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CONCISE ARTICLE

PRIMACINS, *N*-cinnamoyl-primaquine conjugates, with improved liver-stage antimalarial activity†

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Novel primaquine derivatives, obtained by conjugation of the drug's aliphatic amine with different cinnamic acids, resulted in increased *in vitro* activity, compared to primaquine, against liver-stage malarial parasites. The compounds were non-cytotoxic to human hepatoma cells, suggesting that they are a promising new class of agents for the treatment and prevention of malaria.

Effective and safe antimalarial drugs active against both liver and erythrocytic parasite stages will be valuable components of malaria eradication strategies.[1] Mammalian infection by malarial parasites is initiated by an infected mosquito bite, followed by a clinically silent liver-stage infection, during which thousands of merozoites are formed to be released into the bloodstream and trigger the clinically relevant erythrocytic stage of the life cycle.[2] Drugs able to act on the liver stage are highly relevant, as they can be used in chemoprophylaxis to prevent all clinical manifestations of malaria.[3] Moreover, such drugs are particularly relevant in *P. vivax* and *P. ovale* infections, as in these species latent forms that are called hypnozoites are not eliminated by most available therapies, persist in the liver for long periods, and subsequently cause malaria relapses. At present, drugs acting against parasite liver forms are scarce, and primaquine (PQ, **1** in Scheme 1) remains the only drug in clinical use that acts against liver stages of all *Plasmodium* species,[4] including *P. vivax* and *P. ovale* hypnozoites.[5] Unfortunately, PQ's low oral bioavailability and high hemotoxicity hold back its clinical use, especially in vulnerable populations including pregnant women, infants and the elderly. In addition, PQ causes hemolysis in patients with congenital deficiency of glucose-6-phosphate dehydrogenase,[5] complicating its use to treat *P. vivax* and *P. ovale* malaria and limiting its use in chemoprophylaxis.

For the past decade, we have been working on chemical approaches to mask the PQ aliphatic amine in order to obtain analogues resistant to oxidative deamination, the major metabolic pathway underlying the drug's low oral bioavailability (Scheme 1) by conversion into an inactive metabolite, carboxyprimaquine (**2** in Scheme 1).[5] To this end, we have successfully developed imidazoquines (**3** in Scheme 1)[6,7] and primacenes (**4** in Scheme 1),[8,9] peptidomimetic and organometallic derivatives of PQ with promising antimalarial properties, respectively. Now, we disclose a new family of PQ derivatives, the PRIMACINS (**5** in Scheme 1), obtained by coupling PQ to cinnamic acids, as the latter was formerly reported to possess interesting antimalarial properties.[10] PRIMACINS were found to have higher *in vitro* activity than PQ against the liver-stage of the rodent malarial parasite *P. berghei* (Fig. 1).

PRIMACINS were tested against liver stages of the rodent parasite *P. berghei* and against erythrocytic stages of the human parasite *P. falciparum* (Table 1). Compounds **5** were two- or more-fold more potent than PQ against liver stage *P. berghei* and were non-toxic to Huh7 human hepatoma cells *in vitro*, as shown by cell confluency analysis (Fig. 1). The activities present a relatively short activity range (1.38–2.39 μM), so any discussion on structure–activity relationships based on the IC_{50} values obtained would be of no significance. Furthermore, given that the mechanisms of action of PQ against liver-stage parasites are still poorly understood,[5] it is always risky to attempt a justification for activity trends observed, if any. Anyway, lipophilicity may have had some influence on compound's activity, as the two most active compounds, **5h** and **5c**, were also the ones presenting higher log *P* values, 5.77 and 6.15,[12] respectively.

Regarding erythrocytic-stage activity, **5c** was the only compound that displayed an IC_{50} below 10 μM of the eleven compounds **5**, which was nevertheless one order of magnitude higher than that of the reference erythrocytic-stage drug, artemisinin. The broad inactivity of compounds **5** against erythrocytic-stage parasites was not particularly surprising, as (i) PQ is itself a modest blood-schizontocidal[5] and (ii) compounds **5** lack a

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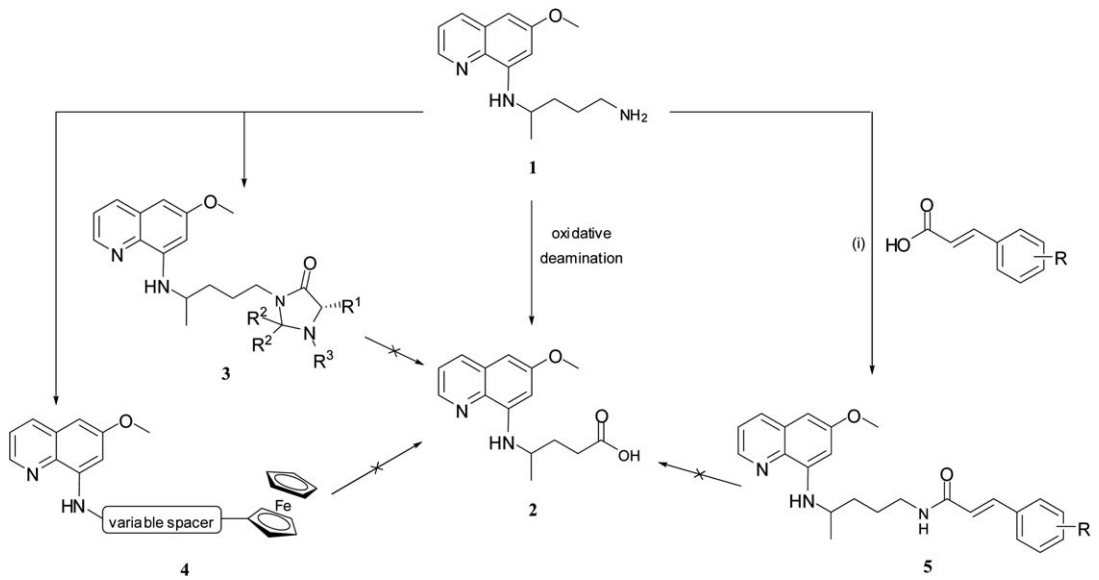

Scheme 1 Chemical structures of primaquine (**1**), its main metabolite, carboxyprimaquine (**2**), PQ derivatives formerly developed by us, namely, imidazoquines (**3**)[6,7] and primacenes (**4**),[8,9] and PRIMACINS herein reported (**5**): (i) PQ was coupled to cinnamic acids in the presence of 1.1 molar equivalents (eq.) of TBTU and 2 eq. of *N*-ethyl-*N*,*N*-diisopropylamine, at room temperature for 24 h, using *N*,*N*-dimethylformamide as solvent.

basic amine: the relevance of a basic amino group linked to the 8-aminoquinoline core through a chain of two to six carbons for the activity of PQ against erythrocytic *Plasmodia* was established three decades ago.[5,13,14] In fact, more recent well-known PQ derivatives displaying some erythrocytic-stage activity have the PQ's basic aliphatic chain either conserved (*e.g.*, tafenoquine) or modified in a way that reasonably preserves its basicity (*e.g.*, bulaquine and sitamaquine).[5] Compared to other cinnamic derivatives, which present low μM activities against Pf Dd2 and W2 strains,[10,15] combining the cinnamic moiety with PQ clearly led to a loss of erythrocytic-stage activity. This suggests that the lack of compounds **5** activity is due to the presence of the 8-aminoquinoline pharmacophore and supports that the absence of the aforesaid aliphatic amine is detrimental for inhibitory erythrocytic activity of PQ derivatives.

As shown here, the absence of the aforementioned aliphatic amine did not preclude the significant activity of PRIMACINS **5** against liver-stage *Plasmodia*. This result agrees with our previous findings on PQ derivatives **4**, as these organometallic compounds were also highly active against liver-stage parasites, regardless of whether a basic aliphatic amine group was present. Yet, compounds **4** which lacked a basic aliphatic amine group

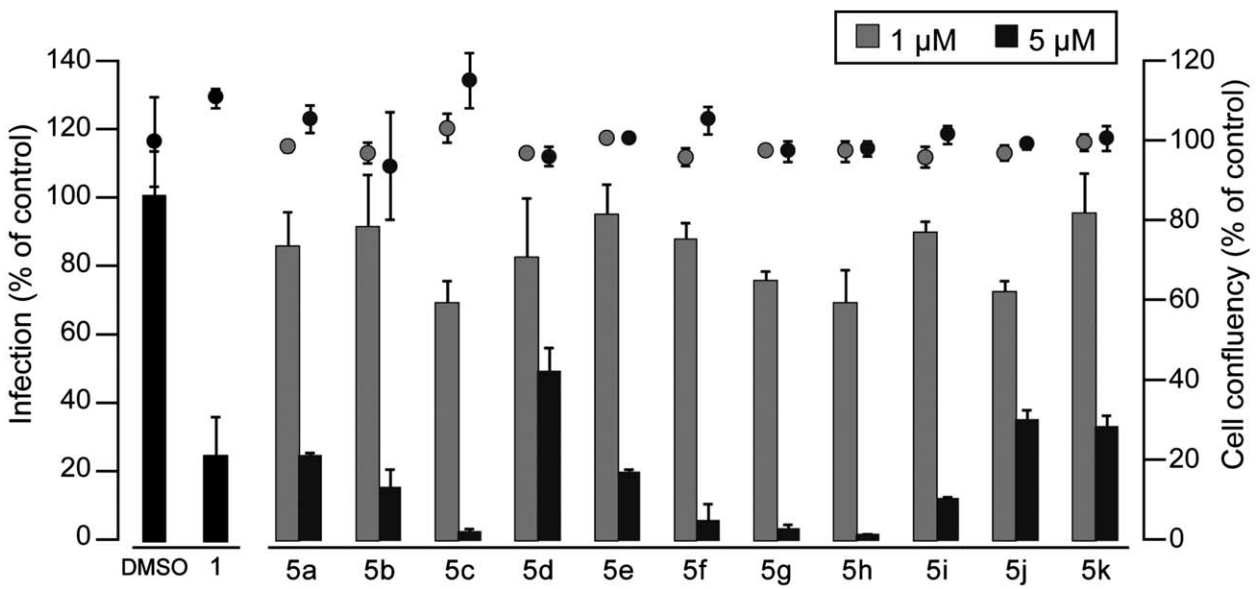

Fig. 1 Activity of PRIMACINS **5** against *P. berghei* liver stages. Anti-infective activity (infection scale, bars) and toxicity to hepatoma cells (cell confluency scale, circles) are shown. Primaquine (**1**) at 10 μM was included for comparison. Infection loads of Huh7 cells, a human hepatoma cell line, were determined by bioluminescence measurements of cell lysates 48 h after infection with luciferase-expressing *P. berghei* parasites.[11]

Table 1 Antiplasmodial activities of compounds **5**

Compound	R	$IC_{50} \pm SD$ (μM) against liver-stage *P. berghei*	IC_{50} (μM) against erythrocytic-stage *P. falciparum*[a]
Reference drug[b]	—	7.50 (PQ)	5.64 nM (ART)
5a	H	2.01 ± 0.27	≥10
5b	*p*-Me	2.13 ± 0.58	≥10
5c	*p*-^{i}Pr	1.54 ± 0.35	4.84
5d	*p*-OMe	2.35 ± 0.19	≥10
5e	*m*-F	1.63 ± 0.16	≥10
5f	*p*-F	2.33 ± 0.47	≥10
5g	*p*-Cl	2.39 ± 0.19	≥10
5h	*p*-Br	1.38 ± 0.33	≥10
5i	*o*-NO_2	2.36 ± 0.58	≥10
5j	*m*-NO_2	2.12 ± 0.49	≥10
5k	*p*-NO_2	2.24 ± 0.27	≥10

[a] Chloroquine-resistant strain W2. [b] PQ for liver-stage activity assays; artemisinin (ART) for erythrocytic-stage activity assays.

were inactive both against erythrocytic-stage parasites and as transmission-blocking agents.[8,9]

Concluding remarks

Novel primaquine derivatives, PRIMACINS **5**, have been synthesized through a cheap and simple one-step condensation of the parent antimalarial drug with a series of cinnamic acids. The compounds were screened *in vitro* for their activity against two major stages of malarial infection in the mammalian host, the liver- and the erythrocytic-stage, and found to have about two- or more-fold higher potency than the parent drug against liver parasites while non-toxic to human hepatoma cells.

Masking the aliphatic amine group of primaquine through conjugation with the cinnamic moiety impairs the metabolic oxidative deamination that underlies its low oral bioavailability, which in turn requires administration of high doses of this hemotoxic antimalarial drug. Thus, PRIMACINS represent a new class of compounds toward the development of more effective and potentially safer liver-stage active primaquine derivatives.

To the best of our knowledge, this is an unprecedented disclosure of primaquine–cinnamic acid conjugates as antimalarial leads.

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