Quinine Conjugates and Quinine Analogues as Potential Antimalarial Agents

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Review article

Quinine conjugates and quinine analogues as potential antimalarial agents

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ABSTRACT

Malaria is a tropical disease, prevalent in Southeast Asia and Africa, resulting in over half a million deaths annually; efforts to develop new antimalarial agents are therefore particularly important. Quinine continues to play a role in the fight against malaria, but quinoline derivatives are more widely used. Drugs based on the quinoline scaffold include chloroquine and primaquine, which are able to act against the blood and liver stages of the parasite's life cycle. The purpose of this review is to discuss reported biologically active compounds based on either the quinine or quinoline scaffold that may have enhanced antimalarial activity. The review emphasises hybrid molecules, and covers advances made in the last five years. The review is divided into three sections: modifications to the quinine scaffold, modifications to aminoquinolines and finally metal-containing antimalarial compounds.

1. Introduction

Malaria is caused by a protozoan parasitic disease transmitted by *Anopheles* mosquitoes. In general, malarial infections in humans are caused by four species of the genus *Plasmodium*, and the majority are caused by *Plasmodium falciparum* (*P. falciparum*) or *Plasmodium vivax* (*P. vivax*) [1]. *P. falciparum*, the life-threatening parasite, is endemic in South and East Asia, South America, the Caribbean, the Middle East and Africa whereas *P. vivax*, which is typically not lethal, is endemic in Central America, India and parts of the Eastern Mediterranean. *Plasmodium ovale* and *Plasmodium malariae* are two rare, nonlethal parasites, most commonly found in Africa and Papau New Guinea [2]. Finally, in parts of Southeast Asia, *Plasmodium knowlesi*, a type of monkey malaria, has been identified recently as a fifth human malaria parasite [3].

In 2012 an estimated 207 million cases were reported, resulting in 627,000 deaths. The vast majority of cases (80%) and deaths (90%) occurred in Africa; where mortality rates were highest (77%) in children under the age of five [4]. There has been a dramatic decrease in malaria cases since the Africa Malaria Report published in 2003, due in part to the growing use of insecticide-treated nets that offer protection against contracting malaria, but also as a result of prompt access to treatment and the prevention of malaria during pregnancy [5].

In spite of the recent success in reducing cases of malaria, there is a continued and growing need for novel antimalarial drugs, since *P. falciparum* has gained resistance to all current drugs, in particular in Southeast Asia, South America and East Africa [6]. In addition, despite time, money and effort expended in the development of malaria vaccines, only limited success has been achieved in this area of research [7].

This review encompasses recent synthetic modifications to quinine, and selected analogues of quinine that show potential antimalarial activity. Due to the importance of novel antimalarial agents, there is increasing activity in this field of research, which has resulted in several extensive review articles [8]. This review is therefore not intended to be comprehensive and particular emphases have been placed on drug-hybrids of quinine and related quinoline heterocycles.

Abbreviations: AQ, aminoquinoline; BH, β-Hematin; CQ, chloroquine; CQR, chloroquine resistant; CQS, chloroquine sensitive; DV, digestive vacuole; FQ, ferroquine; Q, quinine; QD, quinidine; *P.*, *Plasmodium*; PQ, primaquine; WHO, World Health Organisation.

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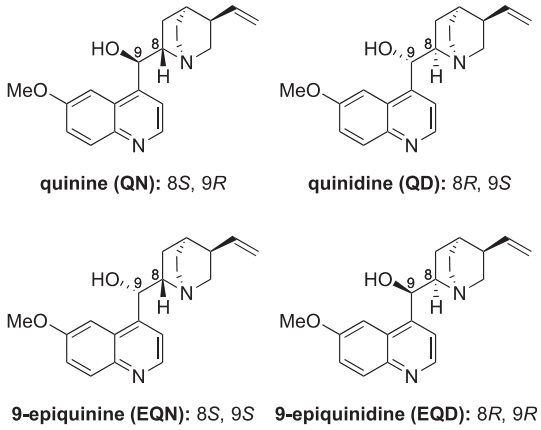


Fig. 1. Quinine and related stereoisomers.

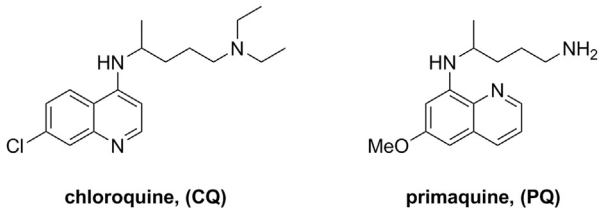


Fig. 2. Quinoline-containing antimalarial drugs.

Table 1
Antimalarial drug resistance [6].

Drug	Drug resistance
Chloroquine-phosphate	Common worldwide
Quinine	Southeast Asia
Mefloquine	Southeast Asia
Artemisinins	Southeast Asia [16]

2. Quinine-derived antimalarial compounds

Quinine (Fig. 1), first isolated in South America by extraction of the bark of the Cinchona tree was, for almost 300 years, the only treatment for malaria. To that end, while the search for novel drug targets and new lead structures for the treatment of malaria are critical objectives, a complimentary strategy is to utilise, and develop by chemical modification, compounds from nature such as quinine with efficacious antimalarial properties [8a].

The quinoline moiety is responsible for the antimalarial properties of quinine, however, understanding of the mode of action of quinoline-containing antimalarial drugs remains incomplete [8e]. It is thought that quinoline-derived antimalarial drugs target the blood stages of the parasite's life cycle that occurs within human erythrocytes. During this stage of the life cycle, digestion of the host's haemoglobin and concomitant accumulation of iron (III) protoporphyrin IX (Fe(III)PPIX) occurs within the digestive vacuole [9]. The parasite then disposes of the heme-complex into the cytoplasm and converts it into hemozoin [10]. It has been demonstrated that quinoline-based antimalarial compounds inhibit the formation of synthetic hemozoin (β-hematin) [11], and hence this is an important area of research in the pursuit of new and improved drugs, and the subject of studies into quinoline drug–heme interactions [9,12].

There are three main types of modification that have been applied to the quinine scaffold. These include substitution at the hydroxyl or vinyl groups, variations to the quinoline or quinuclidine ring and modifications to the stereochemistry. In general, it has been found that both the hydroxyl group and the quinoline ring are essential for the antimalarial activity of quinine by inhibiting the formation of β-hematin (BH) [12a]. It is possible however, to modify the quinuclidine ring without loss of activity [13]. Changes to the stereochemistry of QN and QN derivatives can also be important (Fig. 1) [14]. For example QN and quinidine (QD) are known to have antimalarial properties, quantified *in vitro* against chloroquine sensitive (CQS) strain D-6 of *P. falciparum* (IC_{50} = 29.3 ± 9.5 and 13.4 ± 4.6 nM, respectively) whereas 9-epiquinine (EQN) and 9-epiquinidine (EQD) were shown to be inactive (IC_{50} = 3471 ± 797 and 2700 ± 704 nM, respectively) [15].

As the quinoline moiety is essential for antimalarial activity, various quinoline analogues have been prepared including chloroquine (CQ) and primaquine (PQ) (Fig. 2). However, all known antimalarials have encountered resistance in certain parts of the world (Table 1) and this has led to efforts to produce novel small molecules based on the quinoline moiety that may possess antimalarial activity [8d,8e].

Traditionally, CQ was the most widely used antimalarial due to its effectiveness and relatively low cost, however this is no longer the case due to *P. falciparum* developing either reduced sensitivity or resistance to CQ worldwide [17]. The wide range of possible mutations has resulted in a large number of CQ sensitive (CQS) and CQ resistant (CQR) strains of *P. falciparum* being isolated, used to test the efficacy of novel antimalarial drugs and to study the mechanism of how CQ resistance develops [18].

It is believed that the antimalarial properties of QN and quinoline drugs is due, at least in part, to accumulation of the drug in the parasites' digestive vacuole (DV) [19]. This is possible since the DV is an acidic environment that is able to trap basic QN and CQ. Where resistance to antimalarial drugs occurs, a concomitant reduction of the drug in the DV has been observed which is thought to be caused by changes in the genes that encode membrane transport proteins. Chloroquine resistance transporter (PfCRT) is one of the most well-studied of the proteins found in the membrane of the DV and it has been hypothesised that CQR is due to mutations in PfCRT which result in the drug being transported out of the DV [20]. Therefore, methods to reverse CQ resistance by developing small molecules that can inhibit PfCRT is an important area of research [12d].

2.1. Artemisinin and artemisinin derived-quinine combination therapies

Artemisinin (ART) is a potent treatment for malaria due to its low toxicity, rapid killing of malaria parasites and its nanomolar potency *in vivo* against *P. falciparum* [21]. However, the use of ART is hampered due to its low solubility in water and oil and its short half-life *in vivo* ($t_{1/2}$–2 h) [22] This has led to the design of synthetic analogues of ART (Fig. 3), including dihydroartemisinin (DHA), which is twice as potent as ART [23].

Due to their short half-life, ARTs were initially administered over a weeklong period as a monotherapeutic treatment, but resistance has recently emerged in Southeast Asia (Table 1) [16], partly due to poor patient compliance with the 7-day treatment [4]. To overcome this problem, artemisinin-based combination therapies (ACTs), in which ARTs are simultaneously administered with one or more drugs with independent modes of action are currently recommended by the WHO. Common drugs previously used in ACT include amodiaquine, chlorproguanil/dapsone, lumefantrine, mefloquine, piperaquine and sulfadoxine/pyrimethamine (SP) (Fig. 4) [24]. Currently there are five ACTs recommended for use by WHO:

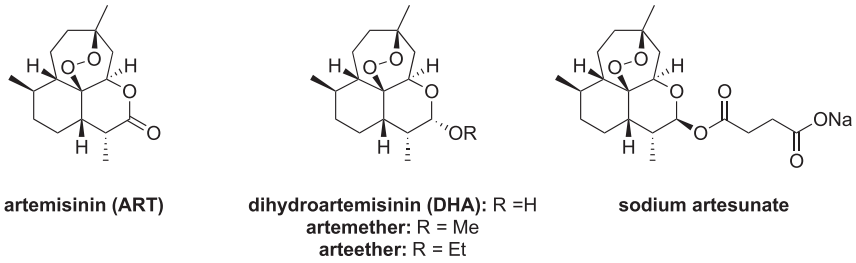


Fig. 3. Artemisinin and common synthetic derivatives showing quick-acting antimalarial activity.

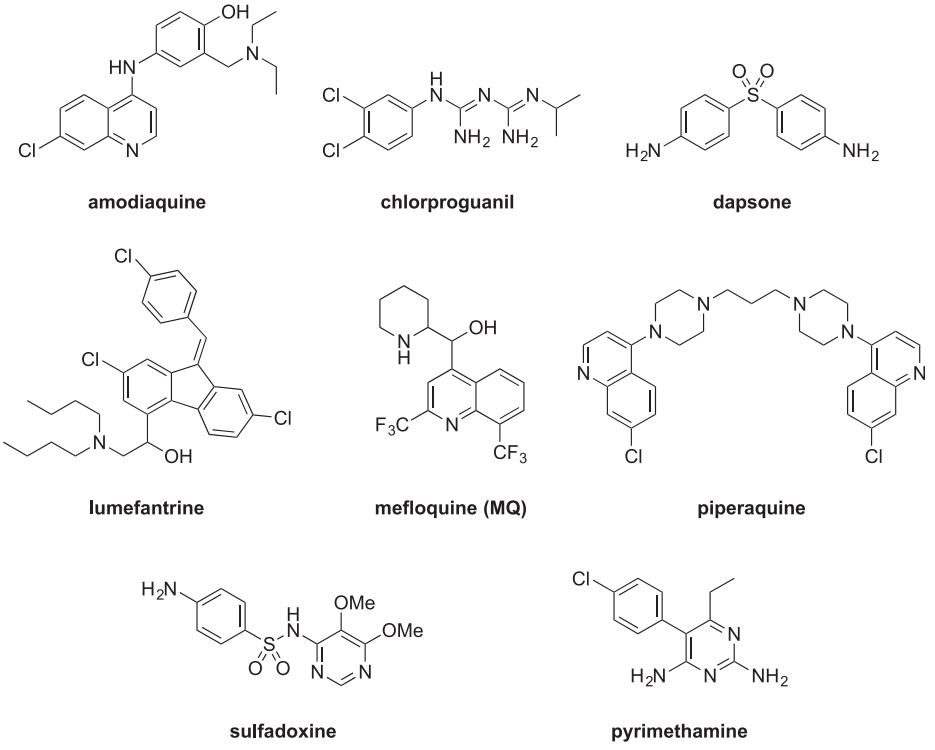


Fig. 4. Slowly-acting antimalarial drugs used in ACTs.

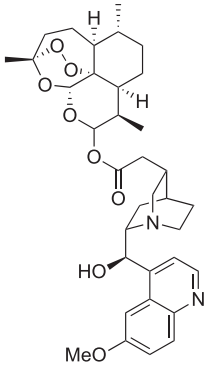


Fig. 5. ART-QN hybrid (**2**), which displayed enhanced antimalarial properties.

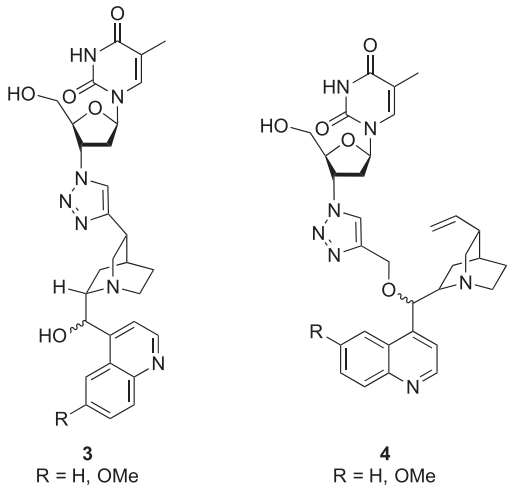
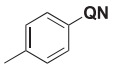
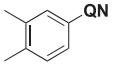
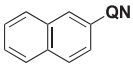
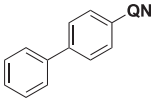
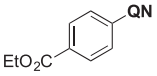
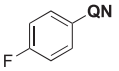
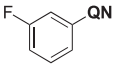
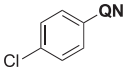
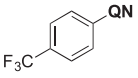
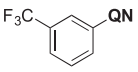
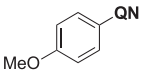
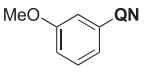
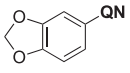
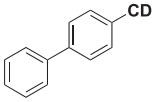
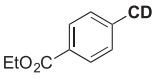
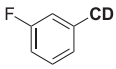
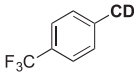
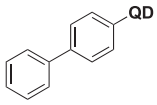
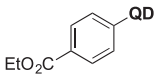
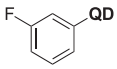
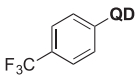
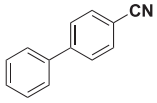
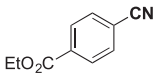
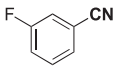
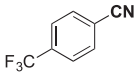


Fig. 6. 3′-Azido-3′deoxythymidinine-*Cinchona* alkaloid conjugates.

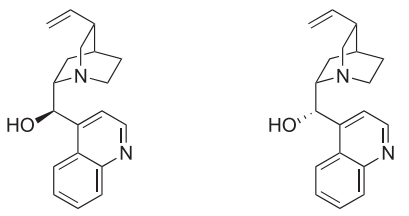
Table 2
In vitro antiplasmodial activities of *Cinchona* alkaloid-derivatives against field-derived CQS and CQR strains of *P. falciparum*.[a,b]

Compound#	Structure	Parasite strain, IC_{50} (nM)	
		HB3	Dd2
QN[c]	–	81 ± 8	320 ± 50
5a		>500	>500
5b		>500	>500
5c		>500	>500
5d		*Data not reported*	
5e		>500	>1500
5f		>500	>500
5g		128 ± 5	184 ± 7
5h[c]		428 ± 4	318 ± 2
5i[c]		>500	>500
5j		145 ± 4	733 ± 9
5k		>500	>500
5l[c]		427 ± 4	485 ± 6
5m[c]		>500	>500
5n		>500	>500
5o		>500	>500
5p		>500	>500
CD[c]	–	70 ± 8	207 ± 11
5q[c]		127 ± 4	176 ± 2
5r		590 ± 17	198 ± 5
5s		297 ± 13	223 ± 8
5t[c]		394 ± 5	928 ± 6
QD[c]	–	18 ± 2	90 ± 8
5u		123 ± 1	202 ± 7
5v[c]		120 ± 5	203 ± 10
5w		165 ± 3	2350 ± 4
5x		98 ± 3	182 ± 2
CN[c]	–	22 ± 5	82 ± 8
5y		90 ± 3	243 ± 2
5z[c]		144 ± 1	263 ± 2
5aa		66 ± 8	983 ± 2
5 ab[c]		154 ± 1	146 ± 2

[a] IC_{50} values are an average of triplicate measurements and are reported ± S.E.M.
[b] IC_{50} values were not calculated for derivatives with low activity (>500 nM).
[c] Compounds that were tested for BH inhibition.

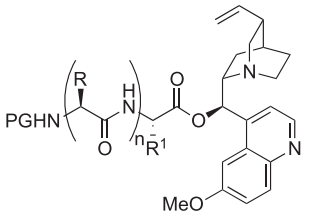
artemether and lumefantrine, DHA and piperaquine and artesunate in combination with either amodiaquine, mefloquine or SP [4].

Combination therapies, whilst effective in the fight against malaria, have associated disadvantages including poor patient compliance and side effects caused by drug–drug interactions [25]. Against this background, drug-hybrids have emerged as an important new class of treatment, which can have far superior activity relative to the individual components of the drug [8c,26].



cinchonidine (CD) **cinchonine (CN)**

Fig. 7. *Cinchona* alkaloids CD and CN.



6
n = 0, 1, 2
PG = Boc, Cbz

Fig. 8. QN-amino acid and QN-peptide conjugates.

Table 3
In vitro antimalarial activities of quinine-amino acid/peptide conjugates against CQS 3D7 strain of *P. falciparum*.

Compound#	Structure	IC_{50} (nM)[a]
6a	Boc-Gly-QN	27 (23–32)
6b	Boc-L-Ala-QN	36 (30–43)
6c	Boc-L-Phe-QN	38 (33–44)
6d	Boc-L-Ile-QN	550 (490–620)
6e	Boc-L-His(Tos)-QN	42 (36–48)
6f	Boc-L-Ser(Bzl)-QN	95 (81–110)
6g	Boc-L-Glu(Bz)-QN	76 (67–86)
6h	Cbz-L-Lys(Cbz)-QN	71 (62–82)
6i	Cbz-L-Asp(Bz)-QN	17 (16–20)
6j	Cbz-L-Cys(Bz)-QN	40 (35–46)
6k	Cbz-L-Ala-L-Phe-QN	120 (100–140)
6l	Cbz-L-Val-L-Leu-QN	74 (60–92)
6m	Cbz-L-Ile-Gly-QN	23 (20–25)
6n	Cbz-Gly-L-Phe-L-Ala-QN	57 (50–64)
–	QN	18 (11–30)

[a] Mean value (95% confidence intervals).

2.2. Modifications of the vinyl group

In the context of quinine-derived drug hybrids showing enhanced antimalarial properties, Walsh et al. [27] recently prepared artemisinin-quinine (ART-QN) hybrid **2** (Fig. 5). The ART-QN hybrid showed higher antimalarial activity against CQS strain 3D7 (IC_{50} = 8.9 nM) than either QN (IC_{50} = 149 nM), ART (IC_{50} = 49.4 nM) or a 1:1 mixture of QN and ART (31.8 nM) [27]. The mechanism of action has not been determined, but it was suggested that the ester is hydrolysed under physiological conditions thus exposing the more potent DHA [27]. Alternatively, it has been proposed that the two covalently bound drugs act synergistically [21c]. Furthermore, it is noteworthy that the combination of QN and ART is more potent than QN or ART individually, thus indicating the importance of ACTs.

In addition to forming carboxylic acid derivatives of quinine, the presence of the vinyl function has provided a site for conjugation *via* both cross coupling [28] and 'Click' reactions [29]. However, in the case of the 3′-azido-3′-deoxythymidine (AZT)-*Cinchona* alkaloid conjugates (Fig. 6), the antimalarial properties of the final compounds were not investigated [29].

Dinio et al. utilised Heck coupling to prepare a library of 16

Table 4
In vitro antimalarial activities of compounds against CQS 3D7 strain of *P. falciparum*.

Compound#	Structure	IC_{50} (nM)[a]
7a	Oxolinic-Phe-QN	115 (65–200)
7b	Oxolinic-Ala-QN	29 (18–46)
7c	Oxolinic-Val-QN	183 (108–313)
7d	Nalidixic-Gly-QN	46 (19–108)
7e	Nalidixic-Leu-QN	50 (31–82)
7f	Nalidixic-Ala-QN	16 (9–27)
7g	Levofloxacin-Gly-QN	12 (9–16)
7h	Levofloxacin-Ala-QN	33 (20–53)
7i	Levofloxacin-Phe-QN	48 (35–65)
7j	Enrofloxacin-Gly-QN	28 (19–44)
7k	Enrofloxacin-Ile-QN	207 (163–263)
–	QN	18 (11–30)

[a] Mean value (95% confidence intervals).

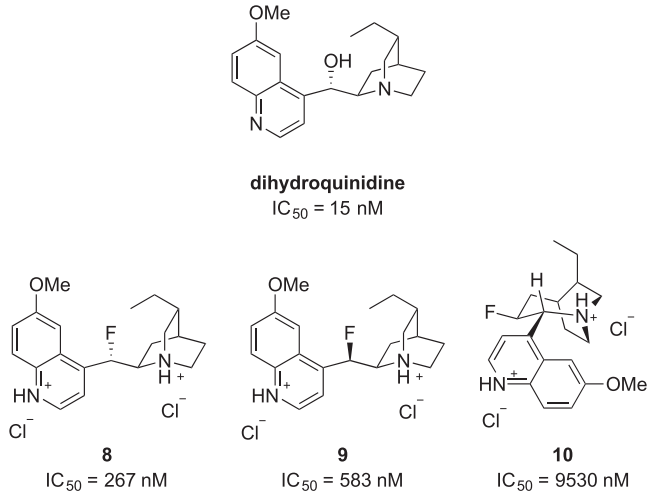


Fig. 10. Fluorinated dihydroquinidine derivatives (**8**–**10**) shown with the parent alkaloid.

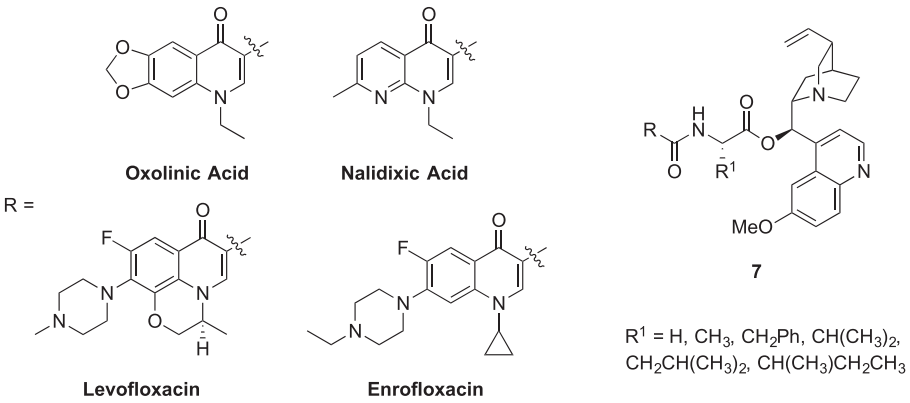


Fig. 9. QN-antibiotic-*bis*-conjugates.

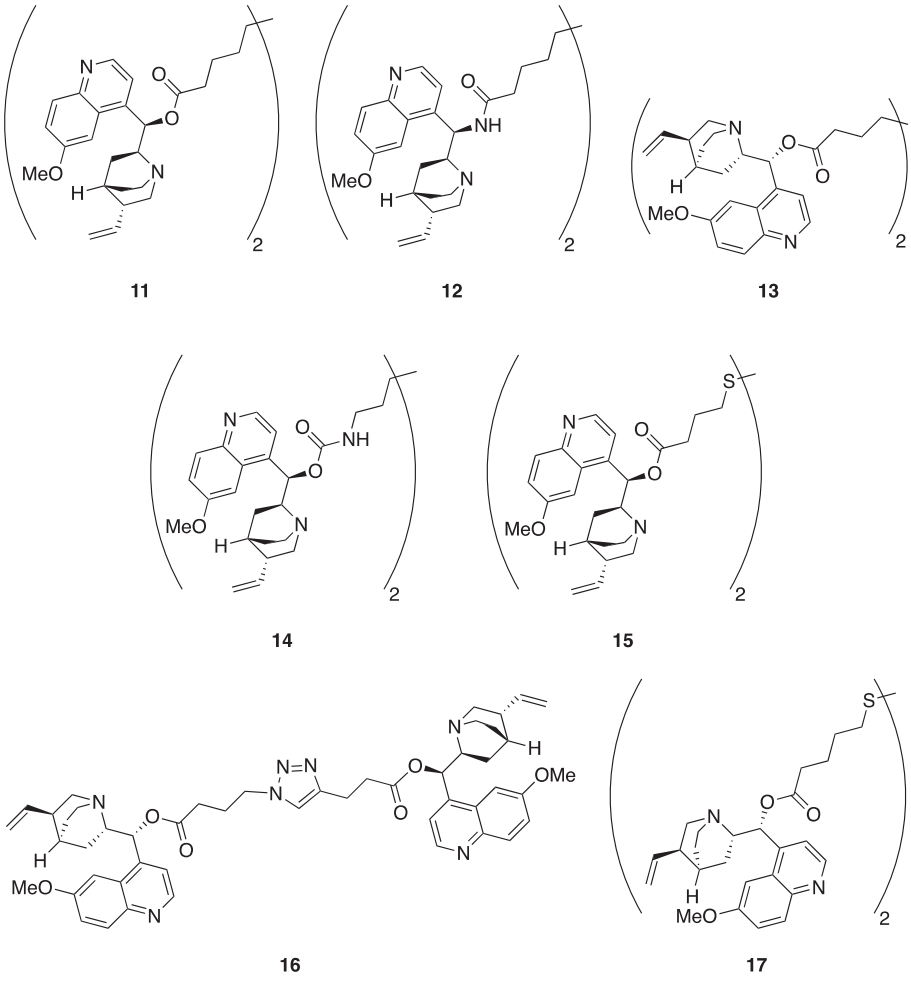


Fig. 11. *Bis*-quinine derivatives.

quinine derivatives (**5**) and investigated their activity towards two strains of *P. falciparum* (CQS/QNS strain HB3 and CQR strain Dd2) [28]. From Table 2 it can be seen that the majority of compounds displayed decreased activity towards *P. falciparum*, however, four compounds (**5g**, **5h**, **5j** and **5l**) with substituents either *para* or *meta* to the alkene were moderately active towards both strains. It is noteworthy that quinine-derivative **5g** was more active than QN against the CQR strain of *P. falciparum*. The Heck coupling was repeated with three further *Cinchona* alkaloids [cinchonidine (CD), QD and cinchonine (CN), (Fig. 7)], to determine whether simple alkene modifications would alter anti-malarial activity. In general QD and CN analogues were less active than QD and CN towards both strains of malaria. The *para*-alkene substituted CD derivatives however, showed comparable activity to CD against CQR strain Dd2 but lower activity against CQS strain HB3. Several of the compounds, both active and less active compounds, were tested for their ability to inhibit BH formation. All tested compounds were able to inhibit BH formation in a manner comparable to QN. This supports data by Alumasa et al. [12a] who proposed that the quinuclidine alkene does not play an important role in the formation of QN-heme complexes. Thus, although the vinyl group is a useful handle for modifying *Cinchona* alkaloids, and tolerant to a wide number of transformations, its inclusion does not appear to be advantageous in potential antimalarial drugs, except for the ART-QN hybrid.

2.3. Modifications at the Hydroxyl position

2.3.1. Amino acid and peptide conjugates of quinine

Amino acids and peptides are used as synthetic vectors due to their ability to act as drug carriers across cell membranes [30]. Drug-peptide conjugates have also been reported for multidrug resistance chemotherapy and for their ability to bind to specific receptors expressed in cancer treatments [31]. Furthermore, Gordon et al. reported amino acids such as L-alanine, L-phenylalanine, L-arginine, L-glutamic acid, and L-lysine may mask the bitter taste of quinine [32]. In the context of antimalarial drugs, it was demonstrated by Portela et al. that amino acid and peptide conjugates of PQ were sensitive to degradation by amino- and endopeptidases *in vitro* [33].

In this context, Panda et al. [34] synthesized 14 quinine-amino acid and quinine-peptide conjugates (Fig. 8) that showed moderate to good antimalarial activity against CQS 3D7 strain compared to quinine (Table 3) [34].

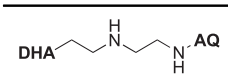
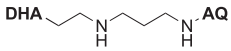
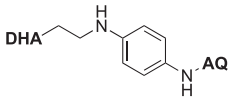
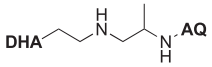
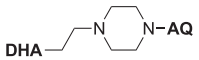
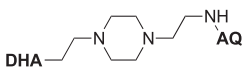
Quinolones and fluoroquinolones have been proposed as treatments for malaria since these drugs have *in vitro* antimalarial activity against CQS and CQR strains of *P. falciparum* [35]. Furthermore, in patients with severe malaria, considerable concomitant bacteremia, septicemia or localized bacterial infections have been observed [36].

Taking into account the importance of quinolones and fluoroquinolones as antibiotic agents, Panda et al. [37] synthesised a

Table 5
In vitro antiplasmodial activities of QN and compounds **11** and **16** against field-derived CQS and CQR strains of *P. falciparum*.

Compound	Parasite strain, IC_{50} (nM)			
	Dd2	FCB	P31	HB3
QN	187.7 ± 28.9	354.7 ± 17.9	535.3 ± 40.8	108.2 ± 11.7
11	132.6 ± 23.8	91.1 ± 5.6	186 ± 12.4	514.1 ± 43.1
16	49.2 ± 6.2	32.0 ± 5.4	232.1 ± 31.1	297.6 ± 30.1

Table 6
In vitro antiplasmodial activities of QN and compounds **17**–**22** against field-derived CQS and CQR strains of *P. falciparum*.

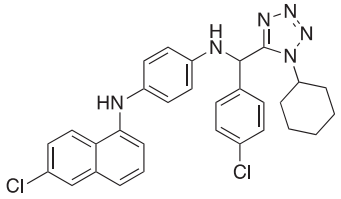
Structure	Compound#	Parasite strain, IC_{50} (nM)	
		D10 (SD)	Dd2 (SD)
	**17**	84.59 (2.22)	152.80 (2.06)
	17a	21.49 (0.13)	25.70 (1.09)
	**18**	117.76 (16.27)	183.49 (5.68)
	18a	14.27 (2.65)	19.75 (0.25)
	**19**	30.39 (2.71)	69.21 (2.02)
	19a	17.25 (1.06)	30.22 (12.22)
	**20**[a]	12.18 (1.21)	17.12 (0.44)
	20a	14.94 (0.05)	20.76 (3.61)
	**21**[a]	30.72 (1.85)	68.49 (4.19)
	**22**[a]	201.38 (2.70)	275.99 (70.52)
	22a	28.99 (2.70)	29.24 (3.26)
CQ ($n = 3$)		21.54 (6.73)	157.90 (52.70)
DHA ($n = 4$)		5.11 (0.64)	2.09 (0.33)

[a] Tested as a suspension. n = number of data sets averaged.

series of QN *bis*-conjugates (**7**) with amino acids linking the two bioactive moieties (Fig. 9). The compounds were tested against CQS strain 3D7 and compared to QN. All the synthesised compounds showed antimalarial activity in the nanomolar range; in particular, all *bis*-conjugates containing levofloxacin (**7g**–**i**) showed activity comparable to quinine, as well as *bis*-conjugates of oxolinic acid and nalidixic acid linked *via* alanine (**7b** and **7f**) and enrofloxacin *bis*-conjugate linked *via* a glycine residue (**7j**) (Table 4) [37].

2.3.2. Fluorinated derivatives of quinine

Bucher et al. generated a library of fluorinated quinine alkaloid salts that were tested for their ability to inhibit the growth of the NF54 strain of *P. falciparum* [38]. The fluorination step resulted in up to three products; substitution of the hydroxyl for fluorine with



25
IC_{50} (CQS strain 3D7) = 156.40 nM
IC_{50} (CQR strain K1) = 138.60 nM
BH inhibition; IC_{50} = 3.81 μg/mL

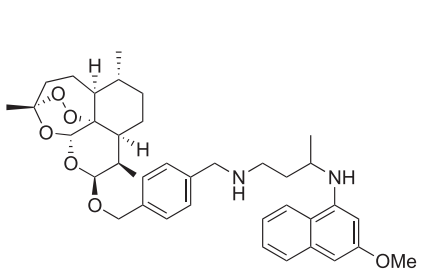
Fig. 13. Tetrazole-CQ conjugate **25**.

either retention (**8**) or inversion (**9**) of stereochemistry and the ring-expansion of the quinuclidine moiety to 1-azabicyclo [3.2.2] nonane **10**. This, however facilitated rapid access to 20 compounds from 6 quinine alkaloids (Fig. 10).

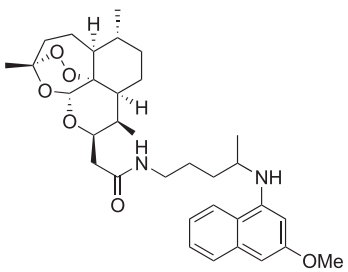
The fluorinated alkaloids were shown to be inhibitors in the nanomolar range, with dihydroquinidine-based inhibitor **8** displaying the highest activity. The antimalarial activity was heavily dependent on the stereochemistry at C-8 and whether or not the quinuclidine unit had ring-expanded. The stereochemistry at C-8 is important for activity; compounds that have an *R*-configuration (QD-like) showed greater levels of bioactivity when compared to the *S*-configured (QN-like) isomers [38]. Indeed, it has been demonstrated previously that QD has higher antimalarial activity relative to QN [15]. The ring-expanded products showed markedly lower antimalarial activity than those bearing the quinuclidine unit (*cf* **10** *vs* **8** and **9**), however all the products demonstrated lower activity than the parent alkaloid. Fluorinated *Cinchona* alkaloids have recently received further attention in terms of their applications in organocatalysis [39].

2.3.3. Bis-quinine derivatives

Piperaquine, a *bis*(4-aminoquinoline) was widely used in China, before resistance developed [40], but it is still used as an adjuvant in ACT [41]. It is thought that quinoline dimers may be more active against CQR strains of *P. falciparum* as the steric bulk in this type of molecule prevents them from fitting adequately into the substrate biding site of PfCRT [8e]. With respect to antimalarial activity, QN dimers have been less widely explored [42]; in 2014 however, Hrycyna et al. prepared seven QN dimers (Fig. 11), of which two (**11** and **16**), were shown to have greater activity against four different field-derived CQS and CQR strains of *P. falciparum* than quinine (Table 5) [43]. Furthermore, the quinine dimers appeared to have a different mode of action to CQ, in that **11** and **16** were not transported out of the parasite's DV by PfCRT, implying that *P. falciparum* may not be able to develop resistance to this type of compound. This is an important factor in the future design of antimalarial drugs.



23



24

Fig. 12. PQ-ART hybrids.

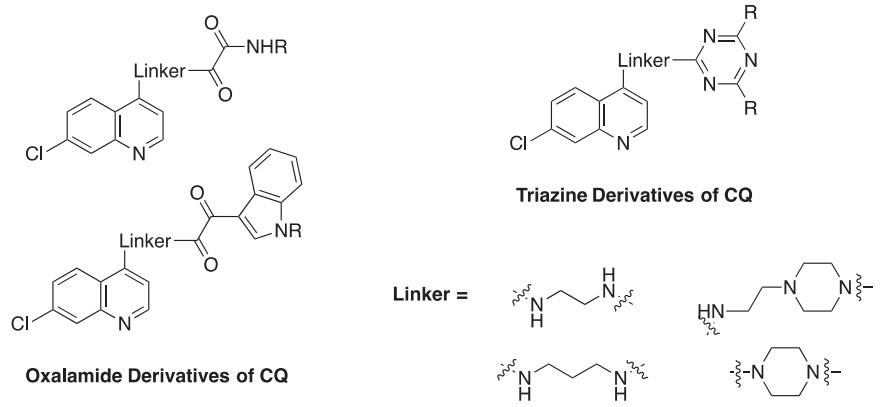
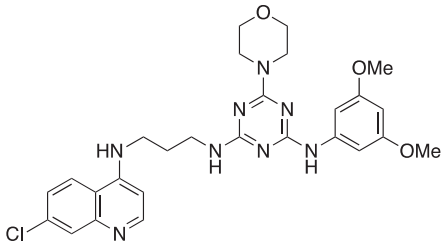
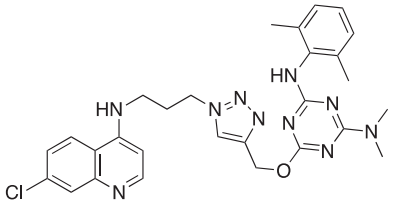


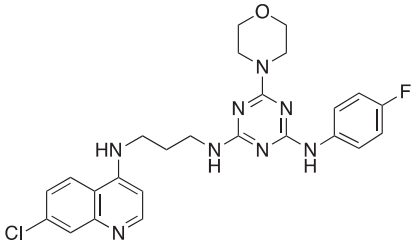
Fig. 14. Oxalamide and triazine derivatives of CQ.



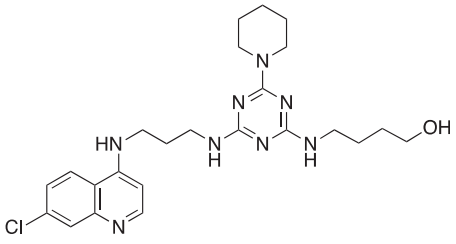
26
IC_{50} (CQS strain D6) = 0.25 μM
IC_{50} (CQR strain W2) = 0.22 μM



27
IC_{50} (CQS strain D6) = 0.58 μM
IC_{50} (CQR strain W2) = 0.73 μM



28
IC_{50} (CQS strain D6) = 0.24 μM
IC_{50} (CQR strain W2) = 0.11 μM



29
IC_{50} (CQS strain D6) = 0.10 μM
IC_{50} (CQR strain W2) = 0.28 μM

Fig. 15. Triazine derivatives of CQ.

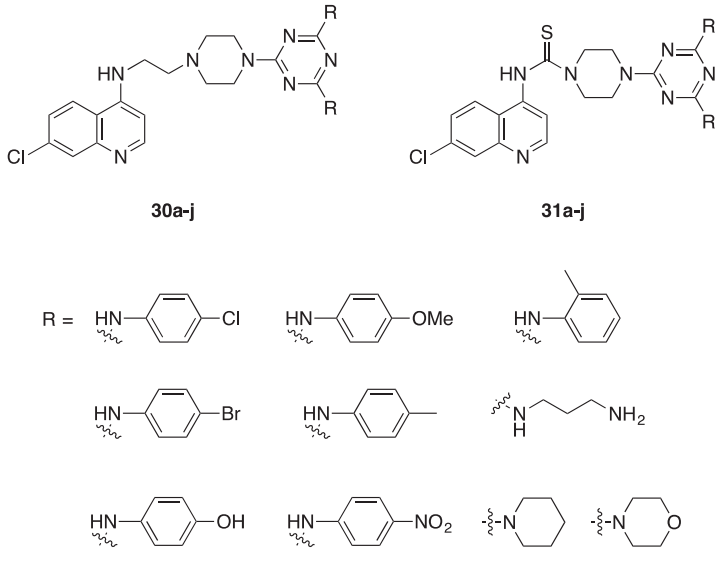
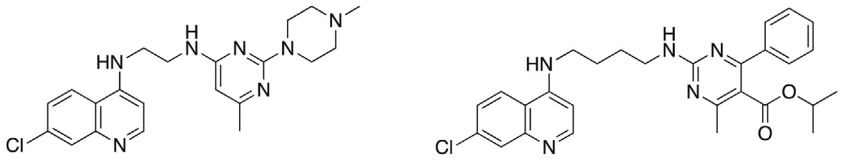
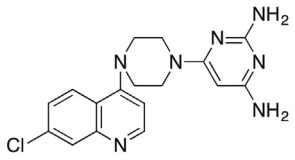


Fig. 16. Piperazine-containing derivatives of CQ.

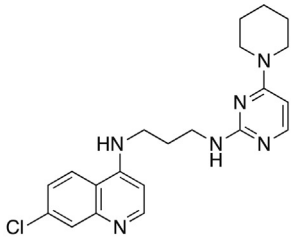


32
IC_{50} (CQS strain D6) = 5 nM
IC_{50} (CQR strain W2) = 30 nM

33
IC_{50} (CQS strain D10) = 156 nM
IC_{50} (CQR strain Dd2) = 153 nM

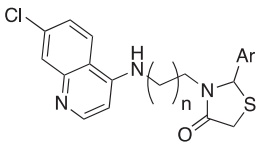


34
IC_{50} (CQS strain D10) = 70 nM
IC_{50} (CQR strain Dd2) = 157 nM



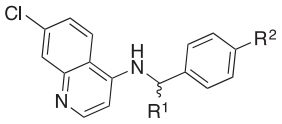
35
IC_{50} (CQS strain D10) = 23 nM
IC_{50} (CQR strain Dd2) = 43 nM

Fig. 17. Selected examples of pyrimidine-CQ conjugates.



36
n = 1-3

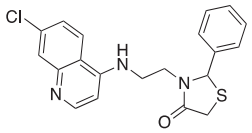
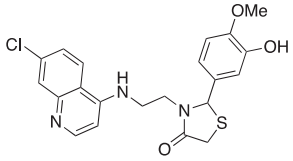
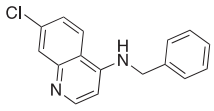
Ar = 2-Thie, 2-Fu, Ph,
3,4-$(MeO)_2$Ph, 3,4,5-$(MeO)_3$Ph,
4-HO-3-MeOPh, 4-MeO-3-HOPh



37
R^1 = H, CH_3
R^2 = H, OMe

Fig. 18. Heterocyclic hybrids based on thiazolidinone or benzylamino fragments.

Table 7
In vitro and *in vivo* antiplasmodial activities of CQ and compounds **36a**–**b** and **37a**.

Compound		Parasite strain, IC_{50} (μM)		% Inhibition of parasite growth murine model
		3D7 (±SD)	Dd2 (±SD)	
CQ		0.027 (0.004)	0.50 (0.03)	100
	**36a**	0.36 (0.02)	0.75 (0.05)	80
	**36b**	0.40 (0.03)	0.54 (0.03)	100
	**37a**	0.30 (0.04)	0.33 (0.05)	25

3. Quinine analogues based on the quinoline scaffold

When designing novel drugs based on the quinine scaffold, it has been observed that the quinuclidine moiety is not always necessary for the observed antimalarial properties. This has resulted in the previously described design and synthesis of quinine analogues with alterations at the vinyl functionality. However, changes to the quinuclidine moiety can often result in a reduction of antimalarial activity observed in the compound. In general, therefore, the bicyclic motif is removed and the quinoline scaffold is used as the basis for the design of novel antimalarial drugs. Modifications of the quinoline moiety of quinine resulted in 4-aminoquinolines (4-AQ) such as CQ and 8-aminoquinolines (8-AQ) including PQ. Unfortunately, *P. falciparum*, the most deadly form of malaria, has developed resistance towards CQ. Despite this, the quinoline moiety remains an attractive scaffold for the design and synthesis of new antimalarial drugs [8e], due to its ease of use, limited host toxicity, its cost effective synthesis and its excellent clinical efficiency [11d]. In particular, drug hybrids of quinoline containing compounds with biologically active heterocycles or known drugs have led to the creation of small molecules with promising antimalarial activity [8d].

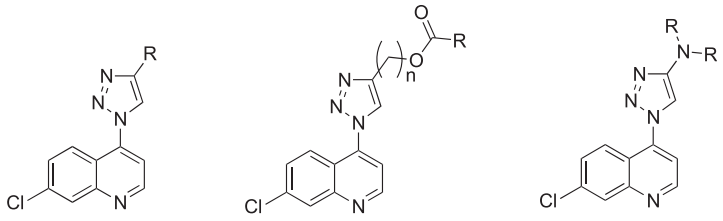


Fig. 19. Modification of CQ with triazoles.

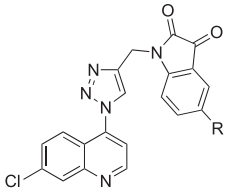
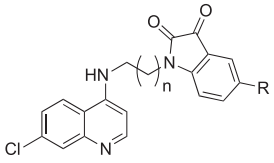
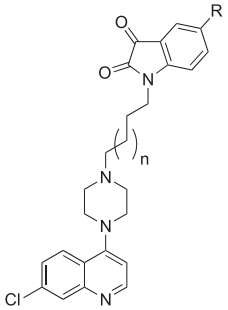
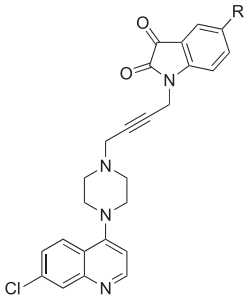
3.1. Artemisinin-quinoline hybrids

The ART-QN hybrid was shown to be an attractive molecule in terms of its enhanced antimalarial activities relative to the two component drugs [27]. In the context of ART derivatives, Lombard et al. [44] and Capela et al. [45] have recently prepared synthetic hybrids of DHA and 4-AQ or ART and primaquine. Lombard et al. [44a] synthesised six DHA-AQ hybrids, and tested the *in vitro* antimalarial activity of both the free amine (**17**–**22**) and the oxalate salts (**17a**–**22a**) (Table 6).

Compounds **17a**, **18a**, **20**, **20a** and **22a** were found to possess the highest activity showing comparable activity to CQ against CQS strain D10 and greater activity than CQ against CQR strain Dd2. Unfortunately, none of the compounds were as potent as DHA, however further testing was carried out using the most active compounds with the aim of determining how the drug hybrids act in the body. To that end, hybrids **17a**, **18a** and **22a** were tested *in vivo* against *Plasmodium vinckei* and it was found that although compound **18a** was the most potent (ED_{50} = <0.8 mg/kg), the survival rate dropped from 100% to 66%, therefore Lombard et al. determined that compounds **17a** and **22a** (ED_{50} = 1.1 and 1.4 mg/kg respectively) were more effective for curing malaria in mice [44b]. The only difference between compounds **17a**, **22a** and **18a** was the presence of an additional carbon atom in the linker between the two hybrids, thus demonstrating the importance in careful choice of linker when designing hybrids drugs.

Capela et al. [45] synthesised two primaquine-artemisinin hybrids **23** and **24** (Fig. 12) (IC_{50} = 12.5 ± 1.1 and 9.1 ± 0.6 nM respectively) that showed superior antimalarial activity to primaquine (IC_{50} = 3330 ± 55 nM) against CQR strain W2 and comparable activity to artemisinin (IC_{50} = 8.2 ± 0.9 nM).

Table 8
In vitro antiplasmodial activities of CQ-isatin conjugates against CQR strain W2 of *P. falciparum*.

Structure	Compound#	R	n	IC_{50} (μM)
	**41a**	H	*n/a*	>5
	41b	F	*n/a*	>5
	41c	Cl	*n/a*	>5
	41d	Br	*n/a*	>5
	41e	Me	*n/a*	>5
	**42a**	H	1	3.07
	42b	F	1	2.30
	42c	Cl	1	1.37
	42d	Br	1	1.73
	42e	Me	1	1.63
	42f	H	2	3.85
	42g	F	2	1.35
	42h	Cl	2	1.21
	42i	Br	2	1.66
	42j	Me	2	1.30
	**43a**	H	1	1.12
	43b	F	1	1.17
	43c	Cl	1	0.27
	43d	Me	1	0.81
	43e	H	2	0.46
	43f	F	2	0.27
	43g	Cl	2	0.42
	43h	Me	2	0.25
	43i	H	3	0.54
	43j	F	3	0.22
	43k	Cl	3	0.92
	43l	Me	3	0.62
	43m	H	5	1.39
	43n	F	5	0.90
	43o	Cl	5	3.79
	43p	Me	5	7.52
	**44a**	H	*n/a*	1.01
	44b	F	*n/a*	0.58
	44c	Cl	*n/a*	0.42
	44d	Me	*n/a*	1.12

3.2. Chloroquine analogues

Since 2009, a large number of publications related to the modification of CQ have been published [8d–f]. Heterocycles play an important role in drug design and discovery [46] and the modification of CQ with heterocyclic moieties is particularly attractive as heterocycles are able to impart beneficial properties to the final compound. In particular, the AQ moiety is present to facilitate inhibition of BH formation and the heterocycle might have improved lipophilicity or heme-binding properties. Furthermore, the linker between the two moieties can have a dramatic effect on the antimalarial properties of the target compound. As CQ analogues have received a great deal of attention in the literature, this is not intended to be an exhaustive review of all CQ-conjugates; rather there is a focus on CQ-heterocyclic conjugates, particularly those containing 5-membered rings, and CQ-drug conjugates.

3.2.1. Heterocyclic derivatives of Chloroquine

The tetrazole ring is able to coordinate with the iron centre in heme and with this in mind, Pandey et al. prepared a series CQ-tetrazole conjugates, which showed promising *in vitro* antimalarial activity against CQS strain 3D7 and CQR K1 [47]. The CQ-tetrazole conjugates were further tested for their ability to inhibit BH formation and although compound **25** showed moderate *in vitro* activity, its ability to inhibit BH formation was comparable with CQ (3.95 μg/mL) (Fig. 13).

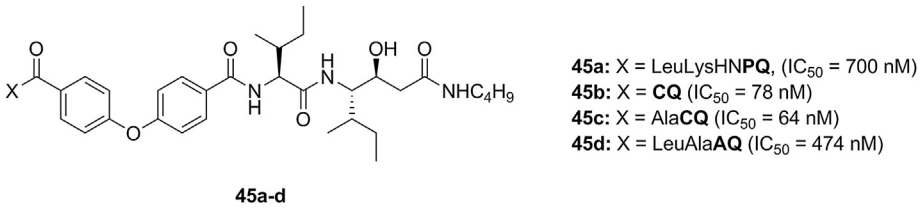


Fig. 20. AQ-Statine Compounds (IC_{50} values are given for CQR strain W2).

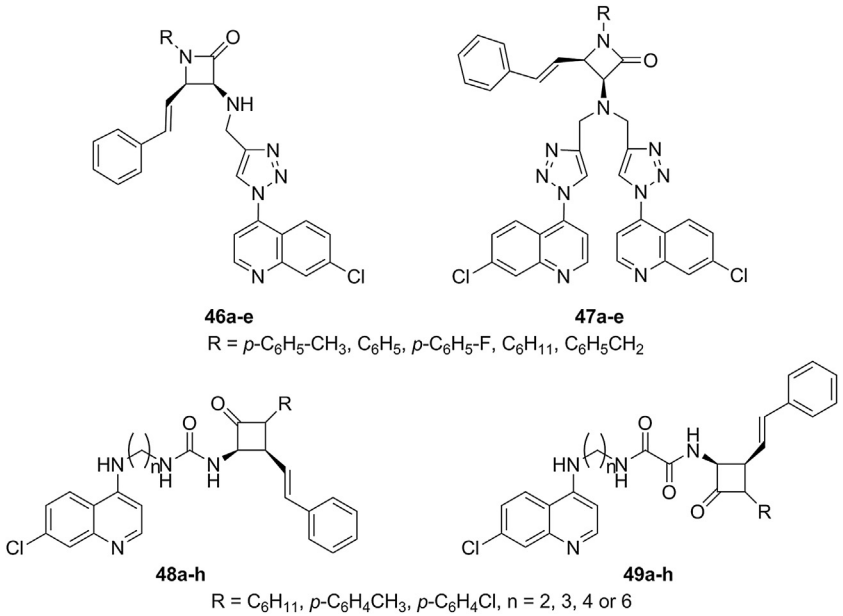


Fig. 21. β-lactam-CQ conjugates.

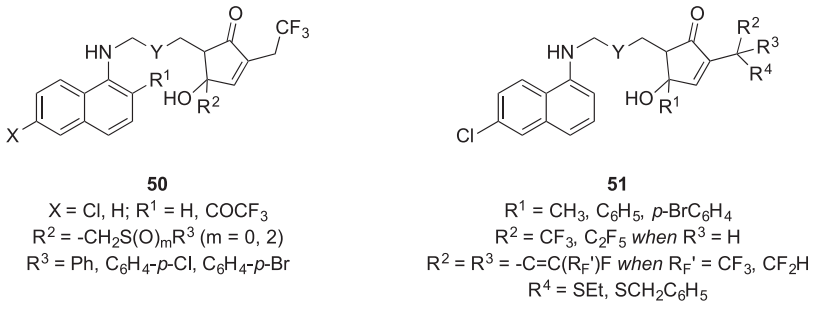


Fig. 22. General structure of γ-lactam-AQ conjugates.

Table 9
In vitro data of selected γ-lactam-AQ conjugates (**50**).

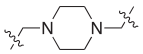
Compound#	X	R^1	R^2	Y	Parasite strain, IC_{50} (nM)	
					3D7	W2
CQ	–	–	–	–	30	750
50a	Cl	H	-CH_2SPh	-CH_2-	107	110
50b	Cl	H	-CH_2SPh	-$(CH_2)_2$-	273	391
50c	Cl	H	-CH_2SPh	-$(CH_2)_4$-	335	395
50d	Cl	H	-CH_2SPh	-CH_2NMeCH_2-	47	55
50e	Cl	H	-CH_2SPh	-CH_2NHCH_2-	89	211
50f	Cl	H	-CH_2SPh		43	51
50g	H	$COCF_3$	-$CH_2SO_2C_6H_4$-*p*-Cl	-$(CH_2)_4$-	>1000	>1000

Table 10
In vitro data of selected γ-lactam-AQ conjugates (**51**).

Compound#	R^1	R^2	R^3	R^4	Y	Parasite strain, IC_{50} (nM)	
						3D7	W2
CQ	–	–	–	–	–	30	750
51a	C_6H_5	CF_3	H	SCH_2H_5	-CH_2-	250	290
51b	C_6H_5	CF_3	H	SCH_2H_5	-$(CH_2)_4$-	326	306
51c	CH_3	CF_3	H	SCH_2H_5	-CH_2NMeCH_2-	56	73
51d	CH_3	CF_3	H	SCH_2H_5	-CH_2NHCH_2-	58	496
51e	*p*-BrC_6H_4	CH_3	H	SCH_2H_5	-CH_2NMeCH_2-	58	74

Table 11
In vitro antiplasmodial activities of CQ-squaric acid conjugates against CQR strain W2 of *P. falciparum*.

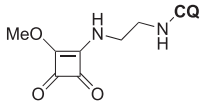
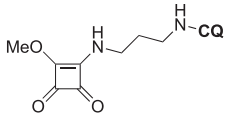
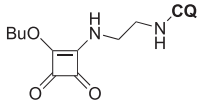
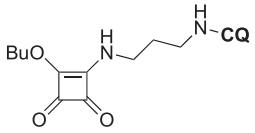
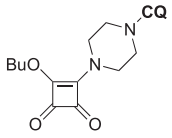
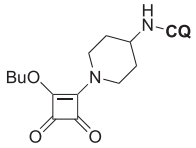
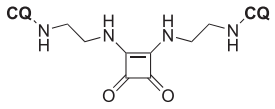
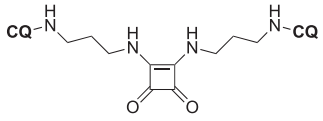
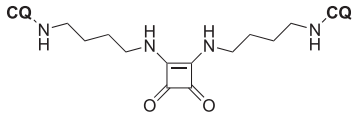
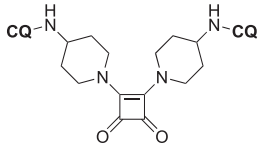
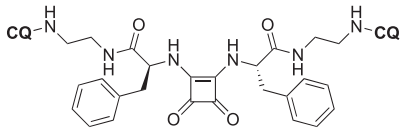
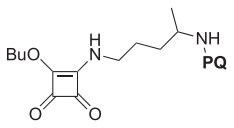
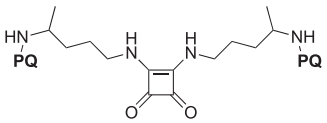
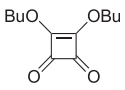
Compound	Structure	W2 IC_{50} (μM)
CQ	–	0.14
52a		1.56
52b		1.84
52c		2.04
52d		>10
52e		7.82
52f		4.71
53a		0.20
53b		0.099
53c		0.095
53d		0.105
53e		0.71
54a		18.59

Table 11 (*continued*)

Compound	Structure	W2 IC_{50} (μM)
54b		2.21
55		8.84
1:1 CQ/55		0.138
2:1 CQ/55		0.091

Sunduru et al. investigated the antimalarial properties of oxalamide and triazine derivatives of CQ. It was envisaged that the oxalamide would impart hydrogen-bonding abilities to the target compound. It was found that although triazine-CQ derivatives were more active *in vitro* than oxalamide-CQ derivatives, studies *in vivo* showed the triazine-CQ conjugates to be toxic (Fig. 14) [48]. Oxalamide-CQ derivatives bearing an indole group showed no antimalarial activity; substituting the oxalamide linker for an alkene restored antimalarial activity [49].

Manohar et al. have also investigated the antimalarial activity of triazine CQ analogues (**26**–**29**) and in this instance their non-toxic analogues showed antimalarial activity against CQS and CQR strains of *P. falciparum* (Fig. 15) [50]. IC_{50} values of the most active compound from each series have been included and it can be seen that the addition of a triazole linker (**27**) between CQ and the triazine resulted in a decrease in activity relative to both CQ and to triazine derivatives of CQ [50b]. Furthermore, changing the 'R' groups on the triazine ring can have dramatic effects on antimalarial activity. For example 4-fluoroaniline groups (**28**) imparted enhanced antimalarial activity against CQR strain W2, where as amino alcohols, bearing a terminal alcohol group (**29**), whilst active against both strains of *P. falciparum* were more active against CQS strain D6. It is noteworthy that in the latest study, the antimalarial activity of the triazine-CQ conjugates was comparable to CQ (CQS strain D6: IC_{50} = 0.05 μM; CQR strain W2: IC_{50} = 0.43 μM) [50c].

Bhat et al. prepared an alternative class of CQ-triazine conjugates in which the triazine moiety was linked *via* piperazine and subsequently to CQ *via* either an alkyl chain (**30a**–**j**) or a thiourea (**31a**–**j**) (Fig. 16). In both cases, only moderate antimalarial activity was observed, however docking studies suggest that the ability of the drug to form hydrogen bonds can be used in the future to predict the activity of potential target molecules [51].

Pyrimidine-derivatives of CQ (**32**–**35**) have also been investigated for enhanced antimalarial activity (Fig. 17) [52]. In general pyrimidine-CQ conjugates exhibited *in vitro* antimalarial activity in the nanomolar range, suggesting that incorporation of this heterocycle is beneficial when designing antimalarial agents. Compound **32** was tested *in vivo* in the *Plasmodium berghei* murine malaria model and cured 80% of treated mice over a twenty eight day period [52a]. Singh et al. conducted a structure-activity relationship study of 18 CQ derivatives; different linkers between pyrimidine and CQ were investigated in addition to different substituents on the pyrimidine heterocycle. Compound **35** was found to be the most active against both CQS and CQR strains of *P. falciparum*. Furthermore, compound **35** displayed the ability to inhibition BH formation [52d].

Thiazolidin-4-ones are rigid, nitrogen containing heterocycles that are considered to be a biologically privileged structure as they are well tolerated in human subjects [53]. With this in mind, Roja Ruiz et al. synthesised twenty one chloroquine-heterocyclic hybrids

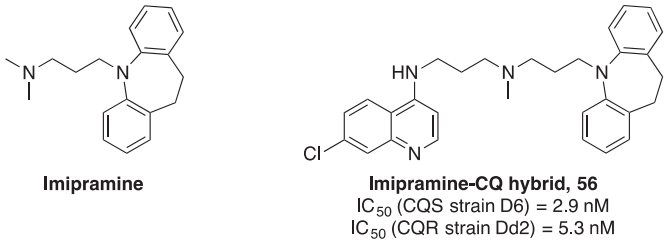


Fig. 23. Imipramine and imipramine-CQ conjugate **56**.

Table 12
In vitro antiplasmodial activity against CQR *P. falciparum* K1 strain and *in vivo* antiplasmodial activity in *P. berghei* mouse model.

Compound Number	IC_{50} (μM)	Dose (mg/kg)	Mean survival time (days)	% Activity
CQ	0.23	4 × 10	17	99
57	0.61	N.D.	N.D.	N.D.
58	0.023	N.D.	N.D.	N.D.
59	0.064	4 × 50	13	99
60	0.037	4 × 20	8	80

in which the heterocyclic moiety was either an *N*-(aminoalkyl) thiazolidin-4-one (**36**) or a benzylamino (**37**) fragment (Fig. 18) [54].

The compounds were tested for antimalarial activity against CQS 3D7 and CQR Dd2 strains of *P. falciparum*. None of the compounds showed enhanced activity when compared to CQ against the CQS strain, but against the CQR strain compound **36a** was more active than CQ and compound **36b** showed comparable activity to CQ. In general the benzylamino-derived compounds were more active *in vitro*, however *in vivo* this was not the case. Compounds **36a**, **36b** and benzylaminoquinoline **37a** were tested *in vivo* against *P. berghei* (Table 7) and compounds **36a** and **36b** inhibited parasite growth in mice by 80% and 100% whereas compound **37a** achieved only 25% inhibition, suggesting that, in this instance, the *N*-(aminoalkyl) thiazolidin-4-one moiety is responsible for the enhanced antimalarial activity.

3.2.2. Chloroquine conjugates containing triazole linkers

Triazole motifs have been introduced into CQ derivatives, as they are stable under *in vivo* conditions in addition to their other highly favoured properties including a moderate dipole character, hydrogen bonding ability and rigidity [55].

Pereira et al. prepared a library of twenty-seven 7-chloroquinolinotriazoles (**38**–**40**), however CQ remained the most active compound (Fig. 19) [56]. In this instance, the decreased activity was attributed to a decrease in the pKa of the quinoline nitrogen; furthermore, the direct attachment of the triazole moiety to CQ may have a detrimental effect on antimalarial activity.

Raj et al. prepared 7-chloroquine-isatin conjugates **41** and **42** as it was envisaged that the π-stacking abilities of isatins might impart beneficial properties to the target compound. Compounds **41a-e** and **42a-j** were tested against CQR strain W2 but relative to CQ (IC_{50} = 0.099 μM) and ART (IC_{50} = 0.014 μM) they showed lower antimalarial activity (Table 8) [57]. The direct attachment of triazole to CQ was more detrimental to antimalarial properties than when the triazole was attached *via* a two or three carbon alkyl chain. Substituting the triazole moiety for an alkyl (**43**) or alkyne (**44**) linker showed an improvement in activity, however this was still lower relative to CQ (Table 8) [58].

3.2.3. Chloroquine conjugates of peptides and lactams

The conjugation of statine to CQ *via* peptide linkers resulted in a series of compounds that were tested for antimalarial activity against CQS and CQR strains of *P. falciparum* (Fig. 20) [89]. Compounds **45b**–**d** displayed greater antimalarial activity against the CQR strain *vs* the CQS strain, whereas compound **45a**, which did not contain the CQ moiety showed reduced activity against both strains. It is noteworthy that compound **45d**, in which the 7-Cl is absent, shows reduced antimalarial activity, thus indicating the importance of this atom when designing antimalarial agents.

Singh et al. were able to prepare bifunctional hybrids of β-lactam and CQ *via* 'Click' chemistry (Fig. 21), but neither the mono- (**46**) nor the *bis*- (**47**) substituted lactam were able to impart enhanced antimalarial activity [59]. The antimalarial activity of the β-lactams was subsequently enhanced by the use of either a urea (**48**) or an oxalamide (**49**) linker (Fig. 21) [60]. The activity of the β-lactam-CQ conjugate was heavily dependent of the length of the

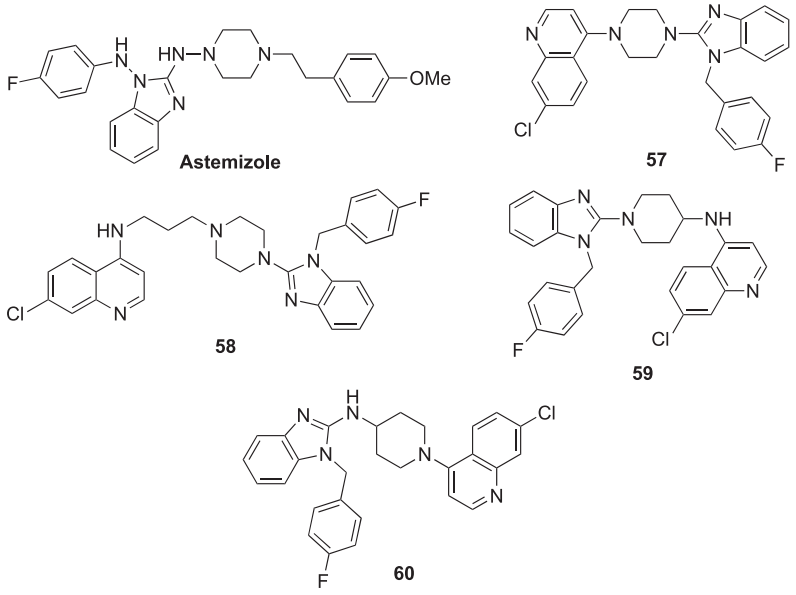
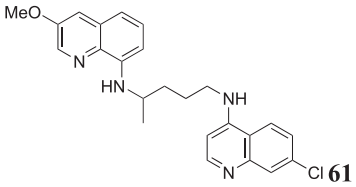


Fig. 24. Astemizole-CQ hybrids.

Table 13
Antiplasmodial activity of hybrid **61** on the asexual blood stages of three *P. falciparum* strains[a] and gametocytogenesis inhibition assay in *P. falciparum* strain NF54.[b]

Compound	IC_{50} (μM)			Relative gametocytemia (%)
	3D7	Dd2	K1	
PQ	3.11 ± 1.536	1.12 ± 0.351	0.46 ± 0.08	0.2[c] ± 0.05
CQ	0.03 ± 0.002	0.26 ± 0.126	0.146 ± 0.02	0.9 ± 0.07
 **61**	0.64 ± 0.046	0.58 ± 0.185	0.08 ± 0.0048	0.7[c] ± 0.04
PQ + CQ	0.03 ± 0.012	0.19 ± 0.035	0.0169 ± 0.055	n/a
DMSO Control	n/a	n/a	n/a	1 ± 0.19

[a] Infected human red blood cells were incubated with serial compound dilutions for a total exposure time of 72 h.
[b] NF54 gametocytes were incubated with hybrid **3** for 48 h. After 7-days of compound-free cultivation gametocytemia was determined.
[c] $p \leq 0.05$ (Student's *t* test). DMSO control set to 1.

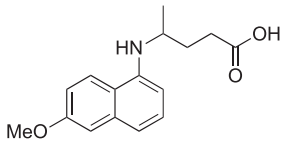
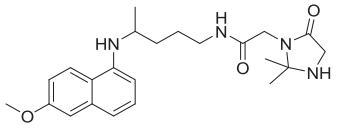
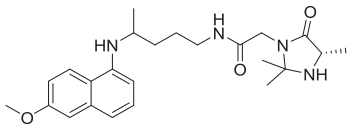
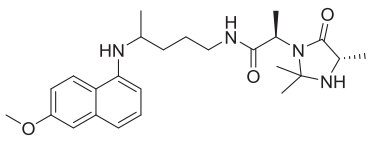

62

Fig. 25. Carboxyprimaquine, a metabolite of PQ.

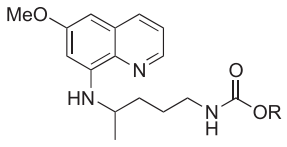
linker with a 6-carbon alkyl chain showing improved activity *vs* shorter chains. In addition, the oxalamide-tethered compounds demonstrated enhanced activity over the urea-tethered compounds. Non-cytotoxic compound **49d** (R = C_6H_{11}, n = 6), considered a good hit, had an IC_{50} of 34.97 nM, lower than CQ (IC_{50} = 59.09 nM) and comparable to ART (IC_{50} = 10.63 nM) and QN (IC_{50} = 18.67 nM).

Cornut et al. synthesised two series of fluoroalkylated γ-lactam-AQ conjugates (**50** and **51**, Fig. 22) and tested their antimalarial activity against CQS strain 3D7 and CQR strain W2 of *P. falciparum* (*see* Tables 9 and 10 *for selected data*) [61]. In general, compounds from series **51** displayed greater antimalarial activity than compounds from series **50**. It is noteworthy that Cournet et al. chose to study the effect that the linker between the AQ and the γ-lactam had on the antimalarial activity of the final compounds. Thus a three-carbon spacer was better than a four- or six-carbon spacer (**50a**–**c** and **51a**–**b**) and the introduction of a basic nitrogen (–NMe, –NH and piperazine) into the linker also had a beneficial effect on antimalarial activity (**50d**–**f** and **51c**–**e**). This analysis corroborates previous studies that demonstrated the importance of the linker and basic nitrogen, capable of intramolecular hydrogen bonding, when designing antimalarials [62]. However, the presence of both a basic nitrogen and a sulfonyl group resulted in decreased activity towards the CQR W2 strain (**51d**). Finally, compounds bearing a trifluoroacetyl moiety (**50g**) showed no discernable antimalarial activity [62].

Table 14
Effect of imidazolidin-4-ones and PQ on the sporogonic development of *P. berghei* ANKA in *A. stephensi* mosquitoes. Counting of oocysts was carried out at day 10 post-feed.

Compound	Dose (μmol kg^{-1})	Mean no. of oocysts per mosquitoes, ±SEM[a]	% Of infected mosquitoes
PQ	10	12.2 (2.9)	14.1
	50	0.2 (0.7)	0.2
 **63**	10	4.8 (1.3)	5.6
	50	3.8 (1.3)	4.4
 **64**	10	81.3 (3.5)	94.2
	50	2.1 (0.2)	2.4
 **65**	10	1.3 (0.2)	1.5
	50	0.05 (0.1)	0.06
Control	0	86.7 (4.0)	100

[a] Mean standard error.



66a-h

Fig. 26. PQ carbamate derivatives.

Table 15
Half-lives, $t_{1/2}$ for non-enzymatic hydrolysis of PQ-carbmates **66a**–**h** at pH 7.4 and the effect of PQ-carbamates **66a**–**e** and PQ on the sporogonic development of *P. berghei* in *A. stephensi* mosquitoes.

Compound	R	pH 7.4 buffer $t_{1/2}$ (d)	% Of infected mosquitoes	Mean no. oocysts per mosquito (±SE)[a]
PQ	–	n/a[b]	3.9	1.6 (1.3)
Controls	–	n/a	91	58.8 (8.9)
66a	Et	NR[c]	19	14.8 (6.8)
66b	$(CH_2)_5Me$	NR	24	24.6 (5.4)
66c	CH_2CF_3	NR	72	41.0 (5.5)[f]
Controls	–	n/a	80	46.1 (9.1)
66d	C_6H_4-4-MeO	>30	74	39.7 (5.5)[f]
66e	C_6H_5	12	65	33.4 (8.9)
66f	C_6H_4-4-Cl	4	ND[e]	ND
66g	C_6H_4-4-NO_2	2.1 min	ND	ND
66h	CH_2CO_2Et	10[d]	ND	ND

[a] Counting of oocysts was carried out at day 10 post-feed.
[b] n/a – not applicable.
[c] NR – no reaction detected.
[d] Ester hydrolysis.
[e] ND - not determined.
[f] $p > 0.05$ versus control by Student's *t*-test.

3.2.4. Bis-conjugates of Chloroquine

Squaric acid derivatives have demonstrated antiplasmodial activities; thus Ribeiro et al. [63] prepared squaric acid-AQ conjugates and tested their antimalarial activity against *P. falciparum* strain W2 (Table 11). Clear trends emerged during this investigation, particularly with regard to the linker. Thus, in general, alkyl linkers imparted higher activity than rigid linkers (compounds **52b** and **52c** *vs* compounds **52d** and **52e**), *bis*-linked squaric acids (**53a**–**e**) showed higher antiplasmodial activity than *mono*-substituted

Table 16
In vitro antimalarial activity (*P. falciparum*) and BH inhibition of 8-AQs.

Compound	R	R^1	R^2	IC_{50} (μg mL^{-1})		BH inhibition IC_{50} (μM)
				D6	W2	
PQ				2.0	2.8	>1000
68a	H	H	H	0.57	0.80	18.5
68b	H	H	$C(CH_3)_3$	0.19	0.12	7.5
68c	OC_4H_9	C_2H_5	H	0.60	0.37	10.2
68d	OC_5H_{11}	C_2H_5	H	0.52	0.18	9.6
68e	OC_8H_{17}	C_2H_5	H	0.92	0.82	15.7
69a	H	H	H	2.6	1.5	75
69b	H	H	$C(CH_3)_3$	NA	NA	>1000
69c	OC_4H_9	C_2H_5	H	0.54	0.58	19.7
69d	OC_5H_{11}	C_2H_5	H	0.57	0.48	20.8
70a	H	H	H	2.3	1.3	75
70b	H	H	$C(CH_3)_3$	0.70	0.56	10.2
70c	OC_4H_9	C_2H_5	H	0.49	0.35	10.7
70d	OC_5H_{11}	C_2H_5	H	0.44	0.42	11.2

NA, not active, '-,' not tested.

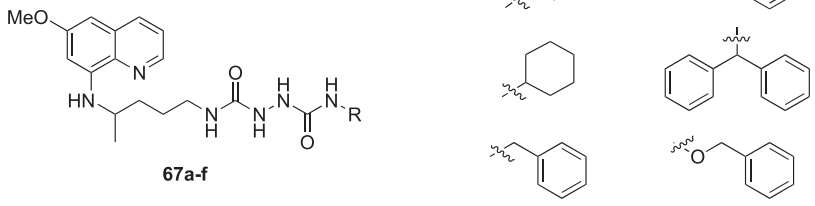
derivatives and substituting the CQ moiety for PQ (**54a**–**b**) resulted in a decrease in activity. It is also interesting that squaric acid **55** in combination with CQ imparted antimalarial activity on a comparable level to the conjugate.

3.2.5. Drug hybrids of Chloroquine

In addition to heterocyclic analogues of CQ and ART-CQ derivatives, drug hybrids of CQ with current prescription drugs have been investigated. For example, imipramine (Fig. 23), a tricyclic heterocycle used to treat depression, can reverse resistance in *P falciparum* when it is taken in combination with CQ [64]. Conjugation of imipramine to CQ afforded hybrid **56** that was more active than CQ against both CQS and CQR strains of *P. falciparum*.

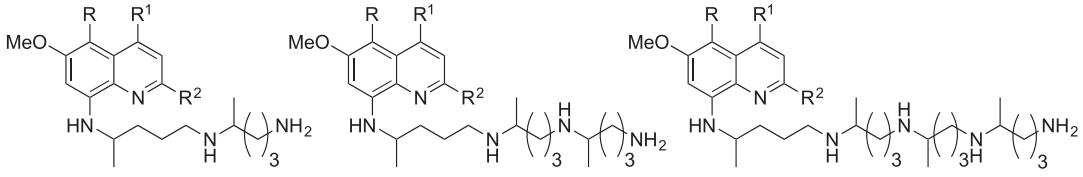
More recently, Chong et al. identified astemizole, an antihistamine drug with a long mode of action, was identified as a potential antimalarial agent (Fig. 24) [65]. This inspired Musonda et al. to design and synthesis four CQ-astemizole hybrids (**57**–**60**), three of which showed improved antimalarial activity relative to CQ (Table 12) [66].

Lödige et al. prepared PQ-CQ hybrid **61** (Table 13) that showed good activity against *P. falciparum* CQR strain K1 and that had a



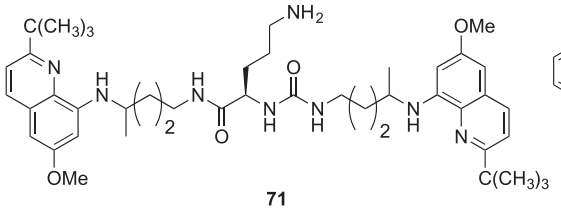
67a-f

Fig. 27. PQ semicarbazide derivatives.

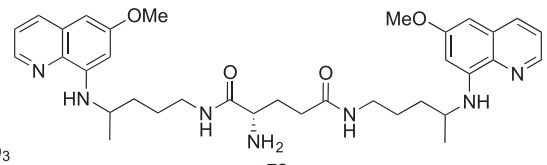


68a-e **69a-d** **70a-d**

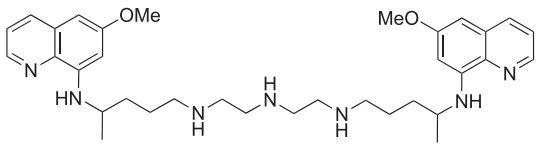
Fig. 28. PQ derivatives bearing longer alkyl chains.



71
(D6: IC_{50} = 0.34 □M)
(W2: IC_{50} = 0.3 □M)

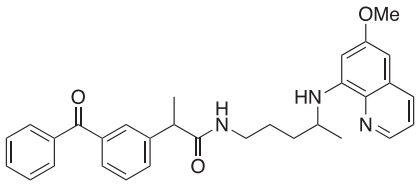


72
(D6: IC_{50} = 2.7 □M)
(W2: IC_{50} = 2.2 □M)

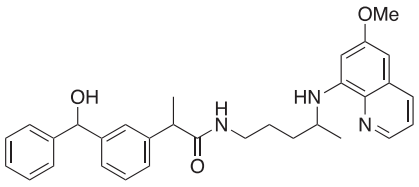


73
(D6: IC_{50} = 2.6 □M)
(W2: IC_{50} = 1.5 □M)

Fig. 29. *Bis*(4-aminoquinolines) that displayed 'curative' *in vivo* antimalarial activity.



74a



74b

Fig. 30. Ketoprofen-PQ conjugates.

modest gametocytocidal effect. Furthermore, hybrid **66** was demonstrated to be active against the asexual blood stages of *P. berghei ANKA in vivo* [67].

3.3. Analogues of primaquine

Primaquine is currently the only known clinically available drug that is active against relapsing malaria caused by both *P. vivax* and *P. ovale* [68]. Its overall effectiveness is however tempered by its short plasma half-life (~6 h) caused by oxidative deamination to carboxyprimaquine (**62**) [69] and high levels of blood toxicity, particularly in patients with 6-phosphate dehydrogenase deficiency (Fig. 25).

The toxicological concerns regarding primaquine not withstanding, due to its ability to block the transmission of malaria it remains an important drug in the worldwide fight against malaria [40,70]. To that end various studies have been initiated to design compounds based on the primaquine scaffold with enhanced pharmacological properties. Masking the terminal amine can prevent oxidative deamination however alternative metabolic pathways emerge. For example, studies by Portela et al. showed that the *N*-acylation of PQ by amino acids or peptides resulted in compounds that were not vulnerable to oxidative deamination, but were sensitive to degradation by amino- and endopeptidases [33].

In order to protect the N-terminal residue against enzymatic induced hydrolysis Vale et al. introduced imidazolin-4-one motifs [71], and prepared a series of PQ-dipeptide derivatives bearing an N-terminal imidazolidin-4-one moiety (**63**–**65**). To investigate the stability of the synthesised compounds, they were hydrolyzed to the parent dipeptide derivative of primaquine in neutral and basic solutions with half-lives ranging from 0.7 to 31 h at 37 °C. The half-life was dependant on both the nature of the substituent present on the imidazolidin-4-one moiety and that on the C-terminus of the amino acid directly coupled to PQ. The PQ-dipeptide conjugates are capable of reducing malaria transmission rates from mosquitos as effectively as PQ, and this was demonstrated by measuring the effect the PQ-conjugates had on the sporogonic development of *P. berghei* in *A. stephensi* mosquitos (Table 14). In particular, compounds **63** and **65** showed comparable numbers of oocysts to PQ and a similar percentage of infected mosquitoes.

Mata et al. developed an alternative pro-drug approach to PQ, by preparing carbamate derivatives of PQ (**66a**–**h**, Fig. 26) [72].

The reactivity of the PQ-carbamate conjugates was investigated in pH 7.4 phosphate buffer at 37 °C and it was observed that *O*-alkyl carbamates were stable, *O*-aryl carbamates hydrolysed very slowly and 4-nitrophenol carbamates (**66g**) hydrolysed within 2 min (Table 15). Compounds **66a**–**e** were then tested for their ability to prevent transmission of malaria, by their effect on the sporogonic development of *P. berghei* in *A. stephensi* mosquitos. All the compounds were less active than PQ, however compounds **66a** and **66b** reduced the percentage of infected mosquitos and the production of oocysts when compared with the controls. It is thought that the gametocytocidal activity of the PQ-carbmate conjugates is not related to release of PQ by carbamate hydrolysis, rather that the compounds have inherent activity.

Perković et al. changed the linker from carbamate to semicarbazide (**67a**–**f**, Fig. 27), however they did not report the antimalarial properties of their compounds [73].

It has been demonstrated that 8-AQs are able to inhibit β-hematin formation; this has important implications for the design of

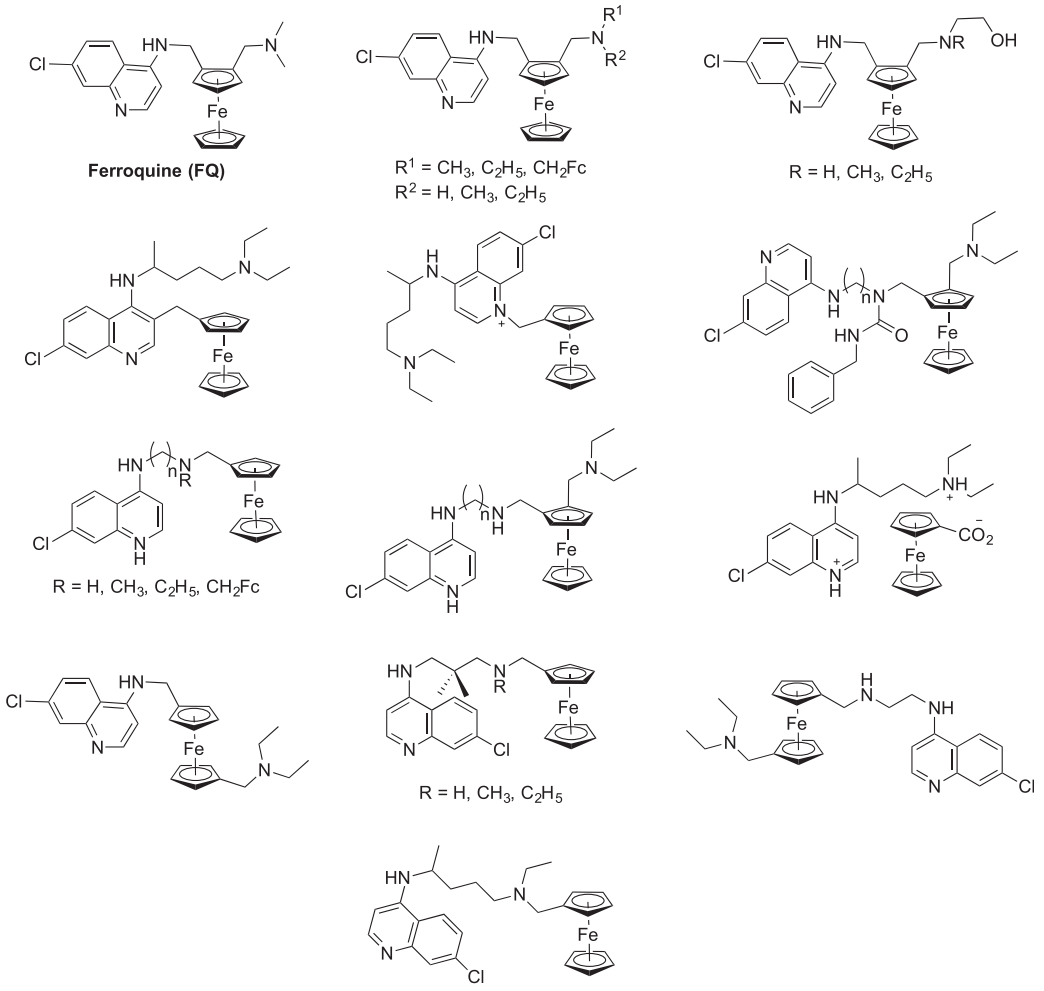


Fig. 31. Analogues of FQ.

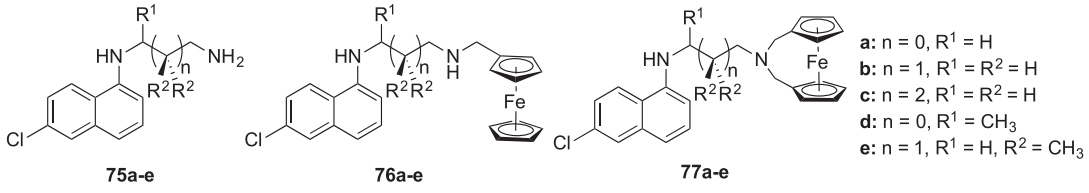


Fig. 32. CQ derivatives (Series **75a**–**e**), Monosubstituted CQ ferrocenyl compounds (Series **76a**–**e**), 1,1′-disubstituted CQ ferrocenyl conjugates (Series **77a**–**e**).

Table 17
In Vitro antiplasmodial activity and resistance indices against *P. falciparum* CQS D10, CQR Dd2 and K1 strains.[a]

Compound#	IC_{50}, (nM)		RI[b]	IC_{50}, (nM)	RI[c]
	CQS D10	CQR Dd2		CQR K1	
77a	176.0	129.7 ± 23.2 ($n = 2$)	0.7	ND	
77b	323.0	224.3 ± 6.73 ($n = 2$)	0.7	307.3 ± 170.5 ($n = 3$)	0.9
77c	91.3	152.2 ± 6.52 ($n = 2$)	1.6	ND	
77d	444.2	269.2 ± 4.48 ($n = 2$)	0.6	291.6 ± 2.24 ($n = 3$)	0.7
77e	669.0	506.5 ± 21.1 ($n = 2$)	0.7	ND	
FQ [81c]	18.0	19.0	1.06	14.0	0.8
CQ	29.1 ± 7.75 ($n = 19$)	180.3 ± 77.4 ($n = 15$)	6.2	758.0	26.0

[a] n = number of data sets averaged. ND = not determined.
[b] Resistance index (RI) = IC_{50}(Dd2)/IC_{50}(D10).
[c] Resistance index (RI) = IC_{50}(K1)/IC_{50}(D10).

antimalarial drugs [74]. Kaur et al. [75] proposed that increasing the length of the side chain in 8-AQ would result in greater accumulation of the compound in the malaria parasite's DV, which is the site for BH formation [76], as a result of an increase in the overall basicity of the 8-AQ. To that end 13 compounds (Fig. 28) were prepared and the *in vitro* activity against CQS strain D6 and CQR strain W2 was tested (Table 16). Nearly all the analogues displayed antimalarial activity and it is note-worthy that, in general, higher activity was observed against CQR strain W2 than CQS strain D6. Furthermore, the majority of analogues showed high BH inhibition. The compounds were tested *in vivo* in a *P. berghei* malaria model and six analogues (**68a**, **68b**, **69a**, **69b**, **70a** and **70b**) were curative whereas the 4,5-disubstituted analogues (**68c**, **68d**, **69c**, **69d** and **70c**) showed no anti-malarial activity.

Kaur et al. also prepared a series of *bis*(4-aminoquinolines) that showed promising antimalarial activity *in vitro* against CQS and

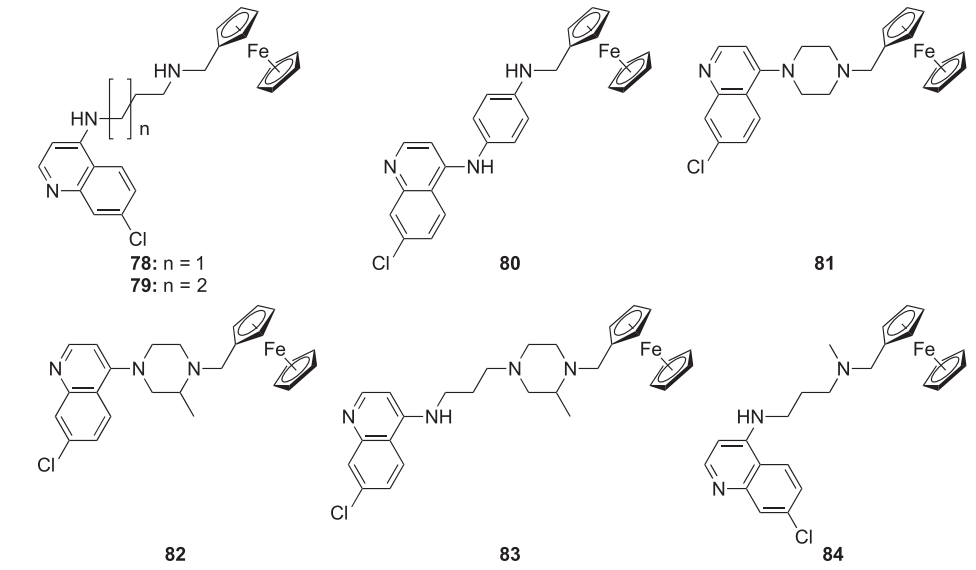


Fig. 33. Ferrocene-chloroquine conjugates with varying linkers.

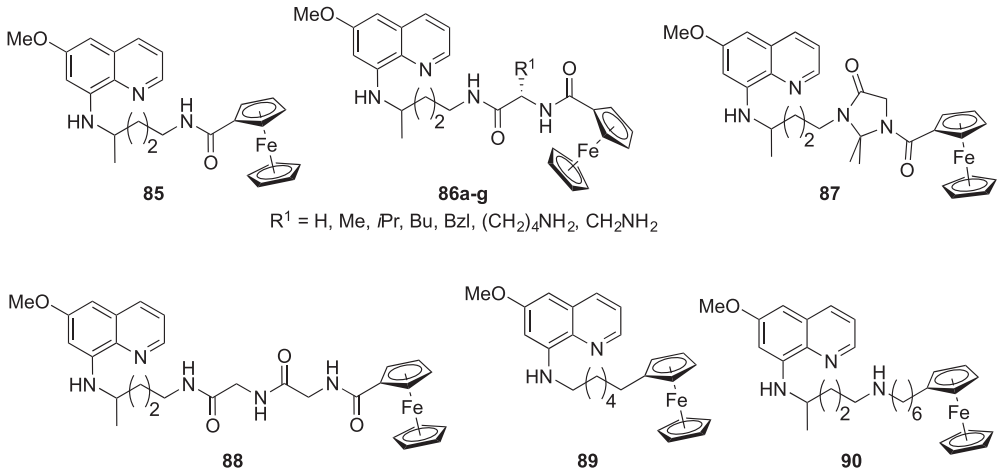


Fig. 34. Primacenes that showed antimalarial activity against the liver stages of *P. berghei*.

Table 18
In vitro antiplasmodial activity of primacenes.

Compound#	Activity against blood stage *P. falciparum* W2 (IC_{50} [μM])	Activity against liver stage *P. berghei* W2 (IC_{50} [μM])	Transmission-blocking activity (infection rate [%]) (mean oocyst burden [no. of oocyst/infected mosquito] ± SEM)	
			10 μmol/kg	50 μmol/kg
PQ	3.3	7.5	45.7 (8.8 ± 3.40)	26.9 (10.0)
85	>10	1.74	98.3 (46.5 ± 6.10)	88.1 (57.9 ± 8.10)
86a	>10	9.33	33.8 (40.8 ± 10.3)	41.8 (80.2 ± 7.60)
86b	>10	6.46	ND	ND
86c	>10	3.09	ND	ND
86d	8.33	1.90	ND	ND
86e	>10	2.40	ND	ND
86f	3.48	6.46	93.3 (106 ± 4.40)	42.4 (26.0 ± 4.40)
86g	ND	ND	68.2 (62.5 ± 6.70)	73.2 (57.6 ± 4.40)
87	>10	2.82	75.6 (30.2 ± 4.2)	86.7 (47.4 ± 4.40)
88	>10	7.41	ND	ND
89	>10	0.17	80.0 (70.7 ± 10.3)	95.8 (85.1 ± 7.70)
90	9.1	ND	65.2 (61.7 ± 6.30)	0.00 (0.00)

CQR strains of *P. falciparum*. However only moderate BH inhibition was observed. Compounds **71**, **72** and **73** (Fig. 29) displayed 'curative' *in vivo* antimalarial activity against the *P. berghei* malaria model [77].

Iron (III) and iron (II) chelators, initially designed for alternative purposes to treating malaria, have demonstrated *in vitro* antimalarial activity, as these compounds can supress the growth of *P. falciparum* in erythrocytes *in vitro* [78]. With this in mind, Rajíc et al. investigated the iron (II) chelating ability of primaquine-nonsteroidal anti-inflammatory drug (NSAID) hybrids and when the NSAID was ketoprofen (**74**, Fig. 30), iron (II) chelation was observed, but no antimalarial properties were investigated [79].

4. Metal-containing antimalarial compounds

The use of metals to induce or enhance cytotoxicity of natural compounds or known drugs has increased in importance since the emergence of platinum-based chemotherapy agents in the treatment of cancer. In the context of antimalarial treatments, it is known that metal-containing compounds may possess antiparasitic activity [80]. Ferroquine (FQ), an analogue of CQ has been developed as an important antimalarial drug; it is currently in the Phase II Sanofi portfolio for uncomplicated *P. falciparum* malaria [81]. It is thought that FQ has two modes of action; firstly FQ is a more potent inhibitor of β-hematin formation than CQ and secondly ferrocene is oxidised to ferrocenium generating a reactive oxygen species (ROS) that is likely to cause irreversible damage to the parasite. The generation of an ROS by FQ was recently investigated by Dubar et al. [82] In terms of developing new drugs based on FQ, this led to the preparation of a large number of structural analogues (Fig. 31). However, to date no analogues have been able to compete with FQ in terms of antimalarial activity. Changes to the metal have also been investigated, and a comprehensive review of the role metallocenes can play in the fight against malaria was published in 2013 [80b].

Salas et al. recently prepared a series of 5 CQ analogues and reacted them with either mono- or di-substituted ferrocene to prepare two sets of ferrocenyl CQ conjugates that may potentially avoid malaria drug resistance mechanisms that occur in current antimalarials (Fig. 32) [83]. The compounds were tested against both CQS (D10) and CQR (D2d and KR10) strains of *P. falciparum* and compared to chloroquine and ferroquine. In general, all compounds showed activity, comparable to chloroquine in CRS sensitive strains. Series **75** and compound **76a** showed greater activity that series **77**, but the activity was not as great as that of FQ. Compounds **77a**–**e** showed greater or comparable activity than CQ and compounds **75a**–**e** and **76a**–**e** against the CQR strains of malaria. The importance of the metal component of the drug is demonstrated by the fact that series **75** showed little activity against CQR strains of malaria. In addition, Salas et al. calculated the resistance index (RI) for the compounds they prepared as a measure of IC_{50}(CQR)/IC_{50}(CQS) and found that the RI values ranged from 0.5 to 24.7 (Table 17, data shown for compounds **77a**–**e** only). The larger RI values indicate a loss of activity as a result of resistance developing so smaller RI values are desired. The disubstituted bridged ferrocenyl compounds had the lowest RI and the fact that they displayed higher activity in CQR strains of *P. falciparum* versus the CQS strains indicates that they have a different mode of activity from CQ, which may prevent resistance developing.

It has been observed that the linker between drug hybrids is an important determinant into whether or not the novel compound retains the biological activity of the component parts [62]. To that end, N'Da et al. recently prepared seven conjugates of quinoline and ferrocene, differing only in the linker joining the two fragments (Fig. 33). It was found that hybrids containing flexible hydrocarbon spacers and a 4-*N* proton (**78**, **79**, **83** and **84**) were the most active against CQS (D10) and CQR (Dd2) strains of *P. falciparum* of which compound **84** demonstrated comparable or improved activity when compared to CQ [84]. These results confirmed previously reported findings demonstrating the importance of the linker and the dependence of the antiplasmodial activity on the conformation of the hybrid. In the case of hybrids **78**, **79**, **83** and **84**, due to the flexibility of the linker, there is the possibility that hydrogen bonding could occur between the 4-*N* and the terminal N which is not possible in the case of rigid linkers (**82**) or those lacking a 4-*N* proton (**81** and **82**). It should be mentioned that Biot et al. have previously synthesised and investigated the antimalarial properties of compounds **78** and **81** [85].

Primaquine-ferrocene (primacenes) conjugates have also been prepared (**85**–**90**, Fig. 34), but in the initial study none of the compounds displayed antimalarial properties against *P. falciparum* (Table 18) [86]. A subsequent study revealed that primacenes containing a basic aliphatic amine group (**86f** and **90**) were able to impair the parasite's sporogonic cycle at 50 μmol/kg, with greater efficacy than PQ. Furthermore it was shown that compound **89**, in which the aliphatic chain of PQ is replaced by ferrocene, is 45 times more active against liver stage malaria than PQ.

Finally, a silver-chloroquine analogue was synthesised and characterised by Dávalos et al., but the antimalarial activity of the complex was not investigated, possibly due to its insolublity in most organic solvents [87]. Non-quinoline containing metal complexes have also been investigated for antimalarial activity, but are beyond the scope of this review [88].

5. Conclusions

Improved vector control, awareness of malaria and a more prudent approach towards antimalarial drugs have all contributed to a decrease in the number of malaria cases over the last ten years. However, emerging resistance to all known antimalarial agents is an increasing problem and as such, there is a continued need for novel compounds bearing antimalarial properties. Against this backdrop hybrid drugs, containing two or more motifs that have biological activity, have emerged as a new and important area of research. The ART-QN hybrid **2** is established as a significant contribution to this field.

In designing hybrid drugs, a number of key features have been noted. The new compounds sometimes show decreased antimalarial activity with respect to QN, CQ, PQ or ARTs, but trends within groups of molecules reveal useful structure-activity relationships, which aid the design and synthesis of subsequent generations of potential antimalarial compounds.

The linker between the two components of a hybrid drug plays an important role in whether or not the target molecule retains its biological activity. In addition, the basicity of the hybrid molecule can determine whether or not it is able to accumulate in the acidic environment of the DV. Where pKa values are known, it has been observed that compounds with a lower pKa show a concomitant decrease in antimalarial activity.

Finally, an understanding of the mechanisms involved in the development of resistance is key to the design of new antimalarial agents. In this respect hybrid molecules having more than one mode of action may prove to be particularly important, and they are expected to make substantial contributions to the control and cure of this devastating human disease.

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