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Antiplasmodial activity of hydroxyethylamine analogs: Synthesis, biological activity and structure activity relationship of plasmepsin inhibitors

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ABSTRACT

Malaria, particularly in endemic countries remains a threat to the human health and is the leading the cause of mortality in the tropical and sub-tropical areas. Herein, we explored new C_2 symmetric hydroxyethylamine analogs as the potential inhibitors of *Plasmodium falciparum* (*P. falciparum*; 3D7) in *in-vitro* cultures. All the listed compounds were also evaluated against crucial drug targets, plasmepsin II (Plm II) and IV (Plm IV), enzymes found in the digestive vacuole of the *P. falciparum*. Analog **10f** showed inhibitory activities against both the enzymes Plm II and Plm IV (K_i, 1.93 ± 0.29 μM for Plm II; K_i, 1.99 ± 0.05 μM for Plm IV). Among all these analogs, compounds **10g** selectively inhibited the activity of Plm IV (K_i, 0.84 ± 0.08 μM). In the *in vitro* screening assay, the growth inhibition of *P. falciparum* by both the analogs (IC_{50}, 2.27 ± 0.95 μM for **10f**; IC_{50}, 3.11 ± 0.65 μM for **10g**) displayed marked killing effect. A significant growth inhibition of the *P. falciparum* was displayed by analog **12c** with IC_{50} value of 1.35 ± 0.85 μM, however, it did not show inhibitory activity against either Plms. The hemolytic assay suggested that the active compounds selectively inhibit the growth of the parasite. Further, potent analogs (**10f** and **12c**) were evaluated for their cytotoxicity towards mammalian HepG2 and vero cells. The selectivity index (SI) values were noticed greater than 10 for both the analogs that suggested their poor toxicity. The present study indicates these analogs as putative lead structures and could serve as crucial for the development of new drug molecules.

1. Introduction

Despite the major advances in technologies, control of malaria remains a critical mission, particularly in endemic regions of the world as they exhibit a great number of deaths annually.[1,2] The malaria elimination related ventures, including insecticide-treated bed nets, insecticide sprays, and artemisinin-based combinational treatments have considerably reduced the malaria incidences and the mortality cases over the years, however the issue of increasing drug resistance remains unsolved.[3–5] The endemic countries are still under the threat of malaria that leads to the loss of many lives, particularly children under the age of five. Alone in 2015, an estimated 438,000 deaths have been reported in Africa.[1] Until recently, Artemisinin-based Combination Therapy (ACT) was considered as a reliable weapon against malaria caused by *P.*

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falciparum. But the present status is a severe distress due to the increasing drug resistance to these front-line therapeutics, artemisinin and ACT.[6] The artemisinin-resistant *P. falciparum* isolates appeared slowly in the area along Thailand-Cambodia border[3,4,7] and now approximately 70% failure cases[8] of ACT therapy are reported as serious threat across the world.[9] New inexpensive and secure drug molecules exhibiting the novel mode of action are highly needed as alternatives to overcome the increasing resistance against artemisinin-based treatments.

Malarial aspartyl proteases, the plasmepsins (Plms), attained considerable attention and have been studied as potential drug targets for many years.[10–17] Digestive Plms (Plm I, II, IV and HAP) are responsible for the degradation of hemoglobin to amino acids; however, these catalytic process limit the synergy between their inhibitors and antiparasitic activity.[18–24] Moreover, several Plm inhibitors have displayed significant *in vitro* and *in vivo* antimalarial profile that advocates their suitability as possible drug targets for the discovery of new antimalarials.[25–27]

To target the aspartic proteases in the parasite, several attempts have been made in the past that led to the identification of hydroxyethylamine (HEA) as the structural unit of choice. As such, many HEA based peptidomimetics have been considered as inhibitors of Plms for more than a decade.[13,14,24,28,29] Besides, C_2 symmetric dipeptides have also been as crucial synthons to build inhibitors of Plms.[30–32] HEA scaffold, a key structural element in most transition state inhibitors, enables hydrogen bonds with aspartic acid side chains present at the catalytic site. Additionally, the secondary hydroxyl group mimics the tetrahedral geometry that is formed in the initial steps of the amide bond hydrolysis of a peptide substrate.[33] The added HEA (P1, and P1′ pockets) also facilitates the inhibitors to fit into the target enzyme (Fig. 1).

Regardless of the strong binding affinity of compound B (Fig. 1) with Plm II, the antiplasmodial effect was unexpectedly poor. On other hand, compound A (Fig. 1) exhibited significant antiplasmodial effect in culture and showed negligible activity against Plm II.[29]

As a part of our ongoing effort to identify new potential inhibitors of plasmepsins (II and IV) with strong activity against *P. falciparum*, we extended our assessments of the phthalimide (Pht)[34–36] scaffold by applying slight chemical modifications at P3 and P3′ sites. It was anticipated that this slight modification could lead to the balanced lipophilicity without the loss of activity. Using this strategy, new C_2 symmetric analogs with or without Pht have been synthesized and assayed against Plms (II and IV) and *P. falciparum* culture. Out of 16 new Pht analogs tested, one analog **10f** exhibited significant binding affinity against Plm II and IV with moderate activity against *P. falciparum*. These new analogs with antimalarial activity targeting Plm II and IV provide the added understanding into the structure-activity relationship and could be highly valuable in the development of new antimalarial agents.

2. Result and discussion

2.1. Compound design and synthesis

In the last decades HEA scaffold has been used to tackle the design of Plms inhibitors including antimalarial molecules. To secure new Plms inhibitors with antiplasmodial activity, we decided to explore the rational construction and expansion of C_2 symmetric HEA-Pht analogs with slight modification at P3 and P3′. From our recent efforts, compound B (Fig. 1) displayed inhibition of Plm II in submicromolar concentration and could arrest the growth of parasite completely.[29] Conversely, compound A (Fig. 1) displayed strong inhibition against the growth of the parasite but negligible effect against the Plms. In this study, we continue to explore these analogs with slight modifications, including a molecular flexibility by adopting two different types of compounds, with or without Pht scaffold. We decided to build new analogs with slight modifications around A and B without losing the activity profile. As such, the easily available and simplest methylated Pht and Boc-protected amino acids were selected to build new analogs. The addition of methyl groups was considered beneficial to increase the binding affinity with the enzymes. The general approach for synthesis of target HEA based Pht analogs **10(a–g)** are depicted in Schemes 1–3.

To obtain the target compounds, we proceed with the first step, regioselective ring opening of phenylalanine epoxide **1** in the presence of piperazine, which is involved as a linker moiety with good nucleophilic sites.[29,35]

It opens the epoxide ring from both sides (Scheme 1) and Boc-protected HEA intermediates **2** were obtained in good yields. Deprotection of **2** was accomplished by using trifluoroacetic acid (TFA) in DCM.[29] The TFA salt **3** formed post deprotection of Boc-HEA was eliminated by TFA scavenger using basic anion exchange resin (Amberlite IRA-402) and it was added until the mixture became basic in nature (pH ~ 10) to give **4** as white solid in good yield. Lastly, intermediate **4** was subjected to the coupling reaction with various phthaloyl-L-amino acids **8(a–g)** that led the desired analogs **10(a–g)** in moderate to good yield (Table 1). For the second series **11(a–d)** & **12(a–c)**, Boc-protected amino acids **9(a–e)** were selected to build C_2 symmetric HEA analogs without Pht scaffold (Scheme 3; Table 2).

2.2. Plm II and IV inhibition

We investigated the Plms inhibitory activities of all the listed new compounds. Plasmepsin II and IV from *P. falciparum* have been investigated as an excellent enzyme model for the screening of new

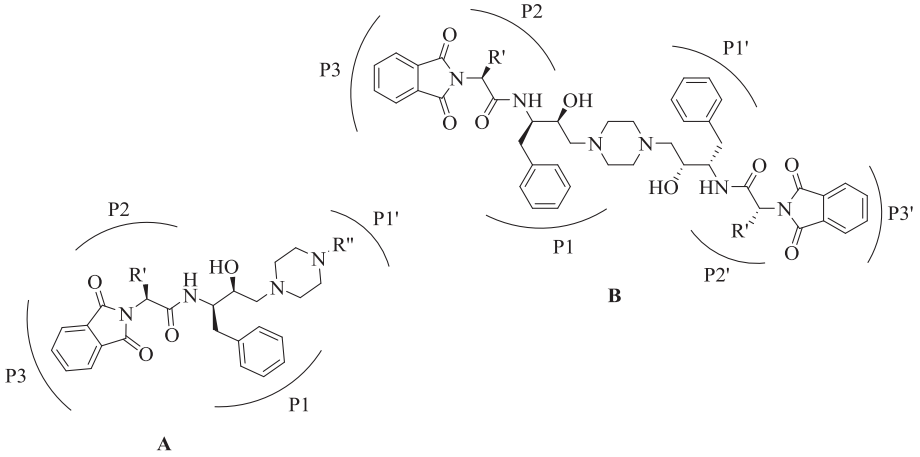

Fig. 1. Functionalized Pht-HEA compounds reported by our group.[29]