Chapter 2

Review of Literature

2. Preamble

This chapter presents different aspects such as epidemiology, life cycle of malaria parasite, symptoms and complications, prevention, diagnosis and treatment of malaria. Further, this chapter discloses mode of action of antimalarial drugs as well as use of hybridization strategy for the development of highly active novel quinoline based compounds to combat malarial parasite resistance.

2.1 Introduction

Human malaria is a tropical, recurring infectious disease which has been a principal concern to humanity for centuries and affects more than 40% of the world’s population of the tropics as well as many temperate regions including parts of the Middle East and Asia (Figure 1). The disease being predominantly fatal amongst children and expectant mothers is responsible for some 3.3 billion cases resulting in 1-3 million deaths per year. In year 2012 alone 627,000 deaths have been reported due to malaria.¹

Malaria is caused by protozoan parasite of the genus *Plasmodium*. Out of 300 known species of plasmodium, mainly five species viz. *P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, and occasionally *P. knowlesi*² cause infection to humans. Of these, *P. falciparum* is the most ubiquitous species as well as fatal to humans. Given a suitable combination of climatic and geographical conditions, the parasites are transmitted by certain species of the female anopheles mosquitoes.

![Figure 1. Approximate geographic distribution of malaria in the world.](image)
2.2 Life cycle of malaria parasite

The life cycle of the malaria parasite is quite complex, consisting sexual reproduction (sporogony) stage, which occurs within the mosquito, and the asexual reproduction (schizogony) stage that is realized in the host (Figure 2). The infection starts when an infected *Anopheles* pregnant female mosquito vector of malaria bites a target human to meet its requirement of blood meal. During the bite, the mosquito releases saliva to prevent blood clotting and sporozoites (present in its saliva) make their way into the blood stream of the host and are further transported to liver (1, Figure 2), in less than 1 hour. This is followed by invasion on hepatocytes and exo-erythrocytic maturation and multiplication over the next few days, where these copy DNA over and over again (2). However, in case of *P. vivax* and *P. ovale*, a part of the liver-stage parasites remain dormant in the liver as hypnozoites (3) and are responsible for malaria relapse. The infected liver cells eventually burst to release *Plasmodium* merozoites into the blood stream, where these invade new target, the red blood cells (erythrocytes) (4). During erythrocytic asexual reproduction, these develop through the stages of rings, trophozoites, and schizonts.

![Figure 2. Life cycle of the malaria parasite.](image-url)
The latter typically divide into a second wave of merozoites, which are released by lysis of the erythrocyte immediately invading new erythrocytes (5). Symptoms of uncomplicated malaria such as fever, convulsions, headache, diarrhea, vomiting and anemia appear at this stage. If malaria remains untreated, erythrocytes filled with mature stages of the parasite can adhere to the walls of capillary veins, causing vascular occlusion (obstruction) which may lead to cerebral death (cerebral malaria). Small number of the merozoites released upon the rupture of RBCs in blood develops into male and female gametocytes (6). Bite of an uninfected mosquito at this erythrocytic stage, results in the transfer of *Plasmodium* gamete cells from human host to mosquito. In the mosquito, the male and female gametes fuse to produce ookinetes which subsequently migrate to the midgut of the insect and the sexual phase of the *Plasmodium* life cycle begins. Ookinetes transform to oocysts which reside in the external membrane of the mosquito midgut and mature (7). Further, oocysts undergo division and rupture to release thousands of sporozoites which travel to the insect salivary glands and get accumulated in the acinar cells. When infected, the mosquito bites a vulnerable human, a new parasite cycle commences (8).3-5

### 2.3 Symptoms and complications of malaria

Common symptoms of malaria include fever and flu-like illness,6 including shaking chills, headache, muscle ache, and tiredness and the events are occasionally accompanied by nausea, vomiting, and diarrhea. Malaria may cause anemia and jaundice (yellowing of the skin and eyes) due to loss of red blood cells. These symptoms usually appear between 10 and 15 days after the mosquito bite. Malaria disrupts the blood supply to vital organs, and if not treated timely, could quickly turn life-threatening. The malarial infection occurring with *P. falciparum* is quite perilous and can result in kidney failure, seizures, mental confusion, coma, and eventually death.

There are several other serious complications associated with malaria. These include development of respiratory distress, which occurs in up to 25% of adults, 40% of children and up to 29% of the pregnant women7 infected with severe *P. falciparum* malaria. Possible causes include respiratory compensation of metabolic acidosis, noncardiogenic pulmonary oedema, concomitant pneumonia and severe anaemia. Further, co-infection of HIV and malaria increases mortality.8 The cerebral malaria involves encephalopathy which is associated with retinal whitening, and constitutes a clinical diagnostic tool in distinguishing malaria from other causes of fever.9 Other associated effects include splenomegaly, severe headache, hepatomegaly (enlargement of liver), hypoglycemia, and hemoglobinuria
with renal failure. Further, the infection of *P. falciparum* and *P. vivax* in pregnant women is a central cause of stillbirths, infant mortality and low weight of infants.

### 2.4 Prevention of malaria

Malaria can be prevented by using insecticides, chemoprophylaxis or vaccination. The use of insecticides as a mosquito vector control approach is considered as an efficient method, which essentially consisted of indoor residual spraying (IRS), use of insecticide (mainly pyrethroids) treated nets (ITN) and/or long lasting insecticidal nets (LLIN), which protect individuals against mosquito bites during night. IRS involves application of at least twelve WHO-approved insecticides (of four principal chemical classes) to the inner surfaces of the dwellings. Although, the ITN and LLIN approaches have shown their efficiency on the field and could lower transmission by 90% and child mortality by 18%, if used by all individuals. However, recent studies have shown that mosquitos are developing resistance even to the latest LLIs. For the population at the highest risk involving pregnant women and infants, in addition to the prevention of malaria by drugs (chemoprophylaxis), WHO established the Intermittent Preventive Treatment (IPT) program, which consisted of periodic administration of an antimalarial drug, regardless of the presence of *Plasmodium* parasites. However, this program has attracted criticism with regard to the emergence of parasitic resistance. Malaria prevention treatment for individuals traveling from malaria-risk-free zones to malaria endemic zones is also strongly suggested. Further, the Centre for Disease Control (CDC) recommends the use of specific medicines for malaria prevention depending on the visited country. Prevention measures also include the use of oral insect repellents, wearing long-sleeved clothing in addition to administration of the antimalarial drugs.

One of the most viable means which could even be described as a decisive weapon for combating infectious diseases is through vaccination. It is unlikely that malaria could be eradicated without a vaccine especially in case of the population residing in areas of high risk such as in tropical Africa which also offers a conducive environment for malaria transmission, although the intricacy of the biology of malaria parasite has rendered the development of a malaria vaccine a formidable task. As a result, there is currently no commercially available malaria vaccine, regardless of several decades of intensive research and development efforts. However, there are several vaccine candidates which are currently in clinical trials or advanced preclinical stage and could turn out to be important future tools for the prevention of malaria. Among these, RTS, S/A S01 is one of the most advanced
vaccine for *P. falciparum* which is in Phase III trial and targets the pre-erythrocyte stage of the disease and is expected to be available in 2016.\textsuperscript{17}

### 2.5 Diagnosis and treatment of malaria

Malaria can be completely cured if diagnosed promptly and treated adequately. However, clinical diagnosis of malaria is challenging because of the non-specific nature of the signs and symptoms, which overlap considerably with other common (*e.g.* common viral, febrile illness), as well as potentially life-threatening (*e.g.* bacterial infections etc.) diseases. Amongst the laboratory techniques, malaria is diagnosed using conventional microscopy by staining thin and thick peripheral blood smears, or other techniques such as Quantitative Buffy Coat (QBC) method\textsuperscript{18} in addition to rapid diagnostic tests *e.g.* OptiMAL,\textsuperscript{19,20} ICT,\textsuperscript{21} Para-HIT-f,\textsuperscript{22} ParaScreen,\textsuperscript{23} SD Bioline,\textsuperscript{24} Paracheck,\textsuperscript{25} and molecular diagnostic methods such as Polymerase Chain Reaction (PCR).\textsuperscript{26-27} Among these, microscopic examination of blood films is the most common (Figure 3A-C), economic, preferred, and reliable diagnosis which distinguishes each of the four major parasite species. However, distinction between *P. malariae* and *P. knowlesi* is made using monoclonal antibody panels as both species look very similar under the microscope.

**Figure 3.** Malaria diagnosis using microscopic examination of blood films (A-C) and rapid diagnosis tests (D).

Where sufficient laboratory facilities and/or technical expertise are not available, malaria is diagnosed using the antigen-based Rapid Diagnostic Tests (RDTs) (Figure 3D),\textsuperscript{28} which could be instantly performed during 15-20 minutes. The malaria antigens suitable as target for RDTs are soluble glycolytic enzyme glutamate dehydrogenase, histidine-rich protein II, *P. falciparum* lactate dehydrogenase (*Pf*LDH) and fructose-bisphosphate aldolase (pAldo). But the rapid tests suffer from the drawback of high cost and low sensitivity in...
comparison to thick blood film test (Figure 3A-C). Once the parasitemia is diagnosed, treatment is accomplished using parasiticidal antimalarial drugs (Figure 4).

Quinine (QN) 1, a natural product isolated from the bark of the tree *Cinchona calisaya* (Figure 5) was the first antimalarial drug used in the 15th century. However, the use of QN as antimalarial drug was limited by its adverse effects such as nausea, headache, ringing in the ears, blurred vision, temporary loss of hearing, and sometimes death in case of over dosage. Later synthetic advances on QN in the late nineteenth century, resulted in the development of derivatives such as mefloquine (MQ) 2, 4-aminoquinolines [amodiaquine (AQ) 3, chloroquine (CQ) 4, and piperaquine 5] (Figure 4)29-30 and 8-aminoquinolines [primaquine (PQ) 6, pamaquine 7 and tafenoquine 8]. Among all these, 4 has been the most popular and a highly effective quinoline antimalarial drug but has turned in effective owing to the development of resistance.

![Figure 4. Structures of quinoline-based antimalarial drugs.](image)

Another very efficient and important traditional antimalarial drug is artemisinin (ART) 9 (Figure 6), which is an endoperoxide–containing natural product isolated from the leaves of the sweet wormwood, *Artemisia annua* (Figure 5). Most potent derivatives of ART such as dihydroartemisinin (DHA) 10, artemether 11, artemotil 12, and artesunate (ASN) 13 were also developed and used in combination with other drugs for the treatment of
uncomplicated infections of *P. falciparum*. These structural analogues of ART (10-13) kill all stages of the parasites in shortest time duration, and are equipped with a rapid symptomatic response apart from rapid absorption and elimination from the human bloodstream ($t_{1/2} < 60$ min). Thus, the pharmacokinetic property of ART is beneficial, since it not only reduces the exposure time but also reduces the chances of resistance. However, in case of non-compliance, high rates of malaria recrudescence are expected.\textsuperscript{31} Thus, to counter this drawback, ARTs are often used in combination of other effective drugs.

Figure 5. *Cinchona calisaya*: source of natural QN and *Artemisia annua*: source of artemisinin (ART).

Antifolates drugs such as sulfadoxine 14, sulfalene 15, dopasone 16, pyrimethamine 17, proguanil 18, chloroproguanil 19, arylaminoalcohols such as atovaquone 20, halofanterine 21, or lumefantrine 22, and antibiotics such as fosmidomycin 23, clindamycin 24, tetracycline 25, and doxycycline 26 are other principal drugs (Figure 7) employed for the treatment of malaria. Discrete use of any of these drugs (monotherapy) is explicitly discouraged by the WHO to counter the development of drug resistance.\textsuperscript{13, 32-33} Thus, the therapies such as ACT (artemisinin combination therapy) that combine ART with some other antimalarial drugs is the preferred regimen for treatment of malaria, which in addition to being effective, is well tolerated by patients.

Figure 6. Structure of ART 9 and derivatives: DHA 10, artemether 11, artemotil 12, and artesunate 13.
The ACTs currently recommended for use by WHO are 11:22, 3:13, 2:3, 13:14:17 and 5:10. The choice of an ACT is entirely dependent upon the therapeutic efficacy of the combination in the country or area of intended use.

The treatment of *P. vivax* malaria is usually accomplished with 4 in areas where this drug is effective while an appropriate ACT (except 13:14:17) is recommended in CQ resistant areas. In order to prevent relapses and for the radical cure of *P. vivax* infection, both 4 and ACTs may be combined with a 14-day course of 6 subject to consideration of the risk of hemolysis in patients with glucose-6-phosphate dehydrogenase (G6PD) deficiency. Severe malaria should be treated with injectable 13 and followed by a complete course of an effective ACT as soon as the patient could take oral medication. Where complete parenteral treatment of severe malaria is not possible, patients should be given pre-referral treatment before further treatment. Options available for pre-referral treatment are: rectal 13 or
intramuscular (i.m.) 1 and 11. Based on a recent assessment, WHO recommended that in areas where there is a threat of ART resistance and in areas targeted for countering *P. falciparum* malaria, and where a single dose of 6 as gametocytocide for *P. falciparum* malaria is not yet implemented, a single (0.25 mg base/kg) dose of 6 should be given to all patients with confirmed *P. falciparum* malaria infection on the first day of ACT treatment, except to pregnant women and infants of less than 1 year of age.\textsuperscript{35}

2.6 Mode of action of antimalarial drugs

The most important classes of antimalarial drugs are the quinolines, antifolates, and ART derivatives (Figures 6 and 7). These drugs target different stages of the malaria parasite’s life cycle especially the erythrocytic phases of development of malarial parasite (Figure 8).\textsuperscript{36} Most of the quinoline-containing drugs (Figure 4) are active only against the erythrocytic stages of the parasite infection and eradicate parasites from the blood fairly rapidly, whereas the 8-aminoquinolines, particularly primaquine 6 is immensely effective against the latent liver forms (hypnozoite) of the relapsing malaria caused by the *P. vivax* and *P. ovale*.\textsuperscript{37}

![Figure 8](image.png) Phases of plasmodial life cycle targeted by antimalarial drugs.

Additionally, 6 not only prevents the maturation of fertile gametocytes but also reduces the malaria transmission. On the other hand, the ART-based drugs effectively target both the asexual pre-erythrocytic (liver stage) and erythrocytic stages inside the host as well as the sexual stage occurring inside the mosquito gut (gametocytocidal agents) and thus
constitute highly potent antimalarials that target all stages of the malaria infection as well as lower the transmission rates.\textsuperscript{38} The sequencing and annotation of the \textit{P. falciparum} genome has facilitated the genomic approaches to the drug discovery. Several new biological targets occurring in the proteolytic parasite organelle, known as ‘food vacuole’ (FV), cytoplasm, membranes, mitochondria, or apicoplast of parasite have been identified for antimalarial therapy.\textsuperscript{39} Among these, the biological process such as heme detoxification, oxidative stress occurring in FV and folate metabolism in cytosol, are the most common and important antimalarial drug targets.

The parasite has a limited capacity to synthesize amino acids and therefore, it feeds on hemoglobin and catabolizes it in acidic FV to obtain amino acids for its growth and maturation. As a consequence of breakdown of hemoglobin, free heme [Fe(III)PPIX, ferriprotoporphyrin IX] is produced as a toxic byproduct as it inhibits enzymes and destabilizes membranes. Since the parasite lacks heme oxygenase, it is, at least in part, detoxified in the FV either by biocrystallization process in which heme is crystalized into insoluble polymerized hemozoin. Also when oxyhemoglobin is released into the FV at pH 5.2, it is rapidly converted into methemoglobin (Fe$^{3+}$) with concomitant reaction of the released electron with oxygen to produce superoxide anion.\textsuperscript{40} The resultant superoxide anion dismutates to H$_2$O$_2$ at pH 5.2 (Figure 9). The latter transformation is aided by superoxide dismutase and glutathione peroxidase enzymes. As these oxidant countering enzymes\textsuperscript{41} are absent in the FV, these are mainly driven from host cytoplasm by heme transporting vesicles. However, these enzymes are degraded by proteases of FV leaving contents of FV susceptible to oxidative damage. Thus, when heme is degraded, it leads to heavy oxidative stress on the parasite.

\begin{center}
\begin{align*}
\text{Fe}^{2+}\text{PPIX} & \rightarrow \text{Fe}^{3+}\text{PPIX} \\
O_2 & \rightarrow \text{O}_2^- \\
\text{H}^+ & \rightarrow 0.5 \text{H}_2\text{O}_2 + 0.5 \text{O}_2
\end{align*}
\end{center}

\textbf{Figure 9.} Generation of hydrogen peroxide in the food vacuole of the parasite.

Further, heme can react with H$_2$O$_2$, displaying both catalase like and peroxidase like activities that regenerate heme molecule (Figure 10). In addition, heme undergo peroxidase like reaction that destroys porphyrin ring.\textsuperscript{42} The quinoline drugs especially 4 (CQ)
predominantly forms $\pi$-$\pi$ complex with heme in FV, prevent (i) the stacking of heme to form hemozoin crystals and also (ii) heme degradation process aided by $\text{H}_2\text{O}_2$ is intercepted. Both of these processes leads to accumulation of toxic heme in the FV and thereby causes parasite death.

![Diagram of heme catalysis mechanism](image)

**Figure 10.** Putative mechanism for the catalase and peroxidase-like activities of heme. Formation of complex with CQ.

In addition to the above mechanisms leading to accumulation of heme, it is also known that drugs like CQ could chemisorb on the growing face of hemozoin and disrupt crystallization through formation of very strong lipophilic and cytotoxic heme-CQ coordination complexes.\(^{43}\) This results in building up of large concentrations of toxic heme perturbing permeability and even causing destruction of FV membrane ultimately leading to parasite death.\(^{36}\) There is yet another controversial proposal that CQ type antimalarials could inhibit a heme-polymerase enzyme, however, several quinoline antimalarials have been shown to specifically inhibit hemozoin formation in the absence of any such enzyme.

Thus, quinoline-containing antimalarial drugs could act in both ways: through inhibition of formation of hemozoin (Figure 11) as well as inhibition of degradation of heme (Figure 10). The differences in the course of their action explain the observed differences in their therapeutic efficacy. For example, quinine and mefloquine bind heme less strongly than does CQ.\(^{44}\) Additionally like CQ, most quinolines such as quinine, quinidine, mefloquine, tefanoquine, except primaquine are known to inhibit hemozoin formation.\(^{45}\)

Further, quinoline-containing drugs, in particular, CQ, are known to intercalate into double helix of DNA through ionic interactions.\(^{46}\) This results in decreasing the twist of the double helix, which in turn alters the conformation of DNA. Distortion of conformation also affects biological and physical properties of the helix.\(^{47}\) However, intercalative mechanism of
action of such drugs is not considered as the primary mode of action of these types of drugs, although it may not be completely ruled out.

Figure 11. Detoxification of heme through hemozoin formation in FV.

Artemisinin 9 group of drugs constitute natural (isolated from Chinese herb *Artemisia annua*) as well as semisynthetic analogs such as dihydroartemisinin 10, artemether 11, artemotil 12, and the water soluble derivative artesunate 13. The endoperoxide pharmacophore based on a 1,2,4-trioxane is essential for the activity of artemisinin analogs, which is considered safe for both children as well as pregnant women. The commonly accepted mechanism of action of artemisinin based drugs is that the heme catalyzed peroxide cleavage generates an alkyl radical at C-4 that results in the alkylation of heme and the formation of covalent heme-drug adduct of the type 27 (Figure 12) and specific parasite proteins. 48 A direct evidence of the antimalarial action of artemisinin through heme alkylation pathway has been presented, 49 wherein, alkylated adducts have been detected in spleens and urine samples of *Plasmodium* infected mice. 5

Even artemisinin based semisynthetic products have inherent limitations that include: availability, purity, cost, poor bioavailability and pharmacokinetics, non-compliance with repeated regimens and recrudescence. Thus, drugs based on synthetic peroxidic molecules have been reported from time to time. Trioxaquinones represented by 28 (DU1301) and 29 constitute synthetic hybrid molecules containing 1,2,4-trioxane motif linked to 4-aminoquinoline unit and have shown promising activity against early erythrocytic stages of *Plasmodium falciparum* as tested against both CQ^{R} and chloroquine sensitive (CQ^{S}) strains. 50 In addition to alkylation of heme by trioxaquine, the aminoquinoline partner of 6 and 12.
promotes heme stacking as well as prevents polymerization of heme into toxic hemozoin and thus act at different stages of parasite’s life cycle through dual mode of action.

![Chemical structures](image)

**Figure 12.** Antiplasmodial compounds containing 1,2,4-trioxane and 1,2,4-trioxolanes motif.

In addition to trioxaquines, some other synthetic antimalarial agents are based on 1,2,4,5-tetraoxanes\(^5\) and 1,2,4-trioxolanes (ozonides) typified by OZ277 (RBx-11160)\(^1\) 30. Atremisinin-dipeptide vinyl sulfone conjugates of the type 31 presents\(^1\) another category of hybrids that target cysteine proteases of the malarial parasite. Cysteine proteases play major role in parasite development. Some derivatives of 31 (Figure 12) had superior activity when compared to both CQ and artemisinin.

The antifolate family of antimalarial drugs (Figure 7) targets folate metabolism pathways of the parasite. The malarial parasite synthesizes (de novo) folate and cannot retrieve folates from the human host. Inhibition of enzymes such as dihydropteroate synthase (DHPS) and dihydrofolate reductase (DHFR) involved in folate metabolism results in depletion of folate pool (Figure 13) and thereby reduces the amount of thymidinemonophosphate available for DNA synthesis. The antimalarials 14 target DHPS, whereas, 17 inhibits DHFR. This inhibition of DNA biosynthesis affects the replication of parasite and consequently its death.

### 2.7 Resistance of antimalarial drugs

A malarial strain turns a resistant strain against an antimalarial drug when its IC\(_{50}\) value exceeds 110 nM.\(^2\) The situation gets still worsened when a drug resistant strain spreads
everywhere just as the chloroquine resistant (CQ-resistant) *P. falciparum* rendered CQ ineffective by year 2000.

The widely accepted mechanism for development of resistance against CQ-containing drugs is complex and not completely understood, however, there is fairly firm evidence that it involves chromosomal mutations.\(^5^3\) Compared to CQ-sensitive strains, a unique characteristic of many CQ-resistant strains is that these strains accumulate four to ten times lower levels of drug in their FV.\(^5^4\) This may primarily be due to lower rates of drug influx and/or higher rates of drug efflux. Further, if the binding affinity of the drug with its target decreases, the drug would show resistance. Generally, CQ-resistance is associated with changes in the DHFR (dihydrofolate reductase) enzymes.

![Figure 13](image.png)

**Figure 13.** Principal (in part) enzymes and substrates of the folate pathway involved in thymidylate cycle (DHFS: dihydrofolate synthase; TS: thymidylate synthase). Drugs (SDX: sulfadoxine 14) and (PYR: pyrimethamine 17) targeting DHPS (dihydropteroate synthase) and DHFR (dihydrofolate reductase) enzymes.
transmembrane proteins of the FV which cause a reduced accumulation of the drug in FV and therefore reduced accumulation of toxic heme as the hemoglobin formation is not interrupted.

One of the most accepted theories suggest that the increased resistance is probably due to mutations in the multiple genes encoding transmembrane proteins that are necessary for efflux of drug from the FV.\textsuperscript{55} For example, the \textit{PfCRT} gene located on chromosome 7 codes one of these transported proteins, termed as \textit{PfCRT} (\textit{P. falciparum} chloroquine resistant transporter) that is involved in drug efflux from FV. A set of mutations [lysine to threonine (K76T) and alanine to serine (A220S)]\textsuperscript{55} were seen in the transporter protein in several \textit{P. falciparum} CQ resistant, but not in the corresponding CQ sensitive strains.

Likewise, in the gene \textit{PfMDR1} (\textit{P. falciparum} multi drug resistance gene), a mutation from asparagine to tyrosine (N86Y) (found in 48 out of 56 CQ-resistance strains) in chromosome 5 is also suspected to be responsible for the emergence of CQ resistance as it alters membrane permeability and hence drug efflux mechanism. This mutation was proposed to lead to enhanced expression of ATP-dependent efflux pump (P-glycoprotein) which causes rapid efflux of CQ from the FV. However, there are strong evidences in favor of K76T mutation compared to that of N86Y, the latter may play only a secondary role in CQ-resistance.

2.8 Strategies used in the design of antimalarials

The strategies deployed for the discovery and development of new antimalarial drugs include the optimization of therapy with available drugs (combination therapy), altering dosing regimens or formulation, development of analogs of existing drugs, recourse to natural resources, use of compounds that were originally developed against other diseases, consideration of new chemotherapeutic targets, validation of novel parasite-specific drug targets as a result of an improved understanding of the parasite biology and the incorporation of two drug pharmacophores in one single molecule (Hybrid drugs).\textsuperscript{56}

Combination therapy involves administration of two or more (cocktail) individual pills. However, the benefits of this approach are often defeated by poor patient adherence to full treatment regimens. On the other hand, another approach involving the co-formulation of two or more individual drugs in a single pill referred as fixed-dose combinations (FDCs) is promptly gaining interest due to the simplified treatment regimens and improved patient compliance. In view of the emphasis on FDCs, medicinal chemists are increasingly considering the concept of hybrid molecules. The hybrid drugs in the current scenario of
research and development of new antimalarial drugs have been purported as the next-generation drugs.

Many structurally diverse molecules are known to inhibit *P. falciparum* chloroquine resistance transporter (*Pf*CRT)-associated CQ export from the FV in CQR parasites. These are termed as reversal agents (RA). An appropriate pharmacophoric constitution for this RA activity has been visualized in the molecules consisting of a pair of aromatic rings, often with an aliphatic nitrogen atom a few angstroms removed from the aromatic rings. Such molecules include the Ca$^{2+}$-channel blocker verapamil and the antidepressant imipramine, among many others. Linking a CQ-like moiety to a RA constitutes a good strategy for obtaining a highly effective drug against malaria. In the following section, we describe the concept of hybrid drugs and their use as potent antimalarial drugs.

### 2.9 Hybrid drugs

In the parlance of medicinal chemistry, a hybrid drug is defined as a chemical entity with two (or more) structural domains which act on different/same biological targets via different mode of action (Figure 14). Dual activity of the hybrid molecule indicates independent action of the constituting pharmacophores inside the biological system.

![Figure 14](image-url) Design strategy of a hybrid molecule.

Hybrid drugs represent an effective rational approach in the design and development of novel therapeutic agents. Hybrid drugs have immense potential to surmount the rapid development of drug resistance, enhance patient compliance and reduce both the cost and the risk of drug-drug interactions compared to cocktails or multicomponent drugs. In view of their encouraging efficacies with good bioavailability and minimized toxicity the concept of hybrid drugs may come up as a potent replacement of the cocktail or multiple drug therapy (MDT) regimens.

### 2.9.1 Classification of hybrid drugs

Hybrid drugs are classified into four categories on the basis of the linkers connecting the two pharmacophores.
1. **Conjugate hybrids**

In conjugate hybrids, the pharmacophores are connected through a *metabolically stable linker*, which is not related to either of the individual pharmacophores. A representative example of this type of hybrids is the “dual-sword” drug trioxaquine 28 that is equipped with a combination of a trioxane and a quinoline pharmacophores.\(^6^0\)

2. **Cleavage conjugate hybrids**

In such hybrids, the linker connecting two pharmacophores is metabolized inside the biological system to release the constituting pharmacophors that interact independently with their respective targets. In the hybrid \(37\), the quinoline based pharmacophore is linked with 1,4-naphthoquinone- a glutathione reductase (GR) inhibitor via a metabolically labile ester linker \(37\).\(^6^1\)

3. **Fused hybrids**

In these hybrids, either the linker is very small or the constituting pharmacophores are fused without a linker as in the hybrid \(38\), which is obtained by fusing hydrophobic gastrin receptor and hydrophilic histamine H2 pharmacophores.\(^5^9\)

4. **Merged hybrids**

These represent the most common, smaller hybrid drugs in which a single pharmacophore is reminiscent of two individual pharmacophores. For example, in the acridone \(39\), a 4-aminoalkylquinoline (chemosensitizer) and 9-acridone (DV targeting unit) could be seen merged.\(^6^2\)

2.10 **4-Aminoquinoline based hybrids in the development of antimalarial drugs**

The rational strategy to design hybrid molecules to prevent emergence of resistance was first employed in antituberculosis chemotherapy, then in cancer chemotherapy and more recently for the treatment of AIDS. In the case of parasitic diseases, the treatment was based on single-drug therapy up to the late 1990s. After 50 years of intensive use of CQ, WHO has
now recommended therapy with two or more drugs as the best way to minimize resistance paving way to the hybrid drug therapy to counter malaria. It has additional benefit of high selectivity and advanced mode of action.

Out of the three main classes of antimalarial drugs discussed in the previous section, quinoline class of compounds particularly, CQ based 4-aminoquinoline drugs have received increasing attention during recent years because of their good activity-toxicity profiles and low cost compared to ART based drugs. This is one of the most extensively studied and highly effective antimalarial classes of quinoline based hybrids. The structure activity relationship studies on the CQ and its derivative revealed that the 4-aminoquinoline nucleus alone provides an Fe(III)PPIX complexing template but is not sufficient for inhibition of hemozoin. The chloro substituent at C-7 position is essential for the antiplasmodial activity and inhibition of hemozoin formation, and the replacement of this group either by an electron donor viz like NH₂, OCH₃ or by an electron-withdrawing group like NO₂, resulted in decreased antimalarial activity. Further, it was also established that the aminoalkyl side chain assists accumulation of drug in FV and strengthen the association with Fe(III)PPIX. Thus, 4-amino-7-chloroquinoline template is essential for antimalarial activity and it has been a focus of attention for the development of hybrid drugs, as reviewed below. In the sections below, synthetic details as well as pharmacology of hybrids bearing core structure of CQ i.e 7-chloro-4-aminoquinolinyl unit as one of the pharmacophores are reviewed.

2.10.1 Trioxane and tetraoxane linked 4-aminoquinoline hybrids

Meunier⁶⁰ developed new chimeric molecules referred to as “trioxaquines” by covalently linking a trioxane motif capable of alkylation of heme, with 4-aminoquinoline molecule, which assist in accumulation of the drug in the FV of the parasite as well as inhibits hemozoin formation. The hybrids constituting linkers of variable (two to four carbon units) length were synthesized (Scheme 1) by reductive amination of trioxane-ketone ⁴⁵ with appropriate 4-aminoquinoline unit ⁴¹. The trioxane-ketone precursor ⁴⁵ was prepared by reacting 1,4-cyclohexanedione ⁴⁴ with the endoperoxide ⁴³, obtained in turn by photooxygenation of 1,4-diphenylcyclopenta-1,3-diene ⁴². The antimalarial data against three strains [Nigerian strain (CQS), FcB1-Colombia (CQR) and FcM29-Cameroon (CQ⁺)] of P. falciparum led to the identification of ⁴⁶a and the corresponding citrate salt ⁴⁷a (DU-1102) with shortest tether (n = 2) as the most active compared to the analogues with longer tethers (n = 3 or 4).
Further, *in vitro* antimalarial studies of 47a against thirty two clinical isolates of *P. falciparum* obtained from Yaounde and Cameroon (regions with high resistance to 4 and 17) showed insignificant difference between the mean IC$_{50}$ values of CQ$_{5}^{8}$ isolates (48 nM) and CQ$_{R}^{8}$ isolates (40 nM). Moreover, the lack of good correlation of the IC$_{50}$ values of 47a with 17 suggested the possibility of cross resistance between them.

![Scheme 1](image)

**Scheme 1.** Synthesis of trioxaquine-quinoline hybrids.

Encouraged by the high, *in vitro* antimalarial activity of 46a, against the laboratory strains (Nigerian strain (CQ$_{5}$), FcB1-Colombia (CQ$_{R}^{8}$) and FcM29-Cameroon (CQ$_{R}^{+}$)) as well as CQ and pyrimethamine-resistant human isolates of *P. falciparum*, another series of trioxaquine citrates 47-51 (Figure 15) were synthesized by varying the length of the tether 47b-c, linking the 4-aminouquinoline and the trioxane fragment, replacing 4-aminouquinoline with 6 (51), changing the nature of diketone (using *cis*-bicyclo[3.3.0]octane-3,8-dione (49a-b) in place of cyclohexane-1,4-dione) and the starting dienes [1,3-cyclohexadiene (48) or α-terpinene (50)]. The antimalarial activities of these hybrids against a set of four different strains of *P. falciparum* [Nigerian strain (CQ$_{5}$), F32-Tanzania (CQ$_{5}$), FcB1-Colombia (CQ$_{R}^{8}$) and FcM29-Cameroon (CQ$_{R}^{+}$)] showed that these hybrids, especially 47a and 50 were highly active against both CQ$_{5}$ and CQ$_{R}$ strains with IC$_{50}$ values in the range 22-27 nM and 6-17 nM, respectively and the data further revealed that the variation of length, diene motif and the
stereochemical features exert less influence on the antiplasmodial activity. Replacing the 4-aminoquinoline 50 [IC$_{50}$ = 6 nM (FcM29-cameroon)] with 6 to create 51 [IC$_{50}$ = 108 nM (FcM29-cameroon)], led to considerable decrease in activity against all the tested strains. Due to high in vitro activity, solubility and the ease of synthesis of 50, the in vivo antimalarial activity on mice infected with P. vinckei revealed that the doses required to decrease parasitemia by 50% (ED$_{50}$) were 5 and 18 mg/kg/day after intraperitoneal (i.p.) and oral administration. Moreover, 50 completely cleared parasitemia without recrudescence at the i.p. dose of 20 mg/kg/day and additionally, did not induce any toxic effects in the mice treated by oral administration of 50 (120 mg/kg/day) over four consecutive days.

Further, the in vivo activities, after oral (by mouth, p.o: per o.s) and subcutaneous (s.c) administration of 50$^{50}$ suggested that the doses of 17 mg/kg/day and 2.8 mg/kg/day were required to reduce parasitemia by 50%. The in vivo administration of 50 by p.o. (CD$_{50}$ = 22 mg/kg/day) and s.c. route (CD$_{50}$ = 3 mg/kg/day) in mice infected with an additional murine strain, P. yoelii nigeriensis revealed better antimalarial activity than ART (CD$_{50}$ = 75 mg/kg/day CD$_{50}$ = 7 mg/kg/day by the p.o. route, s.c. routes, respectively).

Figure 15. Trioxaquine based antimalarial hybrids.

The hybrids 50 and 51 (PQ analogues), were active against all stages of gametocytes of P. falciparum. A single dose of 50 showed toxicity (LD$_{50}$ being 70 mg/kg/day) to the healthy mice that was treated and observed for 60 days. This relatively low toxicity of 50 indicated promising therapeutic index of 23.
Among the 120 active chimeric trioxaquine hybrids synthesized by Muenier, hybrid 29 (PA1103/SAR116242) consisting of two cyclohexyl rings as a linker was identified as a potential drug candidate. The presence of second cyclohexyl linker enhanced the metabolic stability of 29 compared to those bearing a linear aliphatic tether 47-51. Both the diastereomers of 29 showed similar in vitro antimalarial activity. The hybrid 29 had high in vitro activity on several sensitive and resistant strains of P. falciparum isolated from different geographic locations, as well as freshly isolated multidrug resistant strains isolated from patients in Gabon. Also, 29 showed identical effect on CQ$^S$ as well as CQ$^R$ strain of P. falciparum and the in vitro activity (IC$_{50}$ = 7-24 nM) of 29 approached that of the ASN (IC$_{50}$ = 7-10 nM). The in vivo studies on mice infected with two different rodent strains (P. v. petteri and P. vinckei) and on humanized mice infected with the human parasite P. falciparum in the 4-day suppressive test revealed that oral dose of 30-64 mg/kg/day of compound completely reduced parasitemia in all the treated mice. Moreover, the preliminary absorption, metabolism, and safety assay established good drug profile of 29.

Singh et al. prepared a novel series of trioxaquines citrate salts 57-58 (Scheme 2) using allylic alcohol as a precursor. The oral administration of 96 mg/kg of 57a-c resulted in significant improvement in activity (% suppression of parasitemia = 89-96%), compared to 54a (7% suppression of parasitemia) and 4-aminoquinoline dicitrate 59 (65-87% suppression of parasitemia), whereas, the i.m. administration did not show any change in activity. On the other hand, 58a-c exhibited activities (96% suppression of parasitemia) comparable to parent trioxane 54b (96% suppression of parasitemia) following both oral and i.m. administration.

![Scheme 2. Synthesis of trioxaquine-quinoline hybrids from allylic alcohol.](image-url)
Further, the biphenyl derivatives 58 were orally more active than phenyl analogues 57. However, none of the trioxaquines provided significant protection to the treated mice in 28-day survival assay and also, these hybrids had serious limitation of poor stability and poor solubility in both oil and water.

Neill and coworkers\textsuperscript{70} synthesized a library of semi-synthetic 1,2,4-trioxaquines 60a-d and synthetic 1,2,4-trioxolaquines 61a-c (Figure 16) hybrids, which could be readily converted into water soluble salts. The \textit{in vitro} antimalarial evaluation of both the series against 3D7 and K1 strain revealed superior activity of trioxaloquines 61a-c [IC\textsubscript{50} = 6.56-22.6 nM (3D7); 3.60-26.20 nM (K1)] than both ART as well as 1,2,4-trioxaquines 60a-d [IC\textsubscript{50} = 5.40-24.25 nM (3D7); 8.70-16.20 nM (K1)]. The most potent 61a exhibited activity [IC\textsubscript{50} = 6.56 nM (3D7); 3.60 nM (K1)] superior to ART [IC\textsubscript{50} = 9.3 nM (3D7); 12.35 nM (K1)] and CQ [IC\textsubscript{50} = 18.23 nM (3D7); 240.34 nM (K1)] against 3D7 and K1 strains of \textit{P. falciparum}.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{figure16}
\caption{1,2,4-trioxaquines and synthetic 1,2,4-trioxolaquine-quinoline hybrids.}
\end{figure}

Using ART as template, Lombard \textit{et al.} prepared both, dimeric and monomeric ART-aminoquinoline hybrids as potent inhibitors of \textit{P. falciparum} by coupling 62 with appropriate 4-aminoquinolines (Scheme 3). The dimeric hybrids, 63a-b showed excellent antimalarial activity [63a: IC\textsubscript{50} = 7.37 nM (D10), 46 nM (Dd2); 63b: IC\textsubscript{50} = 5.31 nM (D10), 28.43 nM (Dd2)], which was also superior to CQ [IC\textsubscript{50} = 21.54 nM (D10), 157.9 nM (Dd2)], against both the tested strains (D10 and Dd2).\textsuperscript{71} Among the monomeric series,\textsuperscript{72} whereas all the hybrids 64a-d showed activity comparable to CQ, against the CQ\textsuperscript{S} (D10) strain, these were more potent than CQ against CQ\textsuperscript{R} strain. Compound 64c and its oxalate salt displayed good antiplasmodial activity [IC\textsubscript{50} = 12.18 nM (D10), 17.12 nM (Dd2)] as well as cytotoxicity [CC\textsubscript{50} = 3.39 µM, SI = 279]. In the \textit{in vivo} studies on \textit{P. vinckei}-infected mice, hybrid 64b
and 64c cleared parasitemia completely upon oral (50 mg/kg) as well as i.p. administration (15 mg/kg) with 100% survival on day 30 and showed no recrudescence.  

Introduction of ferrocene (Fc) linker between 4-aminquinoline and 1,2,4-trioxane motif led to the development of ‘trioxaferroquines’. The trioxaferroquines 65-68 and their non-quinoline analogues 69-71 (trioxaferrocenes) (Figure 17) were evaluated against two CQR strains, FcB1 and FcM29 in order to check the role of quinoline. The results summarized in Table 1, suggest that like ART, trioxaferroquines 65-68 are highly active compared to trioxaferrocenes 69-71, against both the tested CQR strains. Moreover, the IC50 values of trioxaferroquines 65-68 (Table 1) were close to the potential drug candidate PA1103 \cite{29}: IC50 = 24 nM (FcB1), 10 nM (FcM29). The in vivo evaluation of the most efficient trioxaferroquine 65 in *P. vinckeii petteri* infected mice indicated that when administered orally (10 mg/kg), the parasitemia was cleared only below the detectable level. However, no curative effect was observed at doses below 25 mg/kg.

![Scheme 3. Synthesis of ART-aminquinoline hybrids.](image-url)
Following the rationale of hybridization, Saloja and coworkers linked a radical donor tetraoxane to the 4-aminoquinoline motif (Figure 18) with the objective of enhancing the antimalarial activity as well the penetration of tetraoxanes into the infected erythrocytes.\textsuperscript{75} The resulting hybrid tetraoxaquines \textsuperscript{72-75} were screened \textit{in vitro} against three \textit{P. falciparum} strains [D6 (CQ\textsuperscript{S}), W2 (CQ\textsuperscript{R}, MQ-susceptible), and TM91C235 (multidrug-resistant strain derived from Thailand)].

\textbf{Table 1.} Antimalarial activity of 65-71 against \textit{P. falciparum}.

<table>
<thead>
<tr>
<th>Drug</th>
<th>\textit{In vitro} activity IC\textsubscript{50} ±SEM (nM)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>\textbf{FcB1}</td>
</tr>
<tr>
<td>CQ diphosphate</td>
<td>145 ± 3</td>
</tr>
<tr>
<td>29</td>
<td>24 ± 3</td>
</tr>
<tr>
<td>65</td>
<td>20 ± 3</td>
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<tr>
<td>69</td>
<td>235</td>
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<tr>
<td>66</td>
<td>43</td>
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<tr>
<td>70</td>
<td>334 ± 3</td>
</tr>
<tr>
<td>67</td>
<td>36 ± 2</td>
</tr>
<tr>
<td>71</td>
<td>1697 ± 9</td>
</tr>
<tr>
<td>68</td>
<td>43 ± 2</td>
</tr>
</tbody>
</table>
Out of these, 72 showed the most potent in vitro antiplasmodial activity with an IC$_{50}$ of 2.33 nM, 2.00 nM, and 3.70 nM against D6, W2 and TM91C235 strain, respectively. Antimalarial activity data on three strains also revealed that amide analogues 72-73 were more active than the corresponding amines 74-75 and also the steroidal tetraoxanes 73 and 75 were less active than tetraoxane counterparts 72 and 74. Further, the in vivo studies of 72 and 74 on the mice infected with P. berghei (KBG 173 strain), revealed that both compounds cured five out of five mice at the highest tested dose of 320 mg/kg/day. At lower doses (80 mg/kg), 74 was more active because it afforded complete cure in 3 out of the 5 tested mice, with recrudescence occurring in 2 out of 5.

2.10.2 Reversed 4-aminoquinoline hybrids

In one of the attractive strategies in the design of 4-aminoquinoline-based hybrids, Burgess linked imipramine 78 (RA) to 7-chloroquinoline moiety to generate hybrid 79 termed as ‘reversed chloroquine (RCQ)’ (Scheme 4). The in vitro drug susceptibility assays indicated IC$_{50}$ values [IC$_{50}$ = 2.9 nM (D6), 5.3 nM (Dd2)] of 79 to be lower than that of the CQ [IC$_{50}$ = 6.5 nM (D6), 10.2 nM (Dd2)] and these were equally effective against both CQ(R) (D6) and CQ(R) (Dd2) strains of P. falciparum. A preliminary study of 79 on mice infected with P. Chabaudi demonstrated a 99% suppression of the parasite growth at an oral dose of 64 mg/kg/day for 4 days. The remarkable antimalarial activity of this hybrid molecule rendered this a viable approach to restore the quinolines as a first line antimalarial drug.
Further structure diversification of 79 was achieved by way of varying the aromatic groups of RA and the length of linker connecting RA to quinoline moiety (Figure 19).\textsuperscript{77} The thirteen RCQs thus synthesized exhibited better activity against the \textit{P. falciparum} strains [D6 (CQ\textsuperscript{S}) and Dd2 (CQ\textsuperscript{R})] and also had low toxicities against mouse spleen lymphocytes [CC\textsubscript{50} = 700-62000 nM]. Based upon the structure–activity relationships on these RCQs (Figure 19A), 80 emerged as a lead compound for further development due to its superior antimalarial activity [IC\textsubscript{50} = 5 nM (D6); 13 nM (Dd2)] and therapeutic index [TI = 12000 (D6); 4800 (Dd2)] within the series.

Further, RCQs 81-82 (Figure 20) based on cyclic amine linker of variable chain length/ring size and aromatic RA head groups were synthesized for extensive SAR studies.\textsuperscript{78} The excellent \textit{in vitro} antimalarial activity of these RCQs [81-82: IC\textsubscript{50} = 0.5-9.2 nM (D6); 1.3-15 nM (Dd2) and 1.8-56 nM (7G8)], clearly demonstrated the ability of the RA to overcome CQ resistance. Based on the \textit{in vivo} testing of the selected RCQs on \textit{P. berghei} rodent models, 82b was selected for full preclinical testing as it had no obvious signs of toxicity and completely cured 9 out of 10 mice infected with \textit{P. berghei}. The mode of action of the RCQs is not fully elucidated but the preliminary \textit{in vitro} and \textit{in vivo} hemozoin inhibition studies indicate that the RCQs act in a manner similar to that of CQ.
Figure 19. (A) Structural diversity in 4-aminoquinoline-imipramine RCQs; (B) potent RCQ.

Figure 20. Cyclic amine linker based RCQs.

Kelly and coworkers covalently linked 4-aminoquinoline with the 4-aryl-3,4-dihydropyrimidin-2(1H)-ones (DHPMs, Figure 21), calcium channel blockers to obtain RCQs 83a-c. The antiplasmodial screening of 83a-c and their citrate salts 84a-c indicated that all compounds were more active [IC$_{50}$ = 4-144 nM (K1); 7-100 nM (3D7)] than CQ.
Chapter 2

[IC$_{50}$ = 853 nM (K1); 14 nM (3D7)], against K1 strain, while some of these RCQs yielded activity, comparable to CQ against 3D7 strain. RCQ 83c [IC$_{50}$ = 28 nM (3D7); 4 nM (K1)] and its salt 84c [IC$_{50}$ = 7 nM (3D7); 8 nM (K1)] had both, highest activity as well as therapeutic index [TI = 2223 (83c), 6212 (84c) against K1 strain] within the series. Moreover, the citrate salts (84, IC$_{50}$ = 0.24-0.46 µM) displayed greater inhibitory activity than the citrate-free RCQs (83, IC$_{50}$ = 0.46-0.87 µM) and CQ (IC$_{50}$ = 1.91 µM) in the β-hematin inhibition assay.

![83a: n = 2; 83b: n = 3; 83c: n = 4](image)

![84a: n = 2; 84b: n = 3; 84c: n = 4 (citrate salts)](image)

**Figure 21.** Structure of DHPM based RCQs.

Recently, Zishiri *et al.* linked strong resistance-reversing agent$^{80}$ ‘aminomethyl dibemethins’ (dibemethin is N-benzyl-N-methyl-1-phenylmethanamine) with 7-chloroquinoline nucleus to generate hybrid (Figure 22), which inhibited both, hemoglobin formation and PfCRT. Several hybrids (86a, 87a and 87c) displayed potent *in vitro* antimalarial activity against both CQ$^{5}$ (D10) and CQ$^{R}$ (K1) strains of parasite, with IC$_{50}$ below 100 nM.$^{81}$ The hybrid 87a active against D10 (22 nM) and K1 (26 nM) strains was further tested against three (Dd2, W2 and RSA11) additional CQ$^{R}$ strains. The activity data on three resistant strains confirmed high activity of 87a (IC$_{50}$ = 23-38 nM) in comparison to CQ (IC$_{50}$ = 125-170 nM). The three most active compounds 86a, 87a and 87c showed little or no cytotoxicity in mammalian CHO cell line. Out of the three (85a, 86a, and 87a), hybrids, 85a and 86a reduced parasitemia below 99% in mice infected with *P. berghei* and resulted in survival of the mice beyond 30 days (treated orally with 100 mg/kg/day). Moreover, 85-87 were shown to inhibit hemoglobin formation as well as transport CQ via the *P. falciparum* CQ$^{R}$ transporter in a xenopus oocyte expression system.
2.10.3 Glutathione reductase inhibitors based 4-aminoquinoline hybrids

Glutathione guards *P. falciparum* against oxidative damage and promotes heme detoxification. It was established\(^8^2\) that the increased levels of glutathione enhances CQ resistance and thereby controls the glutathione levels, thus, using glutathione inhibitors, the efficacy of CQ and other aminoquinolines could be restored. Taking the above facts into consideration, 4-aminoquinoline based alcohols were combined with glutathione inhibitor 1,4-naphthoquinone \(88\), via a metabolically labile ester link to give compound \(89\) and hybrid \(90\) (Scheme 5).\(^6^1\) A comparison of the antimalarial activities of the \(89\) and \(90\), against moderately CQR parasite strain FcBIR revealed the latter to be more potent (IC\(_{50}\) = 23.1-144 nM) than the former (IC\(_{50}\) = 10.8-24.1 µM). Moreover, \(90\) was even more active than carboxamate \(92\) (IC\(_{50}\) = 144 nM) synthesized from \(91\) (Scheme 5).

The inhibitor \(37\) (Figure 23) was found to be effective (IC\(_{50}\) below 37 nM) against the six tested strains of *P. falciparum* (THAI, F32, D6, FcB1, W2 and K1). Additionally, it also possessed low *in vitro* cytotoxicity against hRMc-5 cells (human diploid embryonic lung cell line). Moreover, *in vivo* studies in *P. berghei*-infected mice indicated that treatment with \(37\) reduces parasitemia by 99.9 % with concurrent increase in survival period from 8 to 24 days. Further, synthesis and antimalarial evaluation of the glutathione inhibitor based hybrids consisting of amide/amine/ether linkages led to the identification of the tertiary amide \(85\) [IC\(_{50}\) = 7.5 nM (3D7); 9.4 nM (K1)] to be potent as well as relatively less toxic to the human KB cells (CC\(_{50}\) = 37 µM).\(^8^3\) The daily i.p. administration of \(93\) (21 mg/kg) to the mice infected with *P. berghei* resulted in only 18% reduction of parasitemia. The moderate *in vivo* activity of \(93\) results from both, the low bioavailability and low solubility in organic as well
as aqueous medium. Further, combination assay of 93 with ART showed a good degree of synergistic effect.

\[ \text{ROH, DCC, DMAP, CH}_2\text{Cl}_2 \text{ or RCO}_2\text{CHCl}_2, N(C_2\text{H}_5)_3, DMF} \]

\[ X = \text{H}, n = 4 \]
\[ X = \text{H}, n = 5 \]
\[ X = \text{OH}, n = 4 \]
\[ X = \text{OH}, n = 5 \]

\[ X = \text{H}; R = \text{CH}_3,\text{CH}_2\text{OCOC}(\text{CH}_3)_2,\text{CH}_2\text{OCOC}_2\text{H}_2; n = 5 \]
\[ X = \text{H}, \text{OH}; n = 4, 5 \]

**Scheme 5.** Synthesis of 1,4-naphthoquinone-quinoline hybrids.

Figure 23. Potent 1,4-naphthoquinone-quinoline hybrids.

Another series of pro-drugs 97-100 based on inhibition of glutathione were synthesized by linking 4-amino-7-chloroquinoline with 1,4-naphthoquinones 95 or ketones 96, (Figure 24) using analogues 94b-g of ferroquine [FQ: 7-chloro-4-(((2-((dimethylamino)methyl)ferrocenyl)methyl)amino)quinoline {94a}] as a linker. *In vitro* testing against NF-54 (CQ\textsuperscript{5}) and K1 (CQ\textsuperscript{R}) *P. falciparum* strains showed 97-100 to be more potent [IC\textsubscript{50} = 42.2-389 nM (NF54), 26.7-444.8 nM (K1)] than the parent glutathione inhibitors 88b and 95-96 [88b: IC\textsubscript{50} = 43.3 µM; 95-96: 6.2 µM (against K1)] but less active.
than FQ [94a: IC$_{50}$ = 11.6 nM (NF54), 16.1 nM (K1)] and its analogues 94b-g [IC$_{50}$ = 7.5-14.9 nM (NF54), 5.1-15.6 nM (K1)].

Nevertheless, whereas, these hybrids (except 98) were potent against both malarial strains (NF54 and K1) with IC$_{50}$ values between 27 and 161 nM, these were not as active as CQ against NF54 strain (IC$_{50}$ = 11.9 nM). However, these were upto 8-fold more potent than CQ against K1 strain (IC$_{50}$ = 289.2 nM). The preliminary cleavage assays were also conducted to support the pro-drug strategy. The hybrids 97-100 were stable at cytosolic pH (7.4), whereas, at vacuolar pH (5.2), the amide bond hydrolyzed to release the constituting moieties. While contrary to the expectations, inhibition of *P. falciparum* glutathione was not detected, high inhibition of $\beta$-hematin formation was observed.

![Chemical structures](image)

**Figure 24.** Structure of FQ, FQ analogues, 1,4-naphthoquinone, benzoic acid precursors and Fe based dual drugs.

### 2.10.4 Hybrids targeting parasite proteases

The zinc metalloproteases and falcipain family of cysteine is involved in the parasite mediated hemoglobin digestion in the FV of parasite. The inhibition of these proteases affects the heme degradation and is thus identified as valuable targets for hybrid design. Linking of
protease inhibitors with 4-aminoquinoline core yield several hybrids with useful antimalarial properties and are briefly discussed in preceding sections.

2.10.4.1 Zinc metalloproteases

*P. falciparum* zinc metalloaminopeptidase involved in globin hydrolysis are considered as promising molecular targets for antimalarial drugs. PfA-M1 is a zinc-metalloprotease of the M1 family, which preferably cleaves basic, hydrophobic, as well as aromatic amino-acids of globin chain of hemoglobin. In this context, Flipo *et al.* incorporated carboxylic acid or a hydroxamate as zinc chelating groups in the alkyl side chain of piperazine based quinoline.\(^8^5\) Out of 45 analogues, only three compounds 101a-c (Figure 25)\(^8^2\) effectively inhibited zinc metalloprotease (PfA-M1) and also exhibited considerable antimalarial activity against CQ\(^8^\) strains.

![Figure 25. Hydroxamate linked quinoline hybrids.](image)

Steroidal or adamantane based 4-amino-7-chloroquinoline hybrids 102-104 (Figure 26) reported by Saloja *et al.*\(^8^6\) exhibited good to excellent *in vitro* antimalarial activity [IC\(_{50}\) = 884-3.38 nM] against three strains of *P. falciparum* (D6, TM91C235, and W2). Further, these also inhibited a light chain botulinum neurotoxin serotype A (BoNT/A LC), a zinc metalloprotease, at low micromolar levels (7-31 \(\mu\)M). While the steroidal derivatives 102 exhibited high antimalarial activity, the adamantanyl hybrids 103, 104 were less active. Moreover, the antimalarial activity of compound 102a [IC\(_{50}\) = 6.17 nM (D6), 11.01 nM (TM91C235), 3.38 nM (W2)] and 102b [IC\(_{50}\) = 3.83 nM (D6), 7.39 nM (TM91C235), 12.1 nM (W2)] was even greater than ART [IC\(_{50}\) = 9 nM (D6), 13.04 nM (TM91C235), 6.7 nM (W2)]. Compound 102 (IC\(_{50}\) = 7-17 \(\mu\)M) and 103 (IC\(_{50}\) = 11.8-50.1 \(\mu\)M) effectively inhibited the zinc metalloprotease, whereas, 104 was devoid of such activity owing to large steric hindrance. Interestingly, the most potent antimalarials were the most potent inhibitor of the zinc metalloprotease (BoNT/A LC). Thus, these hybrids that target both heme polymerization and the zinc metalloproteases could be good antimalarials.
Figure 26. Hybrids of 4-aminoquinoline and steroid or adamantane based components.

As an extension of work on 4-aminoquinoline-based neurotoxins, Opsenica et al. synthesized structurally simplified hybrids 106 and 109, which were also efficient inhibitors of P. falciparum and BoNT/A LC. These hybrids 106 and 109 were prepared by coupling commercially available aromatic aldehydes with 4-aminoquinolines 105 via reductive amination (Scheme 6). All the hybrids exhibited considerable inhibition of BoNT/A LC (zinc metalloprotease) (% Inhibition = 14-69) at 20 µM. The screening of these hybrids against three P. falciparum strains (D6, W2 and TMC91C235) demonstrated excellent activity in nM range, with compound 106a [IC$_{50}$ = 3.95 nM (D6), 6.18 nM (TM91C235), 5.93 nM (W2)] being as potent as ART [IC$_{50}$ = 6.7-13 nM] and MQ [IC$_{50}$ = 6.5-65.5 nM].
Further evaluation against two virulent multidrug resistant (MDR) strains (TM90C2A and TM90C2B) indicated that **106a** [IC$_{50}$ = 7.16 nM (TMC90C2A), 7.91 nM (TM90C2B)], was 38 times more active than CQ [IC$_{50}$ =249.8 nM (TMC90C2A), 307.38 nM (TM90C2B)], and 10 times more active than MQ [IC$_{50}$ = 51.12 nM (TMC90C2A), 27.98 nM (TM90C2B)]. The SAR profile further suggested that the pyridine hybrids were less potent than the benzene based hybrids against all the *P. falciparum* strains as well as zinc metalloprotease (BoNT/A LC serotypes). Also, the toxicity of **106a** in HepG2 cells (human hepatocellular carcinoma) was low with respect to their potent antimalarial efficacy as indicated by the selectivity index which was greater than 800.

### 2.10.4.2 Cysteine proteases

Cysteine protease falcipains of *P. falciparum* are also essential for degradation of hemoglobin and are therefore interesting antimalarial targets.

#### 2.10.4.2.1 4-Aminoquinoline-isatin hybrids

Chibale group reported synthesis of 4-aminoquinoline-based isatin hybrids **113a-b** and **116** (Scheme 7), consisting of thiosemicarbazone functionalized isatin unit, which inhibit *P. falciparum* derived cysteine proteases. The 4-aminoquinoline motif could additionally help by inhibiting β-hematin formation by promoting accumulation of the drug in the parasite’s FV. Biological evaluation of the hybrids **113** and **116**, specifically **113a** and **113b** against three strains of the malarial parasite *P. falciparum* [CQ$^5$ (D10) and two CQ$^R$ strains (K1 and W2)], and against recombinant falcipain-2 (cysteine protease) showed good *in vitro* activity against K1 and W2 with IC$_{50}$ values of 51 (for **113a**) and 54 nM (**113b**), respectively, while retaining activity against the D10 strain with IC$_{50}$ values of 79 and 95 nM, respectively, for **113a** and **113b**. The introduction of rigid piperazinyl linker to create hybrid **116** (Scheme 7), however, resulted in decrease in activity [IC$_{50}$ = 0.64-1.35 µM (D10); 0.96-1.23 µM (W2)] in comparison to hybrids **113** with flexible ethylene linker [IC$_{50}$ = 0.079-0.32 µM (D10); 0.051-0.24 µM (W2)]. Unfortunately, these hybrids exhibited modest inhibitory activity against falcipain-2 [IC$_{50}$ = 6.07-20 µM] and led to the understanding that the antimalarial activity of these hybrids was not entirely due to inhibition of falcipain-2.
Scheme 7. Synthesis of 4-aminoquinoline-based isatin derivatives 113a-b and 116.

2.10.4.2.2 4-Aminoquinoline-thio/oxo semicarbazone hybrids

Continuing work on malarial falcipain-2 targets, Chibale group introduced hybrids 117 of phenolic Mannich bases and 4-aminoquinoline fragment (Figure 27), that effectively inhibited falcipain-2 [most potent inhibitor (117a) had IC$_{50}$ = 0.63 μM] and possessed good in vitro activity against CQ$^R$ strain (W2) of *P. falciparum*. The most potent antimalarial of these was bisquinoline semicarbazone 117f (IC$_{50}$ = 0.071 μM), which unfortunately exhibited low falcipain-2 inhibition (IC$_{50}$ = 3.16 μM) indicating that its principal antiparasitic activity did not appear to be due to inhibition of this enzyme.

![Figure 27. 4-Aminoquinoline-semicarbazone hybrids.](image)

Further, the thiosemicarbazone hybrids bearing 4-aminoalkylquinoline 118 or a FQ motif 119 (Figure 28) were synthesized and evaluated against falcipain-2 as well as four strains of *P. falciparum* (3D7, W2, FCR3 and BreI). Out of nine hybrids, 119a [IC$_{50}$ = 0.2-1 μM (*P. falciparum*), 2415 nM (falcipain)] and 119b [IC$_{50}$ = 0.2-0.8 μM (*P. falciparum*), 1072 nM (falcipain)] constituted the most active antimalarial hybrids displaying better inhibition of
falcipain-2 than the flexible alkyl quinoline analogues 118 [IC$_{50}$ = 0.3-1.7 μM (P. falciparum)], 4,416-20,000 nM (falcipain)].

![Figure 28. 4-aminoquinoline-thiosemicarbazone hybrid and FQ-thiosemicarbazone hybrid.

2.10.4.2.3 4-Aminoquinoline-chalcone hybrids

Chauhan and coworkers synthesized first chalcone based quinoline hybrids 122 using Claisen-Schmidt Aldol condensation reaction (Scheme 8). The antimalarial activity evaluation against NF-54 strain of P. falciparum revealed that none of the chalcone hybrid was active.

![Scheme 8. Synthesis of chalcone based quinoline hybrids.]

Subsequently, Chibale and coworkers 92 linked quinoline with curcumin based hybrids through triazole to generate quinoline chalcone (125, 127)/dienone (129-130) hybrids (Scheme 9). Among the chalcone-quinoline hybrids 125a-c or 127a-e, three hybrids 125b [IC$_{50}$ = 0.04 μM (D10), 0.07 μM (Dd2), 0.09 μM(W2)], 125c [IC$_{50}$ = 0. 4 μM (D10), 0.4 μM (Dd2), 0.6 μM(W2)] and 127b [IC$_{50}$ = 0.3 μM (D10), 0.3 μM (Dd2), 0.5 μM (W2)] displayed high activity whereas in the dienone series (129 and 130), intermediate 129 [IC$_{50}$ = 0.8 μM (D10), 0.7 μM (Dd2)] was found to be highly active against the three tested strains (D10, Dd2 and W2). The investigation of chalcone-quinoline hybrids (125, 127) for inhibition of hemozoin formation showed that most of the hybrids, especially 125b (IC$_{50}$ = 0. 2 μM) were potent inhibitor of β-hematin formation. Further analogues of 125b and 129 with improved
solubility and antimalarial activity were synthesized by replacing triazole linker with aminoalkyl 131-132 or piperazinyl-based linkers 131-133 (Figure 29).\(^3\)


The physico-chemical properties and ADME (absorption, distribution, metabolism, and excretion) parameters predicted 131-133 to have improved solubility particularly at low pH and high permeability relative to parent hybrids (125b and 129) and were found to exhibit notable in vitro antimalarial activity [IC\(_{50}\) = 0.5-1.7 \(\mu\)M (D10), 0.5-3.1 \(\mu\)M (Dd2), 0.1-0.7 \(\mu\)M (W2)] against all the three tested strains, though none was as active as 125b. Among the hybrids 131-133, hybrid 133c [IC\(_{50}\) = 0.5 \(\mu\)M (D10), 0.5 \(\mu\)M (Dd2), 0.4 \(\mu\)M (W2)] and 133d [IC\(_{50}\) = 0.6 \(\mu\)M (D10), 0.5 \(\mu\)M (Dd2), 0.3 \(\mu\)M (W2)] were the most active hybrids.

Figure 29. Structure of quinoline-chalcone hybrids.
Chapter 2

The *in vitro* metabolic stability assay revealed that most of the hybrids were susceptible to undergo hepatic metabolism via oxygenation, demethylation or N-dealkylation. Moreover, the ability to inhibit β-hematin formation, falcipain-2 and sorbitol-induced lysis was measured in order to predict mechanism of this class of hybrids. The inhibition data demonstrated that β-hematin inhibition is the primary mechanism of action with some contribution from inhibition of falcipain-2.

Inspired by the high activity of the chalcone based quinoline hybrids, Sashidhara *et al* linked various chalcones with 4-aminoquinoline through keto-enamine linker (Figure 30).

Among the 28 hybrids, 134a (IC₅₀ = 3.63 ng/ml) and 134b (IC₅₀ = 4.64 ng/ml) exhibited antiplasmodial activity comparable to CQ (IC₅₀ = 2.45 ng/ml) against 3D7 strain. Furthermore, all hybrids were devoid of cytotoxicity against Vero cell lines as these hybrids had high SI values, particularly hybrid 134a which had SI value (27548.21), three-fold higher compared to CQ, while the rest of hybrids showed SI values ranging between 300 and 3755. The *in vivo* studies in Swiss mice using 134a-b infected with *P. yoelli* indicated that both hybrids rapidly cleared parasitemia below the detectable levels at a dose of 100 mg/kg. Moreover, the mechanistic studies revealed that these hybrids acted through inhibition of heme polymerization.

![Figure 30](image)

**Figure 30.** Structure of keto-enamine linked quinoline-chalcone hybrids.

Further, structural optimization of these hybrids led to the hybrids 135 (Figure 31) [IC₅₀ = 29.06-500 nM (3D7), 82.93-314.86 nM (K1)] possessing activity comparable to CQ [IC₅₀ = 7.68 nM (3D7), 463 nM (K1)] against both the tested 3D7 (CQ⁵) and K1 (CQ⁸) strains. Among the 16 tested hybrids, 135a [IC₅₀ = 82.93 nM (K1)] was found to be most active against K1 strain whereas 135b [IC₅₀ = 29.06 nM (3D7)] displayed highest activity
against 3D7. Furthermore, three hybrids effectively inhibit heme polymerization indicating inhibition of β-hematin formation as their possible mode of their action.

![Chemical structures](image1)

**Figure 31.** Structure of quinoline hydrazones hybrids.

Recently, Smit et al. coupled the carboxylic acid functionalized chalcone 138 with 4-aminoquinolines 139 to create novel series of 4-aminoquinolyl-chalcone amides 140 using 1,1'-carbonyldiimidazole (Scheme 10). In vitro antimalarial evaluation showed that all the hybrids were active with IC₅₀ values ranging between 0.04-0.5 µM and 0.07-1.8 µM against 3D7 and W2 strains of *P. falciparum*. These hybrids depicted moderate to high activity towards parasitic cells in the presence of mammalian cells.

![Synthesis scheme](image2)

**Scheme 10.** Synthesis of amide functionalized chalcone-quinoline hybrids.

Hybrid 140a [IC₅₀ = 0.05 µM (3D7), 0.07 µM (W2)], featuring 1,6-diaminohexane linker was found to be the most active of all, and was two-fold more potent than CQ [IC₅₀ =
0.05 μM (3D7), 0.12 μM (W2)] against the 3D7 and W2 strains, respectively, despite its predicted unfavorable high lipophilicity (log $P = 6$), low solubility, and poor absorption properties.

### 2.10.4.2.4 4-Aminoquinoline-cinnamic acid hybrids

Parez et al. linked 4-aminoquinoline core with trans-cinnamic acid motif either directly 143 or through retro-enantiodipeptide bond 148 to generate series of potential dual action antimalarials which inhibited hemozoin formation as well as enzyme catalytic cysteine residues (Scheme 11). Initial in silico studies suggested that the sequence D-hPhe-D-Leu was favored over the D-Leu-D-hPhe in the catalytic sites of falcipain. In vitro antimalarial evaluation of hybrids 143 and 148 against W2 strain showed that hybrid with dipeptide linker 148 (IC$_{50}$ = 0.83-10.8 μM) was more potent than those without the linker 143 (IC$_{50}$ = >10 μM) and also, the most lipophilic hybrid 148c (IC$_{50}$ = 0.83 μM) displayed highest antimalarial activity within the series. Hybrid 143j (IC$_{50}$ = 14.2 μM) showed the best falcipain-2 inhibition. Thus, the ability to inhibit falcipain-2 does not correlate with antimalarial activity because the poor inhibitors of falcipain-2 are potent antimalarial. Also, some 148 inhibited heme polymerization suggesting its contribution to antimalarial activity.

Second generation cinnamic acid-quinoline conjugates (151a-l, 152) were developed by linking cinnamoyl core 142 with quinoline [4-aminoquinoline (133)/ 8-aminoquinoline] motif through a flexible and more hydrophobic butylamine linker (Scheme 12). All the hybrids 151a-l [IC$_{50}$ = 11–59 nM (W2), 1.44-2.36 μM (liver stage)] and 152 [IC$_{50}$ = 23.7–79.8 nM (W2), 4.02 μM (liver stage)] possessed higher in vitro activity than CQ [IC$_{50}$ = 138 nM (W2), 15.9 μM (liver stage)] with two of them 151c [IC$_{50}$ = 11 nM (W2), 2.50 μM (liver stage)] and 151h [IC$_{50}$ = 11.6 nM (W2), 1.44 μM (liver stage)] were as active as ART [IC$_{50}$ = 9.5 nM (W2)] against the blood (PfW2) stages and as active as PQ against liver stages of *P. berghei*. Replacing 7-chloroquinoline in 151 [IC$_{50}$ = 11 nM (W2)] with 8-aminoquinoline 152 [IC$_{50}$ = 4840 nM (W2)] led to decrease in antimalarial activity as well as loss of heme biocrystallisation activity. The in vivo treatment of mice infected with *P. berghei* using 151c and 151h at the highest dose of 100 mg/kg/day resulted in death of all the mice on day 17. This may be due to low bioavailability and toxicity of both 151c and 151h.

Moreover, most of the hybrids 151a-l inhibited hemozoin formation but none of the hybrids displayed falcipain inhibition upto 50 μM indicating that these hybrids exerted antimalarial effect by heme inhibition pathways.
Review of literature

Scheme 11. Synthesis of cinnamic acid-4-aminoquinoline hybrids.

Scheme 12. Synthesis of second generation cinnamic acid-quinoline conjugates.
2.10.4.2.5 4-Aminoquinoline γ- and δ-lactam hybrids

Chibale and coworkers utilized multicomponent reaction (Ugi three-component reaction of 40, 153 and 154) to carry out parallel synthesis of 4-aminoquinoline γ- and δ-lactams (Scheme 13). The resin-bound macroporous p-toluenesulfonic acid (p-TSA 155) was employed for the purification and isolation of the target hybrid. The antimalarial testing of all lactams 156 against W2 strain indicated that hybrid 156a with an IC₅₀ of 0.096 µM exhibited the best activity, and was found to be 2.5 times more potent than CQ (IC₅₀ = 0.24 µM). Moreover, 156a also inhibited falcipain-2 (IC₅₀ = 17.2 µM) suggesting that antimalarial activity may in part be due to the inhibition of falcipain-2.

**Scheme 13.** Synthesis of 4-aminoquinoline γ-/δ-lactams.

Introduction of different substituents on γ-lactam and 4-aminoquinoline led to the generation of two series of structurally diverse antimalarial hybrids 161 and 163 termed as ‘Quinolac’ (Scheme 14). Out of the 40 compounds tested, two hybrids 161b, 163b exhibited high activity (IC₅₀ = 49 and 42 nM, respectively) against the CQ⁸ W2 strain, while the activity against CQ⁵ 3D7 was of the same order as that of CQ (IC₅₀ = 26 and 19 nM, respectively). None of the tested hybrids were found to be cytotoxic against mammalian cell line (HUVECs), and also, the resistance indices of all the hybrids were usually close to 1.0–2.0. Furthermore, two hybrids (161a and 163a) were found to be quite stable over 48 h at pH 7.4 and 5.2 (LC/MS monitoring). Moreover, the physico-chemical properties of the most potent hybrids calculated using chemaxon software indicated that these hybrids were compliant with Lipinski rule of five criteria (ClogP close to 4.5-6, ClogD 1.2-2.8 at pH 7.4 except for two molecules with values higher than 5).
**Series 1**

![Chemical Structures]

161: X = Cl, H; R³ = H, COCF₃; R⁴ = -CH₂S(O)₆H₂ (m = 0, 2)
Y = (CH₂)₃, CH₂NHCH₂, CH₂NCH₂CH₂

**Scheme 14. Synthesis of quinolac hybrids.**

In order to address the solubility and lipophilicity issues associated with most active hybrids 161a-b and 163a-b, more hybrids with varied substituents were introduced (Figure 32). These hybrids exhibited ClogP lower than 5.0 in contrast to most of the previous active molecules, molecular weight in the range of 508–524 g/mol which was only slightly higher than the accepted 500 g/mol, ClogP at pH 7.4 in the range of -1.10 to +0.45 and were compliant with hydrogen bonding properties. All hybrids were usually 3- to 17-fold less potent (IC₅₀ = 89-499 nM) than CQ (IC₅₀ = 30 nM), but most of them were more active (IC₅₀ = 167-666 nM except 164c and 164h) than CQ (IC₅₀ = 750 nM) on W2 strain. Hybrids 164f [IC₅₀ = 278 nM (3D7), 458 nM (W2), and ClogP = 3.43] and 164g [IC₅₀ = 242 nM (3D7), 274 nM (W2), and ClogP = 2.80] from this series possessed overall good activity on both
strains, good calculated physico-chemical properties and offered sites for deducing possible SAR studies.

\[ \text{R = } C_6H_5, \text{ 164b: } R = p-OCH_3-C_6H_4, \text{ 164c: } R = p-F-C_6H_4, \]
\[ \text{164d: } R = 2-Pyridyl, \text{ 164e: } R = 4-Pyridyl, \text{ 164f: } R = 2-Pyrimidyl, \]
\[ \text{164f: } R = 2-Pyridyl-N-oxide, \text{ 164h: } R = 3-(4-methyl-4H-1,2,4-triazolyl) \]

**Figure 32.** Structure of modified quinoline-lactams hybrids.

### 2.10.4.2.6 4-Aminoquinoline-squaric acid hybrids

Ribeiro *et al.* prepared mono or disubstituted squaric acid 165/4-aminoquinoline 166-167 or 8-aminoquinoline 168-169 conjugates by condensation of 4-/8-aminoquinolines with commercially available squarates 165 (Scheme 15).\(^{103}\) Hybrids 166-169 were found to be inactive against papain [IC\(_{50}\) = >10 µM] and falcipain-2 [IC\(_{50}\) = >10 µM], but had considerably good antiplasmodial activity. Hybrids 167a-d containing two quinoline moieties with IC\(_{50}\) in range 0.2-0.099 µM were more active than their mono quinolinyl analogues 166a-c (IC\(_{50}\) in range 1.5-10 µM) against CQ\(^R\) strain (W2). Hybrid 167b (IC\(_{50}\) = 0.099 µM) displayed highest antimalarial activity 1.5 fold greater than CQ (IC\(_{50}\) = 0.14 µM). Moreover, the substitution of 7-chloroquinoline ring 166-167 by the PQ 168-169 led to loss of activity. Furthermore, most of the hybrids were found to be non-cytotoxic on the NIH 3T3 and HEK 293 cell lines at concentration upto 50 µM.

### 2.10.5 4-Aminoquinoline targeting dihydrofolate reductase

*P. falciparum* dihydrofolate reductase (DHFR), which catalyzes the NADPH-dependent reduction of dihydrofolate to tetrahydrofolate, is a well-known target of cycloguanil (triazine class), and pyrimethamine (pyrimidine class) antimalarial drugs. Inhibition of DHFR is expected to cause a significant decrease of pyrimidine bases of DNA and, consequently, the inhibition of the parasite growth. Thus, conjugation of DHFR inhibitors with 4-aminoquinoline led to the hybrids that acts on dual targets (heme as well as DHFR).
2.10.5.1 4-Aminoquinoline-triazine hybrids

Cycloguanil belongs to the triazine class which exerts its antimalarial activity by inhibiting DHFR enzyme. Inspired by these facts, a diversity oriented library of eighteen 2, 4, 6-triamino-1,3,5-triazine-quinoline hybrids were synthesized as cycloguanil-CQ analogues by consecutive nucleophilic aromatic substitution of cyanuric chloride 170 with suitable amine under controlled conditions (Scheme 16).104


The antimalarial data of hybrid 171 assessed against P. falciparum strains (D10 and W2) cultured in human hematocyte showed that compounds having hydrophobic groups \{NHCH₂C₆H₅, N(C₂H₅)₂, N[(CH₃)₂CH]\} were more active than those bearing polar groups (sulfonyl, carboxyl or guanidine). Compound 171a (IC₅₀ = 32 nM) was the most potent...
hybrid with activity 25 times greater than CQ (IC\textsubscript{50} = 744 nM) against W2 strain. The toxicity profile indicated that the hybrids 171 (Figure 33) are well tolerated by human fibroblast and endothelial cell lines, thus supporting the potential interest for this new class of antimalarial agents.

Systematic variation of substituents around triazine core to create new triazine-quinoline analogues bearing aliphatic diaminoalkyl spacer led to the identification of most potent compound 172 [IC\textsubscript{50} = 0.21 µM (D6), 0.25 µM (W2)]\textsuperscript{105} and 173 [IC\textsubscript{50} = 5.23 ng/ml (3D7)].\textsuperscript{106} Unfortunately, 172 displayed high toxicity on mammalian cells [CC\textsubscript{50} = 11.2 µM (pig kidney epithelial cells), 3.9 µM (human hepatoma cells), and 10.4 µM (monkey kidney fibroblasts)] whereas 173 was toxic during in vivo studies in Swiss mice infected with \textit{P. yoelli}.

Recent research efforts led to the design of novel diaminoalkyl linked triazine-quinoline hybrids which were non-toxic to mammalian cells (Vero, LLC-PK\textsubscript{11} and HepG2) and highly active against D6 clone and W2 clone.\textsuperscript{107} Out of 33 synthesized conjugates, 174\textsubscript{a} [IC\textsubscript{50} = 0.16 µM (D6), 0.15 µM (W2)] and 174\textsubscript{b} [IC\textsubscript{50} = 0.10 µM (D6), 0.28 µM (W2)] were the most potent and identified as future leads. Taking into consideration the basicity of triazine, Kumar \textit{et al.} synthesized nineteen piperazine linked triazine-4-aminoquinoline conjugates by substituting triazine motif with cyclic (morpholine/piperidine/cyclohexylamine/ethylpiperazine/phenylpiperazine/formylpiperazine) or aromatic amines (o/p-flouroaniline, aniline, o/p-toluidine).\textsuperscript{108} These hybrids were evaluated for antimalarial activity against 3D7 strain and for toxicity against Vero cells. The structure-activity relationship studies revealed that combination of piperidine, cyclohexylamine, p-flouroaniline, aniline, and morpholine as substituent on triazine nucleus were well tolerated for antimalarial activity. The most potent compound 175\textsubscript{a} [IC\textsubscript{50} = 7.15 ng/ml (3D7)] and 175\textsubscript{b} [IC\textsubscript{50} = 4.43 ng/ml (3D7)] which displayed high selectivity index (328 and 481) was further evaluated in vivo against CQ\textsuperscript{R} N-67 strain of \textit{P. yoelli} in Swiss mice. Both the hybrids 175\textsubscript{a-b} indicated that i.p administration of dose of 50 mg/kg/day for 4 day resulted in 99% suppression of parasitemia. Further optimization of piperazine linked triazine-4-aminoquinoline hybrids\textsuperscript{109} led to the most potent hybrids 176\textsubscript{a} [% dead parasites = 18 (3D7, 5 µg/ml); 15.5 (RKL-2, 5 µg/ml)] and 176\textsubscript{b} [% dead parasites = 26.5 (3D7, 5 µg/ml); 11 (RKL-2, 5 µg/ml)] bearing more basic 1,3-diaminopropane and 4-bromoaniline substituents.
Replacing the diaminolinker with o/p-diaminophenyl linker resulted in AQ analogue i.e. 4-anilinoquinoline-triazine hybrids. The assessment of \textit{in vitro} antimalarial activity against 3D7 strain led to the identification of five potent hybrids (IC$_{50}$ = 7.03-3.01 nM) superior to CQ (IC$_{50}$ = 8.15 nM). The i.p. as well as oral administration data of selected hybrids shows that hybrid 177a and 177b at highest dose of 100 mg/kg/day completely cleared parasitemia on day 4 in mice infected with \textit{P. yoelli} while the other hybrids managed to exert suppressive effect. Sharma \textit{et al.} further broadened the 4-anilinoquinoline-triazine library by introducing morpholine, 3,4-dimethoxyaniline, \textit{m/p}-methoxyaniline, \textit{m/p}-chloroaniline, \textit{m/p}-fluoroaniline, and N-methylpiperazine as substituents around the triazine motif. Most of the hybrids displayed excellent \textit{in vitro} as well as \textit{in vivo} activity and have
fairly safe selectivity index. One of the hybrid 178 (Figure 33) provided 99.9% protection to mice at dose of 100 mg/kg/day and resulted in survival of 3 out of 5 mice till day 28. In their further research efforts, Manohar et al. incorporated triazole motif into the linker connecting 4-aminoquinoline core with triazine. Among the compounds assayed, hybrid 179 having propyl triazole linker displayed highest activity against D6 clone (IC$_{50}$ = 0.58 µM) as well as W2 clone (IC$_{50}$ = 0.73 µM) and was also found to be non-toxic up to 48 µM to Vero cells.$^{112}$

### 2.10.5.2 4-Aminoquinoline-\(\beta\)-lactam hybrids

Conjugation of the heme binding 7-chloroquinoline with \(\beta\)-lactam containing potential DHFR inhibitors $(180, 182)$ through triazole linker (Scheme 17)$^{113}$ furnished 181 and 183 as useful hybrids. Hybrids 181 and 183 were more active [IC$_{50}$ = 1.1-2.8 µM (W2)] against CQR W2 strain than 182 [IC$_{50}$ = 1.5- >10 µM (W2)]. Moreover, the most potent hybrid 183a (IC$_{50}$ = 1.1 µM) exhibited lowest binding energies [B. E = -61.3 kcal/mol] in the pocket of \(pf\) DHFR. Thus, these hybrids acted as dual inhibitors (DHFR inhibitor and hemozoin formation inhibitor).

![Scheme 17. Synthesis of 4-aminoquinoline-\(\beta\)-lactam hybrids.](image)

Further, the triazole tether was replaced by urea and oxalamide linkers to create hybrids 184 and 185 (Figure 34).$^{114}$ The antimalarial evaluation data against W2 strain showed that 185 linked through an oxalamide group were potent (IC$_{50}$ = 42-193 nM) inhibitors of \textit{P. falciparum} compared to the urea tethered counterparts 184 (IC$_{50}$ = 34-120 nM). The hybrid 185a with suitable combination of oxalamide linker, cyclohexyl substituent,
and alkyl chain of six carbon, displayed the best antimalarial activity ($IC_{50} = 42$ nM) among the tested hybrids.

![Chemical structures](image)

**Figure 34.** Urea and oxalamide linked 4-aminoquinoline-$\beta$-lactam hybrids.

Recently, Raj *et al.* introduced diaminoalkyl as well as amide linkers to further optimize the structure of $\beta$-lactam based quinoline hybrids (Scheme 18). Comparison of the activities of alkyl tethered and amide-tethered series revealed that the alkyl chain-linked hybrids 188 have better efficacy [$IC_{50} = 59.6$-$611$ nM (W2)] in inhibiting the growth of *P. falciparum*, while the introduction of an amide functionality to create 189 [$IC_{50} = 79.5$-$1145$ nM (W2)] adversely affected the activity profile.

![Scheme 18](image)

**Scheme 18.** Diaminoalkyl and amide linked 4-aminoquinoline-$\beta$-lactam hybrids.

Three hybrids [188a: $IC_{50} = 59.6$ nM (W2), 189a: $IC_{50} = 79.5$ nM (W2), and 189b: $IC_{50} = 89.8$ nM (W2)] showed antiplasmodial profile better than CQ [$IC_{50} = 99$ nM (W2)].
The hybrid 188a with a p-fluorophenyl substituent at N-1 and N-ethyl linker exhibited the best activity (IC$_{50}$ of 59.6 nM) among the tested hybrids against CQ$^r$ strain.

### 2.10.5.3 4-Aminoquinoline-pyrimidine hybrids

A series of pyrimidine-quinoline conjugates 190 have been synthesized using rigid p-phenylene linker (Figure 35). The hybrids 190a-c displayed moderate (MIC = 1μg/ml) activity against the NF-54 strain of *P. falciparum*. Further, to improve the antimalarial activity of pyrimidine-quinoline hybrids 190, Rawat and coworkers appended diaminoalkanes on the pyrimidine motif to create hybrid 191a-n (Figure 35). The in vitro antimalarial activity data reveals that all the hybrids are active at nM concentration [IC$_{50}$ = 5-20 nM (3D7), 20-140 nM (K1)] with some of them exhibited 1.6-2.0-fold higher activity than pyrimethamine [IC$_{50}$ = 10 nM (3D7)] and CQ [IC$_{50}$ = 40 nM (3D7), 390 nM (K1)] against both drug-sensitive and drug-resistant strains (D6 and W2) of *P. falciparum*. The cytotoxicity on Vero, LLC-PK11, and HepG2 mammalian cells showed that some of the hybrids were not cytotoxic at all up to the highest tested concentration of 60 μM. The oral administration (30 mg/kg, *P. berghei*-infected mice) of selected potent hybrids 191i [IC$_{50}$ = 5 nM (3D7), 30 nM (K1)] and 191m [IC$_{50}$ = 6 nM (3D7), 60 nM (K1)] resulted in complete suppression of parasitemia with 80% of the treated mice were cured in case of 191i and 20% for 191m.

![Figure 35. 4-Aminoquinoline-pyrimidine hybrids.](image)

Pretorious *et al.* utilized 2,6-diaminopyrimidine core to generate a series of pyrimidine-quinoline hybrids containing flexible aminoalkoxy 192a-e, diaminoalkyl 192f-h, phenylenediamine 192i or piperazinyl 192j linkers (Figure 35). Antimalarial screening suggested that hybrids bearing piperazinyl [IC$_{50}$ = 0.07 μM (D10), 0.157 μM (Dd2)] or aromatic linkers 192i [IC$_{50}$ = 0.22 μM (D10), 0.107 μM (Dd2)] were more active than the
linear chain analogues 192a-h [IC\textsubscript{50} = 0.15-0.85 µM (D10), 0.21-4.10 µM (Dd2)] against both the D10 and Dd2 strains. The most potent hybrid 192j with antimalarial efficacy in the submicromolar range [IC\textsubscript{50} = 0.07 µM (D10), 0.157 µM (Dd2)] was as potent as CQ [IC\textsubscript{50} = 0.04 µM (D10), 0.417 µM (Dd2)] against the D10 strain whereas it is 2-fold more active than CQ against Dd2 strain. However, this hybrid did not display better solubility and distribution coefficient values compared to CQ.

2.10.6 DNA targeting hybrids

Plasmodial DNA is an interesting biological target. Interaction of compound with plasmodial DNA perturbs the conformation of DNA as well as the biological and physical properties leading to the parasite death. These hybrids were created by conjugating suitable known DNA inhibitors with 4-aminoquinoline core.

Manzamine A alkaloids containing a β-carboline moiety are also potent antimalarials due to interaction of β-carbolines with DNA of \textit{plasmodium} through GC-selective intercalation. Based on this rationale, Gupta \textit{et al.} synthesized β-carboline linked 4-aminoquinoline hybrids 194 (Scheme 19) which bind to plasmodial DNA and also inhibit heme polymerization.\textsuperscript{118} The \textit{in vitro} antimalarial screening of twenty three hybrids against NF-54 strain of \textit{P. falciparum} showed that at least five compounds with MIC in range 0.05-0.22 µM were even more active than CQ (MIC = 0.391 µM). The SAR studies indicated that alkylated phenyl ring and propyl chain at 2-position of tetrahydro-β-carboline was well tolerated.

\begin{equation}
\text{Scheme 19. Synthesis of 4-aminoquinoline- β-carboline hybrids.}
\end{equation}

2.10.7 Hybrids of 4-aminoquinoline with drugs

One of the most viable approaches for the antimalarial hybrid design is to link an antimalarial pharmacophore or a drug with the established drugs as described classified below.
2.10.7.1 Clotrimazole linked 4-aminoquinoline hybrids

Clotrimazole (195, CLT), a well-known antimycotic drug, is endowed with low in vitro antimalarial activity [IC\_50 = 0.55 \mu M (W2)], which was thought to be mediated by its ability to interact with Fe(III)PPIX. Based on the peculiar heme binding properties of CLT and antimalarial properties of the previously synthesized quinolinyl CLT 196 [IC\_50 = 85.8-168 nM (D10), 66.9-148 nM (W2), 60-67 nM (K1)], which reduced parasitemia by 99.4\% at a dose of 50 mg/kg in mice infected with *P. chabaudi*, Gemma and coworkers hybridized\(^{120}\) 4-aminoquinoline with CLT–like scaffold (Figure 36). The resulting diarylmethylaminoquinolines 197 and triarylaminquinolines 198 (trityl) were screened against panel of *P. falciparum* strains namely D10, 3D7 or NF54 (CQS) strains and the W2 or K1 (CQR) strains. The low antimalarial activity of trityl 198 in comparison to diaryl 197 was attributed to the decreased pKa, which resulted in less accumulation of triaryl quinolines in the FV. Among the two series, 197a [IC\_50 = 21 nM (D10), 22 nM (W2), 24 nM (3D7), 65 nM (K1), and 12 nM (NF54)] was identified as the most active hybrid against all the tested strains and exhibited very low in vitro toxicity on plasmocytoma murine cells (IC\_50 = 23.8 \mu M) and human lymphoblastoid cells (IC\_50 = 34.6 \mu M). The *in vivo* studies with 197a against *P. berghei* and *P. chabaudi* showed that oral administration of 50 mg/kg daily for 4-days induced 98\% suppression of parasitemia. Moreover, \(\beta\)-hematin inhibitory assay confirmed the accumulation of hybrid in FV and consequent inhibition of heme polymerization as a possible mode of action of this class of hybrid.

![Figure 36. Generalized structure of first generation clotrimazole-quinoline hybrids.](image)

To further optimize the structure of CLT based hybrids in order to increase interaction with heme and enhanced accumulation in FV, more analogues of 197a were synthesized by modifying the protonable heterocycles at the benzhydryl moiety and the secondary amine.
The SAR studies showed that (i) the presence of piperazine or imidazole in the side chain of benzhydryl 199 [IC$_{50}$ = 33-277 nM (W2); 23-114 nM (K1)] increased activity against the CQ$_R$ strains (W2 and K1), (ii) presence of piperazinyl tether reduced activity [200: IC$_{50}$ = 277-6312 nM (D10), 582-735 nM (K1); 201: IC$_{50}$ = 104 nM (D10), 58 nM (K1)], (iii) introduction of halogen substituent on phenyl ring had only little effect on activity, and (iv) hybrids possessing a pyrrolidine [199 (R$^1$ = 3-Cl, R$^2$ = pyrrolidine, R$^3$ = 7-Cl, X = Cl): IC$_{50}$ =19 nM (K1); 199 (R$^1$ = 3-Cl, R$^2$ = morpholine, R$^3$ = 7-Cl, X = Cl): IC$_{50}$ = 107 nM (K1)] proved to be more potent than hybrids carrying a less basic morpholine in the protonatable side chain. Hybrids 199a-b [199a: IC$_{50}$ = 62 nM (D10), 58 nM (W2), 18 nM (NF54), 22 nM (K1); 199b: IC$_{50}$ = 25 nM (D10), 33 nM (W2), 19 nM (NF54), 23 nM (K1)] emerged as the most potent antimalarial agents which exhibited strong in vivo antiplasmodial activity against P. berghei after oral administration. A single dose of 199a or 199b at 30 mg/kg substantially prolonged the mouse survival and completely reduced parasitemia (>99%). All the hybrids interfered with the process of heme detoxification, as demonstrated by a β-hematin inhibition assay and also abolished CQ uptake via PfCRT$_{CQR}$ at 100 μM which led to decrease efflux of hybrid. The evaluation of intrinsic pharmacological properties of 199a indicated that it exhibit optimal half-life in mice, low cytotoxicity and caused a negative inotropic effect in rat hearts when tested at high concentrations.

**Figure 37.** Generalized structure of second generation clotrimazole-quinoline hybrids.
2.10.7.2 4-Aminoquinoline-quinacrine hybrids

Kumar et al. conjugated core component of standard antimalarial drug ‘quinacrine’ with 7-chloroquinoline through aminoalkyl piperazinyl 202a-b or m/p-phenylenediamine 203/204 linker to generate acridine based quinoline hybrids (Figure 38).122 Hybrid 204 bearing m-phenylenediamine linker was most active (MIC = 0.25 µg/ml) against NF54 strain of P. falciparum. Further, the in vivo evaluation of hybrid 204 demonstrated that 204 completely cleared parasitemia on day 4 at the dose of 50 mg/kgx 4 days by i.p. route but none of the mice survived beyond 28 days.

[Chemical structures of 202a-b, 203, and 204]

**Figure 38.** Structure of 4-aminoquinoline-quinacrine hybrids.

2.10.7.3 4-Aminoquinoline-azidothymidine hybrids

In order to develop safer treatment regimens for malaria and HIV co-infections, Chibale and coworkers synthesized123 novel hybrids by covalent fusion of anti HIV drug, azidothymidine (AZT) with 4-aminoquinoline 205-208, DHA 209, or a tetraoxane 210 (Figure 39). Among all the synthesized hybrids, 209[IC$_{50}$ = 0.03 µM (3D7), IC$_{50}$ = 0.01 µM (Dd2)] bearing DHA was the most potent hybrid with antimalarial activity comparable to DHA [IC$_{50}$ = 0.03 µM (3D7), IC$_{50}$ = 0.71 µM (Dd2)] and moderate anti HIV activity [IC$_{50}$ = 2.86 µM (3D7)] with no observed toxicity to HeLa cells [SI 3,333.3 (3D7), >10,000 (Dd2)]. Among the quinoline hybrids (205-208), hybrid 208 with phenyl linker [IC$_{50}$ = 0.16 µM (3D7)] showed maximum activity against 3D7 strain, whereas, hybrid possessing oxolamide linker 205a [IC$_{50}$ = 0.08 µM (Dd2)] was found to be more active against Dd2 strain. Although, 4-aminoquinoline–AZT hybrids 205-208 [IC$_{50}$ = 0.16-3.53 µM (3D7), IC$_{50}$ = 0.08-31.6 µM (Dd2)] exhibited high activity against 3D7 as well as Dd2 strain but were highly toxic to HeLa cells [SI 625 (3D7), 454 (Dd2)] thus limiting further exploration.123
2.10.7.4 4-Aminoquinoline-azithromycin hybrids

A semisynthetic macrolide, azithromycin (AZI) is a slow acting antimalarial that exerts its activity by inhibiting protein synthesis on the prokaryote-like ribosome in *Plasmodium* organelle, the apicoplast. Peric *et al.* modified AZI structure by appending various amine and amide to obtain hybrids 212-216 functionalities at 9a-N position as shown in Scheme 20.\textsuperscript{124} Out of the 42 hybrids, 213\textsuperscript{a} (IC\textsubscript{50} = 22 nM) consisting of azalide and chloroquinoline moiety was the best which was also 120-fold more potent than AZI (IC\textsubscript{50} = 2654 nM) against TM91C235 strain of *P. falciparum*. I.v. administration of 213\textsuperscript{a} resulted in survival of 5/5 mice at dose of 20 mg/kg and 50 mg/kg, whereas, for AZI only 2/5 (20 mg/kg) and 1/5 mice (50 mg/kg) survived. These results confirmed improvement in *in vitro* and *in vivo* activity of these molecules over AZI.

Taking 213\textsuperscript{a} as antimalarial lead, new C-3’-substituted azalide-quinoline hybrids connected through linkers of different length and structure (Figure 40) were devised to enhance antimalarial activity especially against resistant parasites as well as selectivity for the parasite.\textsuperscript{125} The antimalarial screening data showed that decladinosyl hybrids (217-221: R\textsuperscript{1} = H, IC\textsubscript{50} = 11-636 nM) were less active than cladinosyl analogues (217-221: R\textsuperscript{1} = cladinosyl, IC\textsubscript{50} = 3-77 nM). The hybrid 219\textsuperscript{a} (Figure 41) with good antimalarial activity [IC\textsubscript{50} = 77 nM]...
(3D7), 48 nM (W2)], and cytotoxicity [IC$_{50}$ = >50 µM against HepG2 cells] in acceptable range was found to be best candidate for further profiling in the pharmacokinetic study. The in vivo evaluation of 219a showed low oral exposure and oral bioavailability indicating that further development of this hybrid was unlikely.

![Scheme 20. Synthesis of AZI-quinoline hybrids.](image-url)
Based on the above results, novel quinoline-AZI hybrids comprising of favorable pharmacokinetic properties of AZI and superior quinoline activity against *P. falciparum* were created by blocking 2’OH of macrolide with quinoline 222-224 (Figure 42) so as to exclude antibacterial action of AZI.\textsuperscript{126} All the tested hybrids (222-224) exhibited *in vitro* antimalarial activity [IC\textsubscript{50} = 14-1657 nM (3D7A), 9-815 nM (W2)] which was higher than AZI [IC\textsubscript{50} = 11513 nM (3D7A), 2680 nM (W2)], selectivity index was well above hundred for W2 strain (SI: 231-4356) and it did not show any antibacterial activity. Among the 19 hybrids assayed against 3D7A and W2 strains, the best antimalarial activity was observed for hybrids 222a-b and 224a (Figure 41) bearing two sugar moieties and 7-chloroquinoline moiety instead of simple quinoline.
Iron chelation is an interesting target for the treatment of *P. falciparum* malaria. Deferiprone 225 [3-hydroxypyridin-4-one (HPO)] used clinically for the treatment of iron overload, exhibits modest antimalarial activity [GI$_{50}$ = 67 µM (ITG2G1)] against *P. falciparum*. In order to improve the antiparasitic activity of deferiprone, Gehrke *et al.* hybridized 3-hydroxypyridin-4-one scaffold with 4-aminoquinoline ring system via amino or amide linker at N1 (Scheme 21).$^{127}$ All the hybrids 229 and 232 showed enhanced antiplasmodial activity [GI$_{50}$ = 9-2.4 µM (ITG2G1)] compared to the parent 225 [GI$_{50}$ = 67 µM (ITG2G1)] as well as favorable cytotoxicity profile with 16 to 26-fold selectivity for *P. falciparum* over a mammalian cell line. The most potent hybrid 229a [GI$_{50}$ = 2.4 µM (ITG2G1)] within the series is less active than CQ. The evaluation of Fe$^{3+}$ chelation capacity (pFe$^{3+}$) of the hybrid with good solubility profile indicated that pFe$^{3+}$ value of 229a (21.1) at pH 7.4 was between the pFe$^{3+}$ value of potent iron chelators [225 (19.3), desferroxamine (26.6)]. Thus, conjugation with quinoline did not interfere with the iron chelation capacity of HPO, and the synthesized hybrids exerted their antimalarial potency by iron chelation mechanism.

Further, synthesis and antimalarial activity evaluation of 3-hydroxypyridin-4-one-aminoquinoline led to the hybrids 233-234 (Figure 43) which were more active than precursors (HPO and CQ).$^{128}$ Hybrid 233a [IC$_{50}$ = 0.13 µM (K1), 0.004 µM (3D7); 0.1 µM (W2)] 233b [0.08 µM (K1), 0.01 µM (3D7); 0.02 µM (W2)] and 234a (0.07 µM (K1), 0.03 µM (3D7); 0.08 µM (W2)] were the most potent hybrids against K1, 3D7 and W2 strain of *P. falciparum* having activity comparable to CQ [IC$_{50}$ = 0.01 µM (K1), 0.44 µM (3D7), 0.10 µM (W2)]. The β-hematin inhibition activity of all the hybrids was in the range 0.01-1.71 µM.

Figure 42. 2’-AZI linked quinoline hybrids.
which was superior to CQ (1.9 µM). Moreover, the cytotoxicity studies in the mammalian cell lines demonstrated that all the hybrids were non-toxic and exhibited toxicity profile better than that of CQ.

**Scheme 21.** Synthesis of 3-hydroxypyridin-4-one-quinoline hybrids.

**Figure 43.** HPO-aminoquinoline hybrids.

### 2.10.7.6 Primaquine-chloroquine hybrids

Lodge et al. designed ‘primaquine-chloroquine twin’ by combining the terminal amino function of the side chain core moiety of either PQ or CQ (Scheme 22). The hybrid 235 displayed very good activity against the blood stages of three different strains (3D7, Dd2 and K1) of *P. falciparum*. Hybrid 235 (IC\(_{50}\) = 0.08 µM) was six times more active than PQ (IC\(_{50}\) = 0.46 µM) and twice as active as CQ (IC\(_{50}\) = 0.146 µM) against K1 strain. Moreover,
the hybrid 235 also displayed significant activity against both asexual and sexual *P. falciparum* blood stages as well as *P. berghei* sporozoites and liver stages. The hybrid elicits *in vivo* activity against *P. berghei* liver as well as blood stages and prevents blood stage patency, and responded to the symptoms of experimental CM.

\[
\text{H}_2\text{CO}\quad \text{N}
\]

\[
\text{CH}_3\quad \text{6}
\]

\[
\text{NH}_2
\]

\[
\text{Cl}
\]

\[
\text{Cl}
\]

\[
\text{Scheme 22. Primaqune-chloroquine hybrid.}
\]

**2.10.7.7 4-Aminoquinolines conjugated with paclitaxel**

Njogu *et al.* coupled (2R,3S)-N-benzoyl-3-phenylisoserine moiety, a structural component of the antimicrotubular drug paclitaxel with appropriately derivatized ART or quinoline scaffold via ester, amide, or triazole linkage (Figure 44).\(^{130}\) The ART-based hybrids 236 \([\text{IC}_{50} = 0.005 \mu\text{M} (W2)\) and 0.0007-0.0005 \(\mu\text{M} (K1)\)] were more potent than quinoline analogues 236-238 \([\text{IC}_{50} = 0.13-0.56 \mu\text{M} (W2)\) and 0.22-1 \(\mu\text{M} (K1)\)]. Among the quinoline-based hybrids (237c-237d), 237c and 237d were the most active \((\text{IC}_{50} = 0.13 \text{ and } 0.16 \mu\text{M}, \text{respectively})\) against the W2 strain. However, their antiplasmodial activities were considerably lower than that of the control drug CQ \((\text{IC}_{50} = 0.05 \mu\text{M})\). Further, the hybrids bearing triazole linker 238a-d were found to be less active than those having an amide or ester linkage. Moreover, quinoline hybrids 237-238 exhibited low cytotoxicity against L6 cell line \((\text{CC}_{50} = 15.13-88.18 \mu\text{M})\).

![Paclitaxel-quinoline hybrids](image)

**Figure 44. Paclitaxel-quinoline hybrids.**
2.11 Conclusions

Malaria elimination has recently been reinstated as a global health priority but current therapies seem to be insufficient for the task. Elimination efforts require new drug classes that are active against all stages of the parasite life cycle and have new mechanisms of action. To develop these next-generation medicines, hybridization is an innovative approach that targets multiple stages of the parasite life cycle. This review highlighted the development of quinoline based compounds with high *in vitro* and *in vivo* antimalarial activity synthesized using hybridization strategy. The hybridization of CQ with trioxanes led to the highly potent antimalarials “trioxaferroquines” (against resistant strains) which are advanced to phase II in clinical trials. Thus, the hybrids summarized in review may serve as excellent starting points to develop new agents with potential to be used in malaria eradication campaign.

In this thesis, we applied the hybridization strategy for the synthesis of pyrimidine based quinoline hybrids and evaluated their biological activities. The results of these investigations are presented in the chapters 3 to 5 of this thesis.

2.12 References


Chem. Lett.* **2008**, *18*, 6530-6533.


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