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Research paper

Overcoming chloroquine resistance in malaria: Design, synthesis and structure–activity relationships of novel chemoreversal agents

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ABSTRACT

Malaria remains a significant infectious disease with even artemisinin-based therapies now facing resistance in the field. Development of new therapies is urgently needed, either by finding new compounds with unique modes of action, or by reversing resistance towards known drugs with 'chemosensitizers' or 'chemoreversal' agents (CRA). Concerning the latter, we have focused on the resistance mechanisms developed against chloroquine (CQ). We have synthesized a series of compounds related to previously identified CRAs, and found promising novel compounds. These compounds show encouraging results in a coumarin labeled chloroquine uptake assay, exhibiting a dose response in resensitising parasites to the antimalarial effects of chloroquine. Selected compounds show consistent potency across a panel of chloroquine and artemisinin sensitive and resistant parasites, and a wide therapeutic window. This data supports further study of CRAs in the treatment of malaria and, ultimately, their use in chloroquine-based combination therapies.

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1. Introduction

Malaria remains one of the most dangerous infectious diseases, with 214 million cases causing 438,000 deaths in 2015 by WHO estimates [1]. Particularly vulnerable groups include pregnant women [2] and young children [3]. Carried by parasites of the genus *Plasmodium* (*P. falciparum*, *P. vivax*, *P. ovale*, *P. malariae*, *P. knowlesi*), the infection is transmitted by the bite of infected *Anopheles* mosquitoes [4]. Chloroquine (CQ, **1**) has historically been the most commonly used antimalarial drug due to its good efficacy, low toxicity, and affordability. However, resistance against **1** and now nearly all other available treatments has become a major problem, necessitating the development of new treatments on a regular basis [5,6]. Even with artemisinin-based combination therapy (ACT), the current standard treatment, prolonged parasite clearance times have been recently observed in Thailand and Cambodia [7–9], including prominent reports of the failure of the dihydroartemisinin-piperaquine combination [10]. The mechanisms of drug-resistance depend on the drug and are not yet completely understood. Resistance to **1** (CQR) is known to be transporter-mediated, mostly due to mutations in two genes: PfCRT K76T [11] and several mutations in pfMDR1 [12]. These modified transporters are able to remove **1** from its site of action, the digestive vacuole (DV), where it interferes with heme detoxification. Similar point mutations in PfCRT can be traced back to the geographical origin of the *P. falciparum* isolates. For African and most South-east Asian resistant strains, mutation in amino acids at positions 72–76 is a change from CVMNK to CVIET, while the South American resistant strains encode SVMNT [13,14].

Three approaches can lead to new malaria treatments [15]: searching for new compounds with new mechanisms of action, addressing the development of drug resistance with combination therapies, or oppose resistance by developing compounds that restore the drug-sensitivity of resistant parasites. Compounds used for the latter concept are called chemoreversal agents (CRA) or chemosensitizers. Multidrug resistance (MDR) in cancer chemotherapy *via* over-expression of drug efflux pumps or increased DNA

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repair mechanisms is well established, and the concept of reversing this drug resistance has been evaluated in several clinical trials [16,17]. In the case of malaria drug resistance, *in vitro* chemosensitization of resistant *P. falciparum* strains has been shown with, for example, verapamil and promethazine [18–21]. Most of the published CRAs have poor *in vivo* pharmacokinetic and pharmacodynamic properties [22,23], or have a poor safety profile [24]. The only chemosensitizer that we are aware of that has undergone clinical trials is chlorpheniramine [25], but the results were disappointing. More recently reported novel analogues of chlorpheniramine may show greater promise [26]. Clearly novel drug-like candidates with acceptable safety profiles and *in vivo* efficacy for CQ and ART-resistant strains are urgently required.

Potential CRAs can be detected using a fluorescent probe in a straightforward and rapid assay. We have previously published probe **3**, composed of a chloroquine (**1**) portion linked to a coumarin fluorophore (**2**) (Fig. 1) [27].

A high throughout assay employing **3** to screen the LOPAC library (Sigma Aldrich), composed of 1280 drug-like compounds, was used in our program to find new CRA compounds. Four novel CRAs were identified: L-703,606 (**4**) [28], loperamide (**5**) [29], octoclothepin (**6**) [30], and methiothepin (**7**) [31] (Fig. 2).

Analogues of the hit compounds were designed based on a pharmacophore model, synthesized and screened for chemoreversal activity with probe **3**. The potential of the new compounds to sensitise CQR parasite strains to the effects of **1** was then assessed. Cytotoxicity and preliminary *in vitro* DMPK studies confirmed the drug-like potential of the new compounds.

2. Results and discussion

2.1. In silico analysis and design of compounds

All of the hit compounds were observed to fit reasonably well into the pharmacophore model developed by Bhattacharjee et al. [32]. This model describes a 3-point pharmacophore composed of two aromatic hydrophobic sites with a third site being a hydrogen bond acceptor. No protein crystal structure information is available for the mutant or wild type PfCRT, hence any new model must be ligand based and preferably simple to apply as in the case of a 3-point pharmacophore. To assess which parts of the scaffolds of

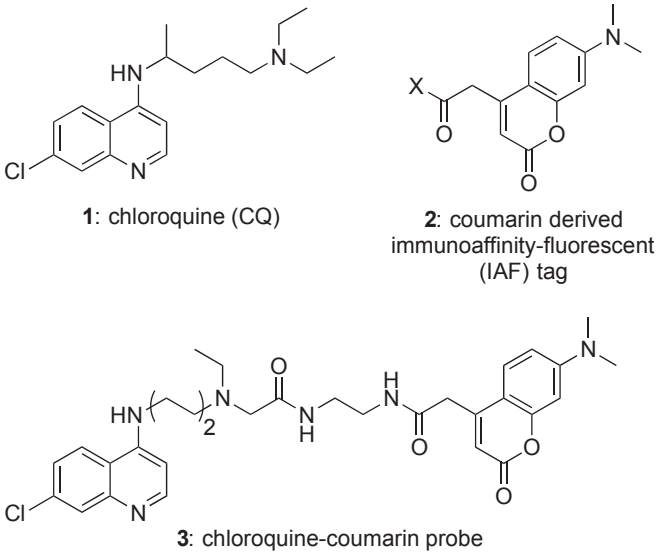

Fig. 1. Chloroquine (CQ, **1**), the fluorescent tag **2** and probe **3**.

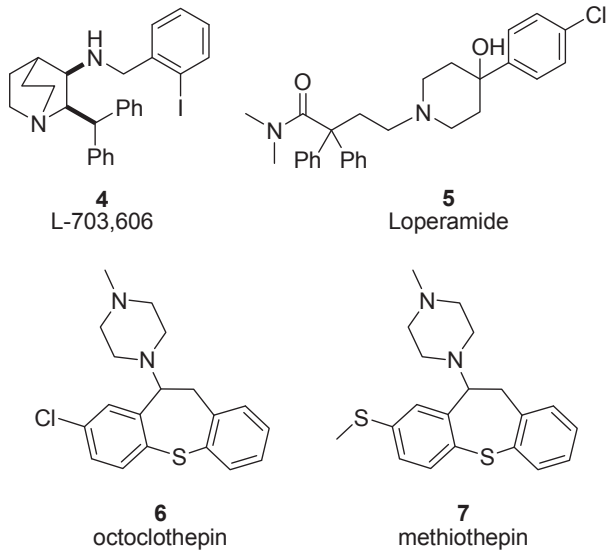

Fig. 2. Structures of the four CRA hit compounds.

our hit compounds are essential for their activity, and if these correspond to the previously published model, we created a 3-point ligand based pharmacophore model with LigandScout [33,34]. Our model comprises of two aromatic hydrophobic interaction sites and a hydrogen bond acceptor, preferably a basic nitrogen atom, similar to the Bhattacharjee model. A database of over 100 compounds was compiled (see Table S1), all being proven chloroquine resistance CRAs targeting the CVIET haplotype of PfCRT in *P. falciparum*. From this database, 77 were used for generation and scoring of the pharmacophore (training set of 22 compounds and test set of 55 compounds, for entire list see Table S2). In order to avoid a predisposition of the pharmacophore model in favor of the hit compounds, they were excluded in its generation. From this model the predicted optimal pharmacophore consists of two hydrophobic regions and a positive-ionizable atom, situated almost equidistant from one another (Fig. 3).

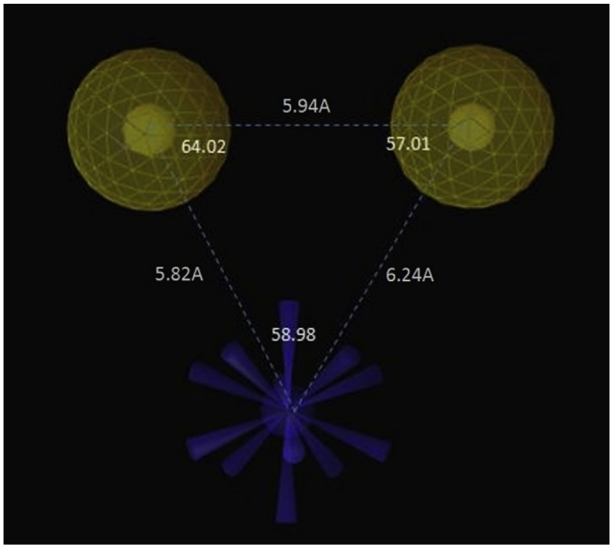

Fig. 3. Optimal chemoreversal agent pharmacophore with distances and angles, consisting of two hydrophobic regions (yellow spheres) and a positive-ionizable atom (blue). Predicted by LigandScout 3.1. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of this article.)

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