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4-Aminoquinoline-Pyrimidine hybrids: Synthesis, antimalarial activity, heme binding and docking studies

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ABSTRACT

A series of novel 4-aminoquinoline-pyrimidine hybrids has been synthesized and evaluated for their antimalarial activity. Several compounds showed promising *in vitro* antimalarial activity against both CQ-sensitive and CQ-resistant strains with high selectivity index. All the compounds were found to be non-toxic to the mammalian cell lines. Selected compound **7g** exhibited significant suppression of parasitemia in the *in vivo* assay. The heme binding studies were conducted to determine the mode of action of these hybrid molecules. These compounds form a stable 1:1 complex with hematin suggesting that heme may be one of the possible targets of these hybrids. The interaction of these conjugate hybrids was also investigated by the molecular docking studies in the binding site of PfDHFR. The pharmacokinetic property analysis of best active compounds was also studied using ADMET prediction.

1. Introduction

Malaria is an infectious disease caused by protozoan parasites of the Plasmodium genus and is transmitted to humans by the infected female *Anopheles* mosquito [1]. According to a WHO report, malaria accounted for 207 million cases and an estimated 627,000 deaths worldwide in 2013 [2]. The African sub-continent is the most badly affected region where almost in every minute one child gets malaria. Though malaria mortality rates have fallen during the last decade, mortality figures are still very high for a disease that is largely preventable and treatable. A major contributor to the problem is the emergence and spread of resistance towards most drugs in clinical use such as chloroquine, amodiaquine, pamaquine and mefloquine (Fig. 1). If new drugs are not introduced onto the market, the spread of malaria could have disastrous consequences. According to the WHO, currently the best available treatments, particularly for *Plasmodium falciparum* related uncomplicated and severe malarial infection, are artemisinin combination therapies (ACTs) where artemisinin or its semi-synthetic derivative dihydroartemisinin, artemether or artesunate (Fig. 1) is administered in combination with a long acting partner drug such as amodiaquine, mefloquine, piperaquine, lumefantrine, sulfadoxine and pyrimethamine. Although, artemisinin combination therapy (ACT) is fast acting, well tolerated and is nearly 95% effective in the treatment of malaria [3], recently cases of resistance to ACTs have been reported in some south-east Asian countries. The other limitations of ACTs are low availability of artemisinin, high cost of production, short biological half-life and reduced pharmacokinetics [4–10]. All these evidences have intensified the synthetic efforts towards development of new drugs for the treatment of malaria. Thus synthesis of new chemical entities for the antimalarial therapy remains a challenging task for the scientists involved in the malaria research.

To combat drug resistant problems, various drug discovery approaches are being engaged and recently the concept of multi-targeted hybrid drugs has been explored. In this approach, two or more different target-selective pharmacophores are linked covalently resulting into one molecule [11]. These dual-drug hybrids have the potential to increase bio-pharmaceutical efficacy, reduce cost, decrease risk of drug–drug interactions and overcome rapid development of resistance problems. With these thoughts, various research groups have synthesized a huge number of hybrid molecules by the combination of chloroquine with other pharmacophores acting on different targets. The most common antimalarial

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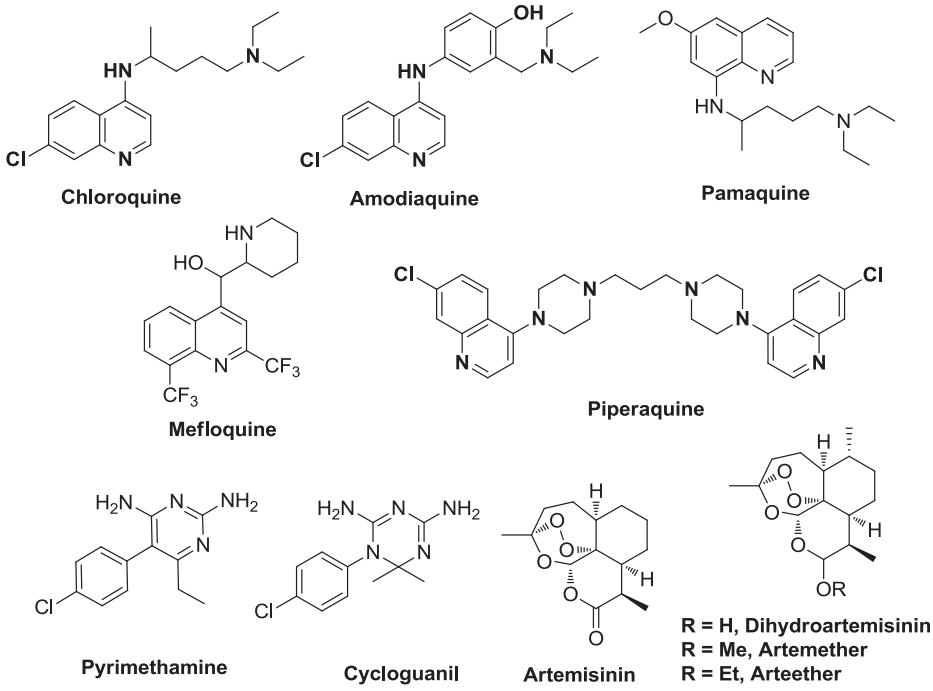

Fig. 1. Antimalarial drugs.

agents from these studies include 4-aminoquinoline-isatin conjugates [12], quinolizidinyl analogs of chloroquine [13], peroxide-based trioxaquine derivatives [14–19], ferrocene-chloroquine hybrids [20–22], 4-aminoquinoline based tetraoxane [23], 4-aminoquinoline-thiazolidinone conjugates [24,25], aminoquinoline-triazine conjugates [26–30], aminoquinoline-pyrimidine conjugates [31–33], substituted triazines [34–36], substituted pyrimidines [37–39]. Many of these derivatives have shown excellent *in vitro* and *in vivo* activity against both chloroquine-sensitive and chloroquine-resistant strains of *P. falciparum* and some of these hybrid compounds have also entered into the clinical trials [11].

Heme and *P. falciparum* dihydrofolate reductase (PfDHFR) are among the most important targets for antimalarial drug discovery. Aminoquinoline based compounds stop hemozoin formation, while pyrimethamine and cycloguanil exhibit antimalarial activity due to their ability to inhibit dihydrofolate reductase enzyme. Pyrimethamine and cycloguanil belong to the triazine and pyrimidine class of compounds, respectively. Based on these studies, we proposed to incorporate pyrimidine moiety (DHFR inhibitor) in to the side chain of 4-aminoquinoline (hemozoin inhibitor) in anticipation that the resultant molecules having two different pharmacophores within one molecule may show potent antimalarial activity and might be able to prevent the drug resistance to certain extent. Recently, we synthesized 4-aminoquinoline-pyrimidine hybrids and studied their antimalarial activity [40]. These conjugates (Fig. 2) showed potent antimalarial activity (IC_{50} = 0.005–0.44 μM) with no cytotoxicity up to 60 μM concentration. Two selected compounds were also evaluated for their *in vivo* activity and showed excellent activity in a mouse model of *Plasmodium berghei* with no toxicity (Fig. 2).

Encouraged by these results and as a part of our on-going research towards the synthesis of novel antimalarial agents [41–46], we designed a new series of 4-aminoquinoline-pyrimidine hybrids so as to develop structurally diverse series of compounds in order to gain structural insight for improved antimalarial activity. The cyclic secondary amines in our previously reported compounds were replaced by various substituted anilines in order to study the effect of phenyl ring in place of saturated heterocyclic ring structure on the antimalarial activity of these compounds (Fig. 2). Thus in the present investigation we report the synthesis, antimalarial activity and heme binding studies of a series of novel 4-aminoquinoline-pyrimidine based molecular hybrids (**7**–**9**). Also we investigated the interaction of these hybrids in the binding site of *P. falciparum* dihydrofolate reductase (PfDHFR) protein structures using molecular docking studies.

2. Results and discussion

2.1. Synthesis

Synthesis of aminoquinoline-pyrimidine conjugates was carried out as outlined in Scheme 1. Firstly, N^1-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**2a**), N^1-(7-chloroquinolin-4-yl)propane-1,3-diamine (**2b**) and N^1-(7-chloroquinolin-4-yl)butane-1,4-diamine (**2c**) were synthesized by the reaction of 4,7-dichloroquinoline (**1**) with the excess of ethane-1,2-diamine, propane-1,3-diamine and butane-1,4-diamine, respectively under neat condition at 120 °C (Scheme 1) [47]. The 4-aminoquinolines (**2a**–**2c**) with free NH_2 group were coupled with aniline substituted pyrimidines (**5a**–**5g**) in the presence of K_2CO_3 and using *N*-methyl pyrrolidone (NMP) as solvent at reflux condition to give 4-aminoquinoline-pyrimidine conjugates (**7**–**9**) which were purified by column chromatography using MeOH/$CHCl_3$ as eluent. The substituted pyrimidines were synthesized by the reaction between commercially available 2,4-dichloropyrimidine (**3**) with different substituted anilines (**4a**–**4g**) at 0 °C to RT in the presence of triethylamines using ethanol as a solvent (Scheme 1) [48]. The reaction of pyrimidine with anilines yielded two regio-isomers **5a**–**5g** and **6a**–**6g**. Compounds **5a**–**5g** obtained as a major product with very less amount of compounds **6a**–**6g** (Scheme 1). The major products **5a**–**5g** was separated by column chromatography.