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Plasmepsin II inhibition and antiplasmodial activity of Primaquine–Statine 'double-drugs'

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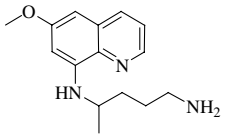
Abstract—Statine-based inhibitors of Plasmepsin II (PLMII) coupled with Primaquine have been designed using the 'double-drug' approach. The IC_{50} values for PLMII inhibition ranged from 0.59 to 400 nM and the best IC_{50} value for inhibition of *Plasmodium falciparum* growth in vitro was 0.4 μM, which represent a remarkable improvement compared to other statine-based PLMII inhibitors.

Malaria is one of the largest parasitic infections in the world and the incidence of malaria is estimated to be 300–500 million clinical cases each year, with a mortality rate of 1.1–2.7 million people, of whom about 1 million are children under the age of 5 years.[1] Drug resistance has spread and intensified over the last 15–20 years, leading to a dramatic decline in the efficacy of the most affordable antimalarial drugs; therefore, there is an urgent need of new antimalarial therapy.

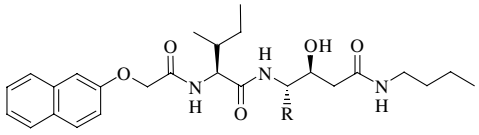
Plasmepsin II (PLM II) is one of the aspartic proteases involved in the degradation of haemoglobin during the intraerythrocytic cycle of *Plasmodium falciparum*.[2] The inhibition of the enzyme, leading to starvation of the parasite, is considered a valid drug target. Several PLM II inhibitors, including molecules with a statine-based core, have been developed. They possess low K_i values against the enzyme (nM order), but their effectiveness in killing the parasite is limited (IC_{50} range 10–20 μM).[3] Differences of this kind may be attributed to low bioavailability, probably because most of these inhibitors derive from peptides by replacement of the scissile peptide bond with a transition state analogue.[3] The peptidic character of these molecules is often the cause of their failure in the cell based assays.

The 'double-drug' approach has been successfully used to improve poor membrane permeation of some HIV protease inhibitors.[4] According to this approach a protease inhibitor unable to permeate cell membrane has been joined to AZT by a cleavable linker, as for KNI 694,[5] with a considerable boost in potency in the cell assay.

We report here the synthesis and the inhibition of PLM II and *P. falciparum* in vitro of molecules designed based on the 'double-drug' approach, in which a statine-based inhibitor of PLM II has been linked to the well known antimalarial Primaquine (PQ).



Primaquine



PS 777621; R=*i*-Butyl; K_i=180nM (Plasmepsin II)
PS 189863; R=Benzyl; Ki=110nM (Plasmepsin II)

Keywords: *P. falciparum*; Plasmepsin II; Statine; Primaquine; Double drug; Chimera drug; In vitro inhibition.
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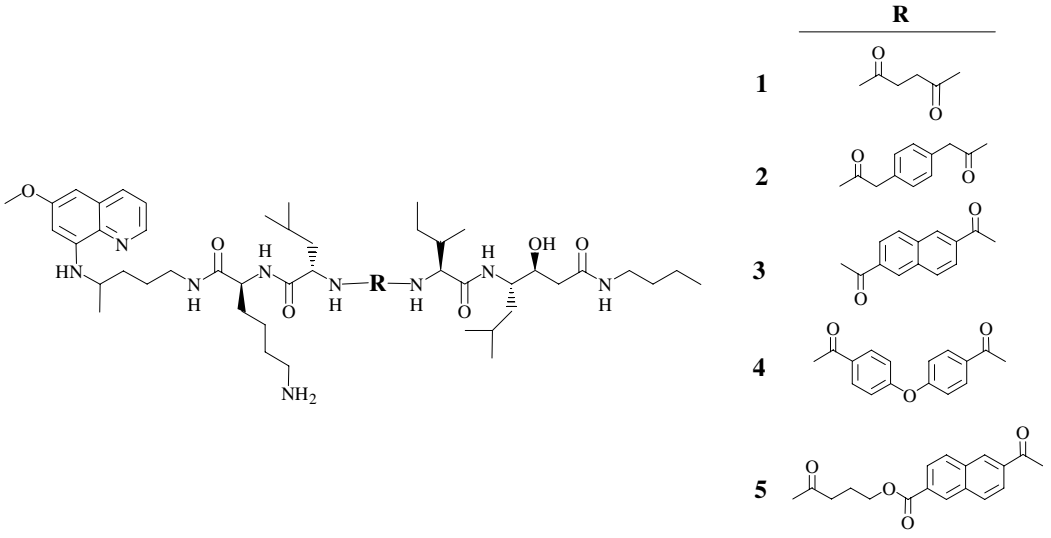


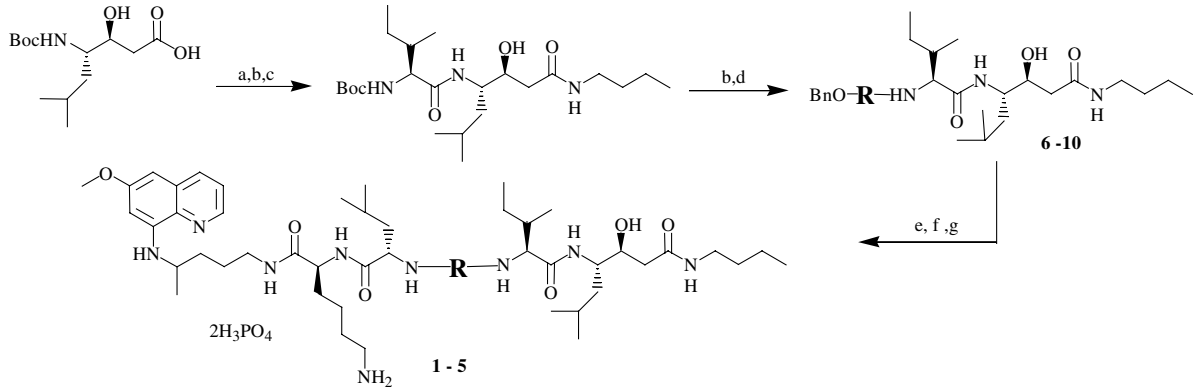
Several statine-based inhibitors, such as PS777621 and PS189863, have been developed.[6] These compounds showed inhibition of PLM II at nanomolar concentrations, but the IC_{50} values against *P. falciparum* growth in vitro were approximately 10 μM.[6]

Primaquine is highly active against the gametocytes of all malaria species infecting humans and against hypnozoites of the relapsing malaria caused by *P. vivax* and *P. ovale*, leading to the eradication of the infection. Concern about toxicity of PQ has limited its use, but recently PQ has been revaluated as a safe and effective agent for the prophylaxis of malaria due to both *P. falciparum* and *P. vivax*.[7] It was shown that the toxicity of PQ is diminished and the activity is increased by the conversion of PQ to peptide prodrugs, that is HVal-Leu-Lys-PQ, which, following cleavage by plasmin, releases the Lys-PQ fragment.[8]

We reasoned that the linkage of the peptide derivative HLeuLysPQ to the Ile residue of PS777621 would provide a potential 'double-drug'. We have thus synthesized compounds **1**–**5** that incorporate linkers derived from dicarboxylic acids that should mimic the P_3 residue of PS777621.

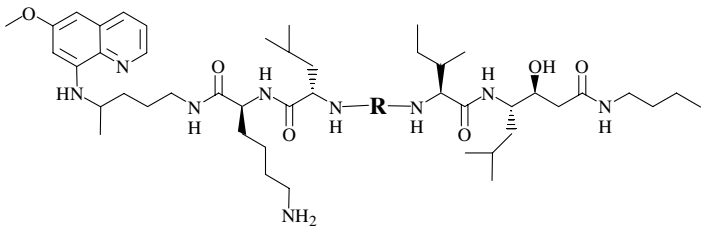
Compounds **1**–**5** were prepared starting from Boc-Sta(3S,4S)OH by coupling with butylamine, followed by removal of the Boc protecting group and coupling with BocIleOH. The dicarboxylic linkers were introduced as monobenzylesters using standard HBTU coupling after Boc deprotection (Scheme 1). Benzylesters **6**–**10** and CbzLeuLys(Boc)Primaquine[8] were hydrogenated and the crude material was coupled using HBTU/HOBt. Boc removal by TFA treatment followed by precipitation of the phosphate salt afforded final products **1**–**5** (Scheme 1).

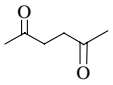
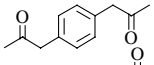
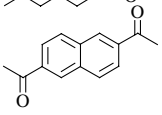
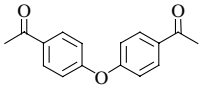
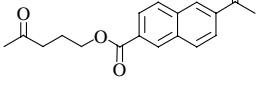
Compounds **1**–**5** were evaluated on PLMII[9,10] and human red blood cells infected with both chloroquine-sensitive (D10) and chloroquine-resistant (W2) strains of *P. falciparum*.[11,12] All compounds inhibited PLM II and parasite growth in a dose-dependent manner and the IC_{50} values are reported in Table 1.



Scheme 1. Reagents and conditions: (a) $C_4H_9NH_2$, HBTU, HOBt; (b) HCl, dioxane; (c) BocIleOH, HBTU, HOBt; (d) BnO**R**OH, HBTU, HOBt; (e) H_2, Pd–C; (f) H-Leu-Lys(Boc)-PQ, HBTU, HOBt; (g) TFA, H_3PO_4.

Table 1

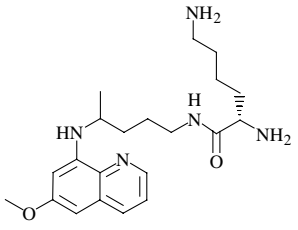


	R	PLM II $IC_{50} \pm SD$ (nM)	*Plasmodium falciparum* $IC_{50} \pm SD$ (μM)	
			D10	W2
1		396 ± 25	20% at 9 μM	40% at 9 μM
2		135 ± 4.4	5.5 ± 1.8	4.2 ± 0.2
3		1.51 ± 0.23	6.2 ± 0.8	4.7 ± 0.3
4		0.59 ± 0.02	0.4 ± 0.1	0.7 ± 0.4
5		9.6 ± 2.1	3.3 ± 0.3	1.0 ± 0.5

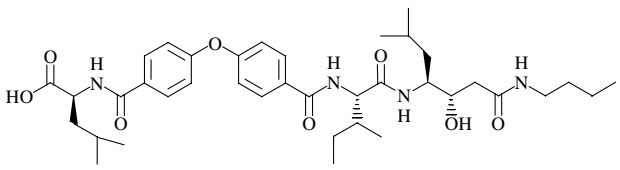
Compound **1** with the linker derived from succinic acid was the least active of the series in both assays, in agreement with the literature that indicates an aromatic substituent as preferred in P_3 for PLMII inhibition.[6] The introduction of an aromatic ring in compound **2** caused an increase in potency, as expected. Compound **3** with a naphthyl linker was considerably more active in the enzyme assay, but the activity against the parasite did not improve. Compound **4** derived from 4,4′-oxybis(benzoic acid) was remarkably active in inhibiting both the enzyme and the parasite growth with IC_{50} values of 0.59 nM and 0.4 μM, respectively. These IC_{50} values are substantially superior compared to the IC_{50} of **3**. In order to ameliorate the activity of the naphthyl linker, by adding more conformational freedom and increasing the distance between PQ and statine, compound **5**, with an alkyl chain and an aromatic moiety joined by an ester bond, was synthesized. This compound was less active against the enzyme, but slightly more active against *P. falciparum* compared to the naphthyl derivative **3**.

To verify the influence of PQ in the in vitro assay, and the possibility that the LysLeu peptide bond of compound **4** could undergo hydrolysis by plasmin, the peptide HLysPQ **11** and the carboxylic acid **12** were prepared.

Compound **11** was found substantially inactive both against PLM II and *P. falciparum*: ($IC_{50} > 1$ μM and approx. 20 μM, respectively). Compound **12**, compared to **4**, showed a drastic reduction in inhibition both of PLM II (IC_{50}: 123 nM) and of parasite growth (IC_{50} approx. 17 μM) indicating that compound **4** is active in the cell assay without being cleaved. In conclusion, these novel PQ–Statine 'double-drugs' have achieved, in comparison with other peptidomimetic inhibitors,[3,6] a significant improvement in the inhibition of both of PLM II activity and *P. falciparum* growth in vitro, including drug-resistant strains. Moreover, a direct correlation was found between the inhibition of PLM II and the antiplasmodial activity, suggesting that PQ–Statine 'double-drugs' kill the parasites mainly by



11



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inhibiting PLM II and consequently the digestion of haemoglobin, that is essential for the survival of *P. falciparum*.

Acknowledgements

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