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Activity of piperaquine and other 4-aminoquinoline antiplasmodial drugs against chloroquine-sensitive and resistant blood-stages of *Plasmodium falciparum* Role of β-haematin inhibition and drug concentration in vacuolar water- and lipid-phases

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ABSTRACT

Chloroquine (CQ), a 4-aminoquinoline, accumulates in acidic digestive vacuoles of the malaria parasite, preventing conversion of toxic haematin to β-haematin. We examine how *bis* 4-aminoquinoline piperaquine (PQ) and its hydroxy-modification (OH-PQ) retain potency on chloroquine-resistant (CQ-R) *Plasmodium falciparum*. For CQ, PQ, OH-PQ and **4** and **5**, representing halves of PQ, β-haematin inhibitory activity (BHIA) was assayed, while potency was determined in CQ-sensitive (CQ-S) and CQ-R *P. falciparum*. From measured pK_as and the pH-modulated distribution of base between water and lipid (log D), the vacuolar accumulation ratio (VAR) of charged drug from plasma water (pH 7.4) into vacuolar water (pH 4.8) and lipid accumulation ratio (LAR) were calculated. All agents were active in BHIA. In CQ-S, PQ, OH-PQ and CQ were equally potent while **4** and **5** were 100 times less potent. CQ with two basic centres has a VAR of 143,482, while **4** and **5**, with two basic centres of lower pK_as have VARs of 1287 and 1966. In contrast PQ and OH-PQ have four basic centres and achieve VARs of 104,378 and 19,874. This confirms the importance of VAR for potency against CQ-S parasites. Contrasting results were seen in CQ-R. **5**, PQ and OH-PQ with LARs of 693; 973,492 and 398,118 (compared with 8.25 for CQ) showed similar potency in CQ-S and CQ-R. Importance of LAR for potency against CQ-R parasites probably reflects ability to block efflux by hydrophobic interaction with PfCRT but may relate to β-haematin inhibition in vacuolar lipid.

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1. Introduction

With the increasing spread of drug-resistance to the 4-amino-7-chloroquinoline base chloroquine (CQ) (**1**) (Fig. 1) in the malaria parasite *Plasmodium falciparum* [1] and the associated raised case-mortality in children [2], a number of related *bis* 4-amino quinolines have been investigated. These compounds contain two 4-amino-7-chloroquinoline moieties connected at the 4-amino group by a variable linker group [3].

One such group, the *bis*-quinolin-4-yl piperazines, yielded several active antimalarials. In particular, the (7-chloroquinolin-4-yl)-*N*-1′ piperazine) dimers piperaquine (PQ) **2** originally reported by Rhone-Poulenc as 13,228 RP and hydroxypiperaquine (OH-PQ) **3** (Fig. 1), in which the *N*-1′ atom of a piperazine linker was directly attached to the C-4 position of each quinoline. PQ and OH-PQ were active ip against *P. berghei* in mice [4]. PQ was orally effective against blood-induced *P. cynomolgi* in rhesus monkeys and was then proved in *P. falciparum* infections in man [5]. There was evidence of persistence of active drug levels in the mouse for 15–30 days after a single ip dose [6,7].

Later, following the extension of the CQ-resistance problem, both PQ and OH-PQ were resynthesized in China [8], and successfully applied by Prof. Chen Lin and colleagues, to the treatment and prevention of infections with CQ-resistant *P. falciparum* on Hainan Island [9]. While as potent as CQ in a CQ-sensitive strain, PQ is reported six times more active than CQ in CQ-resistant *P. falciparum in vitro* [10]. More recent studies have confirmed the value of PQ in multidrug resistance [11] and its utility as a companion drug for the endoperoxides dihydroartemisinin and artesunate [12].

There can be no doubt that the release of free haematin from haemoglobin during digestion [13] is the basis of CQ's selective toxicity for intraerythrocytic malaria parasites [14]. The acidic content of the digestive vacuole (lysosome), where the haematin is released, is the site of concentration of this weakly basic 4-amino-7-chloroquinoline drug [15–18], which binds to haematin and prevents its detoxification by cyclic dimerization [19]. Although the concentration of protonated CQ2H+ into vacuolar water appears to be a requirement for antimalarial activity [15,16,20,21], recent studies indicate that haematin dimerization and detoxication may take place in or closely associated with lipid droplets in the interior of the vacuole [22,23]. The rate of development in cell-free systems of the insoluble crystal of malaria pigment, haemozoin or β-haematin [19], is enhanced by presence in the acidic buffer (pH 4.8) of the long chain alcohol *n*-octanol or other neutral lipids [23,22]. The importance of lipid for haemozoin formation highlights alternative possibilities for different drugs, that drug/haematin complexes may form in the vacuolar water and transfer to lipid sites where inhibition of dimerization takes place, or that haematin within the lipid may complex with uncharged drug already localized there. Experimentally it can be demonstrated that [^{3}H] CQ becomes incorporated into β-haematin during its formation in cell-free systems and in intact infected erythrocytes [24], but it is not yet clear what significance this has for the mode of action. In CQ-R *P. falciparum* blood stages, equilibrium concentration of CQ within the infected cell is diminished [25] probably through efflux [26], currently understood to occur across the membrane of the digestive vacuole [27], and so the drug does not achieve intravacuolar concentrations which inhibit haematin dimerization. Since changes in sequence of vacuolar membrane protein PfCRT, crucially K76T, lysine to threonine, are convincingly linked to CQ-R in *P. falciparum* [28], which replaces a positively charged and polar by a neutral and hydrophobic residue, it is probable that efflux of polar, positively charged CQ2H+ is mediated through the modified protein. Other changes in PfCRT which also increase overall hydrophobicity of the protein, particularly of residues 72–76, are present in field CQ-R isolates. It is recognized that 4-aminoquinolines more hydrophobic than CQ, such as amodiaquine (AQ) and its metabolite mono-desethylamodiaquine (DAQ) retain some activity against CQ-R parasites [29]. This is believed to be due to their interaction with and retention in the hydrophobic lining of a CQ-R PfCRT channel [30–33]. Reversal of CQ-resistance *in vitro* by verapamil and activity of DAQ both vary with hydrophobicity of residues 72–76 of PfCRT [31]. Allelic replacement of a neutral by a positively charged residue at 76 or at some other positions in the PfCRT sequence effectively restores CQ-sensitivity and restores verapamil-insensitivity [33]. A reduction in vacuolar pH has been

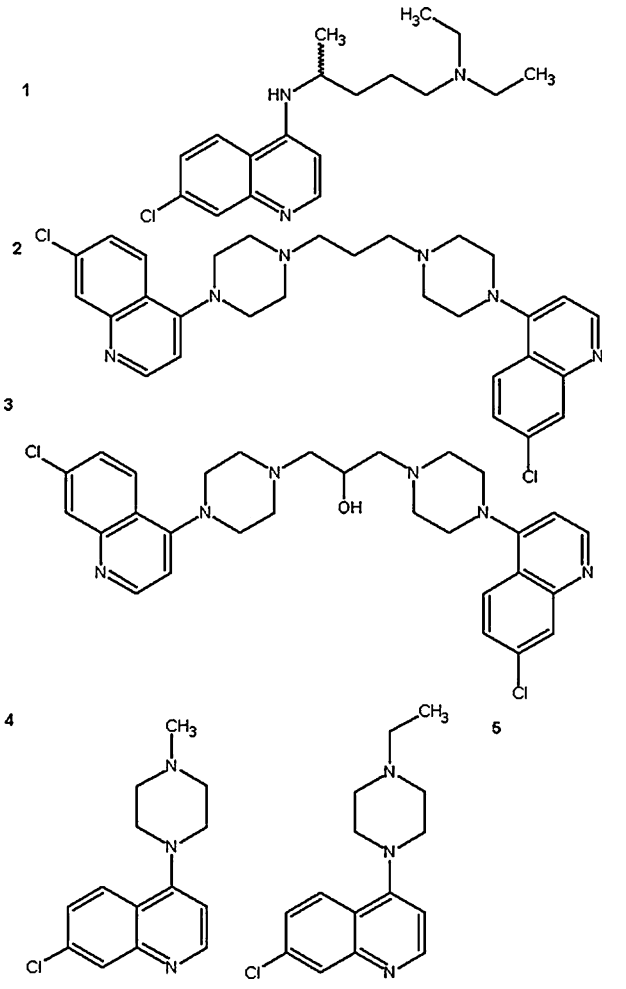

Fig. 1 – Structures of compounds used in the study. Chloroquine, showing asymmetric carbon atom (1), piperaquine (2), hydroxypiperaquine (3), cpd. 4, and cpd. 5.