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Improvement of both plasmepsin inhibitory activity and antimalarial activity by 2-aminoethylamino substitution

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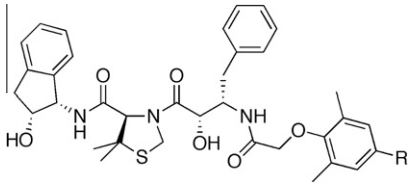
ABSTRACT

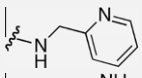
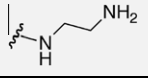
We attached 2-aminoethylamino groups to allophenylnorstatine-containing plasmepsin (Plm) inhibitors and investigated SAR of the methyl or ethyl substitutions on the amino groups. Unexpectedly, compounds **22** (KNI-10743) and **25** (KNI-10742) exhibited extremely potent Plm II inhibitory activities (K_i <0.1 nM). Moreover, among our peptidomimetic Plm inhibitors, we identified the compounds with the highest antimalarial activity using a SYBR Green I-based fluorescence assay.

Malaria is still one of the biggest health problems worldwide; infections with malaria parasites result in one million victims annually.[1] *Plasmodium falciparum*, the most lethal malaria parasite, is increasingly building resistance to available antimalarial drugs such as chloroquine and artesunate.[2] As a result, it becomes more and more necessary to develop new antimalarial drugs with new mechanisms of action.[3] Plasmepsin (Plm) is a specific aspartic protease of the malaria parasite. In the case of *P. falciparum*, 10 Plms are encoded in the parasite genome,[4] and 4 of them, Plm I, II, III (known as HAP), and IV, take part in hemoglobin degradation in food vacuoles.[5] The hemoglobin digestion process is essential for the malaria parasite to obtain amino acids for protein biosynthesis, regulate osmotic pressure in the host cell, and make space for growth.[6] Because inhibition of Plms could knock down malaria parasites,[7] Plms are attractive targets for novel antimalarial development.

We used the hydroxymethylcarbonyl (HMC) isostere[8] in our Plm inhibitor as an ideal transition-state analog. Previously, we developed KNI-10006 (**1**) by optimization of HIV protease inhibitors containing allophenylnorstatine [Apns; (2*S*,3*R*)-3-amino-2-hydroxy-4-phenylbutyric acid] with an HMC isostere and Dmt [(*R*)-5,5-dimethyl-1,3-thiazolidine-4-carboxylic acid] scaffold at the P_1—P'_1 position.[9,10] Compound **1** exhibited highly potent Plm II inhibitory activity (K_i = 0.5 nM). Compound **1** also effectively inhibited three other Plms.[11] Compound **1**, however, exhibited relatively low activity against *P. falciparum* infected in human erythrocytes.[10] We recently reported that attachment of alkylamino substituents to the 4-position of the phenyl group in the 2,6-dimethylphenoxyacetyl moiety of **1** enhanced antimalarial activity.[12] Although

Table 1
Inhibitory activity of Plm inhibitor containing Apns



Compound	R	Plm II K_i (nM)	*Anti-P. falciparum* EC_{50} (μM)
1 (KNI-10006)	H	0.5	5.0
2 (KNI-10538)		10	0.56
4 (KNI-10737)		6.5	0.29

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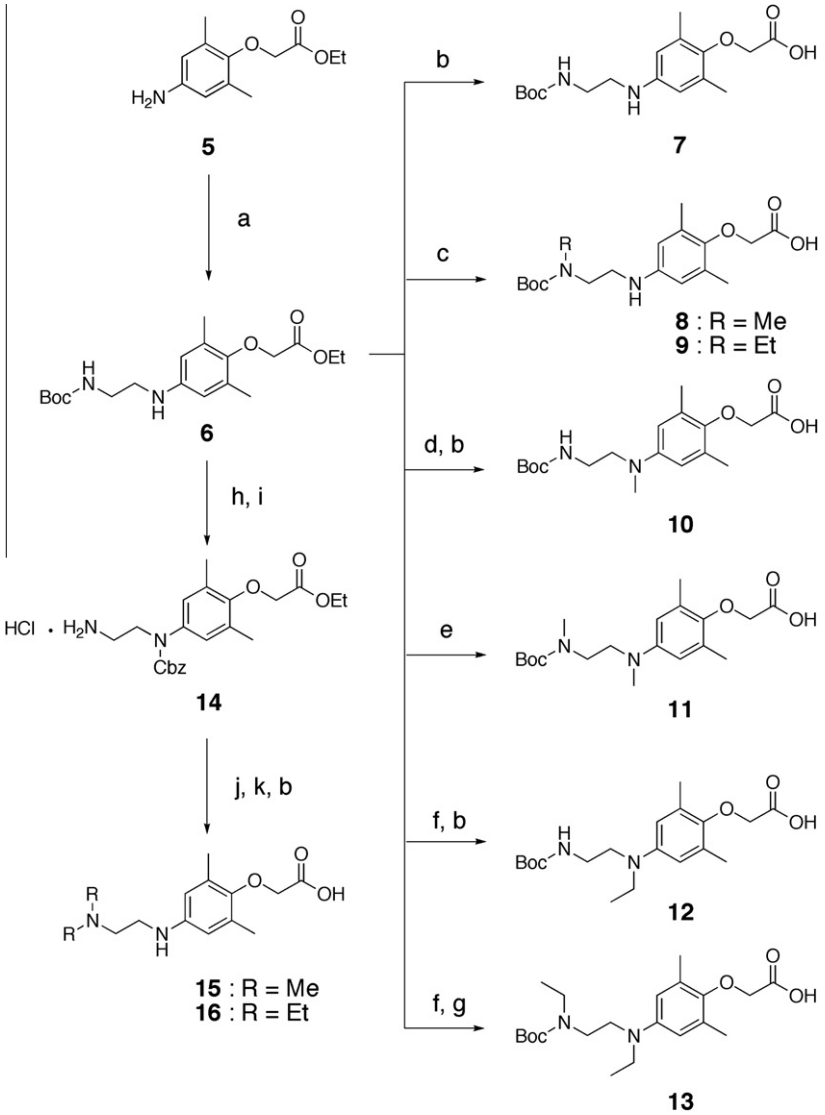
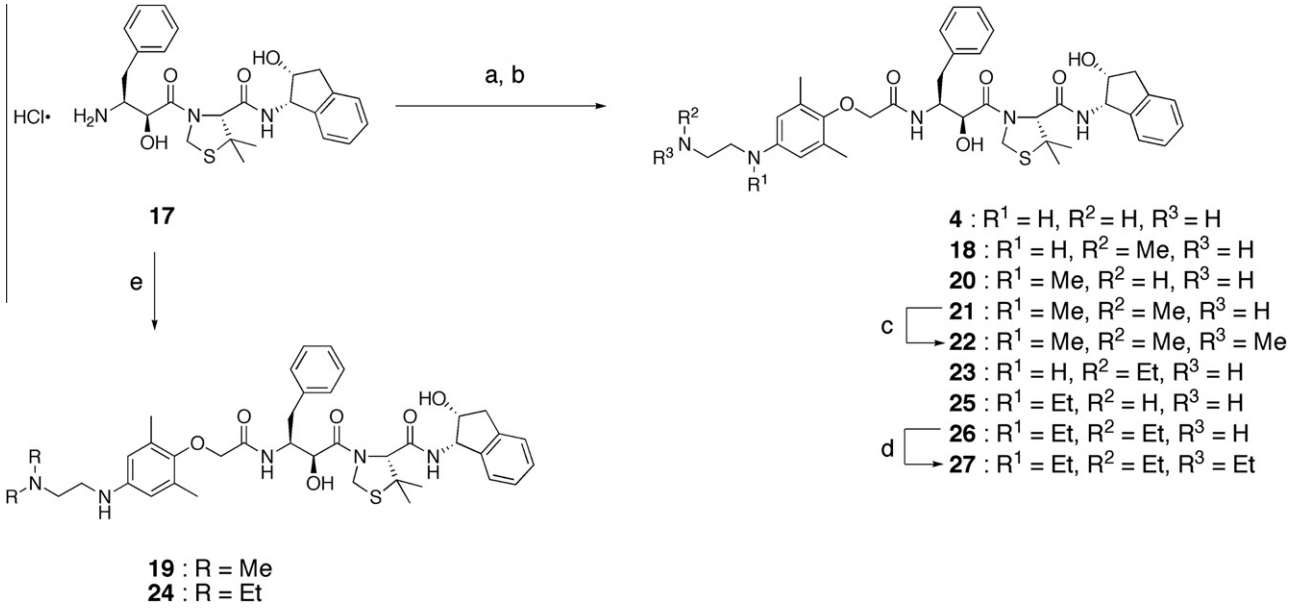
KNI-10538 (**2**), a pyridinylmethylamino analog, exhibited less potent Plm II inhibitory activity (K_i = 10 nM), it exhibited more potent antimalarial activity than **1**. Table 1 shows the antimalarial activity, determined by a SYBR Green I-based fluorescence assay[13] against *P. falciparum* strain D6 with chloroquine-sensitive and mefloquine-resistant phenotypes. X-ray crystallographic studies of KNI-764 (**3**) and **1** in complex with Plms revealed that our Plm inhibitors, containing an allophenylnorstatine structure, bind to Plms in unique manners.[14,15] The binding direction of compound **3** with Plm IV from Plasmodium malariae is opposite to that of pepstatin A. Our binding model based on X-ray crystallographic studies suggested that the pyridinylmethylamino moiety of **2** pointed outside the pockets and was surrounded by water molecules, while maintaining relatively high Plm II inhibitory activity (Fig. 1).[12] We hypothesized that the addition of basic amino groups would not interfere with enzyme binding and would contribute to enhanced antimalarial activity. Therefore, we further investigated other amino substitutions in our Plm inhibitors. Compound **4** (KNI-10737), which possesses a 2-aminoethylamino group at the 4-position of the phenyl function in the 2,6-dimethylphenoxyacetyl moiety, exhibited more potent Plm II inhibitory activity and antimalarial activity than **2** (Table 1). Herein we performed a structure–activity relationship (SAR) study by substituting the amino groups of the 2-aminoethylamino moiety with methyl or ethyl moieties.

The synthesis of phenoxyacetic acid derivatives with 2-aminoethylamino substituents is shown in Scheme 1. Ethyl 4-amino-2,6-dimethylphenoxyacetate (**5**) was converted to intermediate **6** by reductive alkylation with *N-tert*-butyloxycarbonyl(Boc)-2-aminoacetaldehyde. Specific methylation or ethylation and saponification with NaOH or simultaneous hydrolysis with NaH from **6** resulted in phenoxyacetic acid derivatives **7**–**13**. In the case of compounds **15** and **16**, carboxybenzyl (Cbz)-deprotection was additionally performed. These derivatives were coupled with intermediate **17**, which was synthesized from Boc–Dmt–OH as previously reported,[12] using benzotriazol-1-yloxy-tris(dimethylamino)-phosphonium hexafluorophosphate (BOP) as shown in Scheme 2. To obtain compounds **4** and **18**–**27**, additional deprotection of the Boc group was performed except for **19** and **24**. The pure products were obtained after purification by preparative HPLC with >95% purity and identified by MALDI-TOF MS.

The Plm II inhibitory activity and antimalarial activity of 2-aminoethylamino analogs are shown in Table 2. The small change in substitution with methyl or ethyl groups resulted in some differences in Plm II inhibition. For compounds with methyl-

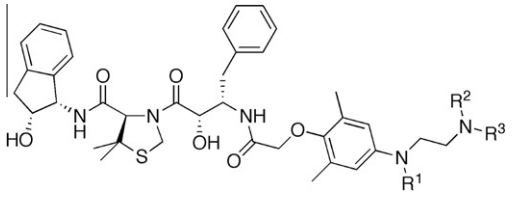


Scheme 1. Reagents: (a) *N*-Boc-2-aminoacetaldehyde, AcOH, $NaBH_3CN$, MeOH; (b) 3N-NaOH aq, MeOH; (c) NaH, MeI (1 equiv) or EtBr (5 equiv), THF; (d) formaldehyde, AcOH, $NaBH_3CN$, MeOH; (e) NaH, MeI (5 equiv), THF; (f) acetaldehyde, AcOH, $NaBH_3CN$, MeOH; (g) NaH, EtBr (5 equiv), THF; (h) Cbz-Cl, Et_3N, DMF; (i) 4N-HCl/dioxane, anisole; (j) formaldehyde or acetaldehyde, Et_3N, $NaBH_3CN$, MeOH; (k) 10% Pd–C, H_2, AcOH, MeOH–H_2O (6:1).



Scheme 2. Reagents: (a) (**7**–**12** or **13**), BOP, Et_3N, DMF; (b) 4N HCl/dioxane, anisole; (c) formaldehyde, Et_3N, $NaBH_3CN$, MeOH; (d) acetaldehyde, Et_3N, $NaBH_3CN$, MeOH; (e) **15** or **16**, BOP, Et_3N, DMF.

Table 2
Structure–activity relationship of 2-aminoethylamino analogs



Compound	R^1	R^2	R^3	Plm II K_i (nM)	*Anti-P. falciparum* EC_{50} (μM)
4	H	H	H	6.5	0.29
18 (KNI-10763)	H	CH_3	H	9.1	1.4
19 (KNI-10761)	H	CH_3	CH_3	17	2.0
20 (KNI-10762)	CH_3	H	H	8.5	1.4
21 (KNI-10740)	CH_3	CH_3	H	1.2	0.19
22 (KNI-10743)	CH_3	CH_3	CH_3	<0.1	0.36
23 (KNI-10741)	H	CH_2CH_3	H	0.68	0.22
24 (KNI-10760)	H	CH_2CH_3	CH_2CH_3	5.1	1.6
25 (KNI-10742)	CH_2CH_3	H	H	<0.1	0.46
26 (KNI-10758)	CH_2CH_3	CH_2CH_3	H	4.2	0.79
27 (KNI-10759)	CH_2CH_3	CH_2CH_3	CH_2CH_3	10	1.1
Chloroquine	—	—	—	—	0.02

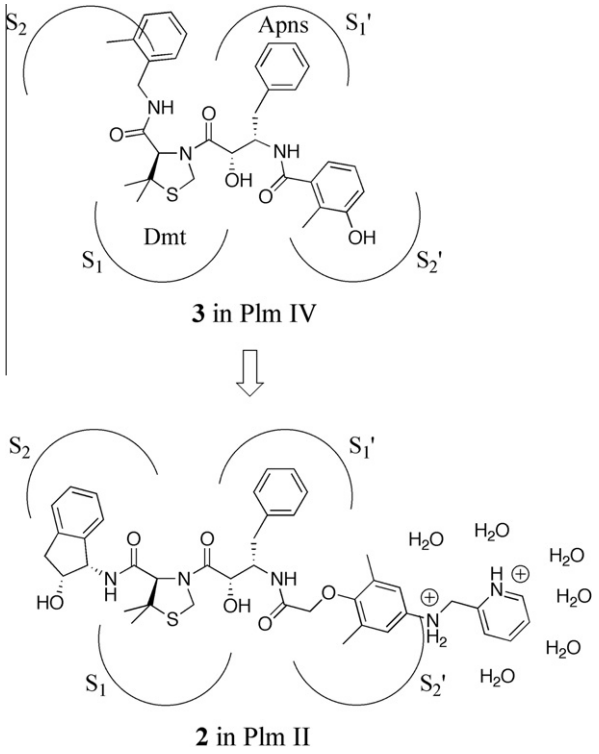


Figure 1. Speculative binding mode of **2** in Plm subsites.

ation, R^1-methylation is important for potent Plm II inhibitory activity. In R^1-desmethylated compounds (**4**, **18**, and **19**), bulkier methyl groups at R^2/R^3 disfavored Plm II inhibition, while in R^1-methylated compounds (**20**, **21**, and **22**), the trend was opposite. Meanwhile, in compounds with ethylation (**23**–**27**), mono-ethylated compounds (**23** and **25**) exhibited more potent Plm II inhibitory activity than di- and tri-ethylated compounds. A tri-ethylated compound (**27**) showed less potent Plm II inhibitory activity than di-ethylated compounds (**24** and **26**). Surprisingly, compounds **22** and **25** exhibited extremely potent Plm inhibitory activities (K_i <0.1 nM). These high potencies were similar to those of other series of Plm inhibitors we have reported.[16] We constructed a binding model of **22** in Plm II (Fig. 2). Energy minimization of **22** bound to Plm II (PDB ID, 1SME) including a water soak around the inhibitor was performed with an MMFF94x force field using a modeling package (*MOE 2008.10*, Chemical Computing Group Inc., Montreal, Canada). The model suggested new hydrogen bonding interactions between the 2-aminoethylamino groups with Asp 130, outside of the enzyme pocket, which may improve Plm II inhibitory activity.

Compounds **4**, **21**, **22**, **23**, and **25** exhibited more potent antimalarial activity against the D6 strain than **2**, especially **21**, which exhibited the most potent antimalarial activity (EC_{50} = 0.19 μM)

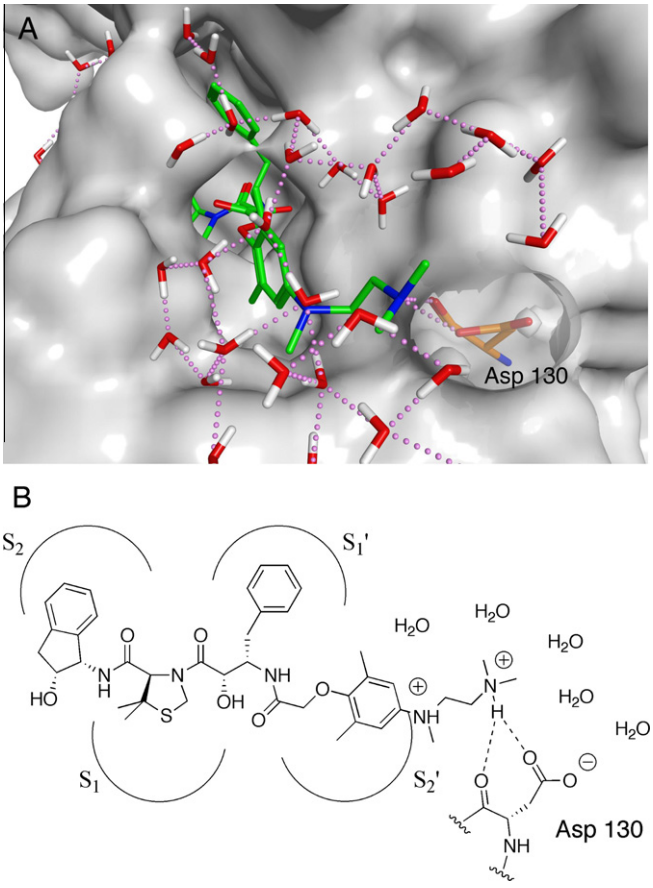


Figure 2. (A) Interaction of 2-aminoethylamino group of **22** (green) with Asp 130 (orange) of the Plm II observed in docking simulation. (B) Schematic representation of H-bonding interaction of **22** with Asp 130.

in our series. Generally, it is difficult for peptidomimetic Plm inhibitors to exhibit highly potent antimalarial activities,[17] because the compounds must permeate four membranes in order to inhibit the target Plms.[5] We interpreted that the membrane-impermeable double protonated forms of 2-aminoethylamino moieties under acidic conditions allowed our Plm inhibitors to accumulate in the acidic food vacuole, as alkaline antimalarial agents,[18,19] and this effect promoted uptake and retention of our Plm inhibitors in food vacuoles. Although the antimalarial activity of chloroquine is about 10-fold more potent than that of **21** (Table 2), we are working on developing more active inhibitors.

In conclusion, we attached 2-aminoethylamino groups to a Plm inhibitor containing an Apns–Dmt scaffold. Investigating SAR of the amino substituents with methyl or ethyl groups, we obtained **22** (KNI-10743) and **25** (KNI-10742) with extremely potent Plm II inhibitory activities. Compound **21** (KNI-10740) showed the most potent antimalarial activity among the series. These results indicated that 2-aminoethylamino groups contributed to both Plm II inhibitory and antimalarial activities.

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