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Original article

Discovery of new thienopyrimidinone derivatives displaying antimalarial properties toward both erythrocytic and hepatic stages of *Plasmodium*

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ABSTRACT

A preliminary *in vitro* screening of compounds belonging to various chemical families from our library revealed the thieno[3,2-*d*]pyrimidin-4(3*H*)-one scaffold displayed a promising profile against *Plasmodium falciparum*. Then, 120 new derivatives were synthesized and evaluated *in vitro*; compared to drug references, 40 showed good activity toward chloroquine sensitive (IC_{50} 35–344 nM) and resistant (IC_{50} 45–800 nM) *P. falciparum* strains. They were neither cytotoxic (CC_{50} 15–50 μM) toward HepG2 and CHO cells, nor mutagenic. Structure–activity relationships were defined. The lead-compound also appeared active against the *Plasmodium* liver stages (*Plasmodium yoelii* IC_{50} = 35 nM) and a preliminary *in vivo* evaluation indicated the *in vitro* activity was preserved (45% reduction in parasitemia compared to untreated infected mice). A mechanistic study demonstrated these molecules do not involve any of the pathways described for commercial drugs and exert a specific activity on the ring and trophozoite stages.

1. Introduction

Malaria is a devastating pathology, which in 2012 affected 207 million people worldwide (range 135–287 million) and caused 627,000 deaths (range 473,000–789,000) [1]. This infection, transmitted *via* the bite of the female *Anopheles* mosquito, is caused by five species of protozoan parasites belonging to the *Plasmodium* genus, namely *falciparum*, *malariae*, *vivax*, *ovale* and *knowlesi*. *Plasmodium falciparum* is the most virulent [2], causing more than 95% of malaria-related morbidity and mortality. According to the WHO [1], important and durable progress has been recorded in recent years, with the estimated incidence of malaria globally

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reduced by 25% since 2000. Moreover, malaria-specific mortality rates were also reduced by 42% between 2000 and 2012. These encouraging statistics indicate that malaria programs are having a good impact, the combined result of vector control, chemoprevention, diagnostic testing and effective malaria treatment. Nonetheless, the growing drug resistance of parasites around the world [3] remains a real and ever-present danger, attributable mainly to *P. falciparum*. Currently, the only fully effective antimalarial drugs utilize artemisinin and its derivatives in combination with several different partner drugs in artemisinin-based combination therapies (ACTs), now recommended as the first line of treatment in endemic areas. However, reduced susceptibility of *P. falciparum* to treatment with artemisinin derivatives identified and confirmed on the Cambodia–Thailand border in 2009 [4,5]. This emerging resistance could lead to a resurgence of more virulent levels of malaria unless new chemical classes of effective drugs be rapidly found. In this context, all innovative practices [6] and encouraging results are being sponsored and shared [7,8], so as to accelerate the development and licensing of new antimalarial drugs. Particularly, as the currently available 8-aminoquinolines (primaquine, tafenoquine) can lead to severe side effects such as acute intravascular hemolysis in individuals with severe glucose-6-phosphate deficiency, the search for original compounds capable of eliminating hepatic stages of the parasite, including *Plasmodium vivax* hypnozoites, is becoming essential [9,10].

This work began by an *in vitro* screening of numerous molecules belonging to our chemical library (part of the CNRS French National Chemical Library), toward *P. falciparum*. This library contained numerous human kinase inhibitor-candidates which had previously been synthesized for a research program centered on the design of new anti-cancer agents [11–13]. Among tested compounds, a thieno[3,2-*d*]pyrimidin-4(3*H*)-one derivative appeared as a hit-compound (IC_{50} < 10 μM against the K1 strain). A closely related scaffold had already been proposed by GlaxoSmithKline as a potential antimalarial structure [7] (Fig. 1). In this context, we investigated the antiplasmodial properties of a series of 120 new derivatives [14]. The very recent work reported by Gonzalez Cabrera et al. also demonstrated the great potential of the 2-aminated-6-arylthienopyrimidine scaffold (Fig. 1) in the research for new antimalarial products [15].

2. Results and discussion

Original thieno[3,2-*d*]pyrimidin-4(3*H*)-one derivatives were synthesized by a one pot procedure including a condensation and a cyclization of methyl-3-amino-5-*p*-tolylthiophene-2-carboxylate with ethoxycarbonyle isothiocyanate in DMF [14]. During this reaction, an intermediary thiourea carbamate was formed. This species then reacted with an alkylamine, added to the crude mixture with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide (EDCI, HCl) [16], and triethylamine [17]. As an example, using terbutylamine, this process led to the formation of 2-(*tert*-butylamino)-6-*p*-tolylthieno[3,2-*d*]pyrimidin-4(3*H*)-one **1** in 70% yield (Scheme 1).

Compound **1** was initially evaluated *in vitro* for its antiplasmodial profile against a chloroquine sensitive (3D7) and a multi-drug resistant (K1) *P. falciparum* strains. The evaluation of *in vitro* cytotoxicity was performed on two complementary adherent cancer cell lines: HepG2 and CHO. HepG2 is a commonly used human-derived hepatocarcinoma cell line expressing many of the hepatocyte-specific metabolic enzymes. The aim of this assay using HepG2 in addition to CHO cells was to evaluate the impact of metabolic activation of the tested compounds on cell viability [18] (Table 1).

Based on these experimental results, compound **1** was used for SAR studies and three regions were probed within this chemical structure: the amine region A, the substitution of the phenyl moiety B and the nature of the cycle C (Fig. 2). All derivatives described below were prepared by using the general route presented for the preparation of compound **1** in Scheme 1.

From the structure of hit-compound **1**, primary, secondary and tertiary amine substituents were explored in region A (Table 2). Compounds including a secondary amine at position 2 were selected as the best derivatives since others (compounds **2**, **14**–**18**) showed poor solubility in the cell culture medium, a high toxicity toward the HepG2 cells and/or no antiplasmodial activity. However, although the compounds including a secondary amine at position 2 (compounds **3**, **8**–**13**) did not appear to be cytotoxic, the substitution of the nitrogen atom of the amine group by a longer aliphatic chain notably increased the cytotoxicity (compounds **4**–**7**). Among all tested substituents, *tert*-butylamine (compound **1**) and isopropylamine (compound **8**) provided the best antiplasmodial profiles.

A SAR study was also conducted for region B (Table 3). The suppression of the methyl substituent of the phenyl ring led to a reduction of the potency (compound **27**). The substitution of the same methyl group by an electron-withdrawing group at the *para* position, such as chlorine (compounds **20**, **24**), bromine (compounds **19**, **23**), fluorine (compounds **21**, **25**), or by an electron-donating group, such as methoxy (compounds **22**, **26**), were tolerated mainly with isopropylamine at region A. However, a significant loss in potency was observed if the phenyl group was suppressed (compounds **28**, **29**) or moved at position 7 of the thieno[3,2-*d*]pyrimidin-4(3*H*)-one ring (compounds **32**–**38**). Finally, two analogs derived from compound **1**, 2-(*tert*-butylamino)-6-*m*-tolylthieno[3,2-*d*]pyrimidin-4(3*H*)-one **30** and 2-(*tert*-butylamino)-6-*o*-tolylthieno[3,2-*d*]pyrimidin-4(3*H*)-one **31** were 3–7 fold less potent than the hit-compound and also more cytotoxic.

We finally explored the SAR based on region C by modifying the heterocyclic core (Table 4). As various substituted quinazolines have already been described as antiplasmodial agents [19–23], we particularly studied the replacement of the thiophene ring by a benzene one, resulting in quinazoline analogs (Table 4). This modification led to a significant loss of activity and widely

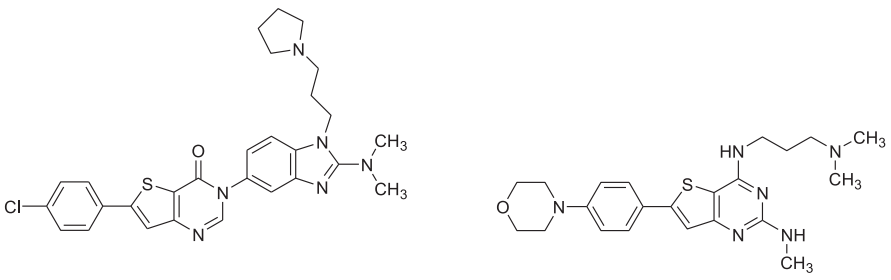

Fig. 1. Thieno[3,2-*d*]pyrimidine derivatives described as antiplasmodial compound by GlaxoSmithKline [7] and Gonzalez Cabrera et al. [15].