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Invited review

Recent developments in the design and synthesis of hybrid molecules based on aminoquinoline ring and their antiplasmodial evaluation

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ABSTRACT

A short history of hybrid molecules based on aminoquinolines gave interesting and important information useful for organic and medicinal chemistry, which are deeply involved in the design and development of new antimalarial agents. The highlights in the preparation of aminoquinoline antimalarials, their protocols and antiplasmodial activity and, specially, the development on hybridization approaches are represented.

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1. Introduction

Malaria is estimated to kill more than 1 million people annually and possibly as many as 3 million, with most of the deaths among children under age six living in sub-Saharan Africa. According to the WHO, between 300 million and 500 million clinical cases of malaria occur every year. Recent empirical estimates have suggested the total could be run as high as 660 million [1]. Despite decades of fighting malaria, the disease gaining ground as the parasite's resistance to drugs and the parasite-carrying mosquito's resistance to insecticides expand [2]. The increasing spread of malaria together with the emergence of resistance against conventional drugs has put enormous pressure on public health systems to introduce new malarial treatments [3,4]. This review does not mean to be comprehensive, and its goal is to highlight the improvements in the preparation of aminoquinoline antimalarials, their protocols and antiplasmodial activity, specially, the development on hybridization approaches allowing a quick access to these compounds. An attempt has been made to present and discuss the more recent contributions in this field over the period 2004–2008, paying a special attention to synthesis, design and biological evaluation of novel aminoquinoline hybrid molecules. However, to provide a good understanding of the scope of the aminoquinoline preparation and their antiplasmodial results, older examples and results were included.

1.1. Development of aminoquinoline antimalarials from quinine to nowadays, historical aspects

Quinoline-containing compounds have long been used for treatment of this disease, beginning with alkaloid quinine isolated as the active ingredient from the crude back of Cinchona trees. The cinchona tree is named after the Countess of Chinchon, who according to legend was cured of malaria in 1630 by a power made from its bark. The pulverized bark was widely distributed in Europe by the Jesuits during the 17th century [5]. The determination of the structure of the cinchona alkaloids became a challenge for chemists throughout the 19th century. Although some attempts for the synthesis of quinine were described [6–8] the true is that the synthetic route is too complex for commercial production. The first attempts at the design and preparation of synthetic antimalarial drugs came from the observation that methylene blue had some antimalarial activity. By modifying its structure the first synthetic antimalarial plasmoquine later called pamaquine (PamQ) was synthesized and was first used in 1926. It was later found to be too toxic, and primaquine (PrimQ) was synthesized as a less toxic analogue. Further systematic modification of quinine and methylene blue led to other simple 6-methoxy-8-aminoquinoline derivatives, historical synthetic quinoline antimalarial agents, pentaquine (PenQ) and isopentaquine (IsopenQ) (Fig. 1).

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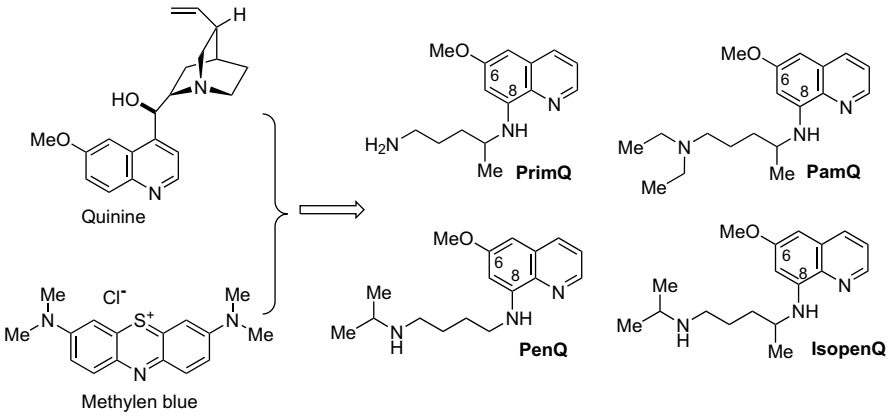


Fig. 1. First 8-aminoquinoline derivatives, historical synthetic quinoline antimalarial agents.

In further work, German scientists at IG Farben attached the basic side chain of PamQ to a number of heterocyclic ring systems, which led to the synthesis of the acridine derivative quinacrine (also known as atebrine or mepacrine), which proved to be active against the blood stages of *Plasmodium falciparum* [9]. Scientists at the Bayer laboratories in Germany also synthesized the 4-aminoquinoline resochin [10]. Resochin initially was thought too toxic for clinical use and was ignored for a decade. However, during World War II, the Allied troops captured a supply of the related drug sontaquine (SQ), and began a re-evaluation of the two drugs (Fig. 2). Resochin was found to be safe at therapeutic concentrations and was renamed chloroquine (CQ) and taken into clinical trials in 1943 [6,10]. Soon it was the mainstay of malarial chemotherapy. Even in the 1950s many authorities thought seriously the possibility of malarial eradication. As an attempt to contribute to eradicate malaria, in the early 1960s, the World Health Organization supplied tons of chloroquine for inclusion in table salt in parts of South America, Africa and Asia. Shortly afterwards, the first cases of CQ resistance were reported from the same areas where this chloroquinized salt had been distributed. Since then, CQ resistance has reached all regions of the world were CQ is used, being a serious problem during the Vietnam War. A massive screening program in the United States at the end of World War II produced another 4-aminoquinoline, amodiaquine (AQ) as an alternative to CQ.

New quest for active compounds against parasite resulted in the development of improved derivatives of first 4(8)-aminoquinoline series. In such a manner new perspective antimalarial agents like *t*-buthylprimaquine, tafenoquine, tebuquine, isoquine, and more others were developed.

1.2. Aminoquinoline antimalarial drugs: advantages and disadvantages

Ideally, an antimalarial candidate should be safe and well tolerated, efficacious and effective, orally, rectally, and parenterally applicable, stable, available as a single dose, effective against all stages of parasite development, not susceptible to parasite resistance, and cheap.

The success of the antimalarial aminoquinoline drugs has been based on excellent clinical efficacy, limited host toxicity, ease of use and simple cost-effective synthesis. Among these antimalarial compounds, especially CQ has been the mainstay of falciparum malarial chemotherapy for decades: it is cheap, safe when used at the correct dose (25 mg/kg in divided doses) and practicable for outpatient use. It remains the most commonly used first-line antimalarial drug in much of Africa, despite failure rates over 70% in some areas have severely limited its usefulness [11]. It remains effective for *Plasmodium ovale*, *Plasmodium malariae* and most cases of *Plasmodium vivax* infections worldwide. Many CQ-resistant strains of *P. falciparum* remain sensitive to its congener AQ alone or in combination with other antimalarial drugs. CQ is rapidly absorbed from the gut and from intramuscular or subcutaneous injections. About half of the absorbed CQ is cleared unchanged by the kidney, the rest being biotransformed in the liver to desethyl- and bisdesethyl-chloroquine [12]. Although clearance is reduced in renal failure, it is not usually necessary to reduce the dose. The terminal elimination half-time is very long (1–2 months). CQ is well tolerated but, when plasma concentrations exceed around 250 μg/mL, unpleasant symptoms (such as headache, diplopia, dizziness

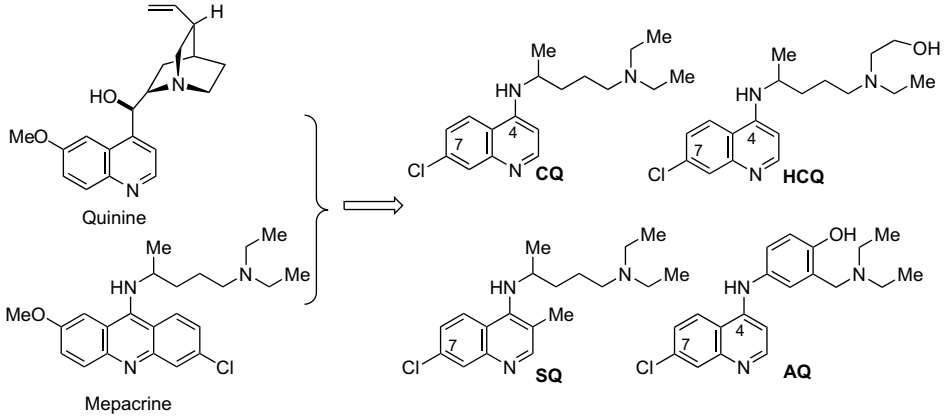


Fig. 2. First 4-aminoquinoline derivatives, historical synthetic quinoline antimalarial agents.

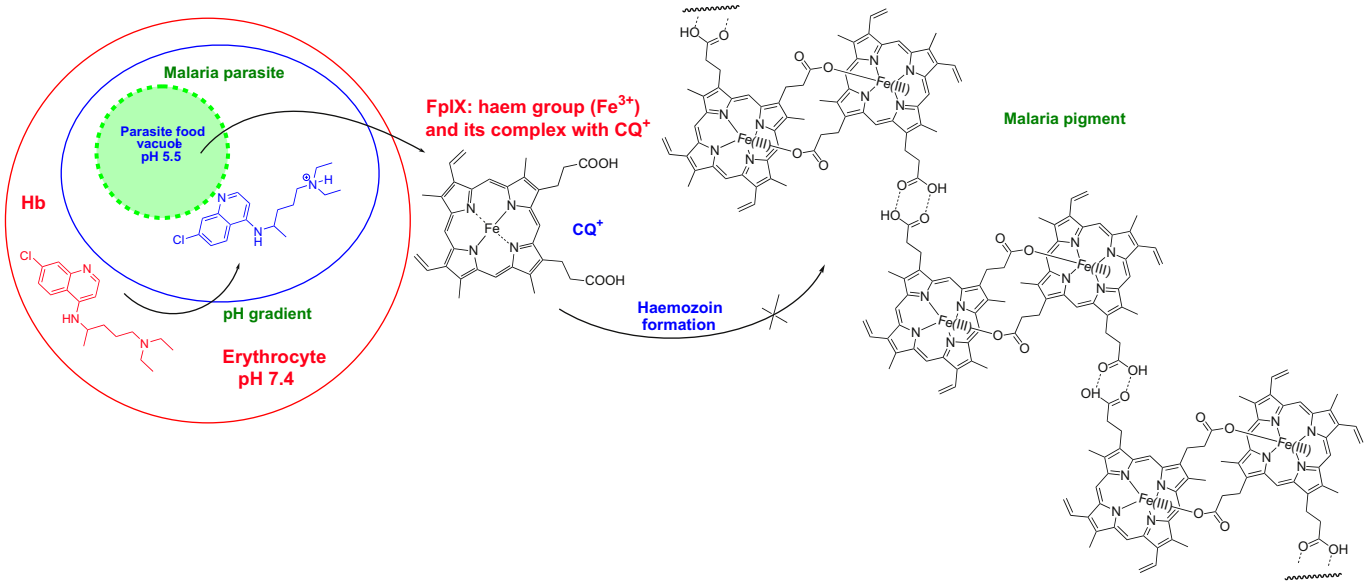
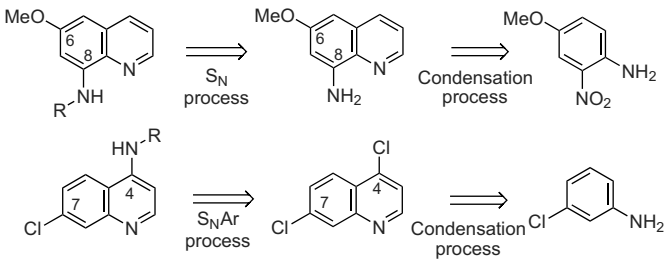


Fig. 3. Possible mode action of CQ.

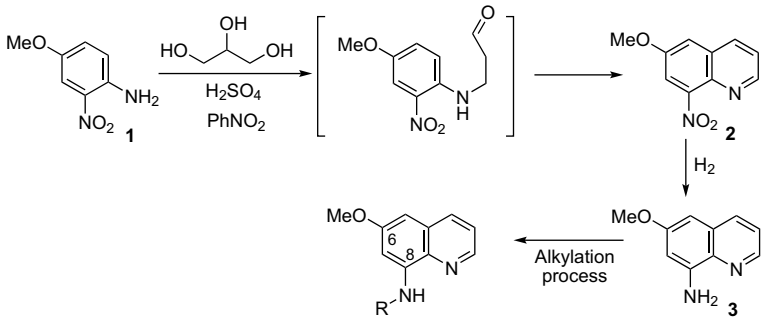
and nausea) may develop [13]. After an overdose, toxicity is manifested rapidly. Coma, convulsions, hypotension, respiratory paralysis and shock and cardiac arrhythmias are common problems and often develop within 1 h of dosing. Of its symptomatic adverse effects, pruritus is worthy to note. Also CQ is eliminated slowly and accumulates in some tissues, notably the retina [14]. So, continuous weekly CQ use (cumulative dose >100 g) may cause retinopathy. In adults toxicity can be seen with doses >1 g, and 5 g (33 tablets of CQ sulphate) is usually fatal without prompt treatment [15]. Some rare toxic effects of chloroquine include photo-allergic dermatitis, aggravation of psoriasis, skin pigmentation and leucopenia. AQ is effective against many CQ-resistant strains of *P. falciparum*. However, clinical use has been restricted because of associations with hepatotoxicity and agranulocytosis. It is extensively converted into its equipotent metabolite desethylamodiaquine, which is responsible for most of the antimalarial activity: desethylamodiaquine achieves much higher concentrations than its parent drug [16]. Another metabolite, AQ-quinoneimine, has an important role in toxic reactions. It is highly reactive and haptenates proteins, generating antigen that may cause organ damage. AQ caused hepatitis and agranulocytosis in patients taking it for prophylaxis. Because AQ retains antimalarial activity against CQ-resistant parasites, initial Biagini's studies involved the design and synthesis of fluoroamodiaquine as a safer alternative to AQ [17].

Primaquine (PrimQ) has been used since the 1940s for the eradication of liver stages in *P. vivax* infections. As a consequence of its specific activity, PrimQ can also be used as a prophylactic. Also



Scheme 1.

prevents the maturation of fertile gametocytes. Unfortunately toxicological concerns have lead to restrictions in the use of PrimQ [3]. PrimQ is absorbed well after oral administration but has a short half-life and needs to be administered daily. Mild gastrointestinal adverse effects are common. Optimization of its structure led to tafenoquine, a new 8-aminoquinoline drug less toxic and with a plasma half-life longer than PrimQ (its terminal half-life is about 14 days), that is also active against erythrocytic stages [18]. The prophylactic activity of tafenoquine was demonstrated in several clinical studies, as well as its capacity to clear liver hypnozoites. In one such studies, patients with acute *P. vivax* malaria were first treated with CQ to eliminate all erythrocytic parasite stages. Then, following treatment with tafenoquine, but not CQ, could relapse the disease to be prevented [19]. Pre-erythrocytic stages ('hypnozoites') of *P. vivax* and *P. ovale* are not eradicated by the drugs used to eliminate erythrocytic infection (mainly chloroquine) and late relapse may result (usually up to 40 weeks after the primary attack). PrimQ is given orally once daily, and courses usually last 14–21 days, although longer courses may be required for some Southeast Asian and western Pacific strains. Its main disadvantage is the more serious toxicity in patients with glucose-6-phosphate dehydrogenase deficiency. PrimQ causes haemolysis in these patients [20], as metabolites of PrimQ undergo redox cycling in the erythrocyte, leading to an oxidative stress that is poorly tolerated by glucose-6-phosphate dehydrogenase-deficient cells [21]. In patients with the severe forms of this deficiency, the risks of PrimQ might well exceed the benefits, and treatment must not be administered since *P. vivax* and *P. ovale* malarias rarely cause life-threatening illness [13]. Also, a haemolytic response was observed in two G6PD-deficient patients who accidentally received tafenoquine, one of whom required a blood transfusion [22]. Unless CQ and the other quinoline antimalarials such as AQ, quinine and mefloquine have supported the malarial chemotherapy for much of the past 40 years, emergence and spread of *Plasmodium* parasites resistant against these drugs have brought the urgency to develop a new generation of safe and effective drugs against malaria. Much of the current effort is directed toward the identification of novel chemotherapeutic targets, but we still do not understand fully the mode of action and the mechanism of resistance to the quinoline compounds.

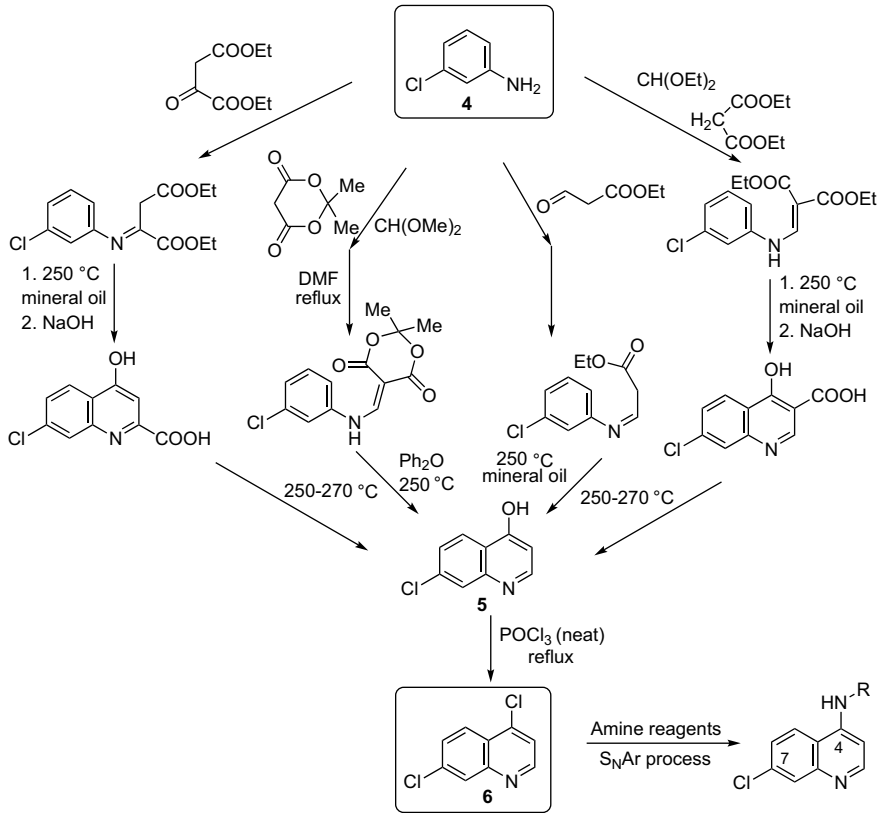


Scheme 2.

2. Mechanism of action, resistance and new targets

CQ is active only against the blood stages of *Plasmodium* actively degrading haemoglobin (Hb) [23]. Predominantly in the trophozoite and early schizont stages, haemoglobin is ingested with the cytoplasm of the host erythrocyte by a phagocytosis-like mechanism and transported into the central food vacuole. It has been assumed, therefore, that CQ interferes with the parasite feeding process [24]. CQ is a diprotic weak base and in its unprotonated form can pass through the membranes of erythrocyte and parasite and accumulate in the acidic food vacuole (pH 5) [25,26] (Fig. 3). Thus, the targets of CQ action are thought to be metabolic processes involved in the uptake or digestion of haemoglobin. So, although a number of enzymes have been proposed as possible drug targets, it is generally accepted that the 4-aminoquinolines interfere with the detoxification of free heme, which is generated during the degradation of Hb [27,28].

The Hb is degraded by a series of proteases within the acidic food vacuole into small peptides, which are subsequently transported to the cytoplasm of the parasite. As a by-product of such degradation, the toxic heme moiety, ferriprotoporphyrin-IX (Fp-IX), is released. The heme molecules are detoxified within the food vacuole of the parasite by polymerization into insoluble granules of hemozoin. CQ forms a complex with heme and inhibits heme polymerization leading to a build-up of toxic heme (FpIX) molecules. All 4-aminoquinolines, including AQ and its active metabolite desethylamodiaquine, appear to act in this way. But the sequestration of the free heme into hemozoin or malarial pigment is not the only way by which heme is detoxified. Two additional mechanisms include a degradation facilitated by hydrogen peroxide within the food vacuole, and a glutathione-dependent degradation which occurs in the parasite's cytoplasm. Some studies suggest that up to 95% of the free iron released during Hb digestion is found in hemozoin [29–31]. X-ray crystallography and spectroscopic analysis indicate that hemozoin has the structure as β-hematin [32]. β-Hematin is a heme dimer formed via reciprocal covalent bonds between carboxylic acid groups on the protoporphyrin-IX ring and the iron atoms of two heme molecules. These



Scheme 3.

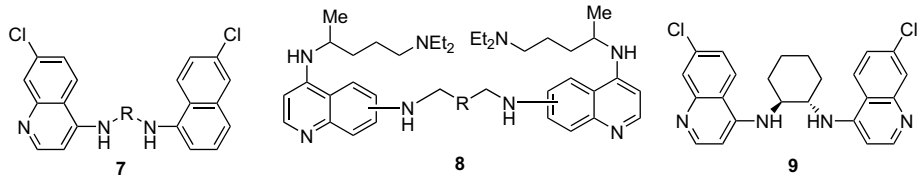


Fig. 4. The bulky bis-quinolines tested against *P. falciparum*.

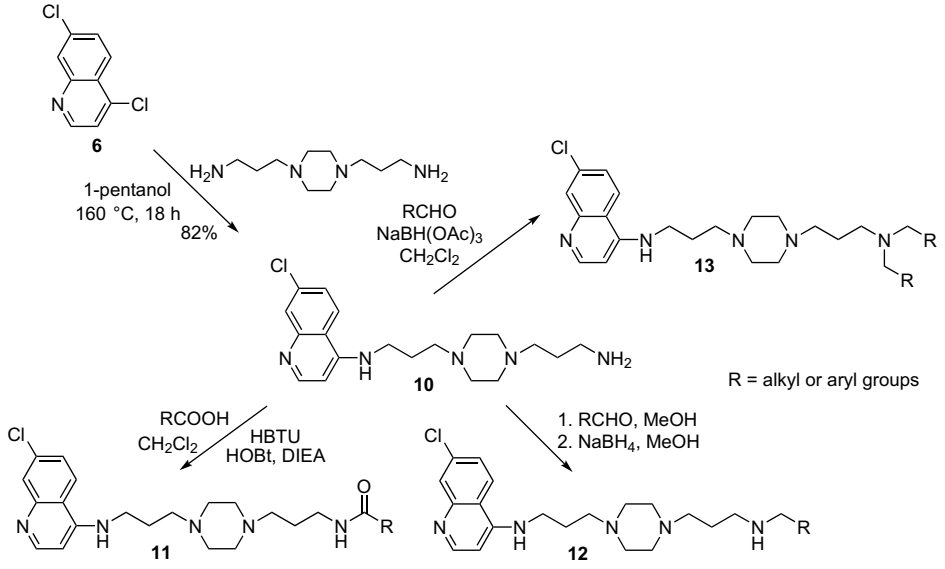
dimers interact through hydrogen bonds to form crystals of hemozoin. By this reason, pigment formation is also known as a biocrystallization or biomineralization process [33]. Regarding to the second mechanism, heme is degraded by reaction with hydrogen peroxide, which is generated by spontaneous oxidation of the released heme from an Fe II to an Fe III stage [34]. This results in the production of reactive oxygen species (superoxide anion, H_2O_2, and hydroxyl radicals), which represent an oxidative stress. In the food vacuole, where the oxidative stress is generated, host derived catalase and peroxidase activities probably contribute to H_2O_2 breakdown, also parasite proteases will rapidly destroy the host enzymes. In addition, it has been shown that free heme, itself, displays both catalase and peroxidase activities [35,36]. However, it also has been shown that CQ is an efficient inhibitor of the catalase activity of heme [37]. The formation of CQ–heme complexes in the food vacuole could inhibit the catalase activity of heme, thereby prolonging the half-life of any H_2O_2 that is produced. Thus, the toxicity of oxidant species will be enhanced in the presence of CQ. If peroxidative damage to membranes or enzymes is the final target of CQ action, this would explain the irreversible nature of CQ activity against the parasite [38]. It is well known that aerobic cells protect themselves against reactive oxygen species using antioxidant compounds and the oxidant defence enzymes, superoxide dismutase, catalase, and gluthatione peroxidase. Some of the heme apparently diffuses into the cytoplasm of the parasite, where it may be destroyed by reduced glutathione [39]. There are quite convincing data that both the heme polymerization and the oxidative and gluthatione-dependent heme degradation are inhibited by the 4-aminoquinolines.

AQ is a more active inhibitor than CQ of the growth of *P. falciparum in vitro* [40], being a useful alternative to chloroquine-resistant *P. falciparum*. The superior potency of AQ to CQ *in vitro* is probably due to differences in the ability to form complexes with heme within the highly compartmentalised infected red blood cell system [13]. Unfortunately, resistance to AQ and its derivatives has followed in the pathway of CQ resistance [41].

The mechanism of action of the 8-aminoquinolines is still largely unknown. The activity of tafenoquine against blood stages possibly depends on the inhibition of heme polymerization by a mechanism similar to 4-aminoquinolines. For the activity against liver stages and gametocytes, an alternative mechanism related to mitochondrial processes has been suggested. Primaquine is thought to be converted in the liver to an active quinine metabolite and exerts its activity by interfering with mitochondrial function [41].

2.1. Resistance

The development of CQ resistance has had a devastating effect on the control of malaria. Eradication efforts faltered in the 1960s, following the development of CQ-resistant parasites. Resistance to CQ was slow to develop, comparing the >20 years taken with ≤1 year for resistance to pyrimethamine or atovaquone [42], suggesting that multiple mutations were required to produce the resistant phenotype. As seen above, the crucial step in the mode of action of CQ is the binding of the drug to FpIX, a by-product of Hb degradation. CQ-resistant parasites accumulate CQ in their acidic food vacuoles much less efficiently than CQ-sensitive strains, suggesting that drug resistance results mainly from exclusion of the drug from the site of action rather than an alteration in the CQ target [25]. Furthermore, that resistance can be "reversed" *in vitro* using drugs such as verapamil, desipramine and chlorpromazine. These reagents can be used to modulate drug resistance in human tumour cell lines, leading to the suggestion that the chloroquine-resistant phenotype may have parallels with tumour multi-drug resistance (MDR) [43]. MDR is thought to be due to the



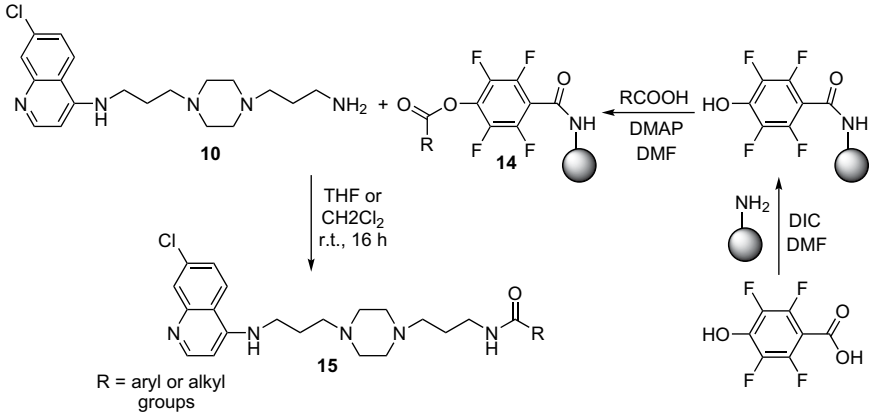
Scheme 4.

Table 1
Biological studies of compounds **11**–**13**: *in vitro* sensitivity of *P. falciparum* FcB1 strain (IC_{50}) and cytotoxicity (CD_{50}) on MRC-5 cells (selectivity indexes are given in parentheses).

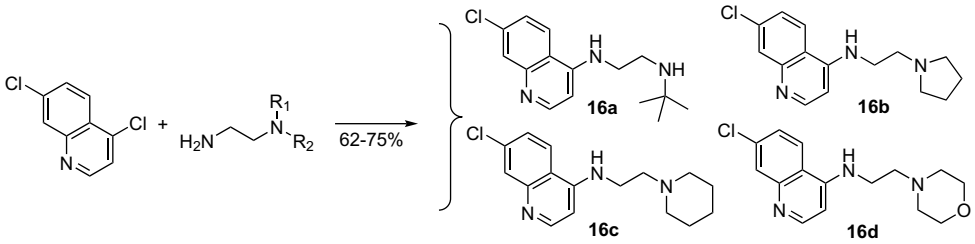
R/compound		**11**		**12**		**13**	
		IC_{50} (nM)	CD_{50} (μM)	IC_{50} (nM)	CD_{50} (μM)	IC_{50} (nM)	CD_{50} (μM)
Cyclohexyl	**a**	215.9	4.0 (19)	43.0	5.5 (128)	16.0	5.1 (319)
Cyclopropyl	**b**	289.2	16.0 (55)	10.2	16.0 (1569)	8.8	4.0 (455)
Hexyl	**c**	34.7	14.5 (418)	9.5	<3.1 (<326)	37.8	<3.1 (<82)
Propyl	**d**	73.4	16.0 (218)	24.8	16.0 (645)	7.5	5.1 (864)
Ethyl	**e**	276.7	32.0 (116)	36.1	16.0 (443)	2.2	0.1 (45)
Methyl	**f**	396.0	32.0 (81)	58.0	32.0 (552)	5.0	0.1 (20)
tert-Butyl	**g**	163.2	20.5 (126)	9.7	4.1 (423)	nd	<3.1 (nd)
Isopropyl	**h**	149.0	17.0 (114)	18.8	4.0 (213)	0.9	8.5 (9444)

overexpression of an ATP-dependent drug effluxer known as P-glycoprotein [44]. In tumour cells, P-glycoprotein pumps are encoded by multi-drug resistance genes, and similar genes (*pfmdr1* and *pfmdr2*) have been found in *P. falciparum*. Resistance to CQ was initially attributed to mutations in the *P. falciparum* multi-drug resistance gene (*pfmdr1* Y86) on chromosome 5 [45] and to a second candidate gene on chromosome 7, *cg2* [46], that encoded a 330 kDa protein associated with the parasite plasma membrane and the food vacuole. However, the level of chloroquine resistance in a number of strains of *P. falciparum* did not correlate with the level of *pfmdr1* expression [47]. Efflux of CQ by a plasmodial P-glycoprotein as responsible for CQ resistance has been so questioned, however other studies have been related this resistance with a diminished level of accumulation rather than a drug export mechanism. This decreased accumulation could result from a modified vacuolar pH [48,49]. Another possibility is that the decreased CQ concentration could result from the loss of an intracellular receptor [50]. Further Sánchez's work implicates a plasmodial Na^+/H^+ exchanger in the facilitated import of CQ [51]. Also, in a genetic cross-experiment, neither resistance gene was linked to resistance [52]. The progeny of a genetic cross between a chloroquine-resistant clone of *P. falciparum* and a chloroquine sentitive clone, has allowed to identify a single gen, *pfcrt*, which is predictive of CQ resistance. This gene encodes a protein, which is located at the parasite's digestive food vacuole, and which has features suggestive of a role as a transporter (CQ-resistance transporter, *crt*). In comparison to CQ-sensitive parasites, resistant parasites carry a number of mutations in *pfcrt*, of which the K76T mutation is predictive of the verapamil-responsive resistance phenotype. An observation with field isolates from a range of geographical settings and transfection studies provides compelling support for the claim that *pfcrt* is the major chloroquine-resistant gene in *P. falciparum*. Presumably these mutations affect the accumulation of CQ in the food vacuole, but the exact mechanism of CQ resistance is not known. Later studies have confirmed that the K76T mutation of the *P. falciparum* chloroquine resistance transporter (*pfcrt*) is the major mutation implicated in resistance to CQ [53]. Recent genetic and genomic advances have allowed approaching the origins and spread of antimalarial drug resistance and the underlying molecular mechanisms. Researchers can now use data from genome sequencing projects to identify genetic regions linked to resistance phenotypes [54]. The gold standard for confirming the identity of a resistance gene involves allelic exchange. If a gene truly confers resistance, then replacing the sensitive allele with the putative-resistant allele, on the sensitive background, should confer resistance. By this way, Sidhu et al. definitively showed that the *pfcrt* allele conferred resistance to CQ by replacing the *pfcrt* allele of a sensitive line with the *pfcrt* alleles of resistant lines from South America, Asia and Africa [55]. Reed et al. also employed allelic exchange to demonstrate that allelic variants of *pfmdr1* could modulate the degree of parasite susceptibility to mefloquine, quinine, halofantrine, CQ, and artemisin [56].

Some of these genetic changes that confer parasite resistance are useful as molecular markers that have been validated as tools for surveillance of trends in parasite drug sensitivity [57]. Changes in drug use policies in Malawi are only an example of the usefulness of these markers. In 1993, Malawi became the first African country to replace CQ with sulphadoxine–pyrimethamine as the first-line antimalarial drug [58]. Molecular surveillance of some molecular markers demonstrated two clear trends. First, the prevalence of the *pfcrt* T76 allele, which is associated with CQ resistance, declined rapidly after the withdrawal of CQ, from 85% in 1992 to undetectable levels by 2001. Also, the prevalence of parasites that carried dihydrofolate reductase mutations (*pfdhfr*) associated with



Scheme 5.



Scheme 6.

resistance to sulphadoxine–pyrimethamine progressively increased [59]. A clinical trial recently confirmed a dramatic increase in CQ efficacy in Malawi, from 50 to 99% in just 12 years, and a similar decrease in the sulphadoxine–pyrimethamine efficacy, from nearly 100–21% during the same time period [60].

2.2. New targets

Resistance of malarial parasite to currently used drugs has led to an urgent need to develop new and effective antimalarials. There are three valid strategies: (1) to generate new drug candidates against validated targets, (2) to identify new potential targets for malarial chemotherapy, or (3) to search antimalarial activity in drugs with other therapeutical uses. There are several potential targets for new antimalarials [61]. Among these, the Hb degradation pathway in *P. falciparum* is a specialized parasite process with a proven history as an exploitable therapeutic target. It probably occurs by a semi-ordered process involving the sequential action of different proteases [62], as aspartic, cysteine and metalloproteases. Among aspartic (acid) proteases, several plasmepsin genes have been identified in the genome of *P. falciparum* and four of these appear to function in the food vacuole [63]. Plasmepsin-1 and plasmepsin-2 are the best characterized and both are capable of cleaving undenatured haemoglobin between phenylalanine and leucine residues located at positions 33 and 34 on the α-globin chains. Cleavage at this site presumably causes the globin subunits to dissociate and partially unfold. The other plasmepsins, as well as plasmepsin-1 and plasmepsin-2, and the falcipains (cysteine proteases) are then able to further degrade these large globin fragments. Among these, four papain-like cysteine proteases have been identified in the *P. falciparum* genome and have been named falcipains 1, 2A, 2B and 3 [64,65]. Falcipains 2A and 3 have been proposed to be involved in Hb digestion, since they have been located in the food vacuole and the corresponding recombinant proteins have acidic pH maxima and can degrade denatured Hb [66,67]. The peptide fragments produced by these digestions are then digested into smaller peptides by falcilysin. Small peptides must be pumped out of the food vacuole into the parasite cytoplasm, where an aminopeptidase carries out the final conversion to amino acids [68,69]. Examples of specific cysteine protease inhibitors, that have been evaluated as candidate drug targets include E64, peptidyl fluoromethyl ketones, and peptidyl vinyl sulfones.

Table 2
Antimalarial activity (IC_{50} nM) and resistance factor of compound (**16**).

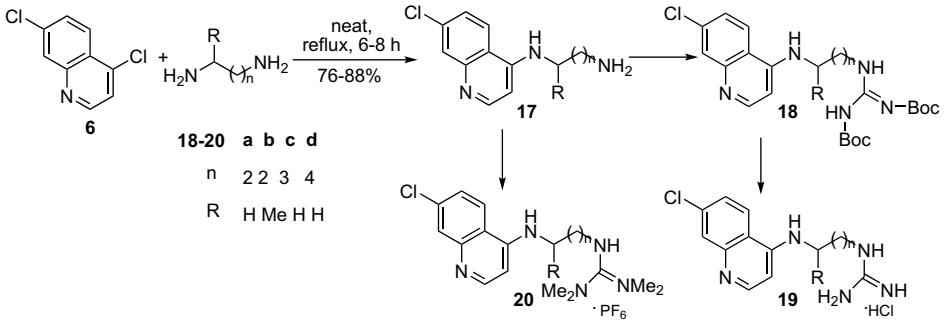
Compound (**16**)	IC_{50} (nM)		Resistance factor (K1/HB3)
	HB3	K1	
a	4.3 ± 1.3 (2)	15.3 ± 2.9 (2)	3.56
b	6.8 ± 0.8 (4)	13.0 ± 2.3 (4)	1.90
c	6.8 ± 1.2 (3)	13.2 ± 1.1 (3)	1.94
d	150 ± 41 (3)	240 ± 29 (3)	1.56
CQ	19.0 ± 2.1 (2)	190 ± 15 (3)	10

They block Hb digestion, causing distension of the food vacuole and inhibition of schizont production [66,70]. The expression of papain-like cystein proteases during gametocytogenesis, which is required for malarial parasite transmission, has not been studied. Data from the work of Eksi et al. demonstrating that gametocytes express falcipain 3 and not falcipain 2A/B, coupled with the possibility that falcipain 3 could be essential for asexual growth, suggest that falcipain 3 is a prime drug target [71]. Falcipain 1 has also been shown to be expressed by gametocytes and to play a role in oocyst production and therefore should also be included in drug development efforts [72].

Membrane biosynthesis has also been exploited as drug target. Infected erythrocytes contain large amounts of phospholipid constituents of the parasite membrane, the food vacuolar membrane and the parasitophorous vacuole membrane. The major parasite phospholipid is phosphatidylcholine, most of which is synthesized by *de novo* synthesis from choline. The rapid multiplication of *P. falciparum* in human erythrocytes requires active synthesis of new membranes. Developing drugs that target membrane synthesis is therefore a valid strategy to fight against malaria. Among these, quaternary ammonium choline analogues block by this way the growth of the parasite. That is the case of G25, a potent antimalarial that inhibits de novo biosynthesis of phosphatidylcholine in the parasite [73].

Oxidative stress is an important mechanism for destruction of intracellular parasites. Most of them have defence mechanism for preventing this oxidative stress. So, *Plasmodium* contains three antioxidant enzymes: superoxide dismutase (SOD), glutathione peroxidase (GPx) and catalase. Functional thioredoxin and glutathione systems participate in antioxidant defence and both could be potential targets for antimalarial chemotherapeutics. Krauth-Siegel and Coombs have shown differences between parasite and host thioredoxin reductase [74]. Regarding to glutathione *S*-transferase, the only isoenzyme of *P. falciparum* acts as a ligand for parasitotoxic hemin and it has been shown to differ considerably from the human enzyme [75]. As it catalyzes the conjugation of glutathione with hydrophobic compounds resulting non-toxic products, the enzyme could be exploited to search for specific inhibitors.

Nucleic acid metabolism pathways also differ between *P. falciparum* and the human host. As most protozoan parasites, *Plasmodium* does not have the *de novo* purine nucleotide pathway and relies exclusively on the salvage of performed host purines for their survival. Two parasitic enzymes concerning the purine pathway, hypoxanthine–guanine–xanthine phosphoribosyltransferase (HGXPRT) and purine nucleotide phosphorylase (PNP) show differences in substrate specificity or structure, respectively, from human analogues. So, parasite HGXPRT has the ability to catalyze the phosphoribosylation of xanthine in addition to hypoxanthine and guanine. Consequently, an approach to developing antimalarial drugs is to use HGXPRT to convert introduced purine base analogues to nucleotides toxic to the parasite. The incorporation of these nucleotides into DNA and RNA will finally result in the



Scheme 7.

cessation of cell replication. Studies from Keough et al. have found three base analogues, 6-chloroguanine, 8-azaguanine and 8-aza-hypoxanthine, which are highly selective as well as effective substrates for *P. falciparum* HGXPRT compared with human analogue HGPRT [76]. The involvement of *P. falciparum* PNP in both purine and polyamine pathways makes also this enzyme an attractive drug target, and rationally designed PNP inhibitors (immucillins) inhibit both parasite and host erythrocyte enzymes to produce purine-less death of *P. falciparum* parasites [77,78].

Pyrimidine nucleotides are also essential metabolites. Unlike human and other mammalian cells, *P. falciparum* cannot salvage preformed pyrimidine bases or nucleosides from human host, but is totally dependent on *de novo* pyrimidine biosynthetic pathway. Because the parasite does not have pyrimidine salvage pathways, the *de novo* pyrimidine biosynthesis enzymes offer potential as targets for drug design. This pathway involves six sequential enzymes, carbamoyl phosphate synthase, aspartate trans-carbamylase, dihydroorotase, dihydroorotate dehydrogenase, orotate phosphoribosyltransferase and orotidine 5′- phosphate decarboxylase that catalyze the conversion of several precursors to uridine 5′-monophosphate [79–81]. Dihydroorotate dehydrogenase is a flavin-dependent mitochondrial enzyme that catalyzes the fourth reaction in this essential pathway and it has been shown as a promising new target for chemotherapeutic intervention [82,83].

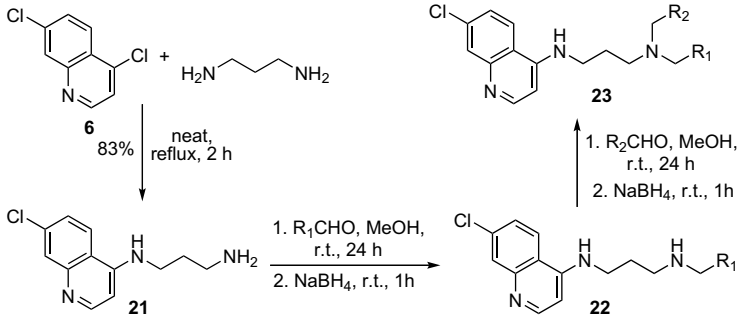
P. falciparum contains a novel, non-photosynthetic plastid organelle of prokaryotic origin called the apicoplast. It is derived from the engulfment of photosynthetic red algae and possesses biosynthetic processes found in plants as well as prokaryotes [82]. So, Bajsa et al. have recently found antiplasmodial activities in natural and synthetic compounds with established phytotoxic action [83]. Among these, semisynthetic derivatives of fusicoccin, a phytotoxin synthesized by the plant pathogenic fungus *Fusicoccum amygdale* were potent antiplasmodial compounds. The organelle has generated immense interest as a putative drug target for malaria and is believed to be the site for type II fatty acid biosynthesis [84], the non-mevalonate pathway of isoprenoid biosynthesis [85] as well as biosynthesis of heme-intermediates [86,87] within the parasite. There is an inherent difference between the fatty acid biosynthesis pathways of the parasite (type II) and the human host (type I), thus making them a promising target for the development of antimalarials. So, triclosan has been shown to be antiparasitic by inhibition of Fab I (enoyl-ACP reductase), one of the several enzymes involved in fatty acid synthesis [88].

Isoprenoid biosynthesis in *Plasmodium* is a mevalonic acid-independent process that occurs inside the apicoplast. Mammals and fungi both depend on mevalonate to generate the intermediate isopentyl diphosphate molecule, but *P. falciparum* and bacteria both utilize 1-deoxy-D-xylulose-5-phosphate as a precursor molecule. Differences between mammals and parasite make this pathway another potential target for antimalarial chemotherapy [85,89]. Phosphonic acid derivatives, such as Fosmidomycin, inhibit DOXP reductoisomerase, enzyme that is absent in humans. Another process related to isoprenoid biosynthesis is the farnesylation of proteins with protein farnesyl transferases (PFTs) as key enzymes. Since the work of Leonard [90], PFT inhibitors have been developed as antitumoral drugs [91]. Later, Nallan et al. have validated *P. falciparum* PFT as a target for the development of antimalarials and have reported tetrahydroquinoline analogues with antimalarial activity both in cultured parasites and in infected mice [92]. Eastman et al. have recently reviewed the progress in developing inhibitors of PFT (PFTIs) as antiparasitic agents [93]. This is a good example of the so-called "piggy back" approach, *i.e.* the investigation of drugs with some other known biological activities and suitable pharmacokinetic properties for the treatment of tropical diseases.

3. Design of novel aminoquinoline hybrids and their antiplasmodial activity

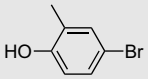
3.1. Starting quinolinic materials

Any design for bioactive molecules starts with the selection of materials prima, which could be commercially available, economic, and non-toxic. Preparation of two distinct structural classes of aminoquinoline antimalarials starts with the appropriated



Scheme 8.

Table 3
IC_{50} values of the aminoquinolines (**23**) against the 3D7, W2 and Dd2 *P. falciparum* strains.

Most active compound (**23**)			IC_{50} (nM) *P. falciparum* strains		
	R_1	R_2	3D7	W2	Dd2
a	Propyl		45	45	45
b	Propyl		50	70	45
c	Propyl		50	50	45
d	Benzyl		50	130	150
CQ			25	>250	>250

quinoline ring construction. The 8-amino-6-methoxyquinolines and 4-amino-7-chloroquinolines are easily obtained from respective key intermediates, which are prepared by condensation of the substituted aniline with appropriated cycling reagents, using distinct alkylation processes (Scheme 1).

The classical Skraup condensation that involves heating *o*-nitroanilines (**1**) with acrolein, generated *in situ* from glycerol and strong acid with requiring an oxidant, allows obtaining key 8-aminoquinoline (**3**) via hydrogenation process of 8-nitro derivative (**2**) (Scheme 2).

In spite of its generality, versatility and simplicity, this synthesis has considerable drawbacks such as harsh reaction conditions and highly acidic medium, which makes them tedious to isolate the product from the crude mixture. Preparation of the key intermediate, 4,7-dichloroquinoline (**6**) for the chloroquinoline series could be achieved through different variations of the Skraup method, in which 3-chloroaniline (**4**) is heated with appropriated condensation agent to form a stable imino/amino derivatives that undergoes cyclization after a high-temperature Friedel–Crafts acylation. Among them, Gould–Jacobs reaction uses ethoxymethylenemalonic ester [94–96], and was the first effective approach to this key quinoline [97]. Instead this condensation agent, various compounds can be utilized, *e.g.* diethyl oxaloacetate [98], methoxymethylene Meldrum's acid [99–101] and ethyl formylacetate [97]. All these agents give finally 7-chloro-4-hydroxyquinoline (**5**) that is treated with $POCl_3$ to afford 4,7-dichloroquinoline (**6**) (Scheme 3).

A disadvantage to all of these reactions is they are usually carried out at very high temperatures (250–300 °C) and are notoriously messy. Moreover, reactions of *meta*- or 3,4-disubstituted anilines normally give a mixture of regioisomers, which are also difficult to separate.

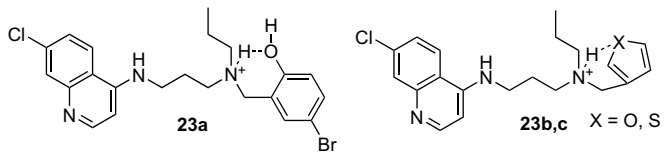


Fig. 5. The intramolecular hydrogen bonding model.

Amino side chains are attached to the 4-chloroquinoline ring by nucleophilic substitution S_NAr. This reaction is often carried out neat, or in the presence of phenol or *N*-methylpyrrolidinone, also at elevated temperatures (100–160 °C) [102,103], and in toxic solvents, *e.g.* MeCN. Isolating the desired product can be difficult since the reaction mixtures tend to solidify upon cooling and require an acid/base extraction to remove the excess phenol. Due to these synthetic problems, they are still needed to develop mild, regioselective and practical syntheses for these key heterocyclic intermediates and subsequent final products or their closer analogues. A mild and convenient alternative for the formation of the C–N bond in 4-aminoquinolines could be palladium-catalyzed amination methodologies [104,105]. Indeed, Beletskaya and coworkers reported the amination of 4-chloroquinoline derivatives under the influence of palladium catalysis employing either BINAP or DPPF derivatives as ligands [106]. Very recently, Margolis and coworkers also reported their investigations on the coupling reaction between an amine and a 4-halo(chloro or bromo)quinoline in the presence of $Pd(OAc)_2$/DPEphos/K_2CO_3/dioxane system at 85 °C [107].

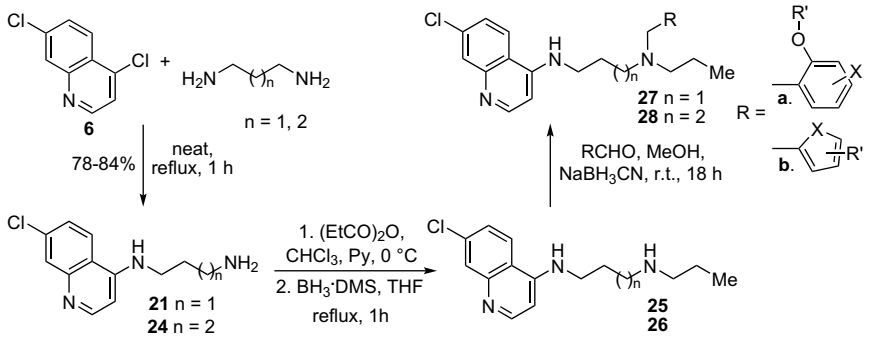
3.2. Design of novel 4-aminoquinoline hybrids

CQ has been mainstays of malarial chemotherapy for much of the past 40 years. The success of this drug was based on limited host toxicity, ease of use, with few effects, low cost and effective synthesis. However, the use of this drug has been seriously eroded in recent years, mainly as a result of the development of parasite resistance to CQ. Only 15 years after CQ were adopted, aminoquinoline monotherapies for *P. falciparum* malaria began to stop working simultaneously in Southeast Asia and in Brazil, and resistance spread rapidly. Global loss of CQ effectiveness has led to intense research on the mode(s) of action of this drug and the development of novel chemotherapeutic agents based on CQ structure that can overcome the parasite resistance mechanism that are important issues for medicinal and organic chemists. Two actual drugs more used against malaria, CQ and AQ are important for target-based antimalarial drug discovery. Both are 4-aminoquinoline derivatives, easily prepared from the 4,7-dichloroquinoline via S_NAr reactions.

As history has already shown, development of antiplasmodial agents aimed at a single parasite target or specialized process has failed to stem the tide of drug resistance. In this sense, double-drug development and/or multi-therapeutic strategies, which utilize heterocyclic skeleton of these two drugs, are valid and perspective [108–110]. These strategies have the potential to overcome this mechanism. So, the design and synthesis of quinoline-containing dual inhibitors or "double drugs" that would potentially inhibit hemozoin formation and another target within *P. falciparum*, and will not be recognized by the proteins involved in drug efflux, are very productive in the generation of new chemical entities that are effective against drug resistant parasites in the long term.

3.2.1. Design of novel CQ hybrids

Using rationale that the bulky bis-quinoline structure may be less efficiently extruded by CQ-resistant *P. falciparum*, this strategy started with the study of *N*,*N*′-bis(7-chloroquinolin-4-yl)(hetero) alkanediamines (**7**) [111–113] and *N*,*N*′-bis[4-((4-diethylamino)-1-methylbutyl)aminoquinolin-8(6)-yl] amides (**8**) [114], which resulted active against both CQ-sensitive and CQ-resistant parasites with similar efficacy, unfortunately, in a general manner, these compounds are known DNA intercalators and often cytotoxic. For this reason the most promising molecule, Ro 47-7737 (**9**) [115] (Fig. 4) has been suspended.



Scheme 9.

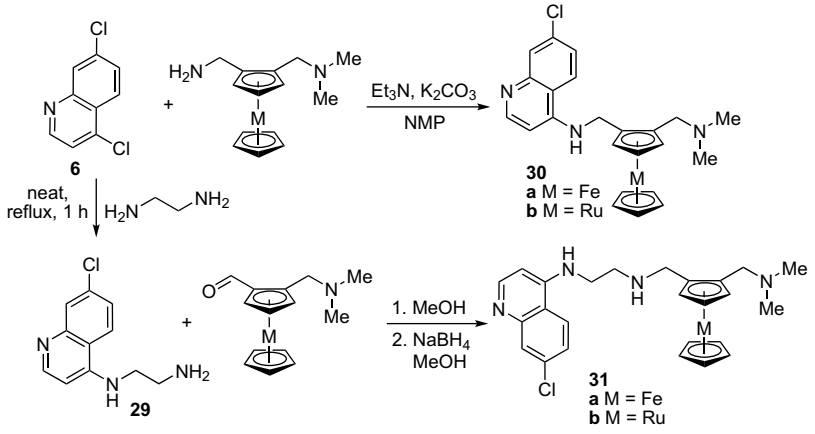
Nevertheless, interest in antimalarial bis-quinoline derivatives continues [116–119]. Results of these investigations on bis-aminoquinoline derivatives with linear or cyclic amino linkers suggested replacing the second quinoline ring by various aromatic and alkyl groups to decrease the cytotoxicity generally reported in bis-quinolines. Thus, new series of monoquinolines (**11**–**13**) consisting of a 1,4-bis(3-aminopropyl)piperazine linker and a large variety of terminal groups were synthesized from intermediate (**10**) that was obtained by condensation of 4,7-dichloroquinoline and bis(3-aminopropyl)piperazine in 1-pentanol without base [117,118] (Scheme 4).

The study of these three series of aminoquinoline hybrids showed that first in bis-quinoline series, the second quinoline ring can be successfully replaced by various aromatic or alkyl groups in terms of activity on CQ-resistant strains (FcB1) and second, a large number of substitutions lead to compounds of reduced cytotoxicity when compared to analogous bis-quinoline [119]. Among compounds synthesized in this study, eleven compounds exhibited a selectivity index superior (ratio CD_{50}/IC_{50} activity) to that of CQ and may be considered as potent agents for therapy. Aliphatic amines (**13a–h**) in particular reveal good activities and greater selectivity indexes than CQ (Table 1), leading to *in vivo* active compounds such as hybrid (**13b**, R = cyclopropyl), a potential therapeutic candidate.

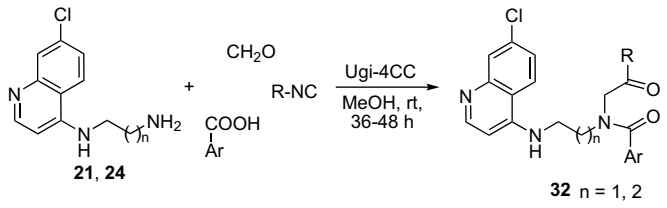
Inspired by this study, Link and co-workers have synthesized a new series of 29 quinoline amides using the parallel acylation reaction of *N*-{3-[4-(3-aminopropyl)pipeazin-1-yl]}-7-chloroquinoline-4-amine (**10**) with polymer-bound carboxylic acids (**14**) in mild condition reaction (THF or CH_2Cl_2, room temperature, 16 h) that opens straightforward access to new monoaminoquinoline derivatives (**15**) (type **11**) [120] (Scheme 5).

Polymer-bound carboxylic acids (**14**) were prepared by condensation of 2,3,5,6-tetrafluoro-4-hydroxybenzoic acid with aminomethylated polystyrene using *N,N′*-diisopropylcarbodiimide (DIC) as activating agent and subsequent by immobilization with commercially available carboxylic acids. Most of the obtained AQ-13 analogues showed activity *in vitro* against both strains (NF54 and K1) in the lower nanomolar range, four compounds (**15**) (R = 4-Et-phenyl, 4-EtO-phenyl, 4-CF_3-phenyl and 4-CF_3O-phenyl) showed an at least fourfold increase in the ratio of inhibition of CQ resistant (K1) to sensitive (NF54) strains over CQ itself. These results suggest that this polymer-assisted solution-phase synthesis is a useful method to speed up structure–activity relationship studies on aminoquinolines toward improved activity *in vitro* versus CQ-resistant strains of *P. falciparum*. Earlier, the design and synthesis of a library of 45 quinoline-based inhibitors of PfA-M1 (neutral zinc aminopeptidase of *P. falciparum*) were reported, indicating that the best inhibitor displayed an IC_{50} of 854 nM [121].

CQ and some aminoquinoline-based drugs including bis-quinoline derivatives with good antimalarial activity inhibit the crystallization of the heme to form hemozoin (and its synthetic equivalent β-hematin). They accumulate at high concentrations in the parasite's acid food vacuole, which is considered their site of action. It is generally admitted that a stronger basicity of the molecule increases the antimalarial activity due to a better uptake in the vacuole owing to the pH gradient between the cytosol and the acidic vacuole. However, *in vitro* activity of these 4-aminoquinoline analogues with altered chain length is less active than CQ in the *in vivo* model that was attributed to rapid N-terminal dealkylation in the biological milieu to metabolites cross-resistant with CQ [122,123]. So, the length and nature of the basic side chain of 4-aminoquinoline



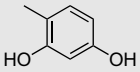
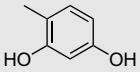
Scheme 10.



Scheme 11.

antimalarials are currently believed to be the primary modulator of activity against CQ-resistant parasite strains and it was assumed that minor modifications of this chain with retained heterocyclic heme binding motif can lead compounds with a dramatically altered resistance profile *in vitro* and *in vivo*. The lipophilicity and nature of these moieties may be important. The increased lipophilicity may aid diffuse freely and rapidly across the biological membrane and lead to a greater affinity for hematin. Thus, there were many studies on new series of CQ-analogues with a shorten side chain and diverse functionalities on the alkylamine side to develop a structure–activity relationship during these years [124–126].

Table 4
Antimalarial activity (IC_{50} μM) against three strains and resistance factor of compound (**32**).

Compound (**32**)				IC_{50} (μM)			Resistance factor	Resistance factor
	n	R	Ar	D10	K1	W2	(W2/D10)	(K1/D10)
a	1	*tert*-Bu		1.064	1.105	0.991	1.04	0.93
b	1	*tert*-Bu		0.712	1.133	1.190	1.60	1.67
c	1	*tert*-Bu		5.655	5.786	4.145	1.02	0.73
d	1	c-Hex		0.540	0.531	0.619	0.98	1.15
e	1	c-Hex		0.793	1.350	>10	nd	nd
f	2	c-Hex		1.672	3.826	5.936	2.29	3.55
g	2	*tert*-Bu		>20	18.15	3.806	nd	nd
h	2	*tert*-Bu		1.379	2.294	1.652	1.66	1.20
i	2	c-Hex		0.237	0.073	1.566	0.31	6.60
k	2	c-Hex		0.242	0.521	0.877	2.15	3.62
CQ				0.037	0.568	0.24	15.5	6.49

Antimalarial activity (IC_{50} μM) against three strains and resistance factor of compound (**32**).

First, a series of short chain CQ-derivatives (**16**) have been synthesized in one step from readily available starting 4,7-dicholoroquinoline and primary diamines. The diethylamine function of CQ is replaced by shorter alkylamine groups (**16a**–**d**) containing secondary or tertiary terminal nitrogen atoms [124] (Scheme 6).

These derivatives inhibited the growth of both the CQ-sensitive (HB3) and the CQ-resistant (K1) *P. falciparum* parasites *in vitro* (Table 2) at concentrations in the nanomolar range. Replacement of the diethylamino function of CQ with a more metabolically inert basic side chain group, such as *tert*-butyl (**16a**), pyrrolidyl (**16b**) or piperidyl (**16c**) led to a substantial increase in antimalarial activity against the CQ-resistant strain. However, when the *tert*-butyl terminal group was replaced with a morpholino group (**16d**), a substantial reduction in drug activity was observed.

Katti and co-workers described the synthesis of new 4-aminoquinoline guanidine derivatives (**18**–**20**) starting with the primary 4-aminoquinolines (**17**) (Scheme 7). All the derivatives were found to form strong complex with hematin and inhibit the β-hematin formation *in vitro*. Four derivatives (**18d**, **19c**,**b** and **20d**) exhibited promising antimalarial activity against CQ-sensitive strain of NF-54 in *in vitro* and CQ-resistant N-67 strain of *Plasmodium yoelii in vivo* (Swiss mice at 30.0 mg/kg by intraperitoneal route) [126].

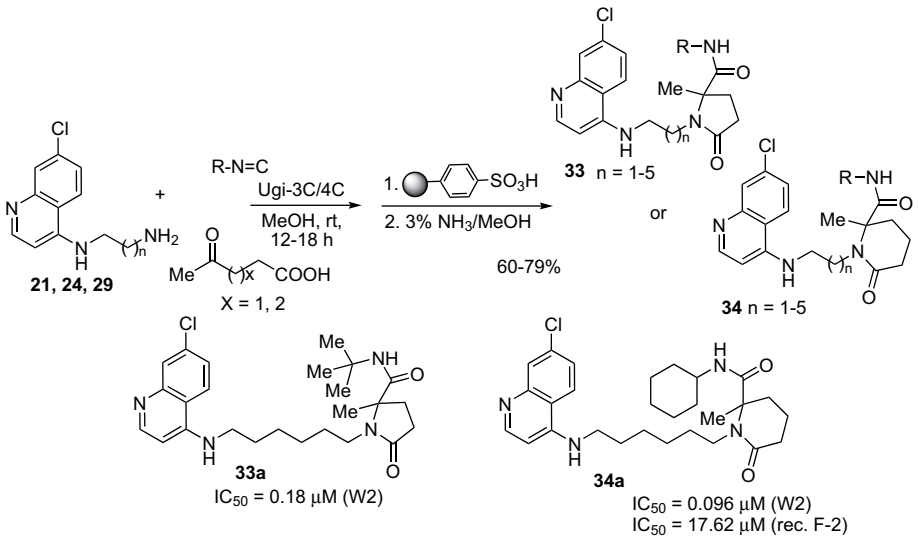
Guy and co-workers reported the development of a robust synthetic method that allows the introduction of two independent points of chemical diversity to the amine of side chain by sequential indirect reductive amination reactions to give final monoaminoquinolines (**23**) from primary amine intermediate (**21**) [125] (Scheme 8).

Biological testing of the compounds prepared in this study revealed that a number of previously unaddressed substitutions at the distal base of CQ afford active antimalarials. Four most active compounds (**23a**–**d**) from initial screen (*in vitro* cultures of *P. falciparum* strain 3D7 at two doses: 30 and 200 nM) were assayed against a panel of three *P. falciparum* strains with varying degrees of drug resistance. The used drug-resistant strains (W2 and Dd2) are known to be resistant to all known quinoline drugs. The IC_{50} values clearly showed the superior activity of compounds (**23a**–**d**) against both of the drug-resistant strains (Table 3).

Analyzing these results authors noted that all of the most active aminoquinolines against drug-resistant strains contain a hydrogen bond acceptor on the propyl substituent attached to the distal basic group, which will be protonated at physiological pH that allows the intramolecular hydrogen bonding between the protonated terminal amine (H-bond donor) and the oxygen (or sulphur) atom of another group (H-bond acceptor) (Fig. 5).

Based on these results, Guy and his team prepared a series of 116 aminoquinolines containing four different alkyl linkers and various aromatic substitutions with hydrogen bond accepting capability to prove this work hypothesis [127]. The synthesis of desired aminoquinolines (**27**, **28**) with linear chains of three and four methylene groups was achieved in a similar fashion obtaining the primary amine intermediates (**21**, **24**) to react with propionic anhydride affording the key secondary amine intermediates (**25**, **26**). The diversity-enhancing step of the library synthesis was the reductive amination of each of these two intermediates with a set of aldehydes that contain an aromatic ring with a hydrogen bond accepting functional group (Scheme 9).

These series showed broad potency against the drug-resistant W2 strain of *P. falciparum*, some of them with α-aminocresol motif



Scheme 12.

(compounds **27** and **28** with sustituent R_a) possess IC_{50} values more that 5 nM. It was noted that the compound **28** with four-carbon diaminoalkyl side chains were slightly higher active than the three-carbon linker equivalents. Such simple modifications, significantly altering the pK_a and sterics of the basic side chain in CQ-analogues, may prove to be part of a strategy for overcoming the problem of worldwide resistance to actual antimalarial drugs. However, it remains unknown that the mode of action for these compounds and more additional experiments are needed to prove the hypothesis of the intermolecular H-bonding between the protonated amine and the functional group in another radical attached to the terminal nitrogen.

From earlier and actual structure–activity relationship studies in CQ-analogues, active against CQ-resistant parasite strains, it seems that the resistance mechanism does not involve any change to the target of this class of drug, but rather involves a compound-specific resistance. As a result, it may be possible to develop new antimalarials based on the same mechanism of action as CQ and new synthesis and biological evaluation of aminoquinoline-like CQ are justified. Besides short chain CQ derivatives, several aminoquinoline metal complexes have been screened against resistant strains of *P. falciparum*. Of these complexes, ferroquine (**30a**) shows the greatest promise, and clinical trials are currently in progress with this candidate [128,129]. The presence of a bulk ferrocenyl moiety (hydrophobic group) in the alkyl side chain of CQ-analogues has a positive effect on the efficacy of these compounds in CQ-resistant strains of *P. falciparum*. The primary mechanism of ferroquine has been reported to be similar to that of chloroquine [130,131], in as far as binding to heme and preventing the formation of hemozoin are concerned. The final step synthesis of this drug and its ruthenoquine analogue (**30b**) is based on S_NAr reaction between 4,7-dichloroquinoline and the appropriate metallocene amine in 1-methyl pyrrolidinone (NMP). Similar metallocene-based CQ analogues (**31**) have been synthesized and evaluated [132–134] (Scheme 10).

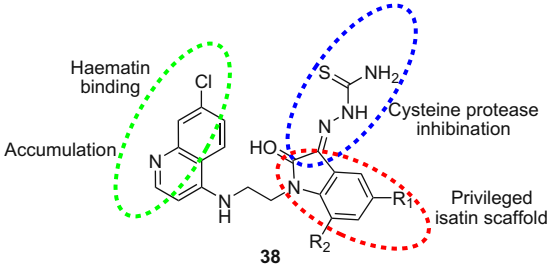


Fig. 6. A new class of 4-aminoquinoline-based isatin derivatives.

Synthesized ferrocene and ruthenocene analogues (**31**) exhibited high antiplasmodial activity against a resistant strain (K1) of the *P. falciparum*. This observation is consistent with the hypothesis that the mechanism for drug resistance in the *Plasmodium* parasites is compound specific. However, no significant difference in antimalarial activity was observed between the ferrocene and ruthenocene analogues.

Why is chlorine required almost always in the 7-position for antiplasmodial activity and what is the effect of replacing in with other groups? Egan and his team [135], and Krogstag and coworkers [96] gave a partial answer to these questions studying structure–activity relationships in 7-substituted (NH_2, NO_2, Me, CF_3, OH, OMe, F, Br and I) CQ-analogues. Based on experimental data on the pK_a values of these aminoquinolines, association constants with hematin, and β-hematin inhibitory activities and taking into account that a relationship between antiplasmodial activity and strength of β-hematin inhibition exists, Egan and coworkers found the linear dependence of the IC_{50}, corrected for pH trapping, on β-hematin inhibitory activity in this aminoquinoline family and included that the 7-group on the quinoline ring would determine the ability of the complex to resist incorporation of the hematin molecule into hemozoin, indicating at the two physicochemical parameters of C-7 functional group such as moderately strong electron-withdrawing capacity and strong lipophilicity. This combination of properties is best exhibited by the halogens chlorine, bromine, and iodine. However, authors noted that it cannot be generalized to aminoquinolines with other lateral chains, other groups attached to different positions on the quinoline ring, or to other aromatic nuclei. Such generalization would require that a deeper theoretical understanding of the interactions be attained using both computational and physical methods.

Nowadays, the development on hybridization approaches is going successfully due to various groups. Among them, Chibale and his team make considerably a contribution to problem of drug resistant. In 2004, it was reported the synthesis and antiplasmodial evaluation of a new class of Ugi adducts based on the 4-aminoquinoline antimalarial pharmacophore. The targeted α-acylamino amides (**32**) were obtained by using the Ugi-4CC reactions between

Table 5
Antimalarial activity (IC_{50} μM) against CQ-sensitive (D10), CQ-resistant (K1 and W2) strains and falcipain-2.

Compound (**38**)			IC_{50} (μM)			
	R_1	R_2	D10	K1	W2	FP-2
a	H	H	0.32	0.71	0.24	14.65
b	Me	H	0.079	0.10	0.051	11.64
c	Cl	H	0.095	0.054	nd	nd
CQ			0.033	0.312	0.240	nd

key primary aminoquinolines, alkyl isocyanides, formaldehyde and hetaryl carboxylic acids in methanol at room temperature in parallel array format [136] (Scheme 11).

The final products were tested against three culture-adapted isolates of *P. falciparum*: K1 and W2 (CQ-resistant), and D10 (CQ-sensitive). Designed new aminoquinoline-containing α-acylamino amides displayed moderate to good activity against CQ-sensitive and CQ-resistant strains of *P. falciparum*. Analyzing biological results, it could be noted that aminoquinoline based on isonicotinic acid with three-carbon linker (**32i**) showed very high activity in the D10 and K1 strains, however, was not active in the W2 (Table 4). Compound based on 2-pyrazinoic acid (**32h**) was the most active against all three strains. Both aminoquinolines have a cyclohexanyl (c-Hex) fragment in α-acylamino amide chain. Several compounds (**32a**, **c**, **d**) with resistance factor values close to 1 were as active in the CQ-sensitive strains as in CQ-resistant strains.

Another parallel synthesis of a new series 4-aminoquinoline γ- and δ-lactams (**33** and **34**) prepared through the Ugi-3C/4C reactions using aminoquinolines with varied carbon linkers, cetoacids (levulinic acid and 4-acetylbutyric acid), *tert*-butyl (or cyclohexanyl) isocyanides and acidic resin-bound *para*-toluene sulfonic acid – MP-TsOH (Scheme 12) was described by the same scientific team [137]. Antiplasmodial tests against CQ-resistant W2 strain of these series showed that aminoquinoline derivatives with the six-carbon spacer (**33a** and **34a**) were generally more efficacious. However, the general activities of these series were modest may be due to their reduced basicities. The most notable result of this study is that for derivatives (**34a**), which act as an inhibitor of falcipain-2, an important cysteine protease of parasites. This is the first report of a 4-aminoquinoline derivative inhibitor of falcipain-2.

A new class of 4-aminoquinoline-based isatin derivatives (**38**) was also designed on the basis of a multi-therapeutic strategy (Fig. 6), taking into consideration that (a) 4-aminoquinoline antimalarials accumulate in the parasite's acidic food vacuole and inhibit β-hematin formation, (b) being a privileged natural product scaffold, isatin unit can be easily functionalized with thiosemicarbazone moiety, which could inhibit *P. falciparum*-derived cysteine proteases [138]. Biological evaluation of target compounds against three strains of the malarial parasite *P. falciparum*, and against recombinant falcipain-2 demonstrated that this strategy worked well, because designed 4-aminoquinoline-based isatin compounds, specially derivatives (**38b**, **c**) showed good *in vitro* activity against K1 and W2 with IC_{50} values of 51 and 54 nM, respectively, while retaining potency against the D10 strain with IC_{50} values of 79 and 95 nM, respectively (Table 5).

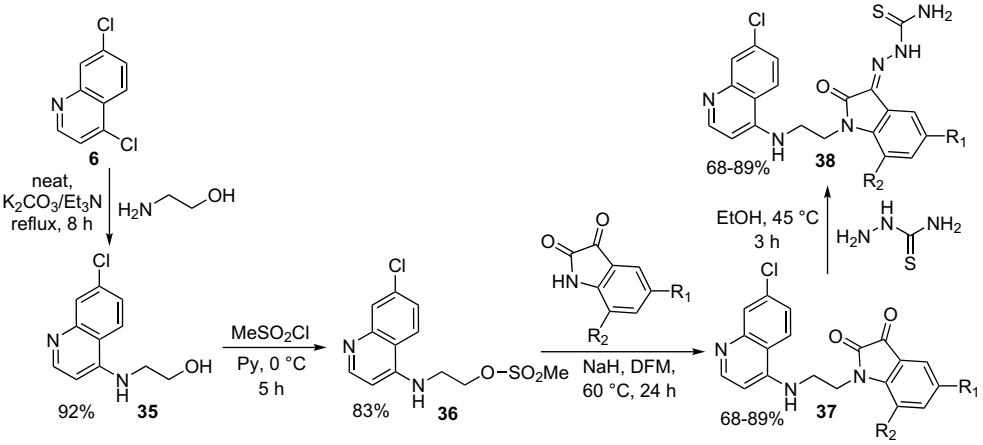
Noteworthy, the inhibitory activity (albeit modest) of these compounds against falcipain-2 indicated that it is important to refine understanding for the mode of action of and for further development of this class of 4-aminoquinolino-isatins based on a flexible ethelene linker and a thiosemicarbazone moiety warrant further investigation as potential antiplasmodial leads. Besides, its preparation from available starting materials through classical reaction of substitution seems easy and real (Scheme 13).

The same synthetic approach was realized to obtain another hybrid molecule (**41**) derived from CQ-skeleton and imipramine ring [139] (Scheme 14).

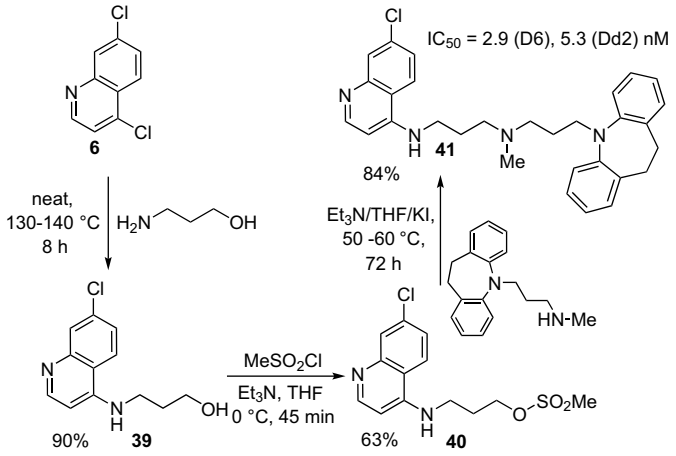
Its design is modelled on strong association with mutations in a parasite digestive vacuole membrane protein, called PfCRT, and on its inhibition by several molecules (reversal agents, *e.g.* imipramine, which is among the better-studied PfCRT reversal agents known [43,140–142]). Speculating that hybrids of CQ-like moiety linked to such reversal agents would provide good activity against CQ-resistant strains of *P. falciparum*, and testing the prepared hybrid (**41**), very effective at low-nM concentrations against CQ-sensitive (D6) and CQ-resistant (Dd2) strains, authors confirmed that this approach is valid and very promising. Moreover, a preliminary study in mice demonstrated oral efficacy against *Plasmodium chabaudi* without obvious toxicity.

Very recently, a set of different modified 4-aminoquinolines having a thiazolidin-4-one (**42**), [1,3]thaizinan-4-one (**43**) or 2,3-dihydrobenzo[*e*][1,3]thiazin-4-one (**44**) nucleus at the terminal side chain amino group was easily prepared via a three component condensation (DCC(dicyclohexylcarboiimide)/THF, rt or PhMe/reflux) between aminoquinolines with varied carbon linkers, chlorine-substituted benzaldehydes and appropriated mercapto acids [143] (Scheme 15).

The *in vitro* activity data (IC_{50} against NF-54 strains of *P. falciparum*) showed that these derivatives, having a nonbasic nitrogen atom at the side chain, showed remarkable antimalarial activity. This suggests that the modification at the lateral side chain nitrogen atom is very well tolerated for antimalarial activity. Moreover, these data indicated that two carbon (or three-carbon) atoms in the side



Scheme 13.



Scheme 14.

chain are best (or appropriate) for the antimalarial activity, suggesting that the length of the side chain is also crucial for the activity of these compounds.

Active 4-aminoquinoline derivatives (**42b**, **43a**, and **44a**, **b**) were selected for *in vivo* activity in Swiss mice against *Plasmodium. yoeli* (N-67 strain). These compounds showed significant activity against *P. yoeli* infections in mice. Compounds (**42b**) and (**43a**) suppressed 76.08 and 81.00% parasitaemia on day four compared to 100% suppression displayed by CQ (Table 6).

The present findings are important for the designing based on CQ-hybrid approach of new antimalarial agents, taking into consideration that basicity of the side chain nitrogen is not very essential for antiplasmodial activity. This approach was also utilized in the development of novel 4-aminoquinolines conjugated with quinolizidinyl or quinolizidinylalkyl alkaloid skeletons (**45** and **46**) and a trioxane motif (molecule **47**) (Fig. 7). The quinolizidine molecules that is directly linked to the 4-aminoquinoline moiety (**45**) or through a chain of 1–3 carbon atoms (**46**) exhibited a high degree of activity on the CQ-sensitive strain (D10) (IC_{50} = 24.2–102.4 nM), some of them also exhibited a strong activity against the CQ-resistant strain (W2) with IC_{50} values as low as 21–24 nM. Moreover, these novel CQ-analogues were either equitoxic or less toxic than CQ on murine cells WEHI (clone 13) [144]. It was noted that this activity was negatively influenced by the increasing number (n) of methylene groups between the 4-aminoquinoline

Table 6
Antimalarial activity (IC_{50} and MIC μM) *in vitro* and *in vivo*.

More active tested compounds	*n*	R	IC_{50} (μM)	MIC (μM)	[(C-T)/C] × 100% on day 4[a]	Mean survival time[b]
42b	1	2,6-DiCl	0.039 ± 0.01	0.55	76.08	14.6 ± 1.4
43a	1	4-Cl	0.013 ± 0.01	0.26	81.00	15.2 ± 1.7
44a	2	4-Cl	0.138 ± 0.02	0.27	40.08	10.6 ± 0.7
44b	2	2,6-DiCl	0.014 ± 0.01	0.28	23.50	13.8 ± 0.9
CQ			0.106 ± 0.01	0.39	99.9	20.0 ± 1.5
Control						10.1 ± 0.7

[a] % Suppression, where C is parasitaemia in control group and T is parasitaemia in treated group.
[b] Calculated for the mice that died during the 28 day observation period, and the mice that survived beyond 28 day were excluded.

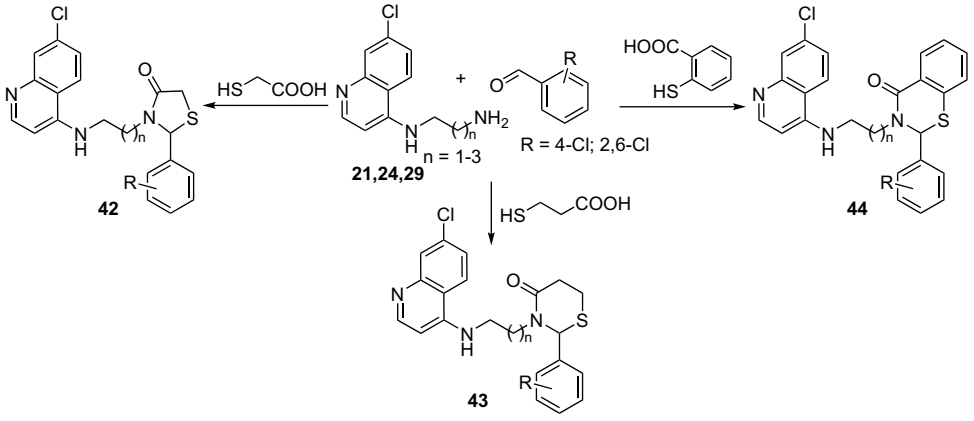
ring and quinolizidine skeleton, which is bulky, strongly basic, and lipophilic byclic moiety.

Agent DU-1302 (**47**) is a new product of combining the structural elements and pharmacological advantages of the artemisinin-like peroxides and the 4-aminoquinolines [110]. It was expected that the CQ-moiety would facilitate the transport to the food vacuole, where the trioxane would be activated by free Fe^{II}-heme liberated during the digestion of haemoglobin. Thus, DU-1302 resulted in highly efficient hybrid against CQ-resistant strain of *P. falciparum* [145], like a promising 4-aminoquinoline (**48**) with a three-carbon side chain.

3.2.2. Design of novel AQ hybrids

AQ, a 4-aminoquinoline Mannich base derivative, which was introduced into the field in the late 1950s, has been shown to be a superior alternative to CQ in areas of high CQ resistance. Unfortunately in the mid-1980s, the use of AQ declined abruptly following initial reports of agranulocytosis and hepatitis when the drug was used in prophylaxis. However detailed investigations have shown that AQ is no more toxic than CQ when used therapeutically to treat uncomplicated *P. falciparum* malaria [146,147]. So, the redesign of new Mannich base antimalarials based on AQ was started with a creation of amopyroquine (**49**, ApQ) and *N-tert*-butylamodiaquine (**50**, TbAQ), both structural analogues to AQ where the diethylamino function at *N*-arylamino moiety is replaced with a pyrrolidine group, or with *N-tert*-butylamino radical, respectively (Fig. 8).

They showed to be more active than both CQ and AQ against 11 CQ-resistant strains of *P. falciparum* isolates [148,149]. Subsequent research into the preparation of AQ-analogues containing side



Scheme 15.

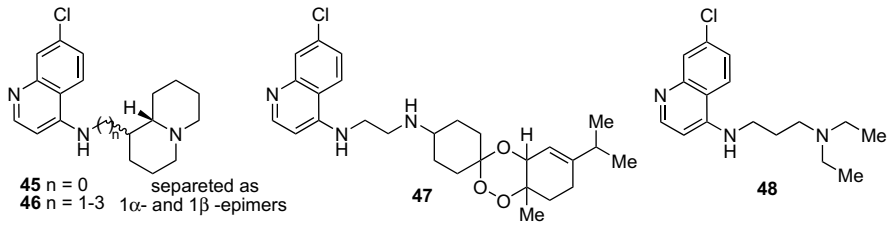


Fig. 7. Novel 4-aminoquinolines conjugated with quinolizidinylalkyl alkaloid skeletons and a trioxane motif.

chains which were less susceptible to metabolism afforded many diverse derivatives [150], one of which was tebuquine (**51**, TQ), a substituted 4-*N*-biphenylylaminoquinoline, exhibited greater antimalarial activity than AQ *in vivo* and *in vitro* [150,151]. Mannich base biaryl moieties of molecules are synthesized via a Suzuki–Miyaura cross-coupling reaction that allows molecular diversification [152]. All these AQ-based analogues contain the 4-hydroxyanilino moiety that was found to be sensitive to a cytochrome P_{450}-catalyzed oxidation reaction which generates a quinone–imine, responsible for binding to cellular proteins, and consequently, for toxic side-effects [153–155].

To avoid the quinone–imine metabolite formation (AQQI), the isomeric series of AQ-analogues (**53**), in which the 4′-hydroxy group on the aniline ring of AQ is interchanged with a 3′-Mannich base side chain [156]. Among them, isoquine (**52**, IsoQ) showed excellent oral *in vivo* ED_{50} activity and did not undergo *in vivo* bioactivation (Fig. 9).

The preparation of isoquine and its analogues (**53**) involves a two-step procedure from commercially available starting materials according to a method originally utilized by Burkhalter and coworkers [157] (Scheme 16).

Thus, starting with the commercially available 3-hydroxyacetanilide, the Mannich products were easily obtained in good yields. Then, a hydrolysis of the amide function of obtained products and coupling reaction corresponding between liberated 3-aminophenols with 4,7-dichloroquinoline key precursor afforded new AQ-analogues (**53**).

Realizing the same idea, other AQ-analogues, *e.g.* tbIsoAQ (**54**) or IsoTQ (**55**) (Fig. 10) were synthesized. First compound is now planned for clinical trials [158], the second is highly active against both CQ-sensitive (D6) and resistant (W2 and TM91C235) clones of *P. falciparum* with $IC_{50} = 0.3$ ng/mL.

However, this molecule showed only marginal antimalarial activity in the Thompson test against *Plasmodium berghei* by oral administration. Moreover, the last stage of its synthesis from *p*-chloro-biarylaminophenol is not selective that is needed an additional careful separation and purification processes [159].

Thus, another synthetic approach and biological strategy are needed. Studying the precise influence of the 4′-hydroxyphenyl ring of AQ, diverse deoxo-AQ-analogues (**58**) were prepared through condensation of 4,7-dichloroquinoline with 3,5-diaminobenzyl alcohol dihydrochloride, according to an S_N2 mechanism, in the presence of *N*-methyl morpholine (NMM) as base and in an EtOH/$CHCl_3$ (55:5) mixture and subsequent amidation reaction to give an amide (**56**) as the first steps. Then, the alcohol group was oxidised (MnO_2/CH_2Cl_2) to the corresponding aldehyde (**57**), presenting a terminal 4-methyl piperidine. The last step of deoxo-AQ-analogues synthesis was a reductive amination between the aldehyde (**57**) and the appropriated amines, using $NaHB(OAc)_3$ [160] (Scheme 17).

Biological results of these series showed that several derivatives (**56**) and (**58**) were more efficient *in vitro* against FcB1R or THAI and K1 strains than AQ (Table 7, all obtained compounds not showed). Compounds (**58a**, **b**) resulted active *in vivo* in the murine *P. berghei* model. Noteworthy, at 40 mg/kg, the morpholino amine compound (58b) completely cured the mice from P. berghei infection and was avoid of toxicity upon-mouse macrophages at 8 μM.

Related 4-anilinoquinolines bearing other amino groups in aryl moiety of AQ were prepared recently, using analogous synthetic strategy [161,162]. These structural modifications demonstrated a decrease of the cytotoxicity and an increase in selectivity index that justifies further synthetic works.

Another approach to the generation of active, no-toxic 4-anilinoquinoline antimalarials was developed by Sergheraert and colleagues [163]. They rationalised that if the tripeptide glutathione (GSH), which is known to guard *P. falciparum* from oxidative damage may have an additional protective role by promoting heme catabolism and its elevation content in parasites leads to increased resistance to CQ, it could be possible to control high intracellular GSH levels by glutathione reductase inhibitors, consequently, to

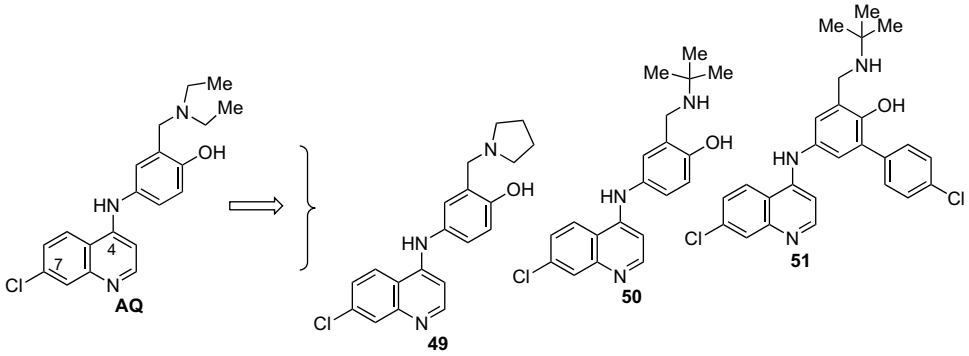


Fig. 8. New structural analogues to AQ.

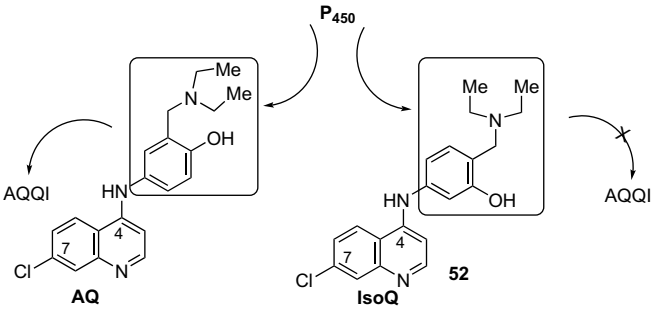


Fig. 9. Bioactivation of AQ and isoQ.

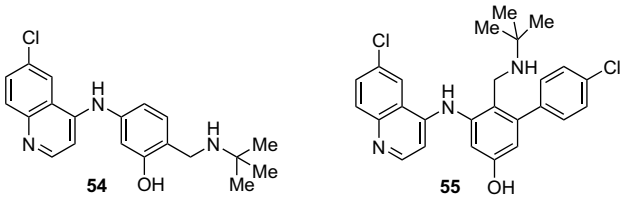


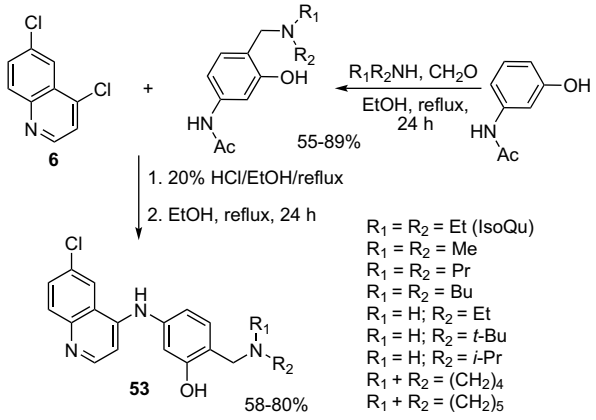
Fig. 10. Structural representation of tbIsoAQ and IsoTQ.

restore the sensitivity to CQ and its 4-aminoquinoline analogues. So, novel molecules based on AQ(CQ)-structures and 1,4-naphthoquinone ring were designed and constructed keeping in mind that these molecules would be easily hydrolyzed releasing two molecular parts, each would find their site(s) of action. Synthesis of target compounds (**60**) that were called double-headed prodrugs was achieved through an esterification of naphtoquinolyl alkanoic acids (**59**) with the alcohol AQ derivative (**56**) in the presence of dicyclohexylcarboiimide (DCC) and 4-(dimethylamino)pyridine (DMAP) (Scheme 18).

Compounds (**60**) displayed potent antimalarial affects *in vitro* in nanomolar range against CQ-resistant parasite strain FcB1R (ED_{50} = 23–56 nM) and % cytotoxicity upon hMRC-5 cells at 25 and 35 μM. It was found that the ether (**60c**) affected on total glutathione content of the parasites. This ether was chosen for their antiplasmodial study *in vivo* that demonstrated a 178% excess mean survival time for the animals treated with 40 mg/kg compound (**60c**) for 4 days. This double-drug model that contain a glutathione reductase inhibitor part and an AQ-moiety affecting heme metabolism appears to be a promising model for the developments of new antimalarial AQ hybrids.

Novel *N*-Mannich base-type AQ-derivatives were also synthesized by reaction of tertiary *N*-chloromethylamides and sodium salt of AQ. These compounds displayed antiplasmodial activity against the multi-drug resistant *P. falciparum* strain Dd2 (IC_{50} values 15–31 nM) and demonstrated no significant loss in activity compared to AQ (IC_{50} 30 nM) [164].

Recently, combining essential elements of CQ and AQ, Chibale and his team reported synthesis of novel hybrids of phenolic Mannich bases (**62**, **63**) linked to CQ-fragment [165]. These novel hybrids were prepared in three or four steps from the key 4,7-dicloroquinoline using the Mannich condensation reactions (Scheme 19).



Scheme 16.

These 4-aminoquinoline hybrids exhibited significant antiplasmodial activity against CQ-resistant strain (W2) of *P. falciparum* and showed inhibitory activity against cysteine protease falcipain-2. Compounds (**63**) were active against both falcipain-2 and cultured parasites. However, there was generally no correlation between the ability of these hybrids to inhibit falcipain-2 and their antimalarial activity against W2 *in vitro*. The most potent 4-aminoquinoline against the W2 strain was the hybrid (**63f**) with an IC_{50} value of 0.077 μM, being 3 times more active than CQ, but at the same time, this hybrid weakly inhibited cysteine protease falcipain-2 (Table 8).

3.3. Design of novel 8-aminoquinoline hybrids

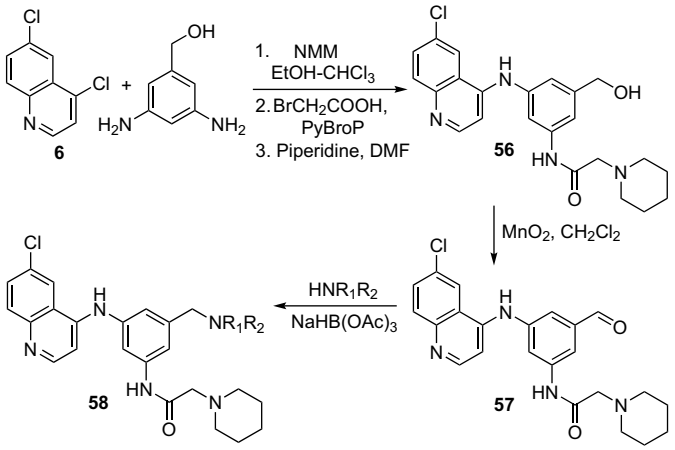
The development of new antimalarial agents based on 8-aminoquinoline skeleton is scarce compared to successful search of novel 4-aminoquinoline molecules with potent and selective antiplasmodial activity. This situation may be associated with the fact that the mode of action of the 8-aminoquinolines is largely unknown.

3.3.1. Design of novel PrimQ hybrids

Although quite toxic, primaquine, PrimQ is an important curative and causal prophylactic antimalarial agent, introduced in the 1940s. It is the only available transmission-blocking antimalarial displaying a marked activity against gametocytes of all malarial parasites, but it is inactive against asexual blood stages. PrimQ causes haemolytic anemia in patients with glucose-6-phosphate dehydrogenase deficiency [166]. Moreover, a rapid metabolism to inactive and toxic metabolites limits PrimQ's usefulness.

Since resistance to the 8-aminoquinolines does not appear to be a problem, numerous efforts to create new PrimQ-analogues with improved potential prophylactic action and/or less toxic [167,168]. Now two promising antimalarial agents, 2-*tert*-butylprimaquine (**64**) and tafenoquine (**65**), are available. They are new improved derivatives of primaquine (Fig. 11). Recently, it was found that the placement of a bulky metabolically stable *tert*-butyl group at the C-2 position of the PrimQ ring resulted in tremendous improvement in the blood-schizontocidal antimalarial activity. 2-*tert*-Butylprimaquine (**64**) exhibits potent *in vivo* blood-schizontocidal antimalarial activities against both drug-sensitive strain (*P. berghei*) and multi-drug resistant strain (*P. yoelii nigeriensis*) [169,170]. Moreover, this 8-aminoquinoline completely devoid of methemoglobin toxicity is associated with PrimQ. Optimization of the structure of PrimQ led to tafenoquine (**65**), which is generally less toxic and has a longer plasma half-life of 2–3 weeks. This compound is also active against erythrocytic stages; CQ- and multiresistant strains are significantly more susceptible than wild-types [171].

Constant research on structural modification of the PrimQ allowed developing these PrimQ-analogues, which have blood-schizontocidal antimalarial activities, and stimulated further



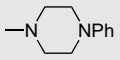
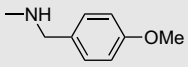
Scheme 17.

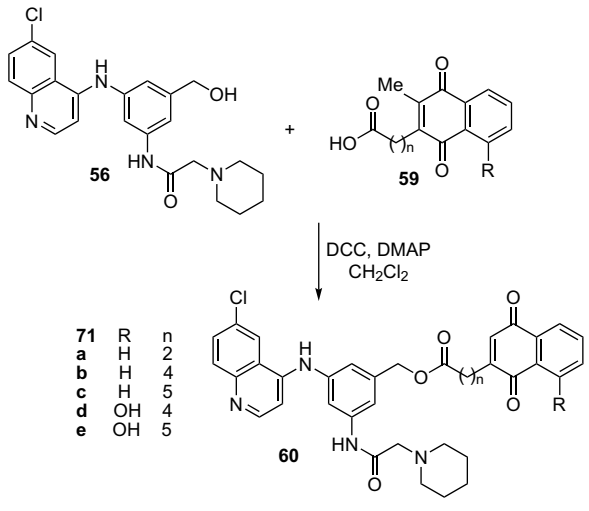
investigations on the PrimQ-analogues. 4-Ethyl-5-pentyloxyprimaquine (**66**) was synthesized to optimize substitution at the C-4 and C-5 positions of PrimQ, known sites of transformation to inactive/toxic metabolites. It was suppressive at 5 mg/kg, *in vivo* against *P. berghei* [172].

Biological evaluation of new 8-aminoquinoline derivatives (**70**) related to previously synthesized compounds (**64**–**66**) did not show promising cytotoxicities against CQ-sensitive and CQ-resistant *P. falciparum* strains, only compound (**70a**) exhibited curative antimalarial activity at a dose of 25 mg/kg/day × 4 in a *P. berghei* infected mice model [173]. These substituted quinoline derivatives were synthesized in eight steps from 4,5-dimethoxy-2-aniline, where the Skraup synthesis and a silver catalyzed radical oxidative decarboxylation reaction were essential key steps to get the 4-alkyl-2-*tert*-butyl-5,6-methoxy-8-nitroquinolines (**68**), which were transformed via four steps into 5-(3-trifluoromethylphenoxy)-8-aminoquinoline derivatives (**69**) in good yields. The latter compounds were then efficiently converted to final substituted 8-aminoquinolines (**70**) in additional two steps (Scheme 20).

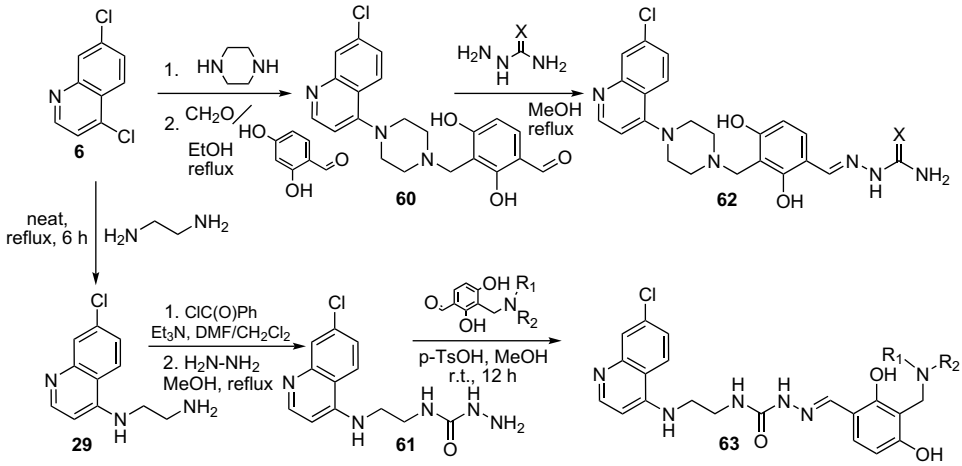
Important structural modifications of the PrimQ are those on the N^8-(4-aminoalkyl)amine group, because its rapid N-terminal deamination reaction in the biological milieu to metabolites, *e.g.* the carboxyprimaquine (**71**) (Fig. 12). So, to reduce this metabolic oxidative deamination pathway, as well as to reduce toxicity of this drug, several peptide and amino acid derivatives based (**72**) on PrimQ structure were prepared early [174–176]. However, it was found that these derivatives are rapidly hydrolyzed to PrimQ by aminopeptidases and endopeptidases. Nowadays, an effort was made to enhance their enzymatic stability using different approaches. For example, N^1-{4-[substituted 8-quinolylamino]-pentyl}-(2*S*/2*R*)-2-aminoamides (**73**) and N^1-[4-(substituted 8-quinolylamino)pentyl]-(2*S*/2*R*)-2-aminoamides (**74**) (Fig. 12) were prepared starting with 6-methoxy-8-nitroquinoline or 4-ethyl-6-methoxy-8-nitro-5-pentyloxyquinoline by the same mode (see, Scheme 20) [177]. Several of the reported analogues have exhibited potent *in vitro* activity against drug-sensitive (D6 strain) and drug resistant (W2 strain) malarial parasites. Quinolyl α-aminoamides (**73a**–**d**) have also displayed promising antimalarial activity *in vivo* in a *P. berghei*–mouse malaria model. In contrast, an analogous series of compound (**74**) resulted less active. The most potent compound (**73a**) was found curative at 25 mg/kg and suppressive at 10 mg/kg. Quinolyl α-aminoamides (**73a**–**d**) were also found to exhibit high selectivity index and significantly reduced

Table 7
Antimalarial activity (IC_{50}) *in vitro* of compounds (**56**) and (**58**).

Compound		IC_{50}, nM, strain		
	NR_1R_2	FcB1	THAI	K1
56	–	44.5 ± 9.7	89.6 ± 13.1	151.6 ± 48
58a		10.0 ± 3.8	6.7 ± 0.9	8.1 ± 0.5
58b		9.6 ± 3.8	12.8 ± 1.1	12.4 ± 1.1
58c		5.5 ± 0.6	nt	nt
58d		4.4 ± 0.1	nt	nt
AQ	–	7.4 ± 2.7	7.3 ± 0.2	14.5 ± 0.5



Scheme 18.



Scheme 19.

Table 8
Antimalarial activity (IC_{50}) against W2 strain of *P. falciparum* and falcipain-2 *in vitro*.

Comp.			IC_{50}, μM	
	NR_1R_2	X	W2	Falcipain-2
62a	–	S	3.75	2.25
62b	–	O	0.25	>20
63a	NEt_2	O	0.44	2.60
63b	Pyrrolidino	–	1.07	0.72
63c	Piperidino	–	0.27	0.63
63d	Morpholino	–	0.11	>20
63e	*N*-Me-piperazino	–	0.38	>20
63f	4-(7-Cl-Quinolinyl)-piperazino	–	0.077	3.16
CQ			0.24	–

methemoglobin toxicity, indicating their better safety profiles than PrimQ. Interestingly, many of these analogues exhibited promising *in vitro* antileishmanial activity against *Leishmania donovani* promastigotes.

Replacement of an amide bond with appropriate isosteres is a commonly used lead-optimization strategy for enhancing enzymatic stability of peptide. It was suggested that imidazolidin-4-ones could serve as these isosteres, because in contrast to peptides, the hydrolysis of imidazolidin-4-ones is not subjected to enzyme (aminopeptidases and endopeptidases) catalysis. Thus, the design of metabolically stable antiplasmodial 8-aminoquinolines containing a 4-imidazolidinone ring is another interesting approach. These molecular hybrids are easily prepared in three steps from PrimQ using standard peptide coupling methods that involved first reaction of N^{α}-Boc protected amino acid (BocAAOH) either DCC or diisopropylcarbodiimide (DIPCDI) in combination with the auxiliary nucleophile 1-hydroxybenzotriazole (HOBt) and hydrolysis reaction of obtained derivatives (**73**) as second step. Deprotection of these N^{α}-Boc protected amino acids based on PrimQ structure led to the amino acid derivatives (**76**) that reacted with acetone and diverse cyclic ketones to afford the final molecules (**77**), which were extracted with chloroform and isolated as yellow-orange waxy oils in 83–97% yields [178] (Scheme 21).

The synthesized double prodrugs (**77a–l**) exhibited potent gametocytocidal activity against *P. berghei*. It was found that those derivatives containing small amino acids (Gly and (S)-Ala) (**77a–c**) were superior to those containing bulky/hydrophobic side chains like (S)-Phe, (S)-Val, and (S)-Leu. Moreover, these hybrids were very stable both in chemical and in enzymatic conditions [179] (Table 9).

These findings indicate that adequate substitution at the C-2, C-4, and C-5 positions of the quinoline ring could be very useful leading to potent modified PrimQ-analogues with blood-schizontocidal activity and no significant blood toxicity, because the computational study [180] and the analysis of the reactivity [181] of these 8-aminoquinoline hybrids may be of advantage to the development of novel antimalarials.

Looking for selective antimalarials based on PrimQ structure that inhibit the aspartic proteases of malaria parasites

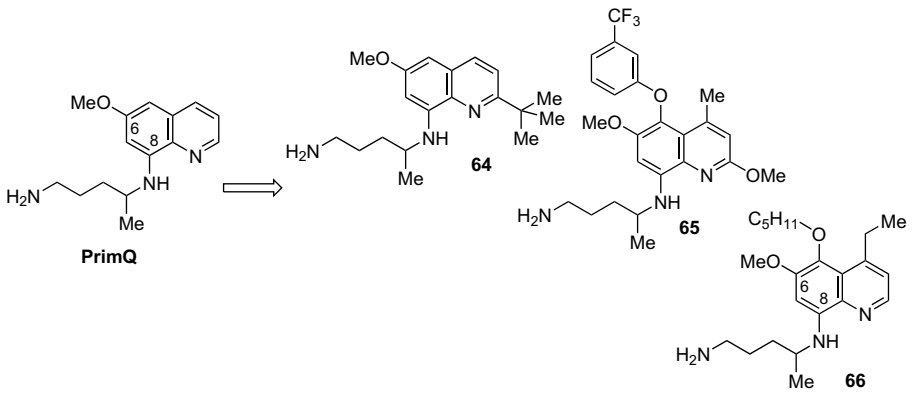
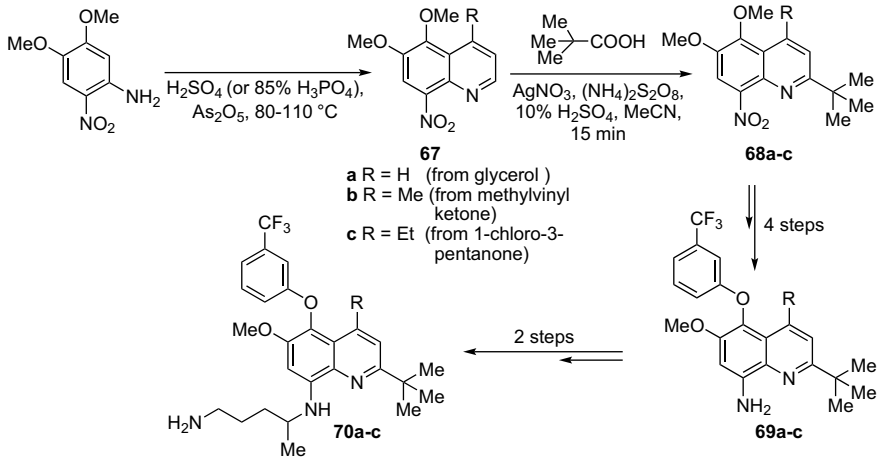


Fig. 11. Promising antimalarial agents based on 8-aminoquinoline ring.



Scheme 20.

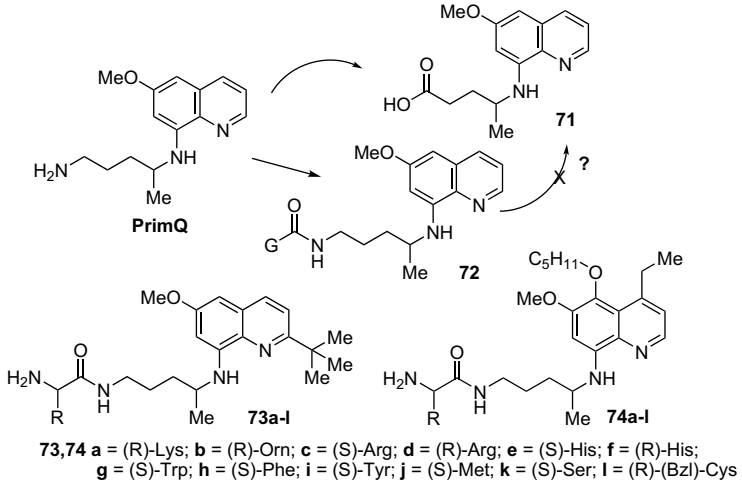
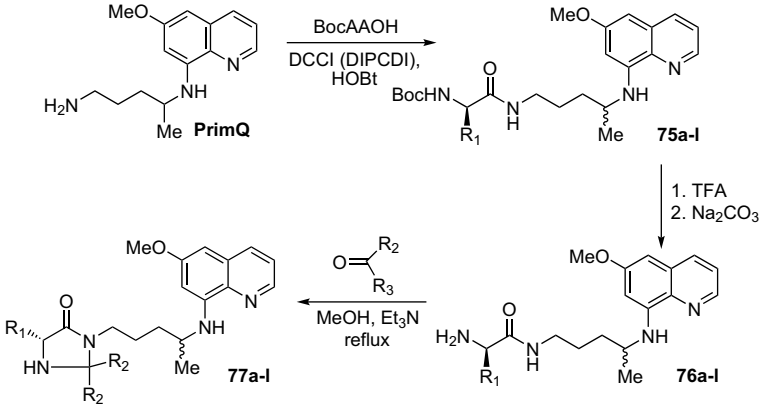


Fig. 12. New PrimQ derivatives with enzymatic stability.

(*e.g.* plasmepsin-2) is another justified approach. Thus, the synthesis of novel molecules and their inhibition of plasmepsin-2 and D10 and W2 strains of *P. falciparum* were reported by Romeo and co-workers [182]. Desired compounds (**80**) were modulated from the structures of statine-based inhibitor PS7777612 (**78**) and PrimQ derivative (**79**) (Fig. 13).

It was postulated that the linkage of the peptide derivative HLeuLysPrimQ to the Ile residue of PS7777612 would provide



Scheme 21.

Table 9
Physicochemical data and gametocytocidal activity of compound (**77**) *in vivo*.

Compound (**77**)				% Infected mosquitoes[a]	Mean no. of oocysts/mosquito (±SE)	% Hydrolysis to (**77**), after 3 days	
	R_1	R_2	R_3			80% Human plasma	pH 7.4 buffer ($t_{1/2}$ (d))
a	H	$(CH_2)_5$		25	7.4 (9.3)	Stable[b]	11 (18)
b	H	Me	Me	21	6.1 (3.5)	16	19 (9.8)
c	Me	$(CH_2)_5$		20	0.8 (1.1)	9	16 (12)
d	CH_2Ph	$(CH_2)_6$		90	78.9 (6.6)	Stable[b]	10 (20)
e	CH_2Ph	$(CH_2)_5$		83	71.0 (6.5)	Stable[b]	17 (11)
f	CH_2Ph	$(CH_2)_4$		65	51.6 (8.8)	3	28 (6.4)
g	CH_2Ph	Me	Me	76	61.2 (7.5)	Stable[b]	6 (31)
h	$CHMe_2$	$(CH_2)_6$		65	17.7 (6.5)	nd	nd
i	$CHMe_2$	$(CH_2)_5$		85	61.1 (6.7)	Stable[b]	16(12)
j	$CHMe_2$	$(CH_2)_4$		35	4.2 (3.3)	5	21 (8.8)
k	$CHMe_2$	Me	Me	25	5.0 (2.5)	Stable[b]	16 (12)
l	CH_2CHMe_2	Me	Me	30	13.7 (10.9)	Stable[b]	10 (20)
PrimQ				28	2.6 (2.0)	nd	nd

[a] At 10 μmol/kg.
[b] No degradation after 3 days of incubation.

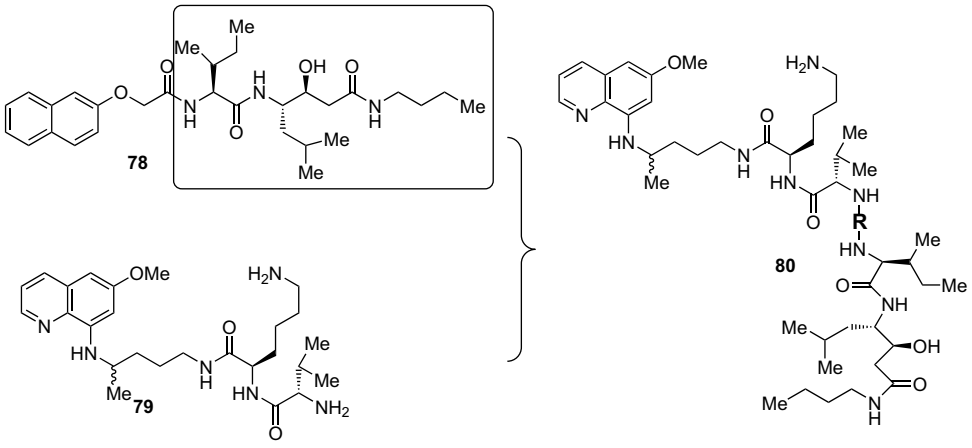
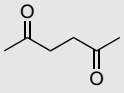
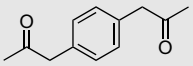
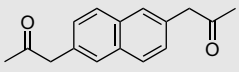
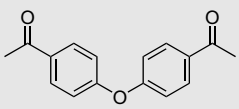
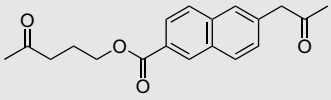


Fig. 13. Development of novel hybrids that inhibit the aspartic proteases of malaria parasites.

Table 10
IC_{50} activity against D10 and W2 strains of *P. falciparum* and plasmepsin-2 *in vitro*.

Hybrids (**80**)		$IC_{50} \pm SD$, μM		
	R	D10	W2	
a		20% at 9 μM	40% at 9 μM	396 ± 25
b		5.5 ± 1.8	4.2 ± 0.2	135 ± 4.4
c		6.2 ± 0.8	4.7 ± 0.3	1.51 ± 0.23
d		0.4 ± 0.1	0.7 ± 0.4	0.59 ± 0.02
e		3.3 ± 0.3	1.0 ± 0.5	9.6 ± 2.1

a potential double drug. Hybrid (**80c**) with a naphtyl linker was active in the enzyme assay, but the activity against D10 and W2 strains of *P. falciparum* was equal to one of the molecule (**80b**). Modified hybrid (**80d**) with 4,4′-oxybis(benzoic acid) linker resulted remarkably active in inhibiting both the enzyme and the parasite growth with IC_{50} values of 0.59 nM and 0.4 μM, respectively (Table 10). Biological tests *in vitro* demonstrated that this hypothesis is noteworthy and needs further investigations.

4. Conclusion

A short history of hybrid molecules based on aminoquinolines gave interesting and important information useful for organic and medicinal chemistry, which are deeply involved in design and development of new antimalarial agents. Nowadays, double-drug development and/or multi-therapeutic strategies, which utilize new chemical entities with two (or more than two) different heterocyclic skeletons (pharmacophores), are valid and perspective to create new antimalarial drugs. These strategies have the potential to overcome drug resistant parasites' problem. So, the design and synthesis of aminoquinoline-containing dual inhibitors or "double drugs" that would potentially inhibit hemozoin formation and another target within *P. falciparum*, and will not be recognized by the proteins involved in drug efflux, are very productive in the generation of new chemical entities that are effective against drug resistant parasites in the long term. The success of this hybridization approach, having a wonderful example of trioxaquines [110] or artemisinin–quinine hybrid [183], stimulates further organic, medicinal and biochemical activities to struggle malaria, the world's most widespread and devastating infectious disease. The hybridization approach will represent more and more a new challenge for medicinal chemists, pharmacologists and biochemists [184–190] because it benefits not only to drug-design efforts, but also to better understand drug resistant parasites' problem.

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