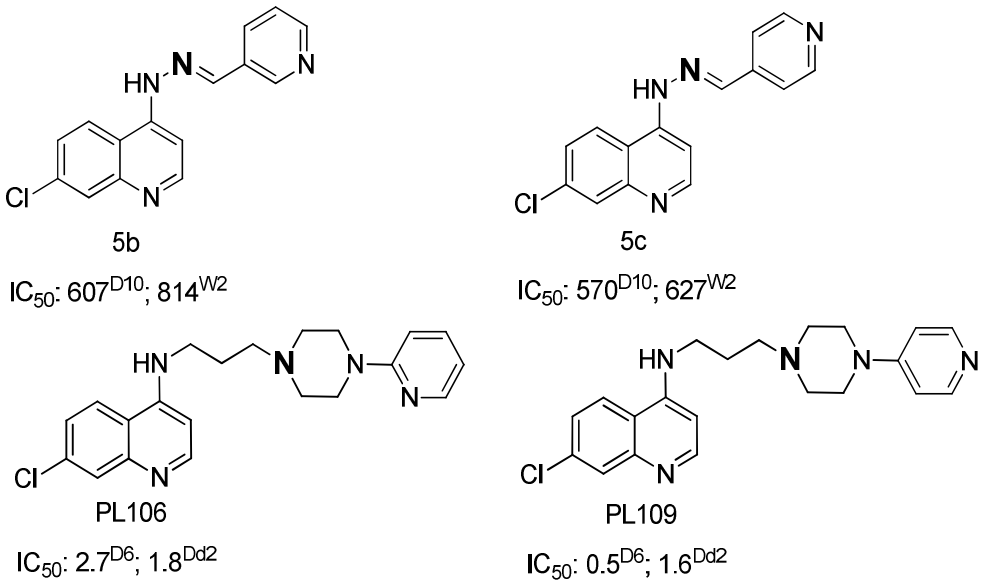


Figure 3.5: Compounds 5b and 5c made by Fattorusso, *et al*.,[5] in comparison with the simplified RCQs PL106 and PL109. D10 is a CQS strain of *P. falciparum*, while W2 is a CQR strain of *P. falciparum*. The bold nitrogen atoms are protonatable and resemble the

CHAPTER 4

Inverse Piperaquine

In this chapter, the focus is on the synthesis of compounds that contain two quinoline rings. Piperaquine (see Figure 4.1) replaced CQ as a first line drug in China from its introduction in 1970 until reports of emergence of resistance in 1990.[79] The Vennerstrom group has investigated the antimalarial effects of a number bisquinolines; one example of such a compound is shown in Figure 4.1.[3] These bisquinolines have been shown to be potent against malaria. For example, piperaquine had an IC_{50} value of 0.7 nM against CQS D6 and an IC_{50} of 1.5 nM against Dd2.

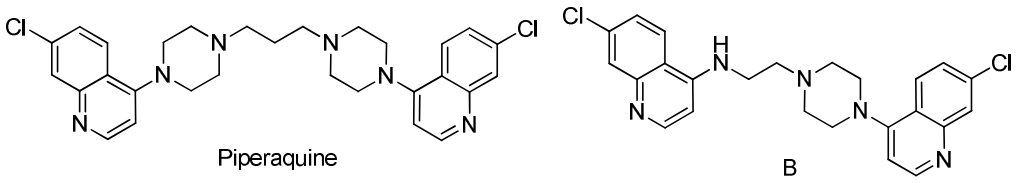
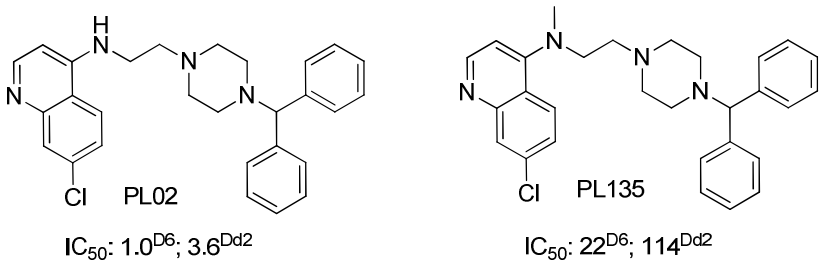


Figure 4.1: Piperaquine a known antimalarial drug. B is a bisquinoline synthesized by the Vennerstrom group.[3]

Although most CQ-like drugs have a 2^o amine at the quinoline 4-position, piperaquine is an exception, having a 3^o amine at this position. Having a 3^o amine at the quinoline 4-position of piperaquine has been pointed out as detrimental by others.[80] In the Peyton Lab, PL02 and PL135 have been synthesized and these compounds are shown in Figure 4.2. From these two compounds it was observed that a 2^o nitrogen on the 4-position of the quinoline ring results in a more potent compound, than if it were a 3^o

nitrogen. For PL02, which has the 2^o nitrogen at position 4 of the quinoline ring, the IC_{50} value for D6 was 1.0 nM for Dd2, and 3.6 nM against Dd2, as compared to 22 nM for D6 and 114 nM for Dd2 for PL135, which has a 3^o nitrogen at position 4 of the quinoline ring.



IC_{50}: 1.0^{D6}; 3.6^{Dd2} IC_{50}: 22^{D6}; 114^{Dd2}

Figure 4.2: PL02 with a 2^o nitrogen on the 4 position of the quinoline ring. PL135 has a 3^o nitrogen on the 4 position of the quinoline ring. Note that the IC_{50} values are in nM.

In the Peyton Lab, work has been done by Simeon Andrews to evaluate the implications of having a piperazine ring on the 4 position of the quinoline ring.[78] Both compounds, PL38 and PL51 shown in Figure 4.3 have reduced antimalarial activities than their analogues PL50 and PL52 shown in Figure 4.3. The activity loss in PL38 and PL51 was postulated to be due to the lack of a 2^o nitrogen at position 4 of the quinoline ring.[78]

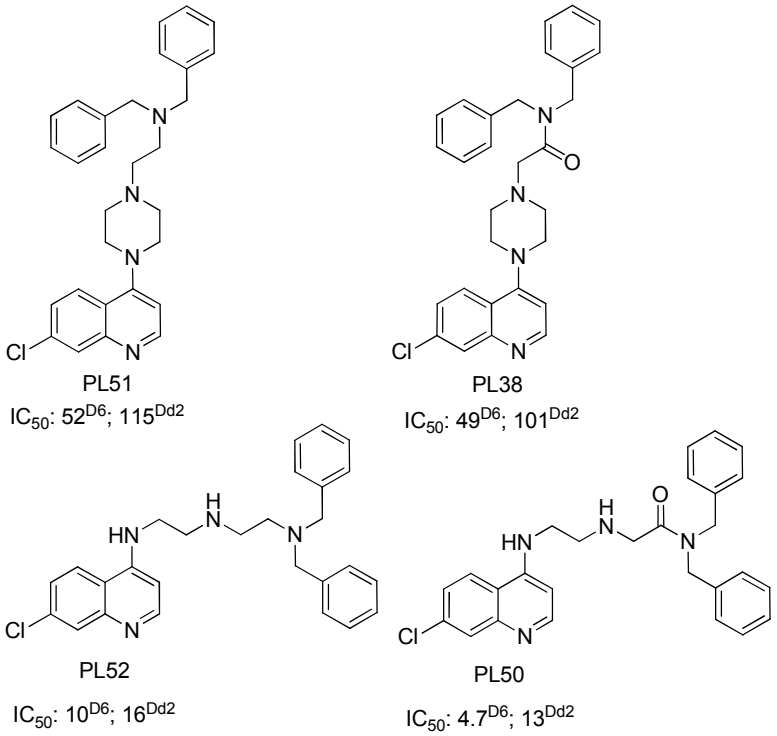


Figure 4.3: PL51 and PL38: compounds with piperazine rings α to the quinoline ring; PL52 and PL50: compounds lacking the piperazine rings. Note that the IC_{50} values are in nM.

Due to these findings, bisquinoline ring system compounds PL192 and PL255 (Figure 4.4) were synthesized. These bisquinoline compounds were expected to have better activity than piperaquine because of the 2^{o} nitrogens on the 4-position of the quinoline ring. Also, the compounds were expected to be more potent because one of the quinoline rings might be acting as a RA, while the other quinoline ring would exert its antimalarial activity. These compounds were synthesized as shown in Scheme 4.1.

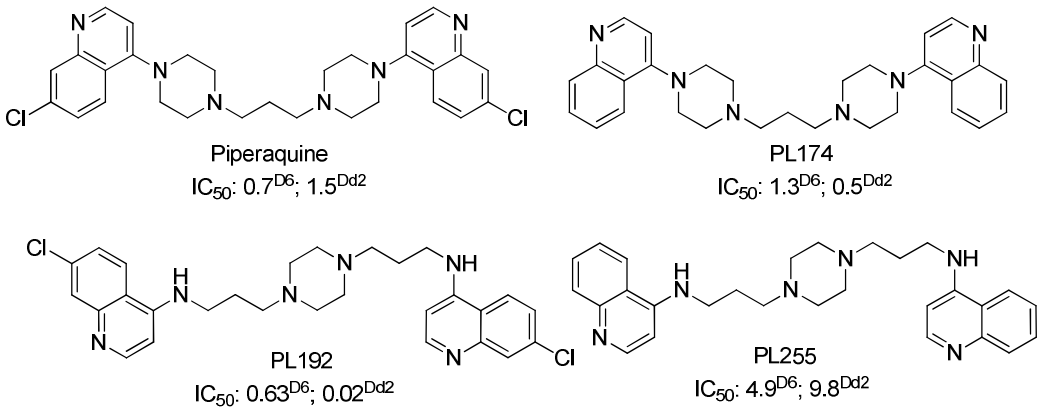


Figure 4.4: Bisquinolines. PL192 and PL255 have the piperazine group in the middle of the compound and hence they have 2^{o} nitrogens at position 4 of the quinoline ring. Piperaquine and PL174 have piperazine rings on the 4-position of the quinoline ring, hence they have 3^{o} nitrogens at position 4 of the quinoline ring.

PL255 was synthesized to show the importance of the 7-chloro group for the bisquinolines. Work done in the Peyton lab has shown that that removing the 7-chloro group for the RCQs is not very detrimental, in contrast to CQ losing the 7-chloro group, as shown in Figure 4.5.[75]

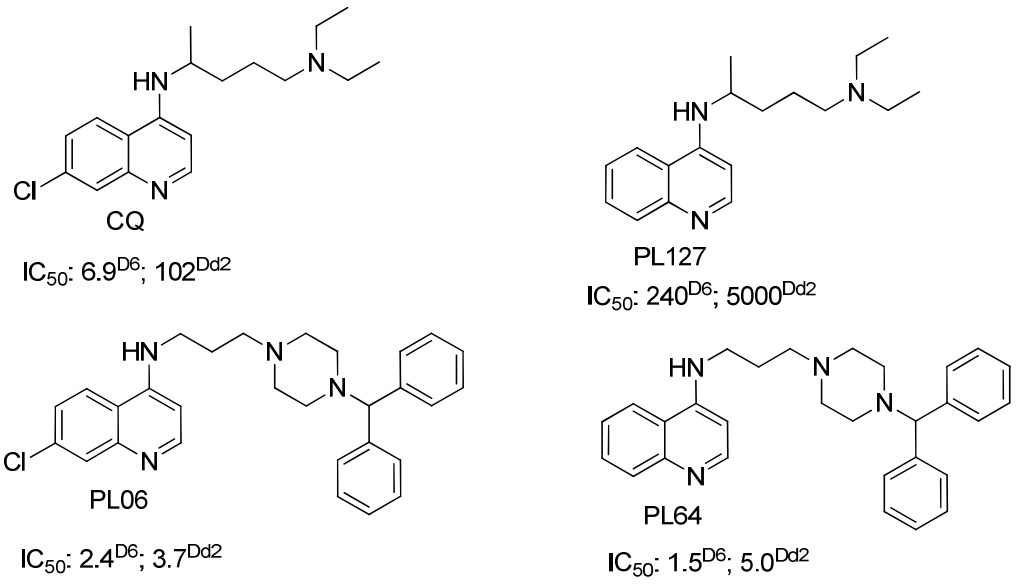
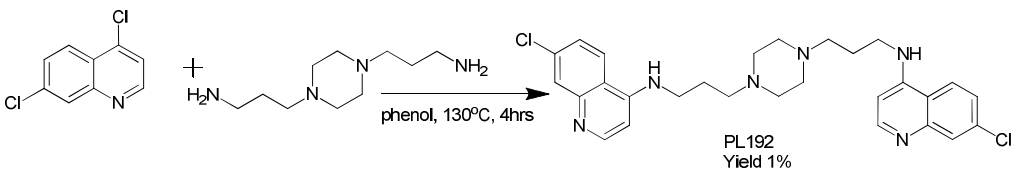


Figure 4.5: Effect of losing the 7-chloro group on CQ and the P.L compounds.

The compounds in Figure 4.3 were made by treating 4,7-dichloroquinoline or 7-chloroquinoline with 1,4-bis(3-aminopropyl)-piperazine, in phenol as reaction solvent as shown in Scheme 4.1. The suspension was heated at 190^{o}C for 4 hrs. The mixture was allowed to cool and then the bisquinoline was extracted with aqueous sodium hydroxide followed by chloroform.

Scheme 4.1



PL192 had an IC_{50} value for D6 of 0.63 nM, for Dd2 it was 0.02 nM compared to 4.9

nM for D6 and 9.8 nM for Dd2 for PL255. This result shows that the 7-chloro group is somewhat important for activity, although not to the same order of magnitude as reported in the Peyton Lab for CQ (Figure 4.4).[75] PL192 was found to be more potent than piperaquine as predicted; this may have been due to the presence of 2^{o} nitrogens at position 4 of the quinoline rings. However, piperaquine and PL174 have activities within the same order of magnitude, showing that removing the chlorine on position 7 of the quinoline ring was not very detrimental. In contrast PL192 and PL255 have a difference in activity that is about 1 order of magnitude, indicating that losing the chlorine on position 7 of the quinoline ring was detrimental to the activities of these compounds as shown in Figure 4.4.

CHAPTER 5

Mode of Action Studies

Missing Quinoline Ring

The previous chapters focused on the antimalarial activities of the simplified RCQs. However, it is important to understand the mechanism(s) by which these compounds work. In this chapter, a more focused investigation of the mechanism of action of these compounds is presented. Compounds PL272, PL260, and PL273 shown in Figure 5.1 were made that lacked the quinoline ring, to assess the possibility of any residual antimalarial activity of the rest of the molecules. The simplified RCQs presented in the earlier chapters were surprisingly potent, and it was hypothesized, that the RCQ head groups may have potential RA activity. PL272 lacks the quinoline ring, the amide is presumed to prevent the nitrogen from being protonatable just like in the parent RCQ compounds.

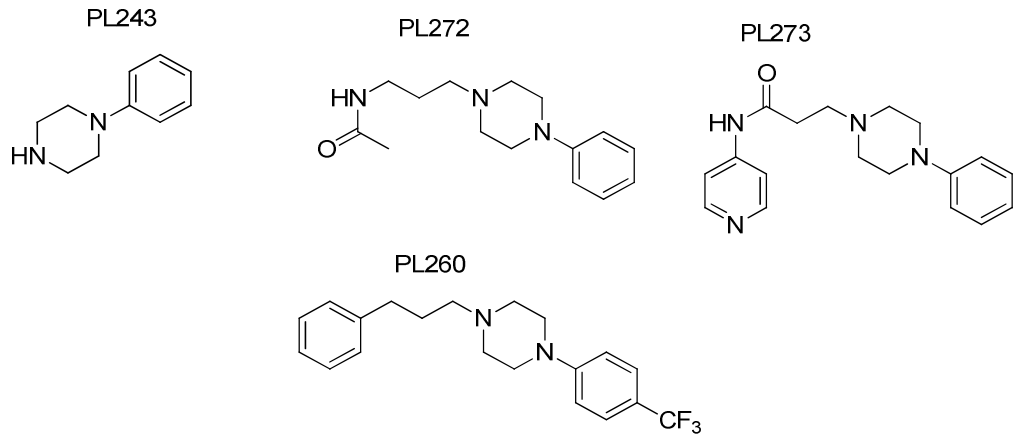


Figure 5.1: Variations of the head group. These compounds will test the reversal agent activity of the head group. PL243 is just the piperazine head group. PL272 has the aliphatic chain linked to the piperazine ring. PL273 has pyridine ring attached to the head group. PL260 contains a head group attached to a benzene ring.

In the Peyton lab, PL74 (Figure 5.2) has been made, and it had an IC_{50} of 185 nM against D6 and 169 nM against Dd2. This result itself was rather surprisingly good; there was activity in this compound despite the missing quinoline ring. As a result, PL273 was made and had IC_{50} values of >2500 nM for both CQS D6 and CQR Dd2. PL273 was less potent than PL74, perhaps because it lacked the biphenyl head group which may be involved in pi bonding with free heme. This was an indication that the simplified RCQs may have a different mode instead of action instead of, or in addition to the unsimplified RCQs.

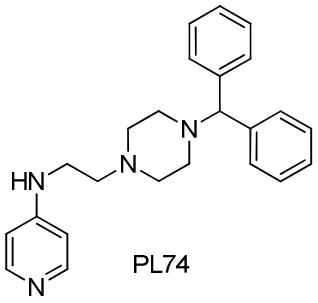
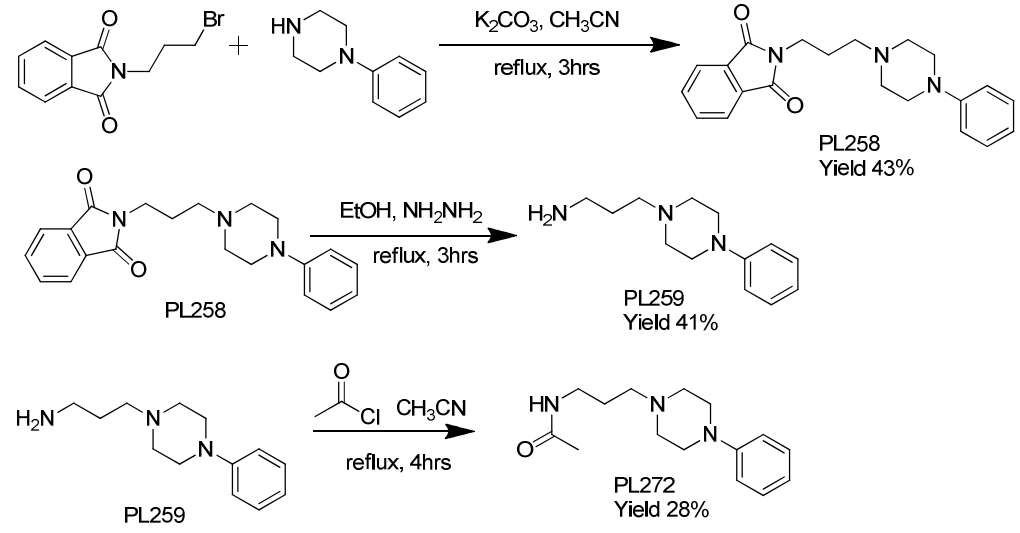


Figure 5.2: PL74, having a pyridine group in place of the quinoline ring.

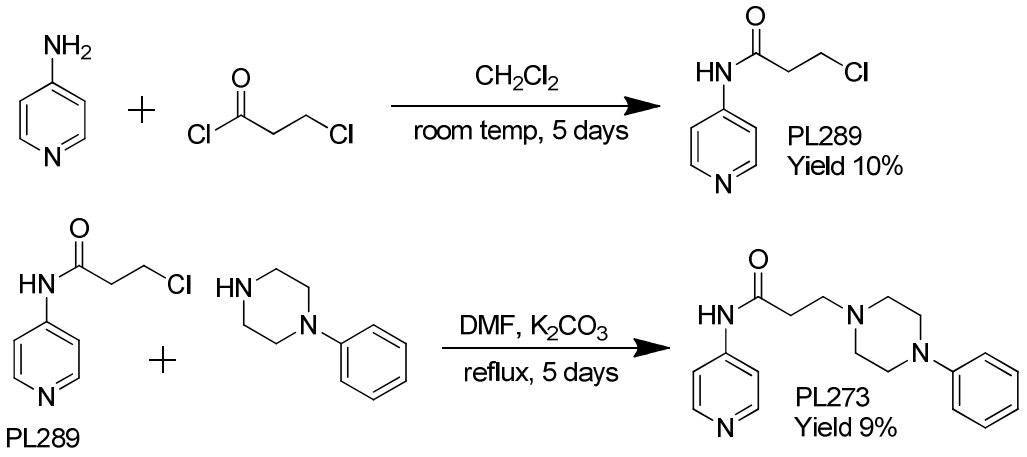
PL272 had activities of >2500 nM for both CQS and CQR strains of *P. falciparum*. PL243 and PL260 were also tested and showed no detectable antimalarial activity up to 2500 nM. This result clearly shows that the simplified RCQ head group does not have significant intrinsic antimalarial activity. PL272 was made via Scheme 5.1.

Scheme 5.1



First the piperazine analogue was treated with N-(3-bromopropyl) phthalimide in chloroform with triethylamine as base. The formed product was treated with hydrazine in ethanol to form the amine. The amine was then treated with acetyl chloride in acetonitrile with triethylamine as base to form PL272.

Scheme 5.2



PL273 was synthesized via Scheme 5.2 by treating the 4-chloropyridine with 3-chloropropionyl chloride in DCM. The synthesized compound PL289 was then treated with the piperazine head group in DMF with potassium carbonate as base.

Accumulation Experiment

A search of the literature showed that CQ accumulates in the DV of *P. falciparum*.[39;81] [82] Previous work in the Peyton Lab has shown that the PL01 behaves like CQ is that it

accumulates in the DV.[75] The experiment used to estimate the uptake of the RCQs by parasitized red blood cells (PRBCs) is similar to the one used by Kelly, *et al*..[83] Thus, 10 mM solution of PL106 was added to a flask containing 5 mL of PRBCs suspended in complete medium (10% parasitemia); the initial medium concentration of PL106 was ~ 5 μM. Samples were removed from the flask at various intervals and centrifuged; the supernatant fluid was then removed and frozen, ready for analysis by HPLC. PL106 was added to flasks containing both CQS and CQR infected RBCs. The negative control for this experiment was a flask containing uninfected RBCs, and the positive control involved infected RBCs interacting with CQ. The experiment was terminated by adding 10 mM ammonium chloride; this resulted in an increase in the pH of the DV,[83] thus PL106 became unprotonated and released from the DV as shown in Figure 5.3.

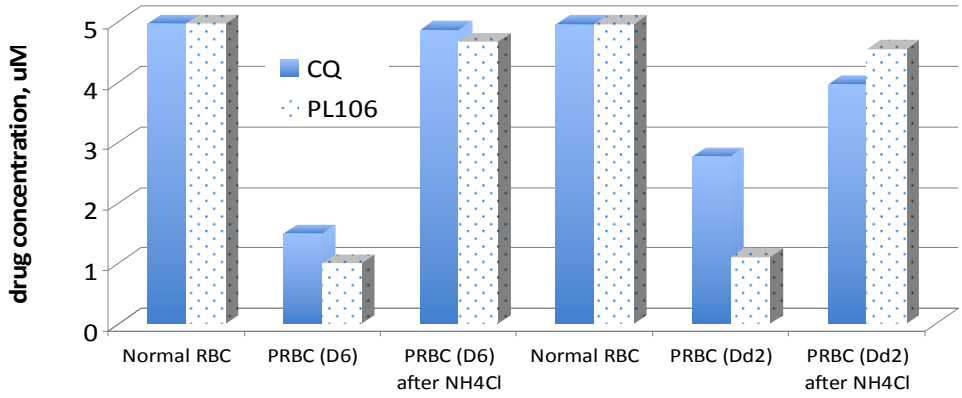


Figure 5.3: Comparison of CQ and PL106 uptake with *P. falciparum* strain combination, showing the drug concentration in the medium after 1 hr with normal RBCs, parasitized RBCs, and with parasitized RBCs after addition of NH_4Cl which liberates the drug from the RBCs. The grey bars indicate CQ and the dotted bars show PL106. The CQR Dd2 strain clearly has enhanced uptake of PL106 relative to CQ, while the CQS D6 strain shows the same effect to a smaller extent.

From this experiment, the amount of drug accumulating in the DV was quantified. Also, the drug was assessed for possible modifications that may have occurred during its uptake (modified compounds would have different HPLC retention times). This experiment was conducted by Jane Xu Kelly (Riscoe Lab, OHSU). From this experiment it was noted that PL106 accumulated more in CQR and CQS PRBCs than in normal RBCs. The retention times of the compounds also showed that PL106 was not modified as a result of its uptake into the RBCs.

Radioisotope Uptake Measurements

The aim of this study was to develop simplified compounds that could overcome CQ resistance as well as have antimalarial activity. CQ resistance has been linked to PfCRT, it has been noted that CQR strains of *P. falciparum* accumulate less CQ in the

DV; this may be because CQ is transported out of the DV by PfCRT.[84] RAs have been proposed to inhibit PfCRT, and thus reverse CQ resistance.[2] The head group for the simplified RCQs deviates significantly from the pharmacophore proposed by Bhattarcharjee, *et al*., hence it may not have optimal RA properties. Despite this deviation, there is marked improvement in the activity of the synthesized compounds (See Table 2.3).

An experiment was conducted by Martin, *et al*., to probe whether the head group of the simplified RCQs was behaving like a RA.[84] In this experiment, the accumulation of CQ in oocytes encoded with PfCRT both sensitive and resistant types were conducted. For the positive control, verapamil was used. The difference in uptake of CQ by the PfCRT CQR oocytes and PfCRT CQS oocytes is small, suggesting that verapamil is acting as a RA (by inhibiting PfCRT) and preventing CQ from being transported by PfCRT. For the negative control, it can be observed that the difference is larger for the uptake of CQ by the PfCRT CQR oocytes than PfCRT CQS oocytes, reflecting the transport activity of the PfCRT. RCQs PL106, PL154, PL158, and PL261 (shown in Figure 5.4) have greater inhibition of CQR PfCRT than the negative control, yet smaller inhibition than verapamil.

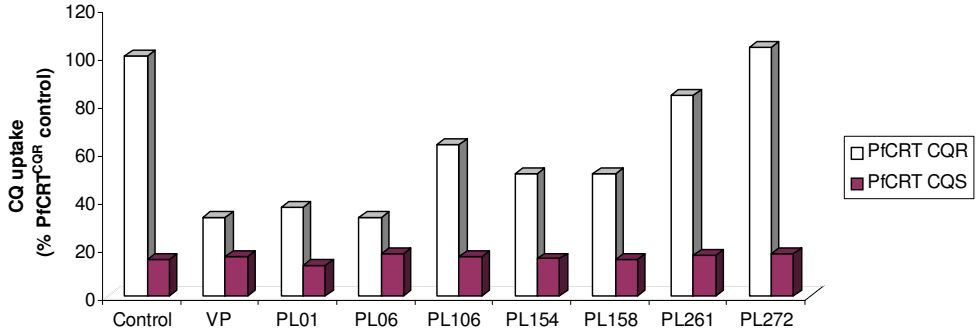


Figure 5.4: Uptake of ^{3}H CQ by PfCRT expressing *Xenopus* oocytes; pH 6.0. The CQ uptake is expressed as a percentage of $PfCRT^{CQR}$. The white bars are for PfCRT CQR oocytes and the grey bars are for PfCRT CQS oocytes.

This result shows that these compounds do have the ability to behave like RAs, yet to a smaller extent than PL01 and PL06. This may be due to the fact that there is a deviation to the pharmacophore proposed by Bhattacharjee *et, al*., with these compounds. However, it is evident that PL272 does not behave as a RA since the difference in CQ uptake for between PfCRT CQR oocytes and PfCRT CQS oocytes is similar to that of the control (suggesting zero inhibition of CQ transport). Yet the simplified RCQs can mimic the RA, if they are envisioned as having the two aromatic head groups, one provided by the quinoline ring and the other provided by the single aromatic head group, as shown in Figure 5.5. PL272 lacks the two aromatic groups, and thus does not behave as a RA. This result suggests that the simplified RCQs act as "mild RAs", in that they can prevent CQ from being transported by the PfCRT protein to a smaller extant than classical RAs such as verapamil.

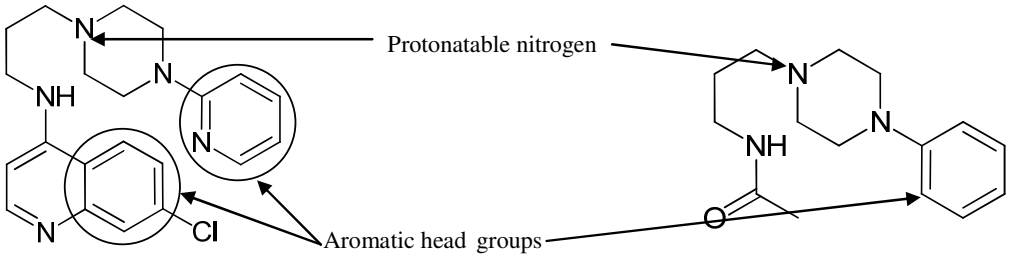


Figure 5.5: PL106 and PL272, PL106 somewhat mimics the RA pharmacophore because it has two aromatic groups PL272 only has one aromatic head group and thus deviates from the pharmacophore proposed by Bhattacharjee, *et al*..

However, PL154 was very potent against *P. falciparum* CQS D6, with an IC_{50} value of 0.3 nM against D6, 0.5 nM against *P. falciparum* CQR Dd2, and 0.1 nM against *P. falciparum* CQR 7G8. Further tests were carried out to evaluate for activity against *P. falciparum* strains C2B and A6 to probe the possibility of respiratory inhibition in the parasite. As can be seen in Table 5.1, PL154 was more potent than both quinacrine (a known respiratory inhibitor)[85] and CQ against all four strains tested. C2B is a well established atovaquone resistant strain.[86] A6 is a mutant of D6 derived by Smilkstein, Risoe, and coworkers, and is resistant to all respiratory inhibitors as far tested.[86] So, it is highly likely that PL154 does not target the mitochondria. Compound PL154 could still have a very different mode of action than these respiratory inhibitors. If PL154 turned out to be truly a DV drug, then it would be a highly potent DV drug (at least *in vitro*).

Table 5.1: In vitro respiratory inhibition studies (Values in nM). There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} (See Chapter 7).

P. falciparum strain	Compound IC_{50} values in nM		
	PL154	quinacrine	chloroquine
D6	0.1	4.3	7.3
Dd2	0.6	11.2	112.0
C2B	0.4	13.2	109.7
A6	1.7	8.0	11.8

PL154 seems to inhibit both the ring stage (0.02 nM for CQS D6 strain and 0.05 nM for CQR Dd2 strain) and the trophozoite stage (0.05 nM for CQS D6 strain, and 0.03 nM for CQR Dd2 strain) of parasite growth by the same order of magnitude. CQ and clotrimazole have been reported in literature, to be more specific to the ring stage by 1 order of magnitude compared to the trophozoite stage.[21;87;88] During the ring stage and the trophozoite stage, there is the highest metabolic activity. At the ring stage the first signs of the malaria pigment are observed. Since PL154 is a CQ-like compound it is expected to have potency by at least partly the same mechanism of action as CQ. CQ is proposed to either cap the ends of growing heme chains or to bind via pi pi bonds to heme dimers. This action is proposed to be most highest during the ring stage and the trophozoite stage.[89]

Heme Binding and β-Hematin Inhibition

CQ is presumed to exert its antimalarial action through binding with heme which is toxic to the parasite.[7] Heme is a by product of hemoglobin digestion in the parasite and is sequestered into hemozoin by the parasite; hemozoin is not toxic to the parasite.[6] The simplified RCQs were thus evaluated for heme binding by Shawheen Shomloo (Peyton Lab, Portland, Oregon),[90] to check whether they behave like CQ. For heme-drug binding studies, CQ or PL compound was dissolved in distilled water, methanol, or dimethyl sulfoxide (DMSO), depending on solubility of the respective compounds; they were also sonicated to ensure complete solubility. A stock solution of heme was prepared by dissolving heme chloride in NaOH. At the beginning of each experiment, the stock heme solution was diluted in phosphate buffer and allowed to equilibrate for four hours. Optical titrations with each compound were performed by successive addition of aliquots of its stock solution to the heme solution. The pH was monitored throughout the procedure with only negligible changes. Equilibrium binding constants were determined by nonlinear least-squares analysis.[91] The results for the heme binding studies are shown in Figure 5.6.

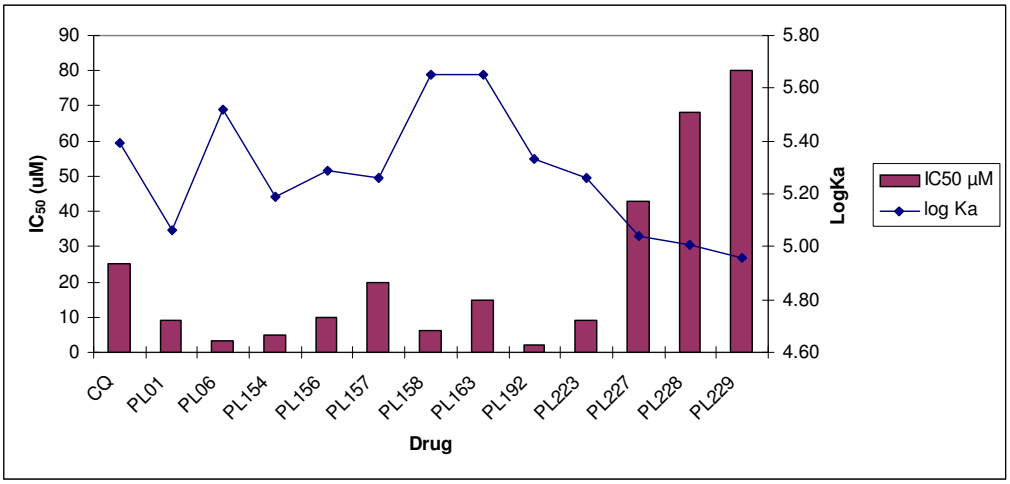


Figure 5.6: Heme binding and β-hematin inhibition studies for the simplified RCQs. The scale for the line graph is on the right and the scale for the bar graph is on the left.

The results show that the PL compounds generally have a LogKa of about 5 which is an indication that there is strong binding with heme. These results also show that these compounds bind to heme within 72 hrs, a similar affinity as does CQ.

β-Hematin, which is synthetic, has an identical crystal structure to hemozoin found in malaria parasites.[92] CQ and CQ-like compounds can prevent the formation of β-hematin *in vitro*.[90] An experiment was thus conducted by Shawheen Shomloo, to evaluate the inhibition constants for β-hematin formation by the simplified RCQ molecules. In this experiment the optimal heme and Tween20 concentrations for promoting heme crystallization were calculated by the procedure described by Huy, 2002.[93] The RCQ compounds were screened for their inhibitory capacity, and IC_{50} values were determined. A series of solutions was made, consisting of varying concentrations of the compound under study in distilled, acetate buffer, heme solution

freshly buffered by sodium acetate, and Tween20 solution. The mixtures were incubated for 24 hours at 37°C,[94] then mixed and transferred to a cuvette for absorbance reading. IC_{50} values were calculated by $(D_{max} - D_{initial}) / 2$ where D_{max} represents the lowest concentration of compound under study to provide maximal absorbance readings indicating maximal free heme, and $D_{initial}$ represents the lowest concentration of drug to provide any increase in absorbance over a solution with no drug. The results for the heme inhibition studies are shown in Figure 5.6. From these results we see that most of the compounds are more potent than CQ in inhibiting β-hematin formation. However PL228 and PL229 (Table 3.1) are less potent than CQ in inhibiting β-hematin formation. This is an anomaly because these compounds have the quinoline ring which is required for inhibition of β-hematin formation. One explanation is that the RA-moiety aromatic head group influences β-hematin formation; this group is absent in PL228 and PL229. This result does not, however, correlate well with the antimalarial IC_{50} values of these compounds, and this may be because these compounds prevent malaria via a different mechanism compared to the single aromatic head group compounds. PL192 has two quinoline rings, which cumulatively result in increased inhibition of β-hematin formation. It is also interesting to note that there is no correlation between heme binding and β-hematin inhibition for the simplified RCQs.

CHAPTER 6

Summary and Conclusions

Malaria is a major health problem, due to the increase in the malaria parasite's resistance to current drugs, there is a need to discover compounds with novel scaffolds that may not have been exposed to the parasites, and so would not exhibit resistance. In addition, there is a need keep remedies for malaria inexpensive, since this disease mainly affects developing countries. CQ was an effective antimalarial drug but lost its effectiveness due to the development of CQR strains of malaria. RAs have been shown to reverse CQ resistance and so the focus of this research has been to develop compounds composed of a RA head group attached to a CQ like portion. In this research, the RA head group has been simplified from the branched aromatic groups described by Bhattacharjee, *et al*., to a single aromatic group. This approach has been shown to be quite viable as a potential drug candidate PL106 was synthesized. This compound was orally available in mice and was shown to have no visible toxicity to human cells as well as mice. Further studies may need to be carried out on PL106 before human trials can be done on it.

From the studies reported in this document, it was noted that the simplified RCQ could accommodate several changes without major losses in activity. It was noted that the linker length between the protonatable nitrogen and the head group could be varied. Also it was noted that the linker length between the quinoline ring and the

protonatable nitrogen could be varied as well. These results were in agreement with previous studies carried out in the Peyton Lab.[78] It was also noted that the head group could tolerate a change from aromatic to aliphatic. Mechanistic studies on the head group showed that the head group on its own does not have RA activity, however when linked to the quinoline ring RA activity was observed. This finding may be due to the fact that the quinoline ring and the single aromatic head group satisfy the condition of two branched aromatic groups proposed by Bhattacharjee, *et al*.. The single aromatic head group on its own does not satisfy the pharmacophore proposed by Bhattacharjee, *et al*..

Further work on this project which is beyond the scope of this dissertation will involve more *in vivo* tests to determine the safety of these compounds.

CHAPTER 7

Materials and Methods

This section gives an outline of the synthesis that was used to make the different antimalarial compounds as well as their intermediates. Names of compounds were generated using ChemBioDraw 11.0.1. The purity of the compounds was detected by a Varian Polaris Binary HPLC system, measuring by UV detection at 254 nm and 325 nm, using a Varian ProStar 325 UV/Vis dual wavelength detector. HPLC method A was done with a Microsorb-MV 100-5 C18 250 mm X 4.6 mm column, eluting with 95 % methanol and 5 % water for 30 minutes. HPLC method B was done with an Ascentis^{TM} 5µm C18 150 mm X 4.6 mm column, eluting with 95 % water and 5 % acetonitrile for 30 minutes. All reagents and solvents were purchased from Aldrich and used as supplied. ^{1}H NMR and ^{13}C NMR and 2D NMR spectra were detected on a Bruker 400 MHz spectrometer. Splitting patterns were described as singlet (s), doublet (d), triplet (t), quartet (q), pintet (p), doublet of doublets (dd), doublet of triplets (dt), doublet of doublet of doublets (ddd), multiplet (m), and broad (br). Mass spectrometry was performed on a Bruker microOTOF-Q instrument. The method use was electrospray ionization (ESI) in the positive mode, at a flow rate of 0.4 mL/min with 1:1 methanol water. Each of the compounds made had a CQ like portion linked RA like portion. Outlined is the method for the synthesis of PL91, the same method was used to make other compounds with varying piperazine head groups.

Synthesis of PL16 3-(7-chloroquinolin-4-ylamino)propan-1-ol

25.48 g (129 mmol) of 4,7-dichloroquinoline was added to 122 mL (157 mmol) of 3-amino-1-propanol and heated at ~135^{o}C for 48 hrs. The reaction was verified by Thin layer Chromatography (TLC) in 100% ethyl acetate (EA). The solution was allowed to cool to room temperature and then poured into 500 mL water with stirring. The precipitate that formed was chilled in an ice bath. The solid was filtered off and washed with water. The solid was allowed to air dry in the fume hood and then recrystallized twice in EA. 22.50 g (95 mmol, 74% yield) of PL 16 was obtained.

Synthesis of PL28 2-(7-chloroquinolin-4-ylamino)ethanol

A mixture of 4,7-dichloroquinoline 4.95 g (25 mmol) and ethanolamine 15.27 g (250 mmol) was heated with stirring at ~135^{o}C for 24 hrs. After cooling, the reaction was poured into 150 mL water and filtered. After air drying the solid was boiled in 100 mL methanol, allowed to cool to room temperature then cooled in ice. The solid was filtered, and then washed with a small amount of ice cold methanol to give PL28 3 g (13 mmol, 54% yield) as an off-white solid.

Synthesis of PL29 3-(7-chloroquinolin-4-ylamino)propyl methanesulfonate

0.5 g (2.1 mmol) of PL16 was treated as a suspension in dry 15 ml dichloromethane with 0.43 g (4.2 mmol) of triethylamine as base. The mixture was chilled over an

ice/salt (<0^{o}C) bath then 0.25g (2.2 mmol) of methane sulfonyl chloride was added to the mixture for ~3 minutes with stirring. The reaction was allowed to proceed for 30 minutes, and then extracted with 30 ml aqueous sodium bicarbonate, then three times with 10 ml dichloromethane. The mixture was dried over magnesium sulfate. Finally, the dichloromethane was evaporated off. The product was weighed and used as starting material for a lot of the compounds made in this article.

Synthesis of PL30 2-(7-chloroquinolin-4-ylamino)ethyl methanesulfonate

To a suspension of PL28 1.5 g (6.7 mmol) in anhydrous 25 mL dichloromethane under a nitrogen atmosphere was added 2 mL (14.3 mmol) triethylamine. The mixture was cooled to bellow 0^{o}C. 0.57 mL (7.4 mmol) of methanesulfonylchloride was added slowly, keeping the temperature below 5^{o}C, and the reaction was stirred in an ice bath for 1 hr. The reaction was added to a 100 mL solution of saturated sodium bicarbonate. The aqueous layer was extracted with 2 X 20 mL dichloromethane. The combined organic extracts were evaporated to leave an off-white product PL30 1.19 g (4 mmol, 59%).

Synthesis of PL91 7-chloro-*N*-(3-(4-phenylpiperazin-1-yl)propyl)quinolin-4-amine

0.3 g (1.0 mmol) of the PL29 was added to the 0.32 g (1.2 mmol) of the 1-phenylpiperazine in 15 ml dry tetrahydrofuran and 0.27 g (2 mmol) of triethyl amine as base. This mixture was refluxed for one day. The mixture was then extracted with 30 ml of sodium bicarbonate, then three 10 ml volumes ethyl acetate. The organic layer was rinsed with 10 mL brine. Then resultant mixture was dried over magnesium sulfate. Finally, the ethyl acetate was removed by rotoevaporation. The solid product was precipitated from an ethanol solvent. The cream compound was filtered, weighed 0.16 g (0.4 mmol, 44% yield) and characterized by ^{1}H-NMR, HPLC & MS. HPLC (method B) t_R = 8.28 (99% Pure). ^{1}H-NMR ($CDCl_3$) δ 1.88(2H, q, *J*=5.4 Hz), 2.59(2H, t, J=5.5 Hz), 2.64(4H, t, J=4.7 Hz), 3.23(4H, t, J=4.7 Hz), 3.31(2H, dt, J=5.6 Hz), 6.85(1H, t, J=7.3 Hz), 6.9(2H, d, J=8.1 Hz), 7.10(1H, dd, J=2.0, 8.9 Hz), 7.25(2H, t, J=8.4 Hz), 7.32(1H, s), 7.71(1H, d, 9.0 Hz), 7.84(1H, d, J=2.0 Hz), 8.42(1H, d, J=5.4 Hz); ESIMS $[M + H]^+$ calcd for $C_{22}H_{25}ClN_4$ 381.1841, found 381.1831.

Synthesis of PL106 7-chloro-*N*-(3-(4-(pyridin-2-yl)piperazin-1-yl)propyl)quinolin-4-amine

0.49 g (1.56 mmol) of PL29 was added to 0.3 g (1.86 mmol) of 1-(2-Pyridyl)piperazine and 0.43 g (3.11 mmol) triethylamine in 30 mL of chloroform. The mixture was refluxed for one day and then extracted with 30 mL sodium bicarbonate and 3 X 10 mL of chloroform. The chloroform was evaporated off and the crude product was columned twice on silica with a 50:50 methanol:ethylacetate solvent mixture. 0.26 g (0.68 mmol, 44 % yield) of tan crystals was obtained as product.

HPLC (method B) t_R = 5.68 (99% Pure). ^{1}H NMR δ (ppm)(CH_3OH-d_4): 8.39 (1 H, d, J = 5.64 Hz), 8.11-8.10 (2 H, m), 7.80 (1 H, d, J = 2.18 Hz), 7.59 (1 H, ddd, J = 8.64, 7.13, 2.00 Hz), 7.40 (1 H, dd, J = 9.01, 2.20 Hz), 6.85 (1 H, d, J = 8.64 Hz), 6.71 (1 H, dd, J = 7.11, 5.03 Hz), 6.59 (1 H, d, J = 5.69 Hz), 3.57 (4 H, t, J = 4.99 Hz), 3.49 (2 H, t, J = 6.80 Hz), 2.64-2.62 (6 H, m), 2.02 (2 H, t, J = 7.01 Hz). ^{13}C NMR δ (ppm)(CH_3OH-d_4): 152.5, 149.7, 148.5, 139.3, 136.4, 127.7, 126.0, 124.3, 118.8, 114.8, 109.2, 99.7, 57.5, 54.2, 46.5, 42.6, 26.1. ESIMS $[M + H]^+$ calcd for $C_{21}H_{24}ClN_5$ 382.1793, found 382.1784.

Synthesis of PL109 7-chloro-N-(3-(4-(pyridin-4-yl)piperazin-1-yl)propyl)quinolin-4-amine

0.44 g (1.4 mmol) of PL29 was added to 0.25 g (1.5 mmol) of 1-(2-Pyridyl)piperazine and 0.28 g (2.8 mmol) in 30 mL of acetonitrile. The mixture was refluxed for 1 day and then extracted with 30 mL sodium bicarbonate and 3 X 10 mL of chloroform. The chloroform was evaporated off and the crude product was columned twice on silica with a 50:50 methanol:ethylacetate solvent mixture. 0.23 g (0.6 mmol, 43 % yield) of tan crystals was obtained as product.^{1}H NMR δ (ppm)(CH_3OH-d_4): 8.26 (1 H, d, J = 5.63 Hz), 8.05-7.99 (2 H, m), 7.98 (1 H, d, J = 9.03 Hz), 7.68 (1 H, d, J = 2.19 Hz), 7.28 (1 H, dd, J = 9.00, 2.19 Hz), 6.74 (2 H, d, J = 6.08 Hz), 6.52-6.45 (1 H, m), 3.40-3.31 (6 H, m), 2.56-2.46 (6 H, m), 1.89 (2 H, p, J = 6.99 Hz). ESIMS $[M + H]^+$ calcd for $C_{21}H_{24}ClN_5$ 382.1786, found 382.1793

Synthesis of PL110 *N*-(3-(4-(biphenyl-4-yl)piperazin-1-yl)propyl)-7-chloroquinolin-4-amine

0.5 g (1.59 mmol) of PL29 was added to 0.45 g (1.91 mmol) of 1-(biphenyl-4-yl)piperazine and 0.32 g (3.17 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL sodium bicarbonate and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product columned on silica with a 25:75 methanol: ethyl acetate solvent mixture. The product was in the form of white crystals and a yield of 0.16 g (0.35 mmol, 21.9 % yield). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.48 (1 H, d, J = 5.77 Hz), 8.09 (1 H, s), 7.91 (1 H, d, J = 8.97 Hz), 7.62-7.57 (4 H, m), 7.44 (2 H, t, J = 7.58 Hz), 7.36-

7.31 (1 H, m), 7.09-7.02 (2 H, m), 6.41 (1 H, d, J = 5.82 Hz), 3.54-3.48 (2 H, m), 3.42 (4 H, t, J = 4.74 Hz), 2.82 (4 H, t, J = 4.69 Hz), 2.80-2.74 (2 H, m), 2.10-2.03 (2 H, m).

Synthesis of PL111 *N*-(3-(4-(biphenyl-3-yl)piperazin-1-yl)propyl)-7-chloroquinolin-4-amine

0.6 g (1.9 mmol) of PL29 was added to 0.55 g (2.29 mmol) of 1-(biphenyl-3-yl)piperazine and 0.39 g (3.81 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL sodium bicarbonate and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product columned on silica with a 25:75 methanol: ethyl acetate solvent mixture. The product was in the form of a white powder and a yield of 0.16 g (0.35 mmol, 18.4 % yield). HPLC (method b) t_R = 11.51 (98% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.52 (1 H, d, J = 5.35 Hz), 7.93 (1 H, d, J = 2.15 Hz), 7.82 (1 H, d, J = 8.94 Hz), 7.63-7.58 (2 H, m), 7.47-7.32 (5 H, m), 7.24 (1 H, dd, J = 8.00, 2.28 Hz), 7.20-7.13 (2 H, m), 6.98 (1 H, dd, J = 8.30, 2.49 Hz), 6.36 (1 H, d, J = 5.41 Hz), 3.43 (2 H, d, J = 5.08 Hz), 3.42-3.37 (5 H, m), 2.77 (4 H, t, J = 4.75 Hz), 2.74-2.68 (2 H, m), 2.05-1.97 (2 H, m).^{13}C NMR δ (ppm)($CHCl_3$-d): 160.9, 152.4, 151.6, 147.9, 145.3, 141.4, 134.6, 129.8, 129.2, 127.5, 125.3, 121.9, 122.3, 119.6, 115.4, 113.7, 112.5, 98.4, 96.4, 58.8, 53.7, 49.5, 44.1, 24. ESIMS $[M + H]^+$ calcd for $C_{28}H_{29}ClN_4$ 457.2154, found 457.2171.

Synthesis of PL112 *N*-(3-(4-(biphenyl-2-yl)piperazin-1-yl)propyl)-7-chloroquinolin-4-amine

0.6 g (1.9 mmol) of PL29 was added to 0.55 g (2.29 mmol) of 1-(biphenyl-2-yl)piperazine and 0.39 g (3.81 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL sodium bicarbonate and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized with 25:75 methanol: ethyl acetate solvent mixture. The product was in the form of a brown powder and a yield of 0.12 g (0.26 mmol, 13.8 % yield). HPLC (method B) t_R = 11.38 (95% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.49 (1 H, d, J = 5.44 Hz), 7.96 (1 H, d, J = 2.13 Hz), 7.88 (1 H, d, J = 8.94 Hz), 7.68-7.63 (2 H, m), 7.60 (1 H, s), 7.45-7.35 (3 H, m), 7.35-7.26 (3 H, m), 7.20-7.11 (2 H, m), 6.32 (1 H, d, J = 5.48 Hz), 3.38 (2 H, q, J = 5.17 Hz), 3.01 (4 H, t, J = 4.72 Hz), 2.65-2.59 (2 H, m), 2.50 (4 H, s), 1.93 (2 H, p, J = 5.46 Hz).^{13}C NMR δ (ppm)($CHCl_3$-d): 151.7, 150.7, 149.9, 141.0, 135.2, 134.9, 131.7, 128.9, 128.6, 128.4, 128.2, 126.8, 124.9, 123.1, 122.3, 118.0, 117.4, 98.4, 58.7, 53.7, 51.1, 44.4, 23.4. ESIMS $[M + H]^+$ calcd for $C_{28}H_{29}ClN_4$ 457.2145, found 457.215

Synthesis of PL154 7-chloro-*N*-(3-(4-(4-chlorophenyl)piperazin-1-yl)propyl)quinolin-4-amine

0.63 g (2 mmol) of PL29 was added to 0.47 g (2.4 mmol) of 1-(4-chlorophenyl)piperazine and 0.39 g (4 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL sodium bicarbonate and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized with 25:75 methanol: ethyl acetate solvent mixture. The product was in the form of an off white powder and a yield of 0.02 g (0.05 mmol, 2.4 % yield). HPLC (method A) t_R = 5.91 (98% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.52 (1 H, d, J = 5.36 Hz), 7.93 (1 H, d, J = 2.15 Hz), 7.78 (1 H, d, J = 8.94 Hz), 7.29-7.27 (2 H, m), 7.24 (1 H, s), 7.20 (1 H, dd, J = 8.91, 2.16 Hz), 6.92-6.87 (2 H, m), 6.36 (1 H, d, J = 5.40 Hz), 3.42 (2 H, q, J = 5.32 Hz), 3.32-3.26 (4 H, m), 2.74 (4 H, t, J = 4.82 Hz), 2.73-2.67 (2 H, m), 2.00 (2 H, p, J = 5.58 Hz). ESIMS $[M + H]^+$ calcd for $C_{22}H_{24}Cl_2N_4$ 415.1451, found 415.1460.

Synthesis of PL155 7-chloro-*N*-(3-(4-(3,4-dichlorophenyl)piperazin-1-yl)propyl)quinolin-4-amine

0.53 g (1.68 mmol) of PL29 was added to 0.47 g (2.0 mmol) of 1-(3,4-dichlorophenyl)piperazine and 0.34 g (3.4 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted

with 30 mL sodium bicarbonate and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in methanol. The product was in the form of gold crystals and weighed 0.15 g (0.3 mmol, 19.8 %). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.52 (1 H, d, J = 5.36 Hz), 7.93 (1 H, d, J = 2.15 Hz), 7.74 (1 H, d, J = 8.94 Hz), 7.33 (1 H, d, J = 8.89 Hz), 7.26 (1 H, s), 7.21 (1 H, dd, J = 8.90, 2.17 Hz), 7.07 (1 H, s), 7.01 (1 H, d, J = 2.87 Hz), 6.78 (1 H, dd, J = 8.91, 2.88 Hz), 6.36 (1 H, d, J = 5.40 Hz), 3.44-3.37 (2 H, m), 3.29 (4 H, t, J = 4.84 Hz), 2.73-2.64 (6 H, m), 2.05-1.95 (2 H, m). ESIMS $[M + H]^+$ calcd for $C_{22}H_{23}Cl_3N_4$ 449.1068, found 449.1061.

Synthesis of PL156 7-chloro-*N*-(3-(4-(4-fluorophenyl)piperazin-1-yl)propyl)quinolin-4-amine

0.58 g (1.84 mmol) of PL29 was added to 0.40 g (2.2 mmol) of 1-(4-fluorophenyl)piperazine and 0.37 g (3.7 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in a 25:75 methanol:ethylacetate solution. The product was in the form of a white powder and weighed 0.26 g (0.7 mmol, 35.4 %). HPLC (method A) t_R = 5.91 (99% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.52 (1 H, d, J = 5.36 Hz), 7.93 (1 H, d, J = 2.16 Hz), 7.80 (1 H, d, J = 8.94 Hz), 7.31 (1 H, s), 7.20 (1 H, dd, J = 8.91, 2.17 Hz), 7.06-6.98 (2 H,

m), 6.97-6.91 (2 H, m), 6.35 (1 H, d, J = 5.40 Hz), 3.41 (2 H, q, J = 5.29 Hz), 3.27-3.22 (4 H, m), 2.77-2.72 (4 H, m), 2.73-2.67 (2 H, m), 2.06-1.96 (2 H, m). ESIMS $[M + H]^+$ calcd for $C_{22}H_{24}ClFN_4$ 399.1746, found 399.1753.

Synthesis of PL157 7-chloro-*N*-(3-(4-(4-methoxyphenyl)piperazin-1-yl)propyl)quinolin-4-amine

0.69 g (2.2 mmol) of PL29 was added to 0.51 g (2.6 mmol) of 1-(4-methoxyphenyl)piperazine and 0.44 g (4.4 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in a 25:75 methanol:ethylacetate solution. The product was in the form of gold crystals and weighed 0.09 g (0.2 mmol, 10 %). HPLC (method A) t_R = 6.88 (98% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.51 (1 H, d, J = 5.36 Hz), 7.92 (1 H, d, J = 2.16 Hz), 7.83 (1 H, d, J = 8.94 Hz), 7.43 (1 H, s), 7.21 (1 H, dd, J = 8.91, 2.17 Hz), 6.98-6.93 (2 H, m), 6.93-6.87 (2 H, m), 6.33 (1 H, d, J = 5.40 Hz), 3.86-3.71 (3 H, m), 3.40 (2 H, q, J = 5.23 Hz), 3.22 (4 H, t, J = 4.73 Hz), 2.78-2.72 (4 H, m), 2.71-2.66 (2 H, m), 2.03-1.95 (2H, m). ESIMS $[M + H]^+$ calcd for $C_{23}H_{27}ClN_4O$ 411.1946, found 411.1949.

Synthesis of PL158 7-chloro-*N*-(3-(4-(4-(trifluoromethyl)phenyl)piperazin-1-yl)propyl)quinolin-4-amine

0.69 g (2.2 mmol) of PL29 was added to 0.61 g (2.6 mmol) of 1-(4-methoxyphenyl)piperazine and 0.44 g (4.4 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in a 25:75 methanol:ethylacetate solution. The product was in the form of white crystals and weighed 0.03 g (0.07 mmol, 3.1 %). HPLC (method A) t_R = 5.88 (99% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.52 (1 H, d, J = 5.35 Hz), 7.93 (1 H, d, J = 2.16 Hz), 7.75 (1 H, d, J = 8.94 Hz), 7.54 (2 H, d, J = 8.60 Hz), 7.20 (1 H, dd, J = 8.90, 2.17 Hz), 7.11 (1 H, s), 6.98 (2 H, d, J = 8.59 Hz), 6.36 (1 H, d, J = 5.39 Hz), 3.46-3.38 (6 H, m), 2.74 (4 H, t, J = 4.91 Hz), 2.72-2.66 (2 H, m), 2.01 (2 H, p, J = 5.65 Hz). ESIMS $[M + H]^+$ calcd for $C_{23}H_{24}ClF_3N_4$ 449.1714, found 449.1729.

Synthesis of PL159 4-(4-(3-(7-chloroquinolin-4-ylamino)propyl)piperazin-1-yl)phenol

0.51 g (1.6 mmol) of PL29 was added to 0.31 g (1.7 mmol) of 1-(4-hydroxyphenyl)piperazine and 0.23 g (1.62 mmol) of potassium carbonate in 20 mL of acetonitrile. The mixture was allowed to reflux for 24 hrs and then the acetonitrile was evaporated off. The remaining residue was dissolved in 20 mL of chloroform and then

extracted with 30 mL saturated sodium bicarbonate solution and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in a 25:75 methanol:ethylacetate solution. The product was in the form of a grey powder and weighed 0.05 g (0.1 mmol, 8 %). ^{1}H NMR δ (ppm)(CH_3OH-d_4): 8.38 (1 H, d, J = 5.64 Hz), 8.11 (1 H, d, J = 9.01 Hz), 7.80 (1 H, d, J = 2.17 Hz), 7.39 (1 H, dd, J = 9.01, 2.19 Hz), 6.94-6.88 (2 H, m), 6.77-6.72 (2 H, m), 6.58 (1 H, d, J = 5.68 Hz), 3.52-3.45 (2 H, m), 3.16-3.08 (4 H, m), 2.72-2.65 (4 H, m), 2.62 (2 H, t, J = 7.16 Hz), 2.05-1.96 (2 H, m). ESIMS $[M + H]^+$ calcd for $C_{22}H_{26}ClON_4$ 397.1790, found 397.1783.

Synthesis of PL163 7-chloro-*N*-(3-(4-*p*-tolylpiperazin-1-yl)propyl)quinolin-4-amine

0.70 g (2.2 mmol) of PL29 was added to 0.47 g (2.7 mmol) of 1-(4-methoxyphenyl)piperazine and 0.45 g (4.4 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution and 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in a 25:75 methanol:ethylacetate solution. The product was in the form off gold crystals and weighed 0.110 g (0.3 mmol, 12.5 % yield). HPLC (method B) t_R = 9.11 (96% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.51 (1 H, d, J = 5.36 Hz), 7.92 (1 H, d, J = 2.16 Hz), 7.82 (1 H, d, J = 8.94 Hz), 7.41 (1 H, s), 7.21 (1 H, dd, J = 8.91, 2.18 Hz), 7.14 (2 H,

d, J = 8.21 Hz), 6.91 (2 H, d, J = 8.35 Hz), 6.34 (1 H, d, J = 5.40 Hz), 3.41 (2 H, q, J = 5.25 Hz), 3.29 (4 H, t, J = 4.77 Hz), 2.75 (4 H, t, J = 4.75 Hz), 2.73-2.67 (2 H, m), 2.32 (3 H, s), 2.06-1.96 (2 H, m). ^{13}C NMR δ (ppm)($CHCl_3$-d): 152.2, 150.5, 149.2, 149.0, 134.7, 129.8, 128.7, 124.8, 122.1, 117.4, 116.6, 98.5, 58.7, 53.7, 50.0, 44.4, 23.6, 20.5. ESIMS $[M + H]^+$ calcd for $C_{23}H_{27}ClN_4$ 395.1997, found 395.1984.

Synthesis of PL192 *N,N'*-(3,3'-(piperazine-1,4-diyl)bis(propane-3,1-diyl))bis(7-chloroquinolin-4-amine)

1.64 g (8.3 mmol) of 4,7-dichloroquinoline was added to 0.79 g of (3.9 mmol)3,3'-(piperazine-1,4-diyl)dipropan-1-amine and 6 g (63.8 mmol) of then heated at ~125^{o}C for 4 hrs(temperature and time control were critical as dark tarry by products formed with large variations in time and temperature). The solution was allowed to cool to room temperature and then diluted with 40 mL dichloromethane. The solution was then washed with 6 X 20 mL 2 M sodium hydroxide and then 30 mL brine. The resulting solution was dried over magnesium sulphate. After the solvent was evaporated the solid product was recrystallized in 25:75 methanol:ethylacetate solution. 0.020 g (0.04 mmol, 1% yield) of an off white crystal powder was obtained. HPLC (method B) t_R = 6.38 (96% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.53 (2 H, d, J = 5.35 Hz), 7.95 (2 H, dd, J = 6.47, 2.15 Hz), 7.91-7.83 (2 H, m), 7.37 (2 H, s), 7.37-7.28 (2 H, m), 6.40-6.32 (2 H, m), 3.47-3.36 (4 H, m), 2.78-2.72 (8 H, m), 2.06-1.96 (4 H, m), 1.71 (4 H, s).^{13}C NMR δ (ppm)($CHCl_3$-d): 152.3, 150.5, 149.2, 134.6,

128.8, 124.7, 122.2, 117.5, 98.7, 59.0, 53.7, 44.3, 23.6. ESIMS $[M + H]^+$ calcd for $C_{28}H_{32}Cl_2N_6$ 523.2138, found 523.2147.

Synthesis of PL223 7-chloro-*N*-(3-(4-(naphthalen-1-ylmethyl)piperazin-1-yl)propyl)quinolin-4-amine

0.70 g (2.2 mmol) of PL29 was added to 0.6 g (2.7 mmol) of 1-(naphthalen-1-ylmethyl)piperazine and 0.45 g (4.4 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution. The aqueous layer was further extracted with 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in a 25:75 methanol:ethylacetate solution. The product was in the form of pale yellow crystals and weighed 0.120 g (0.3 mmol, 12.1 % yield). HPLC (method B) t_R = 9.69 (98% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 1.93 (2 H, p, J = 5.40 Hz), 2.54-2.74 (10 H, m), 3.37 (2 H, q, J = 5.14 Hz), 4.03 (2 H, s), 6.32 (1 H, d, J = 5.39 Hz), 7.35 (1 H, dd, J = 8.91, 2.18 Hz), 7.42-7.60 (4 H, m), 7.64 (1 H, s), 7.79-7.91 (2 H, m), 7.90-7.98 (2 H, m), 8.33 (1 H, d, J = 8.22 Hz), 8.51 (1 H, d, J = 5.34 Hz).^{13}C NMR δ (ppm)($CHCl_3$-d): 23.4, 44.6, 53.3, 53.7, 58.8, 61.4, 98.5, 117.5, 122.6, 124.6, 124.7, 125.2, 125.7, 125.8, 127.6, 128.2, 128.5, 128.7, 132.6, 133.6, 133.9, 134.6, 149.2, 150.6, 152.3. ESIMS $[M + H]^+$ calcd for $C_{27}H_{29}ClN_4$ 445.2154, found 445.2154.

Synthesis of PL227 7-chloro-*N*-(3-(4-phenethylpiperazin-1-yl)propyl)quinolin-4-amine

0.60 g (1.9 mmol) of PL29 was added to 0.44 g (2.3 mmol) of 1-phenethylpiperazine and 0.38 g (3.8 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution. The aqueous layer was further extracted with 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in ethyl acetate solution. The product was in the form of pale tan crystals and weighed 0.29 g (0.7 mmol, 37.2 % yield). HPLC (method B) t_R = 7.6 (99% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 1.91-2.00 (2 H, m), 2.52-2.86 (12 H, m), 2.83-2.90 (2 H, m), 3.39 (2 H, q, J = 5.17 Hz), 6.33 (1 H, d, J = 5.41 Hz), 7.20-7.25 (3 H, m), 7.29-7.35 (3 H, m), 7.56 (1 H, s), 7.89 (1 H, d, J = 8.94 Hz), 7.94 (1 H, d, J = 2.15 Hz), 8.51 (1 H, d, J = 5.36 Hz).^{13}C NMR δ (ppm)($CHCl_3$-d): 152.2, 150.6, 149.2, 140.1, 134.7, 128.7, 128.7, 128.5, 126.2, 124.7, 122.4, 117.5, 98.5, 60.7, 58.8, 53.6, 53.4, 44.5, 33.7, 23.4. ESIMS $[M + H]^+$ calcd for $C_{23}H_{29}ClN_4$ 409.2154, found 409.2156.

Synthesis of PL228 7-chloro-*N*-(3-(4-(cyclohexylmethyl)piperazin-1-yl)propyl)quinolin-4-amine

0.40 g (1.3 mmol) of PL29 was added to 0.28 g (1.5 mmol) of 1-(cyclohexylmethyl)piperazine and 0.26 g (2.6 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution. The aqueous layer was further

extracted with 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in ethyl acetate solution. The product was in the form of pale orange crystals and weighed 0.050 g (0.1 mmol, 9.8 % yield). HPLC (method B) t_R = 7.68 (96% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 0.83-0.98 (2 H, m), 1.14-1.32 (3 H, m), 1.73 (7 H, d, J = 14.81 Hz), 1.82 (2 H, d, J = 13.12 Hz), 1.90-1.99 (2 H, m), 2.23 (2 H, d, J = 7.18 Hz), 2.51-2.70 (8H, m), 3.38 (2 H, q, J = 5.12 Hz), 6.31 (1 H, d, J = 5.43 Hz), 7.33 (1 H, dd, J = 8.90, 2.19 Hz), 7.75 (1 H, s), 7.90-7.96 (2 H, m), 8.50 (1 H, d, J = 5.38 Hz). ^{13}C NMR δ (ppm)($CHCl_3$-d): 23.2, 26.2, 27.0, 31.9, 35.3, 44.6, 53.7, 53.8, 58.6, 66.0, 98.4, 117.7, 122.6, 124.2, 128.6, 134.9, 149.0, 150.5, 152.2. ESIMS $[M + H]^+$ calcd for $C_{23}H_{33}ClN_4$ 401.2467, found 401.2468.

Synthesis of PL229 7-chloro-*N*-(3-(4-cyclohexylpiperazin-1-yl)propyl)quinolin-4-amine

0.45 g (1.4 mmol) of PL29 was added to 0.28 g (1.7 mmol) of 1-cyclohexylpiperazine and 0.29 g (2.9 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution. The aqueous layer was further extracted with 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was

recrystallized in ethyl acetate solution. The product was in the form of pale gold crystals and weighed 0.090 g (0.2 mmol, 15.8 % yield). HPLC (method B) t_R = 6.77 (96% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 1.30 (4 H, d, J = 10.45 Hz), 1.68 (5 H, s), 1.87 (2 H, s), 1.95 (4 H, dt, J = 10.86, 5.61 Hz), 2.34 (1 H, s), 2.61-2.67 (3 H, m), 2.76 (4 H, s), 3.38 (2 H, q, J = 5.14 Hz), 6.32 (1 H, d, J = 5.42 Hz), 7.30 (1 H, dd, J = 8.90, 2.19 Hz), 7.72 (1 H, s), 7.90 (1 H, d, J = 8.93 Hz), 7.94 (1 H, d, J = 2.16 Hz), 8.51 (1 H, d, J = 5.37 Hz). ^{13}C NMR δ (ppm)($CHCl_3$-d): 23.3, 25.9, 26.3, 28.9, 44.5, 48.9, 54.1, 59.0, 63.6, 98.5, 117.7, 122.3, 124.6, 128.5, 134.7, 149.1, 150.8, 152.2. ESIMS $[M + H]^+$ calcd for $C_{22}H_{31}ClN_4$ 387.2310, found 387.2303.

Synthesis of PL255 *N,N'*-(3,3'-(piperazine-1,4-diyl)bis(propane-3,1-diyl))diquinolin-4-amine

1.5 g (9.2 mmol) of 4-chloroquinoline was added to 0.87 g of (4.4 mmol)3,3'-(piperazine-1,4-diyl)dipropan-1-amine and 6 g (63.8 mmol) of then heated at ~125^{o}C for 4 hrs(temperature and time control were critical as dark tarry by products formed with large variations in time and temperature). The solution was allowed to cool to room temperature and then diluted with 40 mL dichloromethane. The solution was then washed with 6 X 20 mL 2 M sodium hydroxide and then 30 mL brine. The resulting solution was dried over magnesium sulphate. After the solvent was evaporated the solid product was recrystallized in ethyl acetate solution. 0.13 g (0.3 mmol, 6.6 % yield) of a white crystal powder was obtained. HPLC (method B) t_R =

4.73 (95% Pure). ^{1}H NMR δ (ppm)($CHCl_3$-d): 8.55 (2 H, d, J = 5.31 Hz), 7.98 (2 H, dd, J = 8.47, 1.18 Hz), 7.94 (2 H, d, J = 8.38 Hz), 7.63 (2 H, ddd, J = 8.45, 6.83, 1.33 Hz), 7.41 (2 H, ddd, J = 8.38, 6.82, 1.28 Hz), 7.25 (2 H, s), 6.38 (2 H, d, J = 5.36 Hz), 3.44 (4 H, q, J = 5.29 Hz), 2.77-2.71 (12H, m), 2.07-1.99 (4 H, m). ESIMS $[M + H]^+$ calcd for $C_{28}H_{34}N_6$ 382.1793, found 382.1784.

Synthesis of PL257 7-chloro-*N*-(3-(4-(4-nitrophenyl)piperazin-1-yl)propyl)quinolin-4-amine

0.67 g (2.1 mmol) of PL29 was added to 0.53 g (2.6 mmol) of 1-(4-nitrophenyl)piperazine and 0.43 g (4.3 mmol) of triethylamine in 15 mL of tetrahydrofuran. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution. The aqueous layer was further extracted with 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was recrystallized in ethyl acetate solution. The product was in the form of bright yellow crystals and weighed 0.06 g (0.1 mmol, 6.6 % yield). HPLC (method B) t_R = 8.93 (95% Pure). ^{1}H NMR δ (ppm)(DMSO-d_6): 211.41 (1 H, d, J = 5.40 Hz), 211.26 (1 H, d, J = 9.03 Hz), 211.11-211.03 (2 H, m), 210.79 (1 H, d, J = 2.25 Hz), 210.45 (1 H, dd, J = 8.97, 2.25 Hz), 210.39 (1 H, t, J = 5.29 Hz), 210.04 (2 H, d, J = 9.14 Hz), 209.52 (1 H, d, J = 5.48 Hz), 206.48 (4 H, t, J = 4.66 Hz), 205.50-205.43 (3 H, m), 204.92-204.81 (2 H, m), 203.01 (4 H, s).^{13}C NMR δ (ppm)($CHCl_3$-d): 159.7,

154.9, 141.4, 139.4, 132.7, 130.9, 129.3, 122.7, 108.4, 103.8, 96.7, 60.8, 58.6, 57.8, 51.6 , 46.7, 4.8. ESIMS $[M + H]^+$ calcd for $C_{22}H_{24}Cl_1N_5O_2$ 426.1691, found 426.1682.

Synthesis of PL258 2-(3-(4-phenylpiperazin-1-yl)propyl)isoindoline-1,3-dione

6.00 g (37 mmol) of 1-phenylpiperazine was added to 9.44 g (35 mmol) of 2-(3-bromopropyl)isoindoline-1,3-dione and 12.17 g (88 mmol) of potassium carbonate in 150 mL of acetonitrile. The mixture was allowed to reflux for 3 hrs. On cooling the acetonitrile was evaporated off and the residue extracted with 200 mL water and 200 mL ethylacetate. The aqueous layer was extracted with 2 X 100 mL ethyl acetate and combined to the organic layer. The solvent was evaporated off to give a pale yellow liquid that solidified after being left in the fume hood. The product was recrystallized in hexane to give a white powder 6.00 g (15 mmol, 43 % yield). ^{1}H NMR δ (ppm)($CHCl_3$-d): 7.87-7.80 (2 H, m), 7.74-7.66 (2 H, m), 7.44 (2 H, d, J = 8.63 Hz), 6.84 (2 H, d, J = 8.61 Hz), 3.80 (2 H, t, J = 6.90 Hz), 3.13-3.08 (4 H, m), 2.55-2.44 (6 H, m), 1.90 (2 H, p, J = 6.85 Hz).

Synthesis of PL259 3-(4-phenylpiperazin-1-yl)propan-1-amine

6.00 g (15 mmol) of PL258 was dissolved in 75 mL of ethanol and 2.25 g (45 mmol) of hydrazine monohydrate was added. The mixture was allowed to reflux for 4 hrs and then the residue was evaporated off. The remaining solid was partitioned in

chloroform and filtered off to get the amine a pale yellow solid 1.34 g (6.1 mmol, 41% yield).

Synthesis of PL260 1-(3-phenylpropyl)-4-(4-(triflouromethyl)phenyl)piperazine

0.5 g (2.8 mmol) of 1-(4-(trifluoromethyl)phenyl)piperazine was added to 0.54 g (2.70 mmol) of (3-bromopropyl)benzene and 0.93 g (6.8 mmol) of potassium carbonate in 20 mL of acetonitrile. The mixture was allowed to reflux for 3 hrs. On cooling the acetonitrile was evaporated off and the residue extracted with 20 mL water and 20 mL ethyl acetate. The aqueous layer was extracted with 2 X 10 mL ethyl acetate and combined to the organic layer. The solvent was evaporated off to give an off white liquid that solidified after being left in the fume hood. The product was recrystallized in hexane to give a white powder 0.51 g (1.7 mmol, 63.8 % yield). HPLC (method B) t_R = 15.88 (95% Pure). ^{1}H NMR δ (ppm)(400MHz, $CHCl_3$-d): 7.47 (2 H, d, J = 8.63 Hz), 6.91 (2 H, d, J = 8.62 Hz), 3.30-3.25 (4 H, m), 2.67 (2 H, t, J = 7.71 Hz), 2.60-2.55 (4 H, m), 2.47-2.39 (2 H, m), 1.92-1.80 (2 H, m). ^{13}C NMR δ (ppm)($CHCl_3$-d): 153.2, 141.9, 128, 128.1, 125.8, 125.2, 114.2, 57.6, 52.6, 48.2, 47.8, 33.7, 28.7. ESIMS $[M + H]^+$ calcd for $C_{20}H_{24}F_3N_2$ 383.1741, found 383.1745

Synthesis of PL261 7-chloro-N-(3-(4-(pyrimidin-2-yl)piperazin-1-yl)propyl)quinolin-4-amine

0.3 g (1.0 mmol) of PL29 was added to 0.17 g (1.1 mmol) of 2-(piperazin-1-yl)pyrimidine and 0.19 g (1.9 mmol) of triethylamine in 15 ml THF. The mixture was allowed to reflux for one day and then extracted with 30 mL saturated sodium bicarbonate solution. The aqueous layer was further extracted with 3 X 10 mL chloroform. The chloroform was evaporated off and the crude product was columned in 1:1 methanol:ethylacetate solution. The product was in the form of beige crystals and weighed 0.16 g (0.4 mmol, 44 % yield). HPLC (method B) t_R = 6.95 (99% Pure). ^{1}H NMR δ (ppm)(CH_3OH-d_4): 8.38 (1 H, d, J = 5.65 Hz), 8.34 (2 H, d, J = 4.78 Hz), 8.11 (1 H, d, J = 9.01 Hz), 7.80 (1 H, d, J = 2.17 Hz), 7.42 (1 H, dd, J = 9.01, 2.19 Hz), 6.64-6.56 (2 H, m), 3.85 (4 H, t, J = 4.99 Hz), 3.48 (2 H, t, J = 6.82 Hz), 2.62-2.56 (6 H, m), 2.01 (2 H, p, J = 7.01 Hz).^{13}C NMR δ (ppm)($CHCl_3$-d): 26.2, 42.6, 44.7, 54.2, 57.5, 99.7, 111.3, 118.8, 124.3, 126.0, 127.6, 136.4, 149.7, 152.5, 152.8, 159.1, 162.9. ESIMS $[M + H]^+$ calcd for $C_{20}H_{24}ClN_6$ 383.1741, found 383.1745

Synthesis of PL272 N-(3-(4-phenylpiperazin-1-yl)propyl)acetamide

1.3 g (6.0 mmol) of the amine was treated with 0.9 g (12 mmol) of acetyl chloride in 30 mL acetonitrile. After 24 hrs the solvent was evaporated off and the solid dissolved in 60 mL sodium bicarbonate solution. 3 X 20 mL of chloroform was used to extract the product which was a beige powder 1.1 g (4.2 mmol, 28 %).

HPLC (method B) t_R = 7.71 (97% Pure).^{1}H NMR δ (ppm)(CH_3OH-d_4): 7.13 (2 H, dd, J = 8.68, 7.20 Hz), 6.87 (2 H, d, J = 8.21 Hz), 6.74 (1 H, t, J = 7.31 Hz), 3.15 (6 H, m), 2.55 (4 H, t, J = 4.92 Hz), 2.36 (2 H, t, J = 7.65 Hz), 1.83 (3 H, s), 1.65 (2 H, t, J = 7.49 Hz). ^{13}C NMR δ (ppm)(CH_3OH-d_4): 173.6, 153.5, 130.4, 121.5, 117.7, 57.7, 54.6, 50.6, 38.9, 27.2, 22.4. ESIMS $[M + H]^+$ calcd for $C_{15}H_{24}ON_3$ 262.1914, found 262.1915.

Synthesis of PL289 3-chloro-N-(pyridin-4-yl)propanamide

12.43 mL (130 mmol) of chloropropionyl chloride was added dropwise to 10.34 g (110 mmol) of 4-aminopyridine. 17.97 g (130 mmol) of potassium carbonate was used as base in 130 mL dichloromethane. The reaction mixture was stirred at room temperature for five days. The residue was evaporated off and the resulting white solid was recrystallized in water. The product was a white powder 2.00 g (11 mmol, 10%).

Synthesis of PL273 3-(4-phenylpiperazin-1-yl)-N-(pyridin-4-yl)propanamide

1.33 g (7.2 mmol) of 3-chloro-N-(pyridin-4-yl)propanamide was added to 1.29 g (8.0 mmol) of 1-phenylpiperazine and 1.49 g (11 mmol) in 45 mL DMF. The mixture was refluxed for 5 days. The residue was evaporated off and the solid was partitioned in 30 mL sodium bicarbonate solution. 3 X 15 mL of chloroform was used to extract the

solution. The solid was obtained by recrystallization in ethanol as off white crystals 0.20 g (0.7 mmol, 9.4%). HPLC (method B) t_R = 5.58 (95% Pure). ^{1}H NMR δ (ppm)(CH_3OH-d_4): 8.40 (2 H, dd, J = 4.98, 1.64 Hz), 7.66 (2 H, dd, J = 4.97, 1.63 Hz), 7.25-7.24 (2 H, m), 7.00-6.97 (2 H, m), 6.86 (1 H, t, J = 7.31 Hz), 3.23 (4 H, t, J = 4.88 Hz), 2.86 (2 H, t, J = 6.96 Hz), 2.74 (4 H, t, J = 4.90 Hz), 2.68 (2 H, t, J = 6.96 Hz). ^{13}C NMR δ (ppm)(CH_3OH-d_4): 173.6, 152.6, 150.8, 148.0, 130.1, 121.2, 117.5, 115.1, 54.7, 54.0, 50.4, 35.0. ESIMS $[M + H]^+$ calcd for $C_{18}H_{23}ON_4$ 311.1866, found 311.1868

Synthesis of PL274 7-chloro-N-(2-(4-phenylpiperazin-1-yl)ethyl)quinolin-4-amine

6 g (20 mmol) of sulphonyl adduct were treated with 3.56 g (22 mmol) of 1-phenylpiperazine and 4.04 g (40 mmol) triethylamine in 50 mL acetonitrile. The mixture was allowed to reflux for 48 hrs. The solvent was evaporated off and the remaining solid was partitioned with 150 mL sodium bicarbonate solution. It was then extracted with 50 X 3 mL of chloroform. The solid was columned on silica with a 50:50 methanol:ethylacetate mixture. The solid product was light brown 2 g (5.5 mmol, 27%). HPLC (method B) t_R = 8.14 (98% Pure). PL274

(CH_3OH-d_4): 8.41 (1 H, d, J = 5.62 Hz), 8.11 (1 H, d, J = 9.01 Hz), 7.82 (1 H, d, J = 2.18 Hz), 7.45 (1 H, dd, J = 9.02, 2.19 Hz), 7.26-7.25 (2 H, m), 7.01-7.01 (2 H, m), 6.86 (1 H, t, J = 7.30 Hz), 6.62 (1 H, d, J = 5.66 Hz), 3.59 (2 H, t, J = 6.67 Hz), 3.25 (4 H, t, J = 4.86 Hz), 2.84 (2 H, t, J = 6.67 Hz), 2.78 (4 H, t, J = 4.88 Hz). ^{13}C NMR δ

(ppm)(CH_3OH-d_4): 40.8, 50.6, 54.2, 57.1, 99.8, 117.4, 121.3, 124.2, 126.2, 127.7, 128.4, 130.0, 131.6, 136.5, 149.8, 152.5, 161.4. ESIMS $[M + H]^+$ calcd for $C_{21}H_{24}ClN_4$ 367.1684, found 367.1679.

In vitro **Inhibition of Growth Studies.** The antimalarial activities of the synthesized compounds were measured versus the CQ sensitive strain D6 and the CQ resistant strains Dd2 and 7G8 using the standardized, malaria SYBR Green assay.[95-97] Continuously maintained cultures of D6, Dd2, and 7G8 were used. The cultures were diluted with complete medium (RPMI-1640 with 0.5 % Albumax II) to achieve 0.2 % parasitemia and 2 % hematocrit. In 96-well micro plates, CQ (positive control) or the respective compound was diluted in complete medium from a 10 mM stock in DMSO which was added to the cell mixture to yield triplicate wells with drug concentrations ranging from 0 to 10^{-4} M in a final well volume of 100 μL. After 72 h of incubation under standard culture conditions, plates were harvested and read by the SYBR Green I fluorescence-based method using a 96-well fluorescence plate reader (Gemini-EM, Molecular Devices), with excitation and emission wavelengths at 497 and 520 nm, respectively. The fluorescence readings were plotted against log [drug], and the IC_{50} values were obtained from curve fitting performed by nonlinear regression using Prism (Graph Pad) software.

Mouse Efficacy Against *P. berghei.*

Compounds were formulated in a solution consisting of 70% Tween-80 (d = 1.08 g/mL) and 30% ethanol (d = 0.81 g/mL), followed by a 10-fold dilution in water. On day 0, heparinized blood (containing 100 µL of 200 u/mL Heparin) was taken from a donor NMRI mouse with approximately 30% parasitemia. The blood was diluted in physiological saline to 10^8 parasitized erythrocytes per mL. From this suspension 0.2 mL was injected intravenously (i.v.) into experimental groups of 3 female NMRI mice, and a control group of 5 mice. Compounds were administered in a volume of 10ml/kg either as single dose 24 hours after infection (day 1) either by oral gavage (p.o.) or subcutaneous injection, or as 4 consecutive daily p.o. doses 4, 24, 48 and 72 hours after infection (days 0-3). On day 3 (with the single-dose regimen) or on day 4 (with the quadruple-dose regimen), 1 µL tail blood was taken and dissolved in 1 mL PBS buffer. Parasitemia was determined with a FACScan (Becton Dickinson) by counting 100,000 RBCs. The difference between the mean value of the control group and those of the experimental groups was calculated and expressed as a percent relative to the control group (= activity). Animals receiving no compound would die typically 5-6 days post-infection and were therefore euthanized right after determination of parasitemia. The survival of the animals was monitored up to 30 days. Mice surviving for 30 days were checked for parasitemia and subsequently euthanized. A compound was considered curative if the animal survived to 30 days post-infection with no detectable parasites by microscopy, with a detection limit of 1 parasite in 10'000 erythrocytes (that is, 0.01%).

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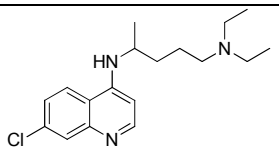
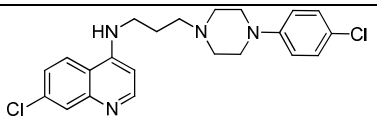
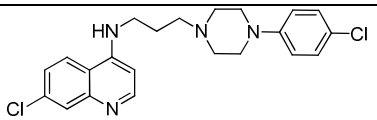
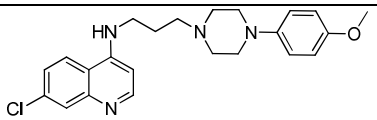
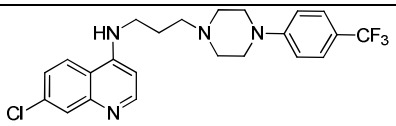
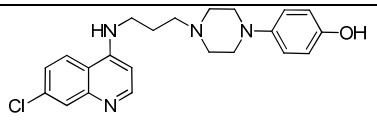
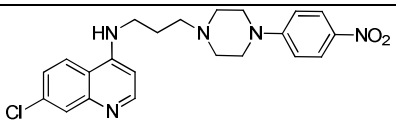
APPENDIX A

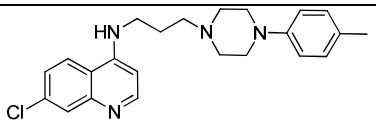
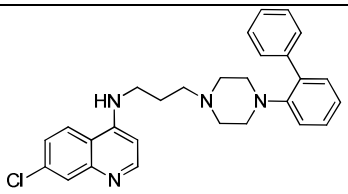
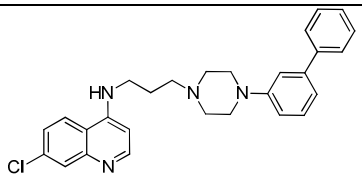
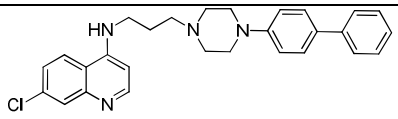
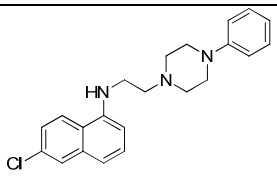
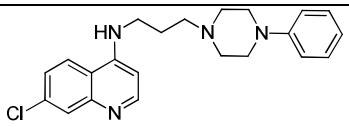
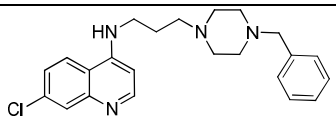
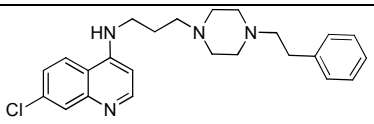
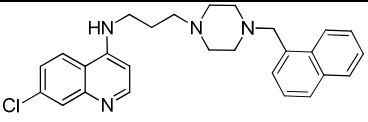
Compound List

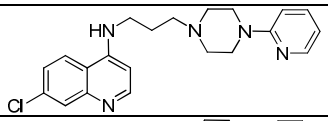
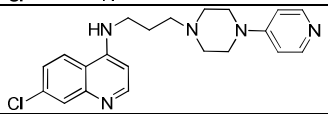
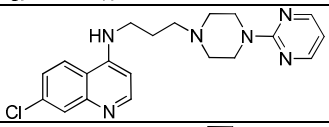
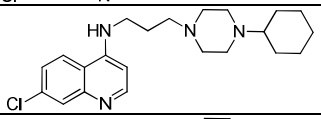
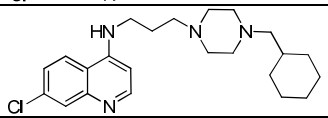
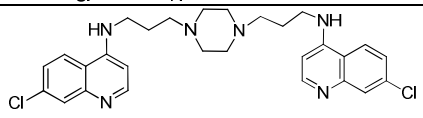
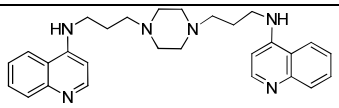
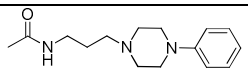
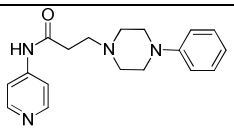
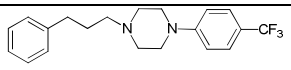
There is a 30% uncertainty in the IC_{50} values that may result from differences in weighing and/or variations in determining IC_{50} as detailed in Chapter 7.

[a]ClogP values were calculated using Chemdraw Ultra 12.0.

[b]IC_{50} values were normalized to CQ values: D6: 6.9 nM, Dd2: 102 nM, 7G8: 106 nM.

Compound	Structure	[a]ClogP	[b]IC_{50}(nM)
CQ		5.1	6.9 D6 102 Dd2 106 7G8
PL154		6.6	0.3 D6 0.5 Dd2 0.1 7G8
PL156		6.0	2.0 D6 0.2 Dd2 0.2 7G8
PL157		5.7	1.3 D6 0.3 Dd2 0.3 7G8
PL158		6.9	0.06 D6 0.2 Dd2 0.3 7G8
PL159		5.0	4.1 D6 4.1 Dd2 2.8 7G8
PL257		5.8	0.9 D6 0.8 Dd2 0.3 7G8

Compound	Structure	[a]ClogP	[b]IC_{50}(nM)
PL163		6.2	0.1 D6 1.3 Dd2 0.5 7G8
PL112		7.0	1.2 D6 2.6 Dd2
PL111		7.0	0.9 D6 1.8 Dd2
PL110		7.0	0.7 D6 0.6 Dd2 0.2 7G8
PL274			0.5 D6 0.5 Dd2
PL91		5.2	0.5 D6 0.5 Dd2 0.5 7G8
		4.1	2.4 D6 7.0 Dd2 9.0 7G8
PL227		5.0	0.5 D6 1.0 Dd2 1.2 7G8
PL223		7.3	1.1 D6 1.1 Dd2 0.8 7G8

Compound	Structure	[a]ClogP	[b]IC_{50}(nM)
PL106		4.3	0.7 D6 1.1 Dd2 0.9 7G8
PL109		4.3	0.5 D6 1.6 Dd2 1.1 7G8
PL261		4.1	1.4 D6 2.3 Dd2 2.3 7G8
PL229		4.8	1.0 D6 2.0 Dd2 2.8 7G8
PL228		5.5	0.2 D6 0.4 Dd2 0.5 7G8
PL192			0.6 D6 0.02 Dd2 0.2 7G8
PL255			4.9 D6 9.8 Dd2 25 7G8
PL272			>2500 D6 >2500 Dd2 >2500 7G8
PL273			>2500 D6 >2500 Dd2 >2500 7G8
PL260			>2500 D6 >2500 Dd2 >2500 7G8

APPENDIX B

Example of Spectra of PL91

All the proton and carbon spectra were run on a Bruker NMR at 400 MHz.

The sample was dissolved in MeOD, and TMS was used as the spectral reference.

Figure A.1 - Proton spectrum with integrals

Figure A.2 - ^{13}C spectrum

Figure A.3 - COSY spectrum

Figure A.4 - NOESY spectrum

Figure A.5 - HMBC spectrum

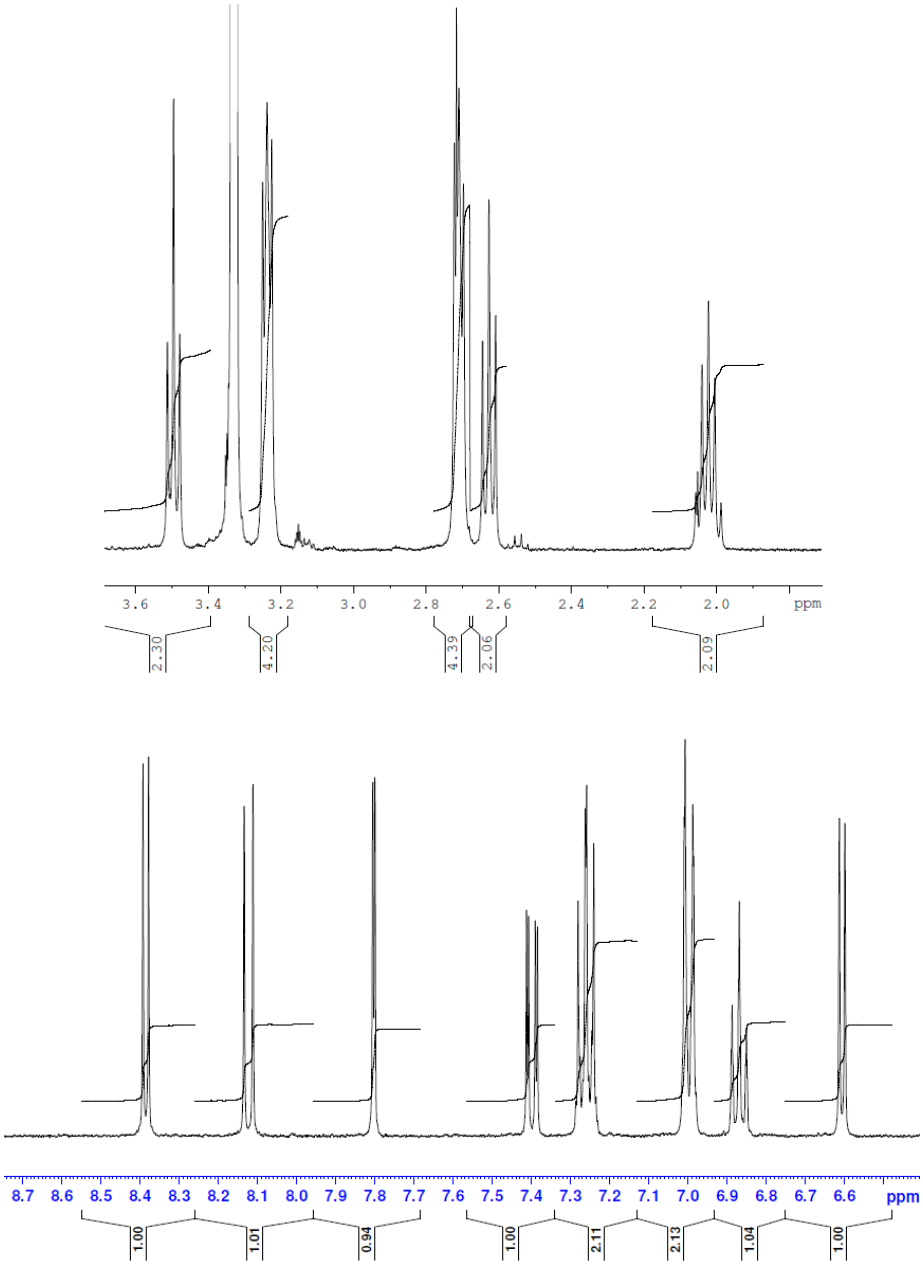


Figure A.1 Proton Spectra of PL91 with integrals. The aliphatic portion of the spectrum is shown at the top and the aromatic portion is shown at the bottom.

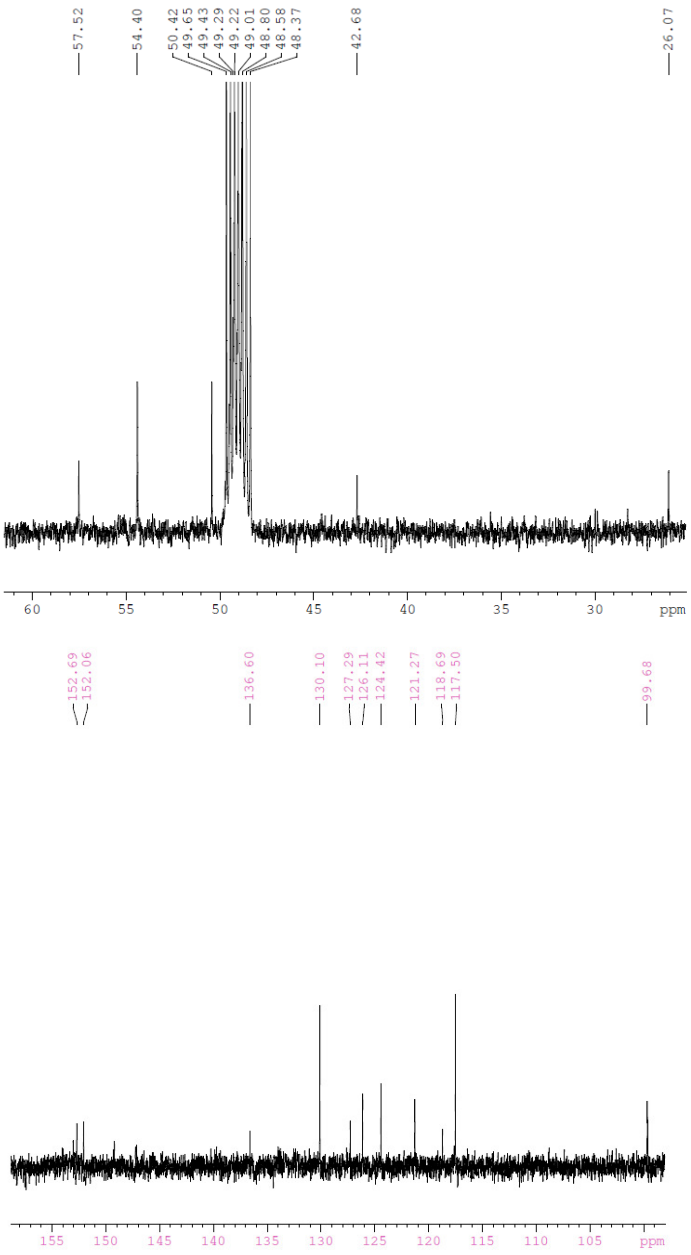


Figure A.2 ^{13}C spectrum.

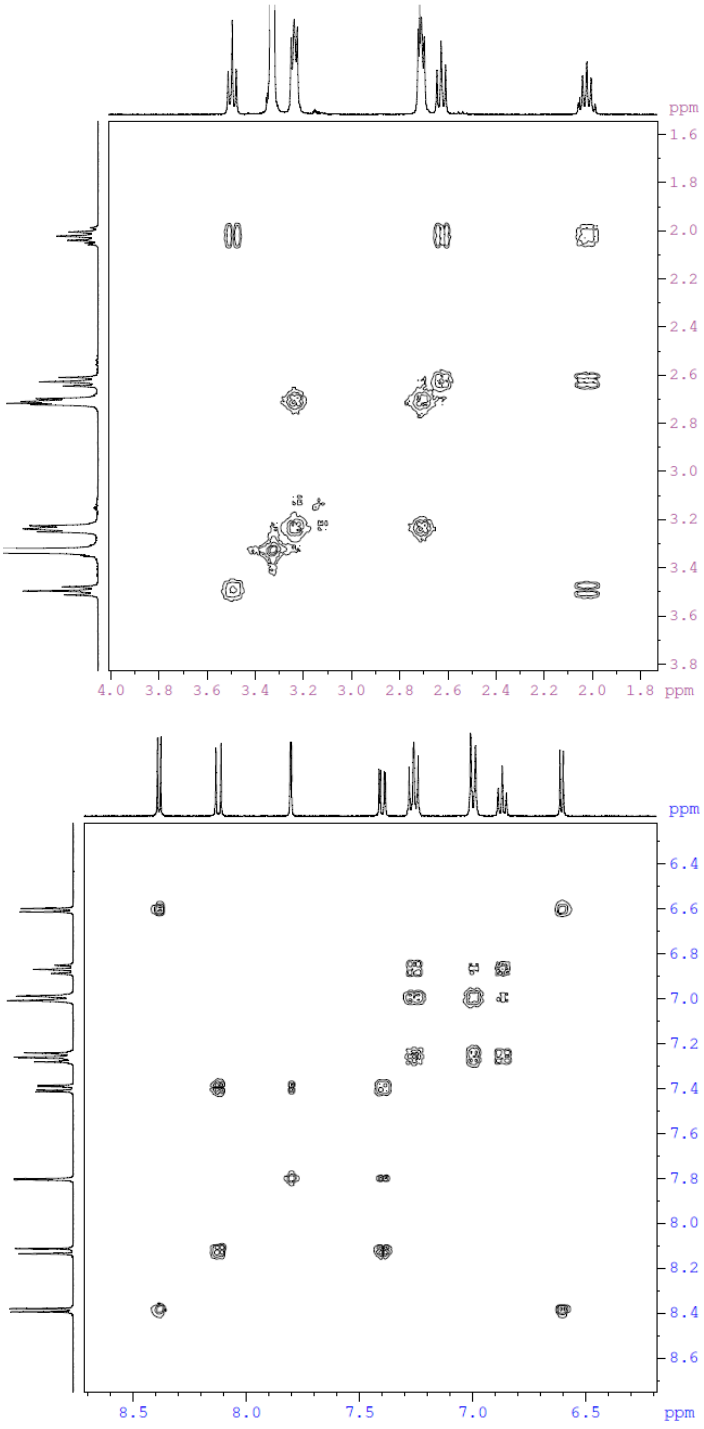


Figure A.3 COSY spectrum showing direct proton-proton interactions.

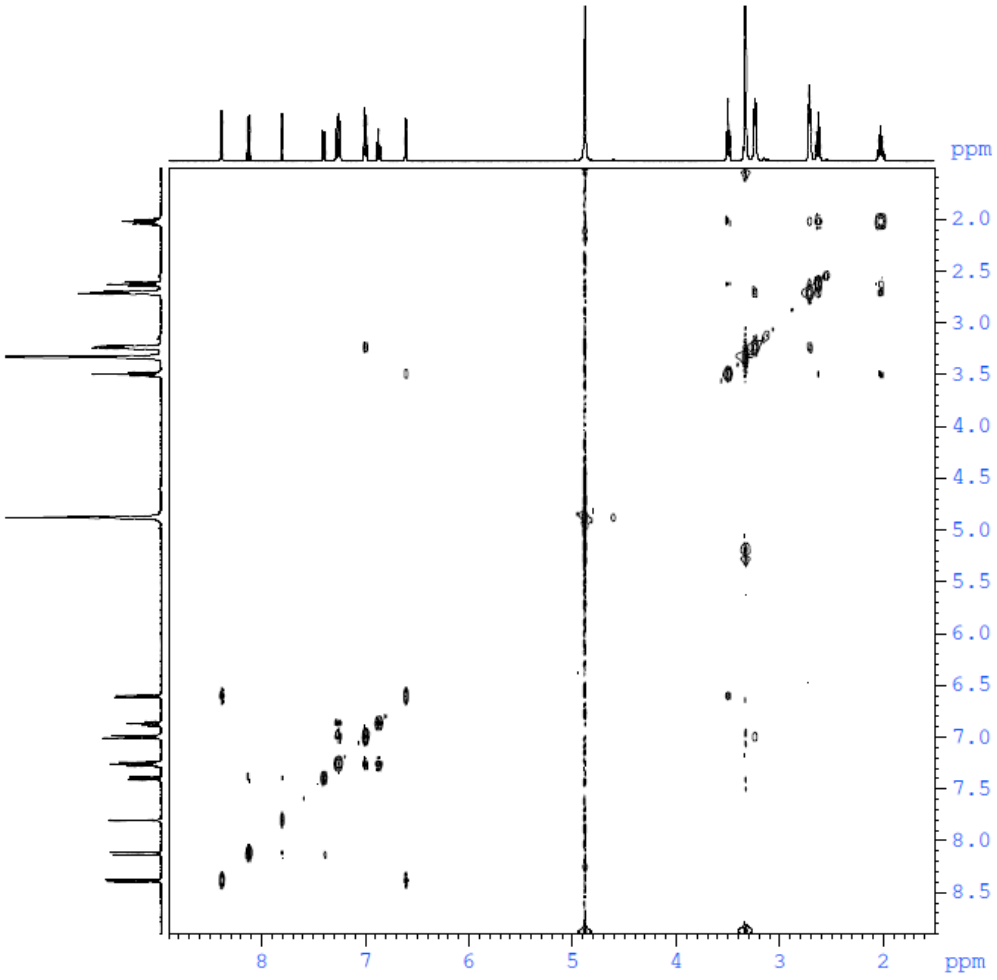


Figure A.4 NOESY spectrum indicating though-space proton-proton interactions.

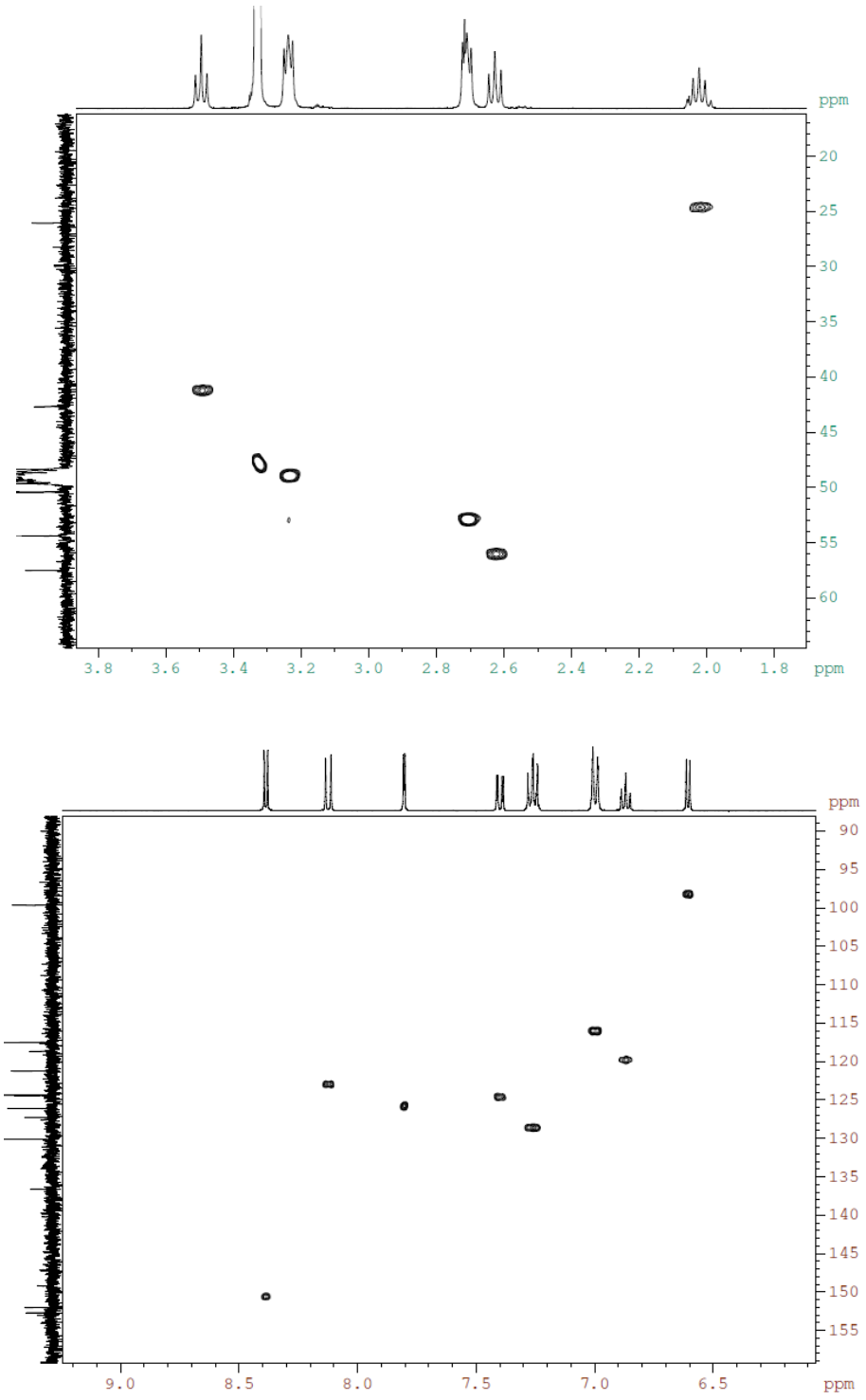


Figure A.5 HSQC spectrum indicating direct proton-carbon interactions.