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Chloroquine–astemizole hybrids with potent in vitro and in vivo antiplasmodial activity

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ABSTRACT

A dual activity, conjugated approach has been taken to form hybrid molecules of two known antimalarial drugs, chloroquine (CQ) and the non-sedating H1 antagonist astemizole. A variety of linkers were investigated to conjugate the two agents into one molecule. Compounds **5**–**8** possessed improved in vitro activity against a CQ-resistant strain of *Plasmodium falciparum*, and examples **7** and **8** were active in vivo in mouse models of malaria.

Between 300 and 500 million clinical cases of malaria are presented each year, resulting in over 1 million deaths.[1] Most of these deaths occur in the poorest regions, sub-Saharan Africa in particular accounting for well over 90% of these cases.[1] The causal agent of the most lethal form of malaria, *Plasmodium falciparum*, has developed resistance to a multitude of drugs including the efficacious, safe and cheap drug chloroquine (CQ, Fig. 1) that was once used as a first line of defence against infection in most endemic regions.[2] This development of resistance has in part been responsible for the global rise of malaria.[2] CQ resistance had largely been attributed to mutations in the PfCRT gene that encodes for the protein believed to mediate efflux of the drug from the digestive vacuole of the parasite,[3] leading to sub-optimal drug concentrations.

To overcome the challenges of multi-drug resistance in *P. falciparum*, combination therapy offers an attractive alternative. In this approach a combination of drugs that target different biological targets/systems within the parasite are co-administered. Imipramine, a drug with no inherent antimalarial activity, has also been shown to reverse CQ resistance in *P. falciparum* when co-administered with CQ.[4]

Burgess et al. have also recently shown that hybridization of CQ and imipramine, to give compound **1**, is a viable strategy to reverse CQ resistance in drug-resistant *P. falciparum*.[5] In addition, a recent report by Chong and co-workers identified astemizole **2** (Fig. 2) as a potent inhibitor against both CQ-sensitive and CQ-resistant parasites in vitro and in vivo.[6]

Based on the CQ–imipramine hybrid data, and astemizole antiplasmodial activity, we were interested in pursuing a hybridization strategy that combined the core portions of the two structurally distinct moieties of CQ and astemizole, each of which possess significant antiplasmodial activities, via an appropriate linker. We hoped this strategy would result in compounds that could overcome *P. falciparum* resistance to CQ, and offer a viable strategy

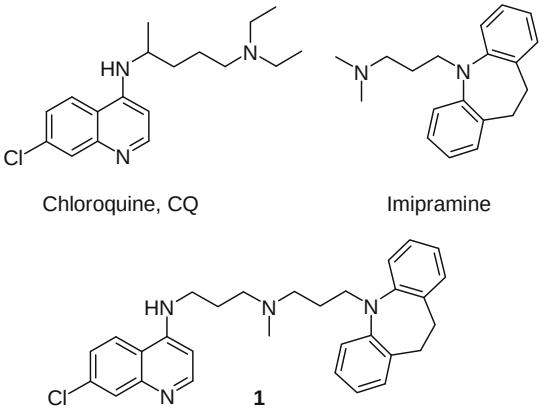

Figure 1. Chemical structures of chloroquine (CQ) and imipramine and CQ–imipramine hybrid **1**.

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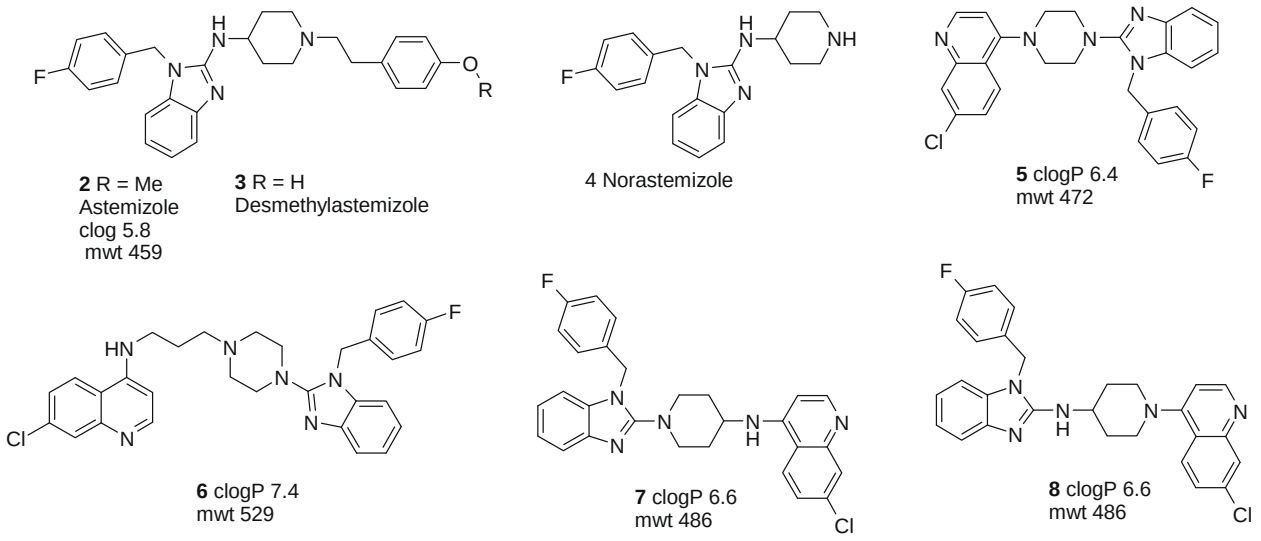


Figure 2. Chemical structures of astemizole and its metabolites (**2**–**4**) and synthetic hybrids **5**–**8**.

for the discovery of new antimalarial therapies. A similar hybridization strategy has also been employed in antimalarial peroxide research, for example, aminoquinoline and trioxanes,[7] artemisinin and quinine,[8] and aminoquinoline and tetraoxanes.[9]

The combination of two separate pharmacological agents into a single molecule is an emerging strategy within medicinal chemistry and drug discovery.[10] The work of Morphy and Rankovic[11] and Hopkins et al.[12] has highlighted both the opportunities within this paradigm, but also the trends towards increased molecular weight and lipophilicity in multipharmacology ligands when compared to known oral drugs.[13] With these challenges in mind, a small set of conjugated CQ–astemizole hybrids **5–8** was designed (Fig. 2), the synthesis and biological evaluation of which are described in this letter.

The plasmodia data disclosed by Chong et al.[6] gave some guidance on the possible strategies for conjugating astemizole and CQ together. Both **2** and **3** had good antiplasmodial activity (in vitro and in vivo), whereas norastemizole **4**, which lacked the phenethyl sidechain, had significantly reduced activity. This suggested to us that alkylation of the piperidine nitrogen was important, and that replacement of the phenethyl unit of astemizole with the quinoline heterocycle of CQ was an approach worth investigating. This strategy had the added advantage of replacing the metabolically vulnerable methoxyphenyl group of astemizole with a more robust heterocyclic moiety. A basic centre was also retained in the new analogues, as CQ is postulated to concentrate in the parasite digestive vacuole by virtue of protonation under the acidic conditions found in that compartment (pH of digestive vacuole measured at 4.7). The calculated pK_as for analogues **5**–**8** were all above 7.0 (using ACD software) and would result in >99% protonation at pH 4.7.

It was a significant challenge to keep these conjugated molecules approaching the Ro5, and in all cases lipophilicity increased compared to astemizole. Clearly this would need to be addressed in further design cycles, due to the potential for high lipophilicity to increase both off-target pharmacology and the potential for adverse toxicological outcomes.[14] However, it was felt the designed set of compounds **5**–**8** would allow for proof-of-principle of this hybrid approach to be established, which could then trigger further work to improve drug-like properties.

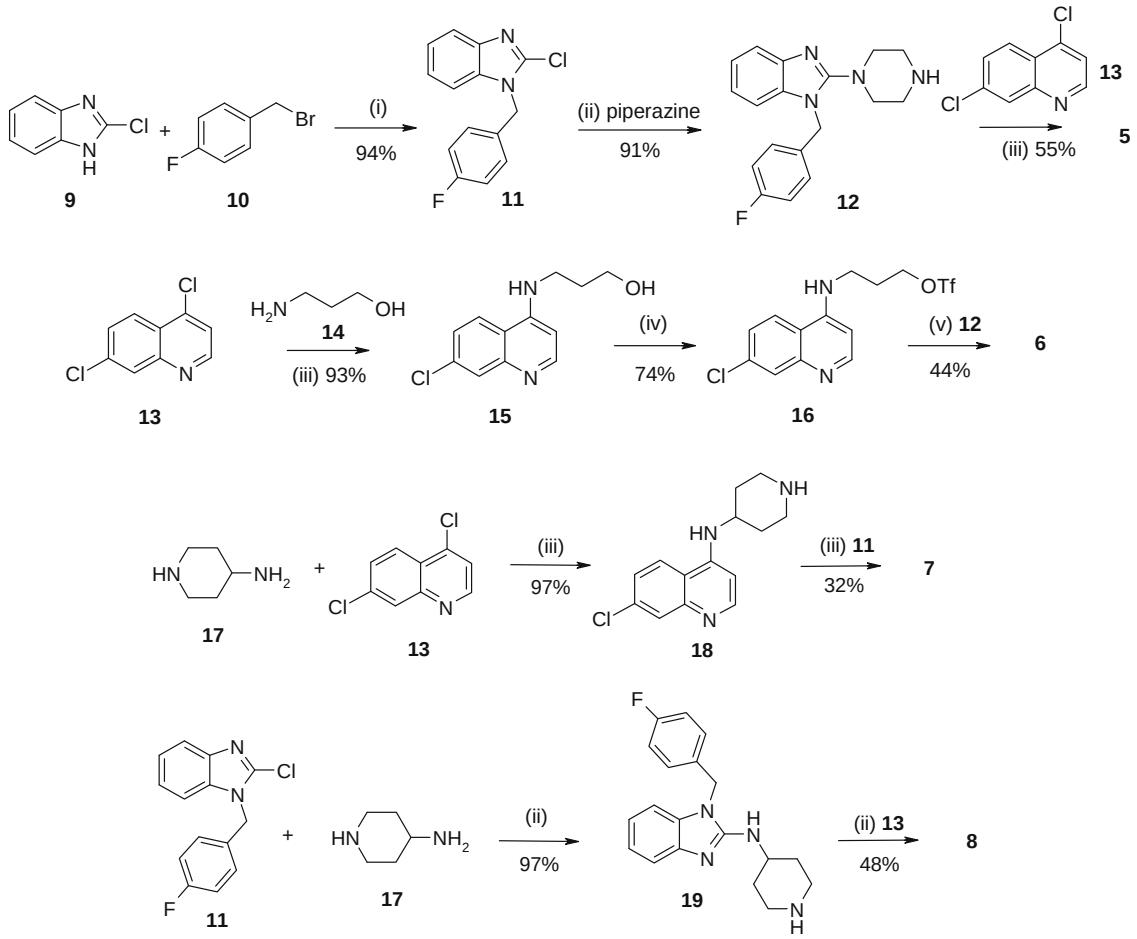
The synthetic route employed to access the target compounds **5**–**8** is outlined in Scheme 1. Alkylation of 2-chlorobenzimidazole **9** was effected with 4-fluorobenzyl bromide **10** under basic conditions to afford **11** in excellent yield. Attempted palladium-catalysed Buchwald–Hartwig type amination of **11** under standard conditions only yielded starting materials, but **12** could be obtained in good yield after treatment of **11** with excess piperazine in the absence of catalyst under microwave irradiation. Final coupling of **12** with 4,7-dichloroquinoline **13** afforded target compound **5**.[15] Amination of **13** with propanolamine **14** gave intermediate **15** in excellent yield. Compound **6** was then obtained by subsequent triflation of **15** to yield **16**, followed by nucleophilic substitution of the triflate with **12**.

Targets **7** and **8** were synthesised in a similar fashion. The analogue **7** was realised via coupling 4-aminopiperidine **17** with **13** to give **18**,[16] and then reaction of **18** with **11** to give the desired product **7**. The synthesis of reverse analogue **8** began with reaction of **11** with 4-aminopiperidine **17** under microwave irradiation, to afford **19** in almost quantitative yield. Coupling of **13** and **19,** again under microwave conditions, gave compound **8**.

Antiplasmodial activity was determined in a CQ-resistant K1 strain of the *P. falciparum* parasite.[17] The results of the antiplasmodial activity of compounds **5–8** are shown in Table 1, along with the value for the standard drug CQ and astemizole **2**. With the exception of **5**, all compounds were 3–10 times more active than CQ with optimum activity being seen in compound **6** (IC_{50} 23 nM). Interestingly, the conformationally constrained aminopiperidine linkers **7** and **8** also delivered potent K1 activity. This is in contrast to a previously described series of CQ analogues where acyclic linkers were found to deliver more potency that those with a conformationally constrained piperidine or pyrrolidine linker.[16] Importantly, the hybrid analogues displayed good cytotoxicity profiles, with all compounds showing >100-fold selectivity for antiparasitic activity over cell-based cytotoxicity.

These results suggested that hybridizing the two different units, each of which possesses significant antiplasmodial activity, via an appropriate linker could overcome resistance to CQ, much of which has been ascribed to mutations in the PfCRT gene.[3]

Based on the in vitro *P. falciparum* activity, analogues **7** and **8** were progressed to a *Plasmodium berghei* mouse malaria model.[18] Both compounds showed in vivo activity although neither was as active as CQ (Table 2). Both **5** and **6** showed prolongation of survival (based on mean survival time) and significant reductions in parasitemia at 4×50 mg/kg (**7**) and 4×20 mg/kg (**8**) ip. At this high dose, **7** reduced parasitemia comparatively to CQ. These in vivo results suggested that the hybridization approach outlined



Scheme 1. Reagents and conditions: (i) NaOH, CH_3CN, reflux, 6 h; (ii) NMP, microwave, 1 h, 180 °C; (iii) DIPEA, NMP, 135 °C, 24 h; (iv) Tf_2O, Et_3N, THF, 0 °C–rt, 5 h; (v) DIPEA, MeCN, reflux, 24 h.

Table 1
In vitro antiplasmodial activity against CQ-resistant *P. falciparum* K1 strain and cytotoxicity in rat L6 myoblasts

Compound	IC_{50} (μM)	Cytotoxicity (μM)
CQ	0.23	—
2	50% at 5 μM[a]	—
5	0.61	126
6	0.023	6.86
7	0.064	9.1
8	0.037	8.8

[a] Single-point determination at 2.5 μg/ml, equivalent to 5.4 μM.

Table 2
In vivo antiplasmodial activity in *P. berghei* mouse model

Compound	Dose (mg/kg)	MST[a] (days)	% Activity
CQ	4× 10	17	99
7	4× 50	13	99
8	4× 20	8	80

[a] MST, mean survival time in days.

herein is a viable strategy to overcome CQ-associated resistance in *P. falciparum*.

In summary, the potential to overcome *P. falciparum* resistance to CQ both in vitro and in vivo has been demonstrated using a hybrid design strategy, which conjugated the antiplasmodial agents, chloroquine and astemizole, into a single molecule. Further work in this area should focus on improving the drug-like properties of the hybrid agents, which may in turn improve the in vivo efficacy of this class of compounds.

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15. Structure determination and purity assessment for test compounds **5**–**8** was carried out by ^{1}H NMR and LC–MS analysis. All data was in agreement with the assigned structures. ^{1}H NMR data for compounds **7** and **8** is detailed below: *Compound* **7**: ^{1}H NMR (400 MHz, $CDCl_3$) δ ppm 1.95–2.06 (m, 2H), 2.16–2.23 (m, 2H), 3.13–3.25 (m, 2H), 3.56–3.61(m, 2H) 3.75 (br s, 1H) 5.25 (s, 2H) 6.33 (d, J = 6.6 Hz, 1H) 7.01–7.10 (m, 3H), 7.14–7.25 (m, 3H), 7.31 (dd, J = 8.9, 1.9 Hz, 1H), 7.41 (br s, 1H), 7.68 (d, J = 7.8 Hz, 1H) 7.85 (d, J = 1.9 Hz, 1H) 8.04 (d, J = 9.4 Hz, 1H) 8.18 (d, J = 6.6 Hz, 1H) 8.53 (s, 1H). *Compound* **8**: 1H NMR (400 MHz, $CDCl_3$) δ ppm 1.80–1.87 (m, 2H), 2.24–2.29 (m, 2H), 3.20–3.27 (m, 2H), 3.54–3.61 (m, 2H), 3.76–3.81 (m, 1H), 5.23 (s, 2H) 6.47 (d, J = 5.5 Hz, 1H) 7.01–7.10 (m, 3H) 7.12–7.19 (3m), 7.23 (t, J = 7.8 Hz, 1H) 7.39 (dd, J = 9.0, 2.3 Hz, 1H) 7.62–7.67 (m, 2H) 7.98 (d, J = 1.9 Hz, 1H) 8.55 (d, J = 5.5 Hz, 1H).
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