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Antiplasmodial activities of 4-aminoquinoline–statine compounds

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ABSTRACT

We report the discovery of new potent inhibitors of the growth of *Plasmodium falciparum* chloroquine (CQ)-resistant W2 strain. These compounds were designed using the double drug approach by introducing a residue able to enhance the accumulation of plasmepsins inhibitors into the food vacuole. Some of the molecules were more active than CQ against CQ-resistant strain and showed good selectivity against cathepsin D.

Malaria is one of the major health problems worldwide, common in the tropics, especially south of Sahara. In 2010 malaria caused about 216 million infections and it was responsible for approximately 655,000 deaths and about 86% of these occurred in children under 5 years of age.[1] Five parasite species are responsible for human malaria: *Plasmodium vivax* (*Pv*), *Plasmodium ovale* (*Po*), *Plasmodium malariae* (*Pm*), *Plasmodium falciparum* (*Pf*) and more recently *Plasmodium knowlesi* (*Pk*).[2] *Pf* infections, if not treated in a timely fashion and with effective drugs, progress to severe forms of disease that are frequently fatal. From the WHO World Malaria Report 2011, *Pf* infection is one of the leading causes of deaths worldwide from a single infectious agent.[1] Due to the rapid spread of parasite resistance to available antimalarial drugs there is an urgent need for new drugs with novel mechanisms of action.[1]

Vacuolar plasmepsins are a family of plasmodial aspartic proteases comprising plasmepsin 1 (PLM 1), plasmepsin 2 (PLM 2), plasmepsins 4 (PLM 4) and a histo-aspartic protease (HAP). These enzymes are localized in the food vacuole of *Pf* and are involved in the degradation of hemoglobin during the erythrocytic stage of the parasite.[3] Vacuolar PLMs are considered promising targets for the development of new antimalarial compounds.[3] However, PLMs inhibitors, including molecules with a statine-based core, have shown potent enzyme inhibition, but limited efficacy in killing the parasite (IC_{50} range 2–20 μM).[3] The current view is that the drug discovery efforts focused on vacuolar plasmepsins should consider the development of compounds that can inhibit two or more of this enzyme family.[4,5]

During the hemoglobin degradation process, the heme group is detoxified by crystallization into hemozoin. 4-Aminoquinolines (Chloroquine (CQ), Amodiaquine, Tebuquine) have been historically among the most popular and effective antimalarials acting by inhibition of hemozoin formation. In vitro antagonism has been demonstrated by the combination of a protease inhibitor with a 4-aminoquinoline, since both molecules interfere with the hemoglobin catabolism process.[6] It is also known that the antiplasmodial activity of the 4-aminoquinolines is increased by their selective accumulation into the acidic parasite food vacuole thanks to their peculiar basic character.[7]

In our previous studies,[8–11] we synthesized a series of PLM(s) inhibitors based on the double drug approach[12] that combines two different moieties, acting against different targets, into a single chemical entity. The synthesized compounds showed a remarkable improvement of inhibition of *Pf* growth with good selectivity towards cathepsin and low toxicity against human dermal fibroblasts. In particular, compound **1**, (Fig. 1) presented relevant activities against CQ-sensitive (D10) and CQ-resistant (W2) *Pf* strains

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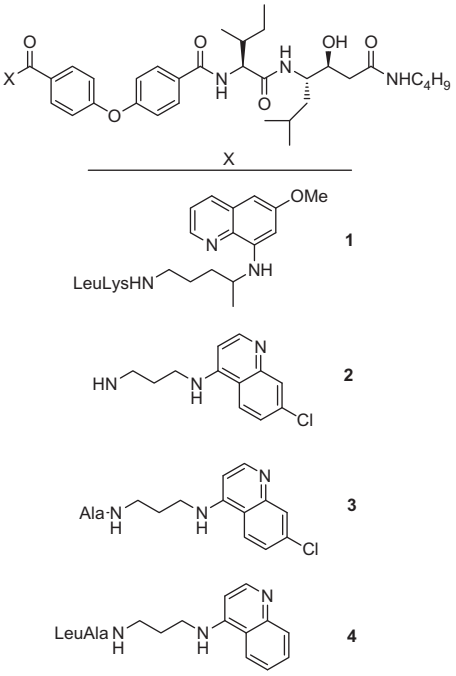
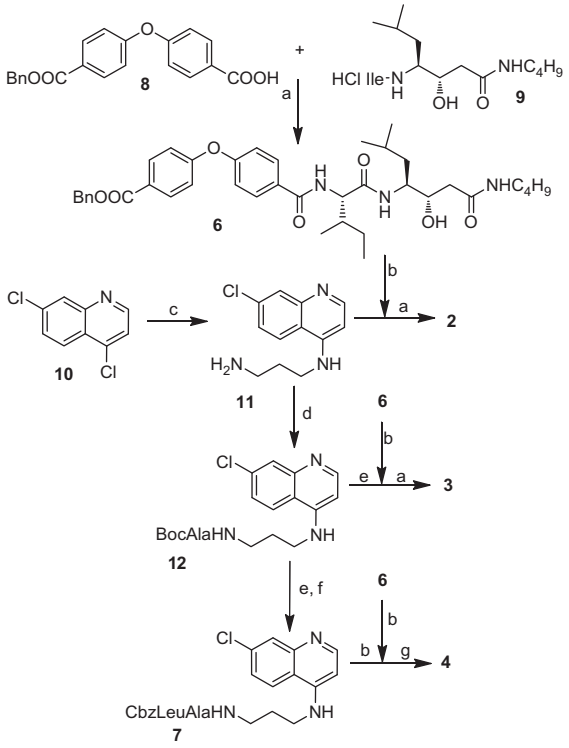


Figure 1. Structures of reference compound **1** and 4-aminoquinoline–statine compounds **2**–**4**.

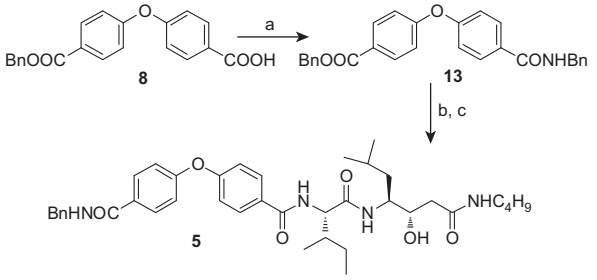
(IC_{50} 400–700 nM) and displayed low cytoxicity against human dermal fibroblasts (50% @ 100 μM).[8]

To further improve the antiplasmodial activities of our inhibitors, we reasoned that the introduction of the 7-chloro-4-aminoquinoline ring system derived from CQ in the statine double drugs could improve the accumulation of plasmepsins inhibitors into the food vacuole. Compounds **2**–**4** (Fig. 1) were designed and synthesized to define the effects of CQ on the double drugs. Compound **2** shows the aminoquinoline ring system bound to the oxybisbenzoic acid through an aminoalkyl spacer analogue to CQ, while compound **3** presents an additional alanine spacer between the two molecules. This substitution was inspired by our previous work on primaquine-statine double drugs where a 10-fold improvement in antiplasmodial activity was observed by introducing a spacer composed of one or two amino acids between primaquine and the aromatic acid.[9] Compound **4** was designed considering that the chlorine atom in position 7 is essential for inhibition of β-hematin formation, but its removal improves vacuolar accumulation.[13] In order to further explore the contribution of PLMs inhibition to the antiplasmodial activity of the double drugs, compounds **5**, **6** and **7** were synthesized (Scheme 1 and Scheme 2). In these compounds the quinolinic ring system has been replaced with a benzylic amide (**5**) or a benzylic ester (**6**) and the statine portion of the double drug has been removed (**7**).

Compounds **2**–**7** were prepared according to Schemes 1 and 2. Compound **2** was obtained by coupling IleStatine hydrochloride **9**[9] with the monobenzylester of 4,4′-oxybisbenzoic acic **8**,[9] followed by coupling the deprotected benzyl ester **6** with amine **11**, obtained by the nucleophilic attack of 1,3-diaminopropane to 4,7-dichloroquinoline **10** (Scheme 1). Compound **3** was obtained by coupling deprotected ester **6** with deprotected compound **12**. Compound **12** was obtained by coupling compound **11** with Boc–Ala. Compound **4** was obtained by coupling deprotected dipeptide **7** with deprotected ester **6** using HATU as coupling reagent. As expected during catalytic hydrogenation of compound **7** the chlorine atom in position 7 was removed (Scheme 1).[14] Compound **7** was obtained by removal of the Boc group from compound **12**



Scheme 1. Reagents and conditions: (a) HBTU,HOBt, NMM; (b) H_2, Pd/C; (c) Diaminopropane, 140 °C; (d) BocAlaOH, HBTU, HOBt; (e) HCl 4N, dioxane; (f) CbzLeuOH, HBTU, HOBt; (g) HATU.



Scheme 2. Reagents and conditions: (a) Benzylamine, HBTU; (b) H_2, Pd/C; (c) **9**, NMM, HOBt, HBTU.

followed by coupling with CbzLeuOH (Scheme 1). Finally compound **5** (Scheme 2) was prepared by coupling monobenzylester **8** with benzylamine followed by removal of the benzyl ester and reaction with IleStatine hydrochloride **9**. All synthesized compounds gave satisfactory analytical (purity ⩾95% by HPLC) and spectroscopic data. ^{1}H NMR and HRMS spectra were consistent with the assigned structures.

Compounds **2**–**7**, have been tested in vitro against recombinant *Pf* PLM 2 and *Pf*, *Po*, *Pv* and *Pm* PLMs 4; and against D10 (CQ sensitive) and W2 (CQ resistant) *Pf* strains (see Supplementary data). [9] The selectivity versus human cathepsin D, which shares a high homology with PLMs, was also investigated for the most promising compounds (Table 1).

As expected, compounds **2**–**6** containing the β-hydroxyamino acid statine inhibited all PLMs at nanomolar concentrations, while CQ and the peptidic derivative of CQ **7** were completely inactive. Considering PLM 2, the K_i values of compounds **2**–**6** are comprised in a small range (1.3 nM–3.8 nM), while for *Pf* PLM 4 the inhibition constants are distributed in a greater range (2.6 nM–20 nM). Among orthologous PLM 4, *Pm* appears to be less susceptible to