4-Aminoquinolines: Chloroquine, Amodiaquine and Next-Generation Analogues

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Abstract For several decades, the 4-aminoquinolines chloroquine (CQ) and amodiaquine (AQ) were considered the most important drugs for the control and eradication of malaria. The success of this class has been based on excellent clinical efficacy, limited host toxicity, ease of use and simple, cost-effective synthesis. Importantly, chloroquine therapy is affordable enough for use in the developing world. However, its value has seriously diminished since the emergence of widespread parasite resistance in every region where *P. falciparum* is prevalent. Recent medicinal chemistry campaigns have resulted in the development of short-chain chloroquine analogues (AQ-13), organometallic antimalarials (ferroquine) and the "fusion" antimalarial trioxaquine (SAR116242). Projects to reduce the toxicity of AQ have resulted in the development of metabolically stable AQ analogues (isoquine/*N-tert*-butyl isoquine). In addition to these developments, older 4-aminoquinolines such as piperaquine and the related aza-acridine derivative pyronaridine continue to be developed. It is the aim of this chapter to review 4-aminoquinoline structure–activity relationships and medicinal chemistry developments in the field and consider the future therapeutic value of CQ and AQ.

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1 History and Development

Quinine **1**, a member of the *cinchona* alkaloid family, is one of the oldest antimalarial agents and was first extracted from *cinchona* tree bark in the late 1600s. The *cinchona* species is native to the Andean region of South America, but when its therapeutic potential was realised, Dutch and British colonialists quickly established plantations in their south-east Asian colonies. These plantations were lost to the Japanese during World War II, stimulating research for synthetic analogues based on the quinine template, such as the 4-aminoquinoline chloroquine (CQ **2**, Fig. 1) [1].

A thorough historical review of CQ (in honour of chloroquine's 75th birthday) is available elsewhere [2]. In short, CQ was first synthesized in 1934 and became the most widely used antimalarial drug by the 1940s [3]. The success of this class has been based on excellent clinical efficacy, limited host toxicity, ease of use and simple, cost-effective synthesis. Importantly, CQ treatment has always been affordable – as little as USD 0.10 in Africa [4]. However, the value of quinoline-based antimalarials has been seriously eroded in recent years, mainly as a result of the development and spread of parasite resistance [5].

Although much of the current research effort is directed towards the identification of novel chemotherapeutic targets, we still do not fully understand the mode of action and the complete mechanism of resistance to the quinoline compounds, knowledge that would greatly assist the design of novel, potent and inexpensive alternative quinoline antimalarials. The search for novel quinoline-based antimalarials with pharmacological benefits superseding those provided by CQ has continued throughout the later part of the twentieth century and the early part of this century since the emergence of CQ resistance.

Comprehensive reviews on the pharmacology [6] and structure activity relationships [7] have been published previously, so will be only mentioned briefly. It is the aim of this chapter to review developments in the field that have led to the next-generation 4-aminoquinolines in the development "pipeline", in addition to discussion of the future therapeutic value of CQ and amodiaquine (AQ). We will begin with studies directed towards an understanding of the molecular mechanism of action of this important class of drug.

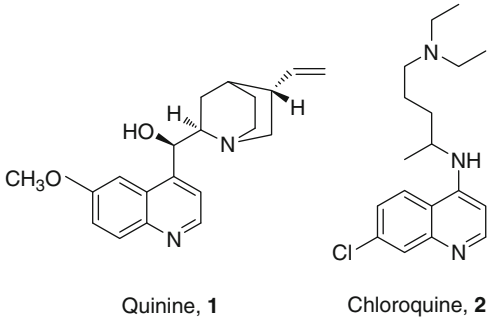


Fig. 1 Quinine **1** and related 4-aminoquinoline antimalarial chloroquine, **2**

2 Mode of Action of Quinoline Antimalarials

The precise modes of action of the quinoline antimalarials are still not completely understood, although various mechanisms have been proposed for the action of CQ and related compounds [8]. Some of the proposed mechanisms would require higher drug concentrations than those that can be achieved in vivo and, therefore, are not considered as convincing as other arguments [9]. Such mechanisms include the inhibition of protein synthesis [10], the inhibition of food vacuole phospholipases [11], the inhibition of aspartic proteinases [12] and the effects on DNA and RNA synthesis [13, 14].

CQ is active against the erythrocytic stages of malaria parasites but not against pre-erythrocytic or hypnozoite-stage parasites in the liver [15] or mature gametocytes. Since CQ acts exclusively against those stages of the intra-erythrocytic cycle during which the parasite is actively degrading haemoglobin, it was assumed that CQ somehow interferes with the parasite-feeding process. Although this is still a matter of some controversy, evidence of proposed mechanisms will be discussed in the following sections.

2.1 Haem–CQ Drug Complexes

To obtain essential amino acids for its growth and division, the parasite degrades haemoglobin within the host red blood cell. Digestion of its food source occurs in an acidic compartment known as the digestive vacuole (DV) (a lysosome-type structure, approximately pH 5). During feeding, the parasite generates the toxic and soluble molecule haem [ferriprotoporphyrin IX, FP Fe (II)] and biocrystallises it at, or within, the surface of lipids to form the major detoxification product haemozoin (Fig. 2) [16].

Slater et al. [17] demonstrated the ability of CQ to inhibit the in vitro FP detoxification in the high micro-molar range. The ability of CQ and a number of other quinoline antimalarial drugs to inhibit both spontaneous FP crystallisation and parasite extract catalysed crystallisation of FP has since been confirmed [18, 19].

Considerable evidence has been presented in recent years that antimalarial drugs such as CQ act by forming complexes with haem (FP Fe (II)) and/or the hydroxo- or aqua complex of haematin (ferriprotoporphyrin IX, Fe (III) FP), derived from parasite proteolysis of host haemoglobin [20–22] (Fig. 2), although the exact nature of these complexes is a matter of debate.

Dorn et al. [23, 24] confirmed that CQ forms a complex with the μ-oxo dimeric form of FP (haematin) with a stoichiometry of 1 CQ: 2 μ-oxo dimers. In other studies, CQ was found to bind to monomeric haem to form a highly toxic haem–CQ complex, which incorporates into the growing dimer chains and terminates the chain extension, blocking further sequestration of toxic haem and disrupting membrane function (Fig. 2) [25, 26].

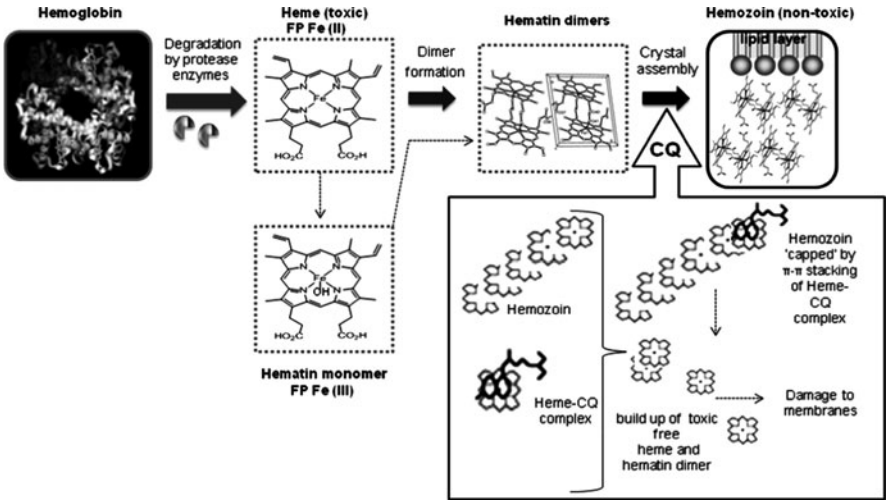


Fig. 2 Degradation of haemoglobin and detoxification mechanisms of the parasite and proposed target of CQ

2.2 Accumulation of CQ in the Acidic Food Vacuole

Due to the weak base properties of CQ and related analogues, their effectiveness has also been shown to be partly dependent upon drug accumulation in the acidic DV. A number of early studies have suggested that CQ accumulation can be explained by an ion-trapping or weak-base mechanism [27, 28]. CQ is a diprotic weak base ($pK_{a1} = 8.1$, $pK_{a2} = 10.2$) and in its unprotonated form, it diffuses through the membranes of the parasitised erythrocyte and accumulates in the acidic DV (pH 5–5.2) [27]. Once inside, the drug becomes protonated and, as a consequence, membrane impermeable and becomes trapped in the acidic compartment of the parasite (Fig. 3).

Various studies have suggested that the kinetics and saturability of CQ uptake are best explained by the involvement of a specific transporter [29, 30] or carrier-mediated mechanism for the uptake of CQ [31]. Another hypothesis by Chou et al. [32] suggests that free haematin (FP) in the DV might act as an intra-vacuolar receptor for CQ. Work by Bray et al. also strongly supports this hypothesis [33].

3 CQ Resistance Development

The first incidences of resistance to CQ were reported in 1957. The reasons for the emergence of resistance are multi-factorial: uncontrolled long-term treatment regimes, travel activity resulting in spread of resistant strains and frequent feeding of mosquitoes from several different hosts, to name but a few [34]. The mechanism by which resistance is acquired is discussed below.

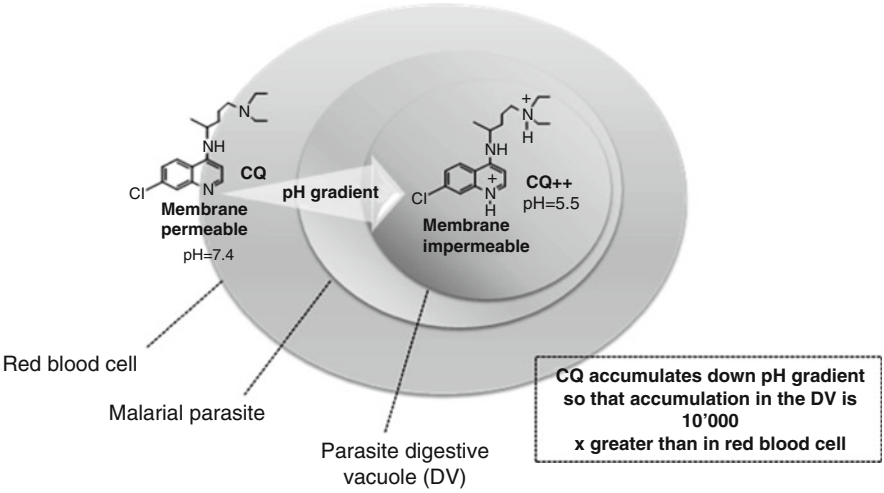


Fig. 3 Ion trapping; diffusion of CQ due to the pH gradient leads to increased concentration of CQ in the DV

3.1 Parasite-Resistance Mechanisms

It was soon proven that the concentration of CQ inside the DV was reduced in parasite-resistant strains. The powerful accumulation mechanism of CQ was therefore less effective, suggesting mutations in transporter proteins in these resistant strains. Resistant isolates also have reduced apparent affinity of CQ–FP binding in the DV, therefore CQ-resistant isolates have evolved a mechanism whereby the access of CQ to FP is reduced [35].

3.1.1 PfCRT

Another characteristic of CQ-resistant isolates is that their phenotype can be partially "reversed" by the calcium channel blocker verapamil so that the isolates become resensitised to CQ [35]. Verapamil was shown to act by increasing the access of CQ to the FP receptor and this effect is considered a phenotypic marker of CQ resistance. The characteristic effects of CQ resistance (reduced CQ sensitivity, reduced CQ uptake and the verapamil effect) have all been attributed to specific amino acid changes in an integral DV membrane protein, the *P. falciparum* chloroquine resistance transporter (PfCRT) [36, 37]. PfCRT mutated at amino acid 76 appears to be central to the chloroquine resistance phenotype. Mutant PfCRT seems to allow movement of drugs out of the DV; therefore blocking of PfCRT by verapamil restores sensitivity.

In brief, there are three proposed models for the resistance mechanism of PfCRT:

- *The partitioning model*: CQ was found to flow out of the DV of CQ-resistant strains much faster that CQ-sensitive strains, by a verapamil-blockable route [38]. Initially, this was attributed to changes in DV pH for CQ-sensitive and CQ-resistant strains. However, it was later shown that CQ-resistant parasites have a similar resting DV pH, and, therefore, must possess a CQ efflux mechanism in the DV membrane, increasing the permeability of a particular form of CQ [39].
- *The channel model*: In this model, mutated PfCRT acts as a channel, providing a leak pathway for the passive diffusion of protonated CQ, allowing it to flow freely from the DV [40, 41].
- *The carrier model*: In this alternate model, mutated PfCRT acts as a carrier, transporting protonated CQ by facilitated diffusion or active transport across the DV membrane [42, 43].

The issue of exactly how PfCRT confers this phenotype has been recently reviewed, although it remains a matter of debate [44].

3.1.2 PfMDR1

A multi-drug resistance homologue in *P. falciparum* (PfMDR1) has also been implicated in CQ resistance. PfMDR1 has been demonstrated to reside in the parasites' DV membrane with its ATP-binding domain facing the cytoplasm [45]. This suggests that PfMDR1 directs drug movement into the DV. Loss of this drug import capability could be advantageous to the parasite when the drug targets the DV. Irrespective of the specifics of MDR1-mediated chloroquine transport, the protein has been shown to contribute to chloroquine resistance. Sanchez et al. functionally expressed a number of different polymorphs of *pfmdr1* (the gene that codes for PfMDR1) in *Xenopus laevis* oocytes in order to characterize the transport properties of PfMDR1 and its interaction with antimalarial drugs. They demonstrated that PfMDR1 does indeed transport CQ and that polymorphisms within PfMDR1 affect the substrate specificity; wild-type PfMDR1 transports CQ, whereas polymorphic PfMDR1 variants from parasite lines associated with resistance apparently are not as efficient [46].

3.2 Recycling of CQ

CQ still remains the treatment of choice in a few geographical areas where it can still be relied upon, although guidelines now instruct the use of combination chemotherapy to slow the development of resistance to the partner drug [47]. In some resistance "hot spots", CQ was completely abandoned for a combination of sulfadoxine–pyrimethamine almost two decades ago. In such cases, there is evidence to suggest that CQ sensitivity can be restored [48]; 8 years after discontinuation of CQ in Malawi, the *pfcrt* T76 mutation [49] had disappeared from nearly

every isolate analysed. Similar observations have been made in Tanzania, South Africa, China and parts of Thailand [50]. These results have given some hope that "drug-cycling" may be an option for the future and CQ combinations may be used effectively again in disease-endemic areas where it was once abandoned [2]. However, the concern with this strategy is that re-selection of resistance mutants is likely to be very rapid.

Ursing et al. have reported that the failure rate of CQ treatment can be decreased by giving the drug twice per day rather than as a once daily treatment regimen [51–53]. Doubling the dosing frequency in this way achieved a high cure rate despite underlying CQ resistance and without any adverse side effects [51]. This increase in efficacy can be explained by the pharmacokinetics of CQ; the second daily dose of CQ acting to raise plasma concentrations to levels where they have activity against resistant parasites [54]. It has also been shown that the use of this type of treatment regimen can stabilize the spread of CQ resistance [53, 55]. One major drawback with this type of double-dose treatment regimen is the narrow therapeutic index for CQ and, in order for such treatment to be widely used, extensive safety re-evaluation would need to be performed in large populations to ensure safety at the population level.

4 Modifications to Improve CQ

CQ, **2** contains a 7-chloroquinoline-substituted ring system with a flexible pentadiamino side chain. The haem-binding template, 7-chloro- and terminal amino group are all important for antimalarial activity, as detailed in Fig. 4.

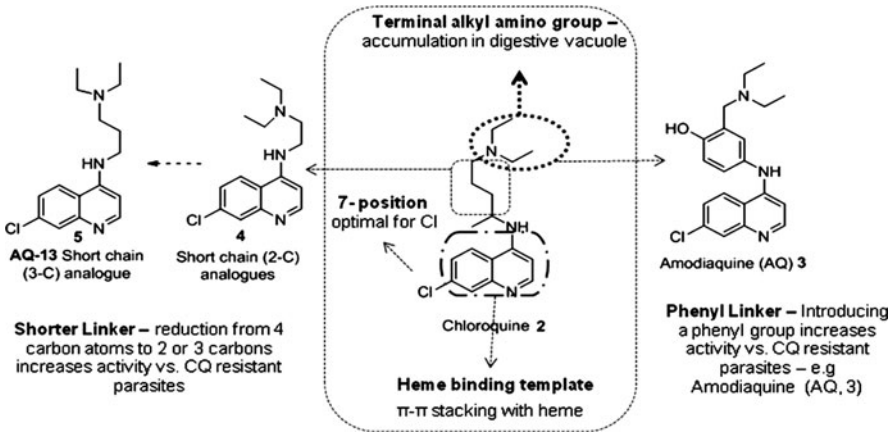


Fig. 4 Exploring the structure–activity relationship (SAR) of CQ: modifications shown led to the development of new analogues AQ (**3**), AQ-13 (**5**) and other short chain analogues (**4**) which have good activities against CQ-resistant strains

Since CQ's discovery, numerous attempts have been made to prepare a superior antimalarial quinolone-based drug. The following section briefly summarizes some of the more important recent advances in the field, with particular emphasis on 4-aminoquinolines that are in clinical and pre-clinical development. For a more in-depth discussion of 4-aminoquinoline analogue development over the last 10 years, Kaur et al. have recently published an extensive review [56].

4.1 Modifications to Overcome Resistance: Short-Chain Analogues

4.1.1 AQ-13

Studies on 4-aminoquinoline structure–activity relationships (SARs) have revealed that 2-carbon side-chain CQ analogues such as **4** retain activity against CQ-resistant *Plasmodium* parasites [57, 58]. Krogstad et al. have synthesized a series of analogues with varying diaminoalkane side chains at the 4-position [57]. Interestingly, compounds with diaminoalkyl side chains shorter than four carbon atoms or longer than seven carbon atoms were active against CQ-susceptible, CQ-resistant, and multi-drug-resistant strains of *P. falciparum* in vitro (IC_{50} values of 40–60 nM against the K1 multi-drug resistant strain) and exhibited no cross-resistance with CQ.

One of these analogues, AQ-13 **5**, a short-chain aminoquinoline antimalarial drug, underwent Phase I clinical trials. The mode of action is suggested to be the same as CQ but the presence of the short linker chain is believed to enable the molecule to circumvent the parasite-resistance mechanism (PfCRT), making **5** active against CQ-resistant parasites.

Preliminary pharmacokinetic studies indicate that AQ-13 has a similar profile to that of CQ [59] and the Phase I clinical trials were positive [60], concluding minimal difference in toxicity compared with CQ. However, since AQ-13 exhibited increased clearance compared with CQ, dose adjustment is required and an initial dose-finding Phase II (efficacy) study of AQ-13 in Mali is planned. Since clinical trials have shown that oral doses of 1,400 and 1,750 mg AQ-13 are as safe as equivalent oral doses of CQ and have similar pharmacokinetics, more recent trials were performed to determine if a 2,100 mg dose of AQ-13 (700 mg per day for 3 days) was safe to include as a third arm in Phase II studies in Mali and to investigate the effects of food (the standardised FDA fatty meal) on the bioavailability and pharmacokinetics of AQ-13. Based on the results, it is proposed to compare the 1,400, 1,700 and 2,100 mg doses of AQ-13 with each other and with Coartem in an initial dose-finding efficacy (Phase II) study of AQ-13 in Mali [61].

A possible drawback with these derivatives is the potential to undergo side-chain dealkylation (for short-chain CQ analogues such as **5** (AQ-13), deethylation is a particular problem in vivo) [62]. This metabolic transformation significantly

reduces the lipid solubility of the drug and significantly increases cross-resistance up to and beyond that seen with CQ [63].

4.1.2 Ferroquine: An Organometallic Antimalarial

Metal complexes have been used as drugs in a variety of diseases [64]. Incorporation of metal fragments into CQ has generally produced an enhancement of the efficacy of CQ with no acute toxicity. Three novel CQ complexes of transition metals (Rh, Ru, Au) have been synthesized (**6**, **7** and **8**, Fig. 5) [65, 66], with the Au–CQ complex **8** in particular, displaying high in vitro activity against the asexual blood-stage of two CQ-resistant *P. falciparum* strains.

Four new ferrocene-CQ analogues were developed by Biot and co-workers, where the carbon chain of CQ was replaced by the hydrophobic ferrocenyl group [67]. Some of the compounds showed potent antimalarial activity in vivo against *P. berghei* and were 22 times more potent against schizonts than CQ in vitro against a drug-resistant strain of *P. falciparum*. The same group reported two new ferrocene-CQ compounds in 1999, one of which (**9**) showed very promising antimalarial activity in vivo against *P. berghei* and in vitro against CQ-resistant strains of *P. falciparum* [68].

Now named ferroquine (SSR-97193, FQ), **9** is the first novel organometallic antimalarial drug candidate to enter clinical trials. A multi-factorial mechanism of action is proposed including the ability to target lipids, inhibit the formation of haemozoin and generate reactive oxygen species [69]. The ferrocene group alone does not have antimalarial activity but possibly utilises the parasites' affinity for iron to increase the probability of encountering the molecule [69, 70]. In addition to its activity against CQ-resistant *P. falciparum* isolates, FQ is also highly effective against drug-resistant *P. vivax* malaria [71]. A Phase II clinical trial in combination with artesunate is to be completed by October 2011 to assess activity in reducing parasitaemia and to explore the pharmacokinetics of ferroquine and its metabolites [72].

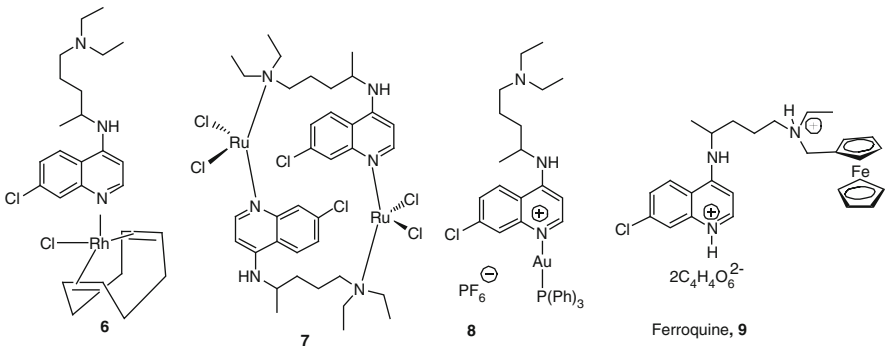


Fig. 5 Organometallic antimalarials

4.1.3 Piperaquine

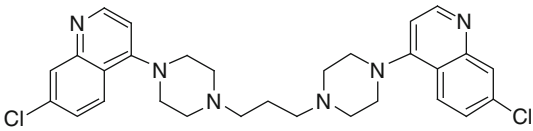
Other notable work in the chloroquine SAR field has involved the preparation of bisquinoline dimers, some of which possess excellent activity against CQ-resistant parasites. This activity against resistant parasites may be explained by their steric bulk, which prevents them from fitting into the binding site of PfCRT. Alternatively, the bisquinolines may be more efficiently trapped inside the DV because of their four positive charges.

Early examples of such agents include bis(quinolyl) piperazines such as piperaquine, **10** (Fig. 6). Piperaquine was first synthesized in the 1960s and used extensively in China for prophylaxis and treatment for the next 20 years. With the development of piperaquine-resistant strains of *P. falciparum* and the emergence of the artemisinin derivatives, its use declined during the 1980s [73].

During the next decade, piperaquine was rediscovered as one of a number of compounds suitable for combination with an artemisinin derivative. The pharmacokinetic properties of piperaquine have now been characterised [74], revealing that it is a highly lipid-soluble drug with a large volume of distribution at steady state, good bioavailability, long elimination half-life and a clearance rate that is markedly higher in children than in adults. The tolerability, efficacy, pharmacokinetic profile and low cost of piperaquine make it a promising partner drug for use as part of an artemisinin combination therapy (ACT).

Initial results were encouraging [73, 75], and Phase III clinical trials were completed in 2009 [76]. A recent report analysing individual patient data analysis of efficacy and tolerability in acute uncomplicated falciparum malaria, from seven published randomised clinical trials conducted in Africa and South East Asia concluded that dihydroartemisinin (DHA)-piperaquine is well tolerated, highly effective and safe [77]. Although not currently registered in the UK, a fixed combination called Duo-cotecxin is registered in China, Pakistan, Cambodia and Myanmar in addition to 18 African countries. Concerns with this combination lie in the fact that the calculated terminal half-life for piperaquine is around 16.5 days [78], compared with that of DHA (approximately 0.5 h) [79]; hence, the development of resistance could be a possibility due to prolonged exposure of piperaquine at sub-therapeutic levels effectively as a monotherapy.

A 1,2,4-trioxolane (RBx11160/Arterolane) has also been recently partnered with piperaquine and progressed to Phase III clinical trials. The clinical trials of RBx11160 alone identified its tendency to degrade relatively rapidly due to high levels of iron (II) in infected red blood cells, leading to a clinical efficacy of 60–70% [80]. The combination with a longer lasting drug such as piperaquine,



Piperaquine, **10**

Fig. 6 Structure of piperaquine **10**

with a completely different mechanism of action, may reduce the possibility of resistance and recrudescence [81]; recent results suggest the combination is highly active, with patients being free from recrudescence on day 28 after treatment [76]. This combination may also offer an advantage over DHA-piperaquine in the sense that the artemisinin-based component of the combination is a totally synthetic 1,2,4-trioxolane. This avoids over-reliance on the natural product artemisinin, whose cost and availability has been shown to fluctuate in recent years [82].

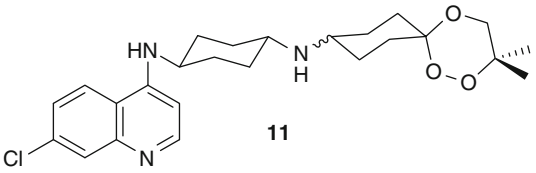
4.1.4 Trioxaquine SAR116242

Combination chemotherapy is now the mainstay of antimalarial treatment; each novel artemisinin-based antimalarial that reaches clinical trials is usually employed in an additional trial with an appropriate partner drug. However, a relatively novel approach is the concept of "covalent biotherapy" – a synthetic hybrid molecule containing two covalently linked pharmacophores [83]. The hybrid is designed to target the parasite by two distinct mechanisms thus circumventing resistance development. The hybrid also has several advantages over multi-component drugs such as:

- Expense – in principle, the risks and costs involved with a hybrid may not be any different when compared with those of a single entity.
- Safety – lower risk of drug–drug adverse interactions.
- Matched pharmacokinetics (i.e. a single entity)

A possible disadvantage, however, is that it is more difficult to adjust the ratio of activities at different targets [84]. Recent examples include trioxaquines developed by Meunier and co-workers, containing a 1,2,4-trioxane (as the artemisinin-based component) covalently bound to a 4-aminoquinoline [85]. These novel trioxaquines were found to be potent against CQ and pyrimethamine-resistant strains, and have improved antimalarial activity compared with the individual components. Several trioxaquines were developed over a number of years culminating in the selection of a drug-development candidate known as SAR116242, **11** (Fig. 7).

The superior antimalarial activity in both CQ-sensitive and CQ-resistant isolates ($IC_{50} = 10$ nM) has been attributed to its dual mechanism of haem alkylation and haemozoin inhibition. In addition, incorporation of a second cyclohexyl ring within the linker that joins the two pharmacophores increased the metabolic stability of this molecule compared with other trioxaquines containing a linear tether [86].



11

SAR116242

Fig. 7 Structure of SAR116242 **11**

The drug was synthesised as a mixture of diastereoisomers, but each diastereoisomer was found to be equipotent in their in vitro antiplasmodial activities and also displayed similar pharmacological profiles. However, it is not clear whether the pharmacokinetics and safety profiles of each individual diasteroisomer are the same. SAR 116242 is undergoing pre-clinical assessment by Sanofi-Aventis to determine its potential as the first "fusion" antimalarial.

4.1.5 Amodiaquine

Amodiaquine **3** (AQ), a phenyl substituted analogue of CQ, was first found to be effective against non-human malaria in 1946. Its mechanism of action is thought to be similar to CQ, but this is again a matter of some controversy [87].

Clinical use of AQ has been severely restricted because of associations with hepatotoxicity and agranulocytosis. Due to this toxicity, WHO withdrew recommendation for the drug as a monotherapy in the early 1990s. The AQ side chain contains a 4-aminophenol group; a structural alert for toxicity, because of metabolic oxidation to a quinoneimine (Fig. 8). Although cross-resistance of CQ and AQ has been documented for 20 years [88], AQ remains an important drug as it is effective against many CQ-resistant strains. Therefore, many drug design projects have since focussed on reducing this toxicity [87].

4.2 Modifications to Reduce Toxicity of AQ

4.2.1 Metabolism of CQ and AQ

CQ is highly lipophilic, as well as being a diacidic base. After oral administration, CQ is rapidly absorbed from the gastrointestinal tract, having a high bioavailability of between 80 and 90%. CQ undergoes *N*-deethylation to give the desethyl

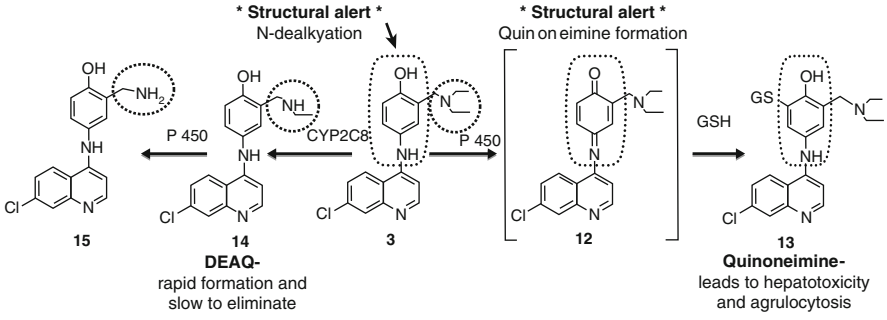


Fig. 8 Metabolism of AQ to toxic quinoneimine and DEAQ metabolites

compound as a major metabolite which has the same activity as CQ against sensitive strains, but reduced activity versus CQ-resistant strains [89].

Upon oral administration, AQ is rapidly absorbed and extensively metabolized. Although AQ has a high absorption rate from the gut due to a large first pass effect, AQ has a low bioavailability and is considered a pro-drug for desethylamodiaquine (DEAQ, **14**) [90]. In contrast to the metabolism of CQ, AQ also produces a toxic quinoneimine metabolite **12** (Fig. 8). The metabolites have been detected in vivo by the excretion of glutathione (GSH) conjugates (such as **13**) in experimental animals [91, 92]. It has been postulated that AQ toxicity involves immune-mediated mechanisms directed against the drug protein conjugates via in vivo bioactivation and covalent binding of the drug to proteins [93].

The main metabolite of AQ is DEAQ **14**, with other minor metabolites being 2-hydroxyl-DEAQ and *N*-bisdesethyl AQ (bis-DEAQ **15**) [94] (Fig. 8). The formation of DEAQ is rapid and its elimination very slow with a terminal half-life of over 100 h [95], as a result the mean plasma concentration of DEAQ is six- to sevenfold higher than the parent drug. Recent studies have established that the main P450 isoform catalysing the *N*-dealkylation of amodiaquine is CYP2C8 [96]. Mutations in PfCRT have been found in resistance isolates and correlate with high-level resistance to the AQ metabolite DEAQ in in vitro tests.

4.2.2 Modification of Metabolic Structural Alerts

Since AQ retains antimalarial activity against many CQ-resistant parasites, the next focus was to make a safer, cost-effective alternative. Initial studies involved the design and synthesis of fluoroamodiaquine (FAQ, **16**, Fig. 9) [97] since this analogue cannot form toxic metabolites by P450-mediated processes and retains substantial antimalarial activity versus CQ-resistant parasites. However, the resulting *N*-desethyl 4′-fluoro amodiaquine metabolite has significantly reduced activity against CQ-resistant parasites [97]. Concerns about cost led to the preparation of

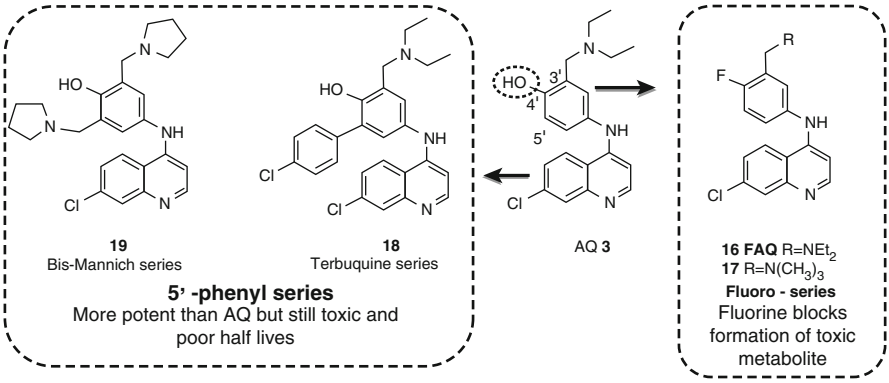


Fig. 9 Modification of structural alerts to reduce toxicity of AQ

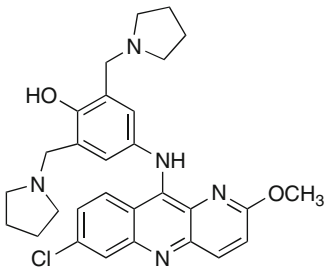
other synthetically accessible analogues; the tebuquine series [98] and the bis-Mannich series [99] (Fig. 9).

Tebuquine (**18**), a biaryl analogue of AQ discovered by Parke-Davis, is significantly more active than AQ and CQ both in vitro and in vivo and has potent antimalarial activity and reduced cross-resistance with CQ [100, 101]. Both the bis-Mannich and terbuquine series were expected to offer advantages over AQ in the sense that they contain Mannich side chains that are more resistant to cleavage to *N*-desalkyl metabolites. A potential drawback with the bis-Mannich class of antimalarial compounds was recognized by Tingle et al. [102]. They demonstrated that such compounds have long half-lives, raising concerns over potential drug toxicity and resistance development. Compounds in the tebuquine series have also been shown to have unacceptable toxicity profiles that is exacerbated by the long half-lives [102].

Pyronaridine

Pyronaridine **20** (Fig. 10) is another member of the class of Mannich-base schizontocides; however, the usual quinoline heterocycle is replaced by an azaacridine. Like AQ **2**, pyronaridine **20** retains the aminophenol substructure which can be oxidised to the respective quinoneimine. Since pyronaridine contains two Mannich-base side chains, it has been suggested that the second Mannich base moiety prevents the formation of the hazardous thiol addition products by sterically shielding the quinoneimine from the attack of the sulphur nucleophile [103].

Pyronaridine **20** was developed and used in China since the 1980s, but has not been registered in other countries. In a clinical study performed in Thailand, high recrudescence has been observed and in vitro assays revealed the presence of pyronaridine-resistant strains [104]. Another study in Africa showed high activity against CQ-resistant field isolates (IC_{50} values of 0.8–17.9 nM) [105]. Data suggest there may be some in vitro cross-resistance or at least cross-susceptibility between pyronaridine **20**, CQ **2** and AQ **3**. The combination of pyronaridine **20** and the artemisinin analogue artesunate (Pyramax) is in clinical development and began Phase III clinical trials in 2006. In terms of safety, pyronaridine-artesunate was well



Pyronaridine **20**

Fig. 10 Structure of pyronaridine **20**

tolerated in Phase II trials. However, a few patients exhibited raised liver enzymes, therefore the risk of toxicity to the liver still needs to be closely monitored [106]. Pyramax was submitted to the European Medicines Agency (EMA) for regulatory approval at the end of March 2010 [107].

Isoquine

An approach to circumvent the facile oxidation of AQ involves the interchange of the 3′-hydroxyl and the 4′-Mannich side-chain function of AQ. This provided a new series of analogues that avoid the formation of toxic quinoneimine metabolites via cytochrome P450-mediated metabolism (Fig. 11) [108].

While several analogues displayed potent antimalarial activity against both CQ-sensitive and resistant strains, isoquine **22** (ISQ), the direct isomer of AQ, displayed potent in vitro antimalarial activity in addition to excellent oral in vivo ED_{50} and ED_{90} activity of 1.6 and 3.7 mg/kg, respectively, against the *P. yoelii* NS strain (compared with 7.9 and 7.4 mg/kg for AQ) [109]. Subsequent metabolism studies in the rat model demonstrated that **22** does not undergo in vivo bioactivation, as evidenced by the lack of glutathione metabolites in the bile. Unfortunately, pre-clinical evaluation displayed unacceptably high first pass metabolism to dealkylated metabolites, which complicated the development and compromised activity against CQ-resistant strains [110].

Since the metabolic cleavage of the *N*-diethylamino-group was an issue, the more metabolically stable *N-tert*-butyl analogue was developed in the hope that this

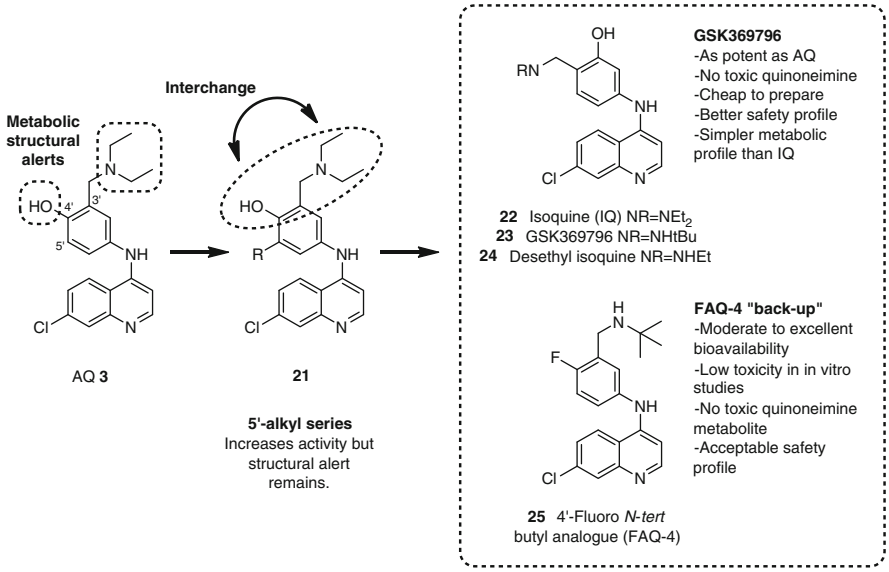


Fig. 11 Modifications of AQ to reduce toxicity of metabolic structural alerts

would lead to a much simpler metabolic profile and enhanced bioavailability. Development of the *N-tert*-butyl analogue **23** (GSK369796) followed (Fig. 11), which has superior pharmacokinetic and pharmacodynamic profiles to isoquine in pre-clinical evaluation studies performed by Glaxo SmithKline pharmaceuticals [110]. In spite of the excellent exposures and near quantitative oral bioavailabilities in animal models, development of **23** has been discontinued due to the inability to achieve exposures at doses considered to demonstrate superior drug safety compared with CQ.

4′-Fluoro-*N-tert*-butylamodiaquine FAQ-4 (**25**) was also identified as a "back-up" candidate for further development studies based on potent activity versus CQ-sensitive and resistant parasites, moderate to excellent oral bioavailability, low toxicity in in vitro studies, and an acceptable safety profile, and this molecule is undergoing formal pre-clinical evaluation [111].

5 The Future of CQ and AQ

5.1 CQ/AQ Next-Generation Candidates in Clinical Development

4-Aminoquinoline-based drug development projects continue to yield promising drug candidates and several molecules have entered into pre-clinical development or clinical trials over the last few years. Projects to reduce resistance development of CQ have resulted in the development of short-chain chloroquine analogues (AQ-13), organometallic antimalarials (ferroquine) and a "fusion" trioxaquine antimalarial (SAR116242). Projects to reduce the toxicity of AQ have resulted in the development of metabolically stable amodiaquine analogues (isoquine/*tert*-butyl isoquine) and aza-acridine derivatives (pyronaridine) (Table 1).

5.2 CQ/AQ Combinations: ACTs and Non-ACTs

The 4-aminoquinolines CQ and AQ have had a revival over the last 20 years due to the development of ACT. Artesunate-amodiaquine (Coarsucam) was approved for the WHO pre-qualification project in October 2008. It is expected to have a 25% share of the ACT market, with another ACT, Coartem (artemether/lumefantrine) taking the remaining 75% [76].

Methylene blue (MB), a specific inhibitor of *P. falciparum* glutathione reductase was the first synthetic antimalarial drug ever used in the early 1900s. Interest in its use as an antimalarial has recently been revived, due to its potential to reverse CQ resistance and its affordability [112]. It is thought that MB prevents the crystallisation of haem to haemozoin in a similar mechanism as the 4-aminoquinolines.

Table 1 Summary of 4-aminoquinolines entering or in clinical trials, modified and updated from recent reviews [4, 76]

Active ingredients (product name)	Partnership	Phase/ status	Strengths	Weakness
Artesunate 50 mg Amodiaquine 135 mg (Coarsucam®)	Sanofi-Aventis, DNDi	Prequalified 2008	• Soluble tablets for paediatric use. • 1 tablet a day – 3 days • WHO prequalified • Three dose strengths • Has 25% of the ACT market	• Resistance to AQ – GI side effects • Not used as prophylactic due to toxic effect of AQ • Reports of resistant strains • No approval yet but WHO prequalified
DHA 10 mg piperaquine 80 mg (Eurartesim™), Artekin, also Duocotexin (fixed dose Holley and Cotect)	Sigma-Tau, MMV, Chongquing, Holley	III	• 1 tablet a day for 3 days • Piperaquine longest half life of all ACTs partners. • Long post-treatment prophylaxic effect • Extensive safety data	• On WHO treatment guidelines but not approved • Long half life of piperaquine could lead to resistance (16.5 days – DHA approximately 0.5 h)
Pyronaridine 60 mg artesunate 20 mg (Pyramax)	Shin Poong, MMV	III	• 1 tablet a day for 3 days • End point achieved in Phase III trials, submitted to EMEA (late 2009) • Clinical data and registration also for *P. vivax*	• Possible hepatotoxicity from pyronaridine – needs to be investigated • Long half life pyronaridine may lead to resistant strains • Paediatric formula in development (2012 release)
Azithromycin 250 mg Chloroquine 150 mg	Pfizer/MMV	III	• Fixed dose combination (four tablets) for prophylactic use during pregnancy • Long post-treatment prophylaxic effect • Extensive safety data • High efficacy in Phase III trials, even in CQ-resistant areas	• Prohibitively expensive for malaria control programmes • Regimen requires partial self-administration • Anti-CQ campaigns in some areas – may be problem with patient compliance
Rbx11160 150 mg Piperaquine 800 mg (Arterolane)	Ranbaxy	II	• No embryotoxicity concern as with artemisinin combinations • Synthetic so costs kept low • Potential activity against artemisinin-resistant strains to be established	• Efficacy concerns (poor activity of Rbx11160as a monotherapy) • As yet no studies in children, or juvenile toxicology data • Phase III India 2009 – no launch until at least 2011

(continued)

Table 1 (continued)

Active ingredients (product name)	Partnership	Phase/ status	Strengths	Weakness
			• Phase III study as a combination planned India 2009	
SSR-97193 (Ferroquine) artesunate	Sanofi-Aventis	II	• Also effective against *P. vivax* chloroquine resistant strains	• Cost of goods for metal based drugs – may be expensive
Methylene blue, chloroquine	Ruprecht-Karls-University, Heidelberg, DSM	II	• Reports of combination with AQ or artesunate planned. • MB/AQ Cost-effective	Methylene blue/chloroquine did not meet WHO criterion of 95% efficacy
AQ-13	Immtech	I	• Similar to CQ in its efficacy and PK	• Very similar structure to CQ-possible parasite could develop resistance very quickly? • AQ-13 exhibits increased clearance compared with CQ therefore higher dose required
N-tert-butyl Isoquine	GSK, MMV	I	• Excellent exposures • Near quantitative bioavailabilites • Superior PK data to ISQ	• *N-tert* discontinued due to problems with inadequate exposure levels • Phase I back-up molecule being evaluated
SAR116242 (Trioxaquine)	Sanofi, Palumed	Preclinical	• Totally synthetic, metabolically stable and cost effective	• Synthetic route produces diastereomers • Molecule has potential to express both established safety concerns of 4-aminoquinolines (narrow TI) and endoperoxides (embryotoxicity, neurotoxicity) requiring careful safety evaluation

MB was entered into clinical trials with CQ as a partner drug but this combination was not sufficiently effective, even at higher doses of MB [113]. More recent trials with AQ or artesunate as a partner drug provided more optimism; MB-artesunate achieved a more rapid clearance of *P. falciparum* parasites than MB–AQ, but MB–AQ displayed the overall highest efficacy. As MB and AQ are both available and affordable, the MB–AQ combination would be an inexpensive non-ACT antimalarial regimen. A larger multi-centre Phase III study is now planned for the near future.

Another non-ACT combination in Phase II clinical trials is azithromycin/chloroquine (AZ/CQ). Azithromycin is a newer member of the family of macrolide antibiotics. This combination has entered Phase III clinical trials and is currently the most promising non-artemisinin-based prophylactic therapy for Intermittent Preventative Treatment in Pregnant Women (IPTp) [76] and a fixed-dose combination tablet of AZ/CQ is being developed specifically for this use. The combination is synergistic against CQ-resistant strains of *P. falciparum* and has already shown efficacy in the treatment of symptomatic malaria in sub-Saharan Africa, an area of high CQ resistance [76]. Both AZ and CQ have demonstrated safety in children and pregnant women over a number of years and azithromycin provides an additional benefit in treating and preventing sexually transmitted diseases [114]. A pivotal study comparing AZ/CQ IPTp with the current adopted therapy sulfadoxine–pyrimethamine IPTp began in October 2010 and is expected to be completed by January 2013 [115].

6 Conclusions

Due to the increasing spread of malaria resistance to drugs such as CQ and AQ, current treatment regimes rely heavily on artemisinin-based therapies. This could lead to an overdependence on artemisinin availability and may influence cost, so it is extremely important that 4-aminoquinoline drug development programmes continue. Costly lessons have been learnt from the loss of sensitivity to one of the most important drugs for malaria treatment and extreme caution is now taken to ensure that with every new antimalarial developed, a partner drug is found and co-administered to reduce the spread of parasite resistance. Increased understanding of 4-aminoquinoline SARs, mechanisms of toxicity and parasite resistance has aided development of what will hopefully be the next generation of 4-aminoquinolines. The future of 4-aminoquinolines relies heavily on strong partnerships between the public health sectors, MMV (Medicines for Malaria Venture) academia and private pharmaceutical/biotechnology companies to yield a continuing pipeline of 4-aminoquinoline candidates, which not only overcome resistance development but also demonstrate increased efficacy compared with CQ. Equally important is a consideration of the safety attributes of this class since the animal toxicities observed in industry standard pre-clinical development of next-generation analogues such as NTB-isoquine (**23**) **8**, in the absence of any prior human

experience, might have precluded the further development of any 4-aminoquinoline and indicates limitations of our current pre-clinical testing strategies to accurately predict human risk in malaria treatment [110].

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