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Novel squaramides with in vitro liver stage antiplasmodial activity

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ABSTRACT

A structure–activity relationship study was performed with ten 8-aminoquinoline-squaramides compounds active against liver stage malaria parasites, using human hepatoma cells (Huh7) infected by *Plasmodium berghei* parasites. In addition, their blood-schizontocidal activity was assessed against chloroquine-resistant W2 strain *Plasmodium falciparum*. Compound **3** was 7.3-fold more potent than the positive control primaquine against liver-stage parasites, illustrating the importance of the squarate moiety to activity.

1. Introduction

Malaria is the most deadly human protozoan infection. It is caused by parasites belonging to the genus *Plasmodium*, with an estimated 1.1 billion people at high risk of being infected and developing disease. There are five *Plasmodium* species that infect humans, with *Plasmodium falciparum* and *Plasmodium vivax* contributing the most to the public health burden posed by malaria.[1,2]

The life cycle of *Plasmodium* parasites includes infection of mammalian hosts and an invertebrate vector. In their mammalian host, sporozoites injected by an infected mosquito migrate to the liver, where they undergo an asymptomatic, yet obligatory phase of development inside hepatocytes.[3] This results in the formation of merozoites that emerge from hepatocytes and invade red blood cells to cause the symptoms of malaria.

Most drugs employed in the treatment of malaria act mainly as potent erythrocytic stage antimalarials.[4,5] However, to achieve malaria eradication, targeting the *Plasmodium* liver stage will be helpful.[6,7] Given the asymptomatic, yet obligatory nature of the liver stage, effective prophylaxis can be achieved by targeting *Plasmodium* liver forms. In addition, anti-liver stage drugs are essential to control *P. vivax* and *Plasmodium ovale* infections, since in these species latent hypnozoites may persist in the liver after *Plasmodium* bloodstream clearance, resulting in relapse and, therefore, representing a potential reservoir of infection.[8]

The 8-aminoquinoline derivative primaquine (**1**, Fig. 1) is the only available drug that is active against all *Plasmodium* exoerythrocytic forms, including latent hypnozoites and gametocytes that are transmitted to the *Anopheles* vector. In fact, the World Health Organization recommends primaquine for radical treatment of *P. vivax* infections and as a single dose for treatment of *P. falciparum* as a gametocidal agent.[1] More recently, other 8-aminoquinoline derivatives were developed, displaying better efficacy and lower toxicity, such as bulaquine (**2**), whose use has already been approved in India.[9] This scaffold continues to represent one of the most studied families of liver stage-targeting drugs, especially employing a hybridization strategy approach.[10–19] Nevertheless, finding new scaffolds capable of targeting the liver stage of infection continues to be challenging, due to technical difficulties in studying liver stage forms, as well as significant problems in the identification of valuable *Plasmodium*-specific liver stage targets.[8,20]

Squaramides represent a unique moiety capable of establishing up to four H-bonds, by acting as H-bond acceptor and donor and, therefore, they are widely used in organocatalysis,[21–24] in chemical biology (bioconjugation to proteins and carbohydrates, and as ion receptors)[22,23] and in medicinal chemistry (antiplasmodial,[25] antichagasic,[26] anticancer,[27,28] and antibacterial[29] activity).

We previously reported the antiplasmodial activity of squaramide derivatives.[30–32] In particular, squaramides linked to 4-aminoquinolines and squaramides linked to primaquine (**3**, **4**) were tested against the chloroquine-resistant W2 strain of *P. falciparum*.[30] The similarity of compound **3** to bulaquine prompted us

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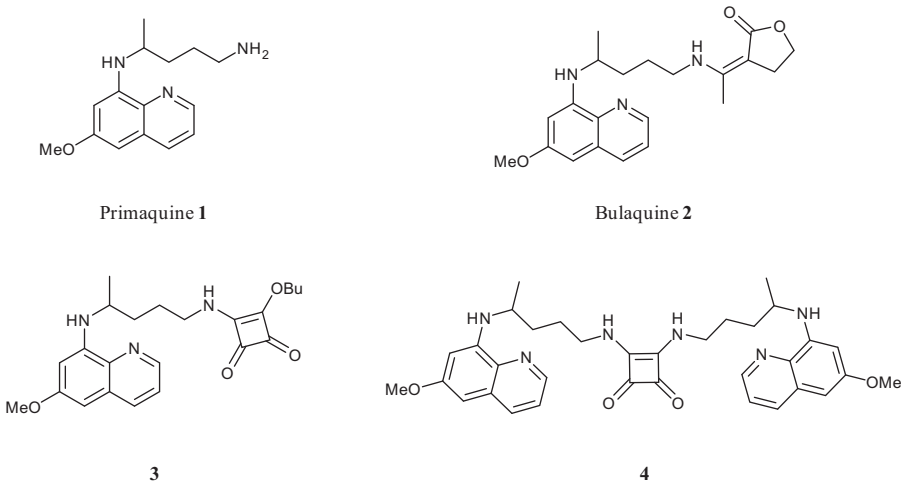

Figure 1. Chemical structure of 8-aminoquinoline derivatives with antimalarial activity.

to further explore the antiplasmodial potential of hybrid compounds containing an 8-aminoquinoline moiety linked to squaramide and to evaluate these novel compounds for their activity against *Plasmodium* liver stages. In order to perform a structure–activity relationship (SAR) study, particularly directed to understand the structural features for optimal liver stage activity, we synthesized compounds containing different linkers between the squaramide and the 8-aminoquinoline moieties, and different side chains attached to the squarate ring (Fig. 2).

2. Results and discussion

2.1. Chemistry

Compounds **5a**–**h** were synthesized starting from commercially available 6-methoxy-8-nitroquinoline (**6**), which was reduced by Pd/C catalyzed hydrogenation, forming 6-methoxy-8-aminoquinoline (**7**) in quantitative yield. Mono-squaramides **5a**–**d** were synthesized by reacting 8-aminoquinolines, with different linkers, with 3,4-dibutoxy-3-cyclobutene-1,2-dione (**8a**). To obtain the required starting materials, compounds **10a**–**d** were synthesized by reaction of compound **7** with different 2-bromoalkyl-isoindoline-1,3-diones (**9a**–**d**) in the presence of trimethylamine, as described previously[33] (Scheme 1). Starting materials **9a**–**b** were obtained commercially, while compounds **9c**–**d** were prepared by reacting the corresponding dibromoalkyl derivative with potassium isoindoline-2,3-dione in acetone.[34] Reflux of compounds **10** and hydrazine hydrate in ethanol led to compounds **11a**–**d** in 86–98% yields.[33] Mono-squaramides **5a**–**b** were synthesized by reacting compounds **11a**–**b** with **8a** in methanol, in low yields (21% and 39% yield, respectively), and compounds **5c**–**d** were synthesized by reacting **8a** with compounds **11c**–**d** in butanol, in the presence of triethylamine (45% and 35% yield, respectively). We also tried to synthesize the mono-squaramide **5** with R^1 = H, n = 3 (Scheme 1), but the corresponding starting material did not react with 3,4-dibutoxy-3-cyclobutene-1,2-dione (**8a**) (step d of Scheme 1).

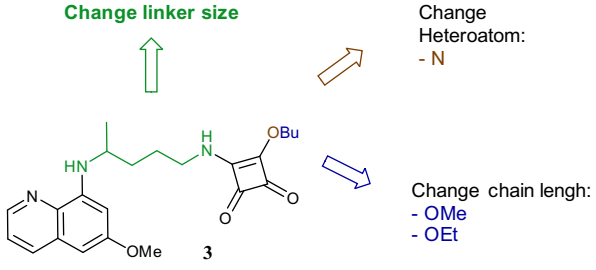

Figure 2. Synthetic strategy scope.

Compound **5e** was obtained by reaction of compound **7** with 3,4-dibutoxy-3-cyclobutene-1,2-dione (**8a**) in 50% yield (Scheme 1).

Mono-squaramides **5f**–**g**, with different alkoxy side chains, were also synthesized (Scheme 2). Compound **5f** was synthesized in 74% yield by reacting primaquine with 3,4-dimethoxy-3-cyclobutene-1,2-dione (**8b**) in methanol. The reaction of primaquine with 3,4-dibutoxy-3-cyclobutene-1,2-dione (**8a**) in ethanol gave rise to mono-squaramide **5g** as major product (61% yield) and as minor product, compound **3**.

Compound **5h**, with a *N*-butoxy side chain, was obtained by reaction of compound **5f** with *n*-butilamine, in the presence of triethylamine, with 86% yield (Scheme 2).

2.2. Biological activity and SAR study

The squaramide derivatives were evaluated for their ability to inhibit infection of the Huh7 human hepatoma cell line by *Plasmodium berghei* parasites. We started our work by testing the previously reported squaramides **3** and **4**. Both compounds had an IC_{50} value of ~1.3 μM, which represents a 7.3-fold increase in potency when compared to primaquine (Table 1). In addition, the IC_{50} of the starting compound **8a** was higher than 10 μM (Fig. 3), showing that linking primaquine and squaric moieties in the same molecule resulted in a more active compound. Furthermore, the initial observation that **3** and **4** are equipotent suggested that adding a second primaquine moiety to the squarate scaffold would not improve activity against the liver stage parasite. Therefore, our subsequent derivatizations focused only on the mono-squaramide derivative **3** (Fig. 2).

To investigate the importance of the linker between the 8-aminoquinoline and squaramide moieties, we synthesized and evaluated compounds **5a**–**e** (Fig. 3, Table 1). Derivative **5e**, without linker, was inactive up to 10 μM concentration. All derivatives with a linker were more active than **5e**, with an IC_{50} value lower than 5 μM. Varying the linker length from two to five carbon atoms revealed that the most potent derivatives were compounds **5a** (R^1 = H, n = 2) and **5d** (R^1 = H, n = 4), with an IC_{50} of 1.8 and 2.2 μM, respectively. Next, we evaluated the importance of the