European Journal of Medicinal Chemistry 52 (2012) 82–97

Contents lists available at SciVerse ScienceDirect

European Journal of Medicinal Chemistry

journal homepage: http://www.elsevier.com/locate/ejmech

Original article

2-Aminopyrimidine based 4-aminoquinoline anti-plasmodial agents. Synthesis, biological activity, structure–activity relationship and mode of action studies

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ARTICLE INFO

Article history:
Received 1 February 2012
Received in revised form
2 March 2012
Accepted 2 March 2012
Available online 13 March 2012

Keywords:
2-Aminopyrimidine
4-Aminoquinolines
DHFR inhibitor
CT DNA interaction
DHPM

ABSTRACT

2-Aminopyrimidine based 4-aminoquinolines were synthesized using an efficacious protocol. Some of the compounds showed *in vitro* anti-plasmodial activity against drug-sensitive CQ^S (3D7) and drug-resistant CQ^R (K1) strains of *Plasmodium falciparum* in the nM range. In particular, 5-isopropyloxycarbonyl-6-methyl-4-(2-nitrophenyl)-2-[(7-chloroquinolin-4-ylamino)butylamino] pyrimidine depicted the lowest IC_{50} (3.6 nM) value (56-fold less than CQ) against CQ^R strain. Structure–activity profile and binding with heme, μ-oxo-heme have been studied. Binding assays with DNA revealed better binding with target parasite type AT rich pUC18 DNA. Most compounds were somewhat cytotoxic, but especially cytostatic. Molecular docking analysis with *Pf* DHFR allowed identification of stabilizing interactions.

1. Introduction

Faced with the challenges of drug resistance, poor health systems, lack of affordable, safe and convenient treatment options, efficient treatment of malaria, one of the most devastating parasitic diseases, represents an unmet medical need. Malaria is a major public health concern in more than 90 countries inhabited by more than 2.4 billion people – 40% of the world's population and is responsible for almost 1 million deaths every year [1]. The majority of malaria victims in developing countries are pregnant women or children under the age of five, possessing little or no immunological protection. The disease is estimated to result in ~250 million new annual infections worldwide. Though the majority of the cases and approximately 90% of the malaria deaths are found in sub-Saharan Africa, the disease is now increasing in Asia and Latin America. Malaria is caused by protozoan parasites of the genus *Plasmodium* that infects and destroys red blood cells eventually leading to death, if untreated. The persistent threat of emergence of multidrug resistant *Plasmodium falciparum,* universal chloroquine resistance [2,3], suspected resistance to artemisinins [4,5], and lack of effective, appropriate and affordable treatment options have given a new impetus to the research leading to broadening of the range of therapeutic targets. Thus, creating a new armamentarium of drugs with promising antimalarial activity coupled with understanding of their mode of action may lead to the development of a new generation of treatments both for malaria control and eradication.

The common feature of the drugs based on quinine **1** is the presence of a quinoline unit, usually a 7-chloroquinoline (chloroquine **2** and amodiaquine **3**, Chart 1), and are known to cause parasite death by blocking the polymerization of the toxic heme, into an insoluble and non-toxic pigment, hemozoin, resulting in cell lysis and parasite cell autodigestion [6–8]. The mode of action of 2,4-diaminopyrimidine based drugs, typified by pyrimethamine **4** [9] and the lead compound WR99210 **5** [10] is through the inhibition of *P. falciparum* Dihydrofolate reductase (*Pf* DHFR) enzyme, required for the biosynthesis of tetrahydrofolate involved in the biotransformation of thymidylate synthase-catalysed deoxyuridylate to deoxythymidylate (dUMP → dTMP), through a methyl group transfer reaction during DNA biosynthesis [11–16]. In addition, a number of polyamines inhibit ornithine decarboxylase activity in *P. falciparum* through binding with plasmodial DNA

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doi:10.1016/j.ejmech.2012.03.007

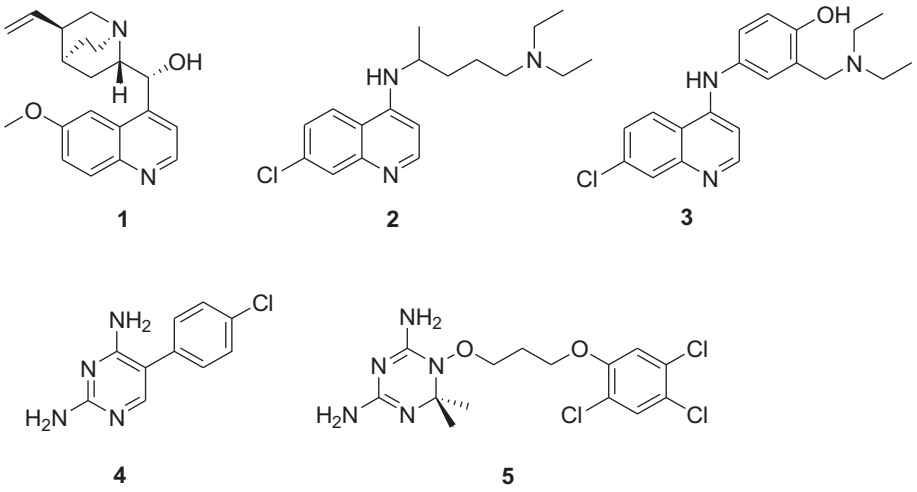

Chart 1. Antimalarial agents: quinine (**1**), chloroquine (**2**), amodiaquine (**3**), pyrimethamine (**4**) and WR99210 (**5**).

[17,18]. Recently, investigations in hybrid antimalarial agents, combining 4-aminoquinoline with other pharmacophore having antimalarial activity have been found to be less prone to resistance to parasite and has thus offered an effective means to overcome the problem of drug resistance.

We envisaged that linking 7-chloro-4-aminoquinoline unit, critical for antimalarial activity through a diversely functionalized lateral side chain with other antimalarial moiety such as aminopyrimidine, might furnish *conjugate hybrids* [19] capable of showing useful antimalarial activity. In this communication, we report synthesis of a set of compounds that possess basic, hydrophobic as well as hydrogen bonding substituents, required for targeting either or both heme as well as DNA, thus providing new antimalarial agents active against chloroquine resistant strains of *P. falciparum*. We have evaluated their anti-plasmodial activity, cytotoxicity and cytostatic activity, binding studies with DNA and heme (monomeric as well as μ-oxo dimeric) using UV–visible, fluorescence spectrophotometry as well as NMR analysis.

2. Chemistry

Compounds **10a**–**s** were synthesized as outlined in Scheme 1, *via* a common intermediate **8**. 3,4-Dihydropyrimidin-2(1*H*)-ones **6** were prepared through HCl-catalyzed Biginelli condensation of appropriate aldehyde (R^3CHO), alkylacetoacetate ($R^2CH_2COOR^1$) and urea [20]. Dehydrogenation of **6** using pyridinium chlorochromate in DCM furnished pyrimidinones **7** [21]. Refluxing **7** with $POCl_3$ yielded **8** which upon nucleophilic substitution reaction with appropriate 4-amino-7-chloroquinoline **9** gave **10a**–**s** in a synthetically useful manner [22]. Structures of **6**–**10** were established on the basis of spectral (^1H NMR, ^{13}C NMR, MS, FT IR) as well as microanalytical analysis. The yields of the 2-aminopyrimidines **10** are reported in Table 1.

3. Results and discussion

3.1. In vitro anti-plasmodial activity and structure–activity relationships (SARS)

Antiplasmodial activity of pyrimidines linked to CQ as in **10** has not been described in literature albeit the related dihydropyrimidin-2(1*H*)-ones (DHPMs) have previously been reported [23]. Using the synthetic protocol shown in Scheme 1 allowed considerable diversification around the pyrimidine core for conducting SAR analysis. The *in vitro* anti-plasmodial activities of **10a**–**s** were determined in primary and secondary screening against CQ^S and CQ^R strains of *P. falciparum*. The half maximal inhibitory concentration (IC_{50}) of **10a**–**s** are summarised in Table 2 (Fig. 1). Evidently, the compounds have anti-plasmodial activity in the nM range and against the CQ^R strain of *P. falciparum*, in some cases activity was found to be even superior to CQ. Systematic variation of the length as well as nature of the spacer connecting the pharmacophores discerned useful trends in the anti-plasmodial activity of these analogs.

Comparing **10a**–**g**, bearing linear alkyl spacers, revealed an increase in the anti-plasmodial activity with increase in length of the spacer up to 4 methylene groups (**10a**–**10c**, Table 2). Further lengthening of the spacer chain length resulted in significant

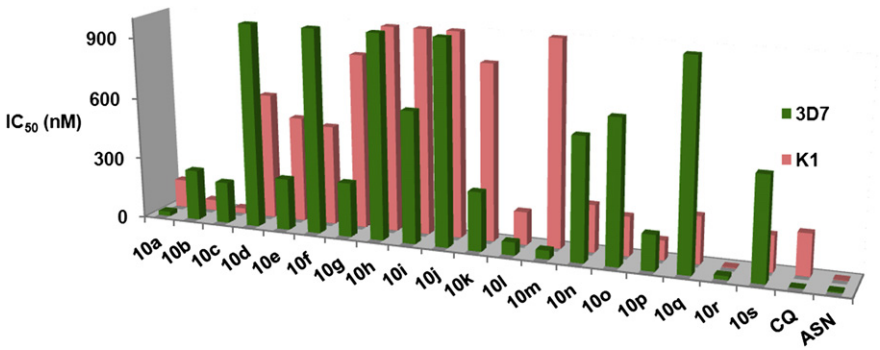

Fig. 1. Antimalarial activity of compounds **10a**–s against the CQ^S and CQ^R strains of *P. falciparum*.