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Research paper

Evaluation of 7-arylaminopyrazolo[1,5-*a*]pyrimidines as anti-*Plasmodium falciparum*, antimalarial, and *Pf*-dihydroorotate dehydrogenase inhibitors

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ABSTRACT

Malaria remains one of the most serious global infectious diseases. An important target for antimalarial chemotherapy is the enzyme dihydroorotate dehydrogenase from *Plasmodium falciparum* (*Pf*DHODH), which is responsible for the conversion of dihydroorotate to orotate in the *de novo* pyrimidine biosynthetic pathway. In this study, we have designed and synthesized fifteen 7-arylpyrazolo[1,5-*a*]pyrimidine derivatives using ring bioisosteric replacement and molecular hybridization of functional groups based on the highly active 5-methyl-*N*-(naphthalen-2-yl)-2-(trifluoromethyl)- [1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine. The compounds were tested against *Plasmodium falciparum*, as antimalarials in mice with *P. berghei*, and as inhibitors of *Pf*DHODH. Thirteen compounds were found to be active against *P. falciparum*, with IC_{50} values ranging from 1.2 ± 0.3 to 92 ± 26 μM in the anti-HRP2 and hypoxanthine assays. Four compounds showed the highest selective index (SI), which is a ratio between cytotoxicity and activity *in vitro*. The inhibition of *Pf*DHODH showed that compound **30** ($R_2 = CH_3$; $R_5 = CF_3$; Ar = 7-β-naphthyl) displayed higher and selective inhibitory activity, with $IC_{50} = 0.16 \pm 0.01$ μM, followed by **25** ($R_2 = CH_3$; $R_5 = CH_3$; Ar = 7-β-Naphthyl) and **19** ($R_2 = CF_3$; $R_5 = CF_3$; Ar = 7-β-naphthyl), with $IC_{50} = 4 \pm 1$ μM and 6 ± 1 μM, respectively. The trifluoromethyl group at the 2- or 5-positions of the pyrazolo[1,5-*a*]pyrimidine ring led to increased drug activity. The docking results agreed with the values obtained from enzymatic assays.

1. Introduction

Among the current devastating infectious diseases, malaria is the third leading cause of death in the world after tuberculosis and AIDS [1]. According to epidemiological data, it is present in more than 90 countries [2,3]. This means that approximately half of the world's population is exposed to this disease, which has resulted in approximately 198 million cases of malaria in 2013, leading to 584,000 deaths [4].

The emergence of *Plasmodium falciparum* multidrug resistance to chloroquine (CQ) and mefloquine (MQ) [5,6] and the confirmation of cases of artemisinin derivatives resistance [7] justifies the need for research to expand the range of therapeutic targets [8].

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The enzyme dihydroorotate dehydrogenase (DHODH) has been investigated as a pharmacological target for new therapeutic agents to treat cancer, rheumatoid arthritis and parasitic diseases such as malaria, Chagas disease and leishmaniasis [9–12].

The DHODH enzyme participates in the fourth step of the *de novo* pyrimidine biosynthesis pathway. This flavoenzyme catalyses the stereospecific oxidation of (S)-dihydroorotate (DHO) to orotate based on the ping-pong mechanism of catalysis [13]. In the first half-reaction comprising the oxidation of dihydroorotate to orotate, electrons are transferred to the flavin mononucleotide moiety (FMN) that is reduced to dihydroflavin mononucleotide ($FMNH_2$). After dissociation of orotate from the enzyme, $FMNH_2$ is regenerated by an appropriate electron receptor [13].

There are two classes of DHODHs that are classified based on the difference in their amino acid sequences and the preference for electron acceptors. Both human (*Hs*DHODH) and *P. falciparum* (*Pf*DHODH) enzymes belong to class 2, that use ubiquinone as a terminal oxidant agent and are located in the inner mitochondrial membrane [10–12].

*Hs*DHODH inhibitors are not clinically significant and have demonstrated high potency as anti-inflammatory and anti-proliferative activity [14]. Unlike human cells, which can acquire pyrimidine bases through pathway reuse, the *P. falciparum de novo* pyrimidine biosynthetic pathway is essential for the maintenance of life. The inhibition of *Pf*DHODH has been proved to be an attractive strategy to search for new substances with anti-plasmodial activity [9,10,12]. Synthetic compounds have been identified as inhibitors of class 2 DHODH enzymes (Fig. 1) such as brequinar and its analogues **1** [15]. Some triazolopyrimidine compounds such as 5-methyl-*N*-(naphthalen-2-yl)- [1,2,4]triazolo[1,5-*a*]pyrimidin-7-amine **(JZ8; 2)** and **3**, and *N*-phenylbenzamide **4** were discovered by Phillips and co-workers through enzyme-based high-throughput screening [11,16,17]. Other compounds, such as **5** and **6**, were obtained through pharmacophoric group modification [18,19].

In 2012, we evaluated a series of 2-(trifluoromethyl) [1,2,4]triazolo[1,5-*a*]pyrimidines against *P. falciparum* that were designed based on bioisosteric replacement of functional groups in the antimalarial compounds mefloquine and amodiaquine (**11**) [20]. We observed that a trifluoromethyl group at the 2-position of the triazolopyrimidine ring plays an important role in antiplasmodial activity (Fig. 2).

Following the good results obtained with 2-(trifluoromethyl) [1,2,4]triazolo[1,5-*a*]pyrimidines [20], in this study, we planned to use the ring bioisosterism replacement (green) of triazolopyrimidine **10** (Fig. 3) to obtain 7-arylaminopyrazolo[1,5-*a*]pyrimidines. . Molecular hybridization (blue) of 7-β-naphtylamino of **10** was also used. Different arylamines (blue) were incorporated into the structure to investigate the importance of the substituent at the 7-position of the pyrazolopyrimidine nucleus. Investigation of the impact of the substituents CF_3 and CH_3 (red) in the 2- or 5-positions of the pyrazolo[1,5-*a*]pyrimidine scaffold was prioritized. These compounds were evaluated *in vitro* against *P. falciparum*, *in vivo* as antimalarial, and *in vitro* as *Pf*DHODH inhibitors.

In addition, a molecular docking study was performed to evaluate the possible binding mode of the 7-arylaminopyrazolo[1,5-*a*] pyrimidine compounds to *Pf*DHODH [21].

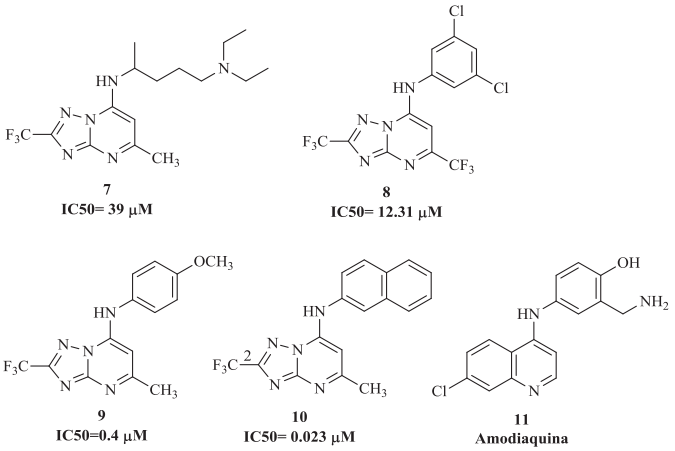

Fig. 2. 2-(trifluoromethyl) [1,2,4]triazolo[1,5-*a*]pyrimidines **7**–**10** reported as having antiplasmodial activity.

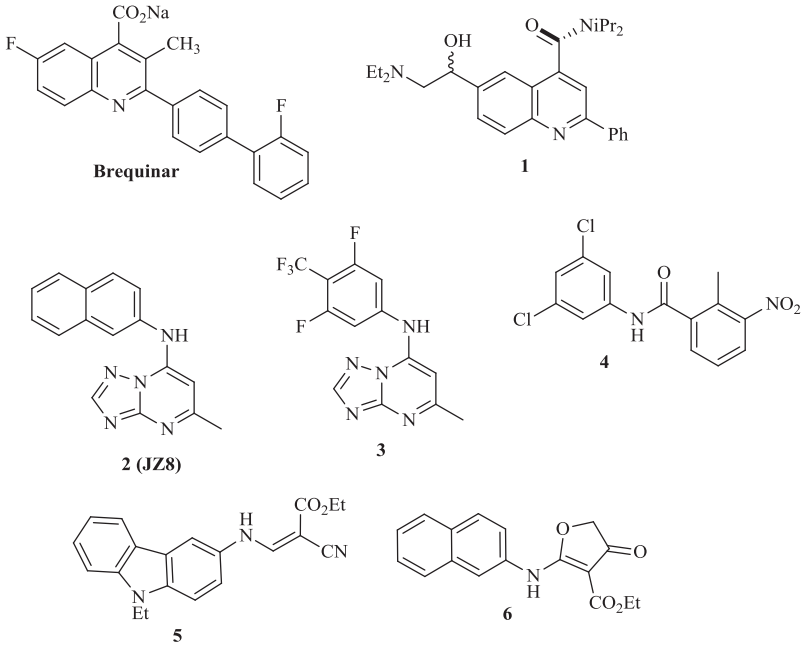

Fig. 1. Brequinar and compounds **1**–**6** as *Pf*DHODH inhibitors.