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Optimization of plasmepsin inhibitor by focusing on similar structural feature with chloroquine to avoid drug-resistant mechanism of *Plasmodium falciparum*

Takuya Miura [a], Koushi Hidaka [b], Yukiko Azai [a], Keisuke Kashimoto [a], Yuko Kawasaki [c], Shen-En Chen [c], Renato Ferreira de Freitas [c], Ernesto Freire [c], Yoshiaki Kiso [d,*]

[a] Center for Frontier Research in Medicinal Science, Kyoto Pharmaceutical University, Yamashina-ku, Kyoto 607-8412, Japan
[b] Faculty of Pharmaceutical Sciences, Kobe Gakuin University, Chuo-ku, Kobe 650-8586, Japan
[c] Department of Biology and Johns Hopkins Malaria Research Institute, Johns Hopkins University, Baltimore, MD 21218, USA
[d] Laboratory of Peptide Science, Nagahama Institute of Bio-Science and Technology, Nagahama, Shiga 526-0829, Japan

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ABSTRACT

The plasmepsins are specific aspartic proteases of the malaria parasite and a potential target for developing new antimalarial agents. Our previously reported peptidomimetic plasmepsin inhibitor with modified 2-aminoethylamino substituent, KNI-10740, was tested against chloroquine sensitive *Plasmodium falciparum*, D6, to be highly potent, however, the inhibitor exhibited about 5 times less activity against multi-drug resistant parasite (TM91C235). We hypothesized the potency reduction resulted from structural similarity between 2-aminoethylamino substituent of KNI-10740 and chloroquine. Then, we modified the moiety and finally identified compound **15d** (KNI-10823), that could avoid drug-resistant mechanism of TM91C235 strain.

The most lethal malaria parasite *Plasmodium falciparum* is building resistance to common malaria medicines such as chloroquine and artesunate, which is used in artemisinin based combination therapy (ACT) recommended by WHO.[1] For this reason, development of novel antimalarials effective against drug-resistant parasites is a critical issue for global health. *P. falciparum* genome contains ten plasmepsin (Plm) genes,[2] parasite specific aspartic proteases, and four of them, Plm I, II, IV and HAP (Plm III) are involved in hemoglobin degradation to obtain amino acids essential at trophozoite stage.[3] Therefore, many groups reported Plm inhibitors as novel antimalarial agent,[4] however, a difficulty was turned out to develop Plm inhibitors with high antimalarial activity.[5,6]

Otherwise, we have been engaged in development of peptidomimetic Plm inhibitors containing allophenylnorstatine [(2*S*,3*S*)-3-amino-2-hydroxy-4-phenylbutyric acid] with a transition state analogue of aspartic protease, that is, hydroxymethylcarbonyl (HMC) isostere.[7,8] We recently reported allophenylnorstatine-containing inhibitors with structurally modified with 2-aminoethylamino substituents exhibited potent antimalarial activities against *P. falciparum* strain D6, which is African origin with chloroquine-sensitive and mefloquine-resistant phenotype.[7f] Especially, the antimalarial activity of KNI-10740 (**1**) was the most potent among these analogues with $EC_{50\ D6}$ value of 0.19 μM (Fig. 1).

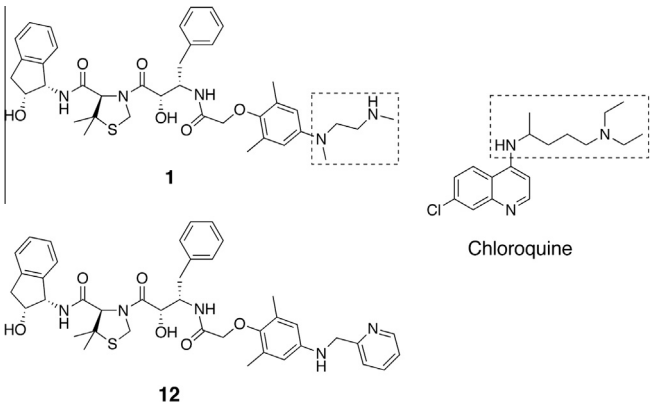

Figure 1. Structures of compounds **1** (KNI-10740), **12** (KNI-10538), and chloroquine. Similar partial structures between **1** and chloroquine are highlighted.

* Corresponding author. Tel.: +81 749 64 8113; fax: +81 749 64 8140.
E-mail address: y_kiso@nagahama-i-bio.ac.jp (Y. Kiso).

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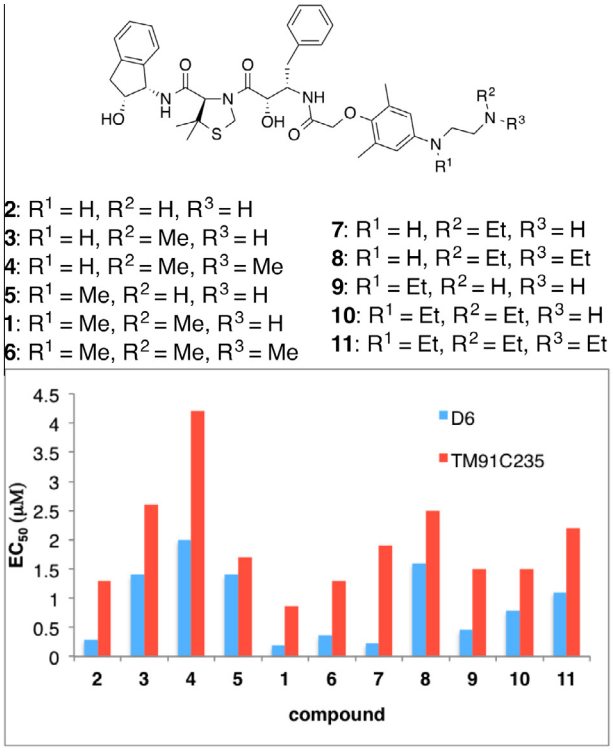


Figure 2. Antimalarial activities of 2-aminoethylamino analogues against D6 and TM91C235.

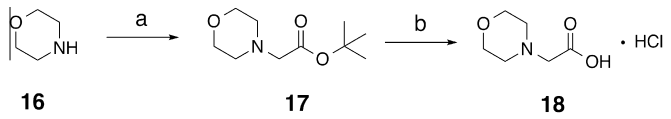
Herein, we report the result of antimalarial activities of 2-aminoethylamino analogues against multidrug-resistant TM91C235 strain, which is Thai origin with chloroquine, mefloquine and pyrimethamine-resistant phenotype, and optimization of the antimalarial activity by avoiding drug-resistant mechanism of *P. falciparum*. As far as we know, this is the first report on Plm inhibitor tested against multidrug-resistant malaria parasite.

The result of 2-aminoethylamino analogues against both D6 and TM91C235 is illustrated in Figure 2. The antimalarial activities of Plm inhibitors were determined by SYBR Green I inhibition assay.[9] The antimalarial activities against TM91C235 strain were obviously less potent than those against D6 in all cases. Resistance index (RI) represents a ratio of the IC_{50} value between TM91C235 and D6 ($RI = IC_{50\ TM91C235}/IC_{50\ D6}$). RI value of **1** is 4.5, that is, **1** exhibited about 5 times less potency compared to that against D6 strain ($EC_{50\ TM91C235} = 0.86\ \mu M$). Additionally, the highest RI was observed in **7** ($EC_{50\ D6} = 0.22\ \mu M$, $EC_{50\ TM91C235} = 1.9\ \mu M$, RI = 8.6), whose RI value was close to that of chloroquine (RI = 10).

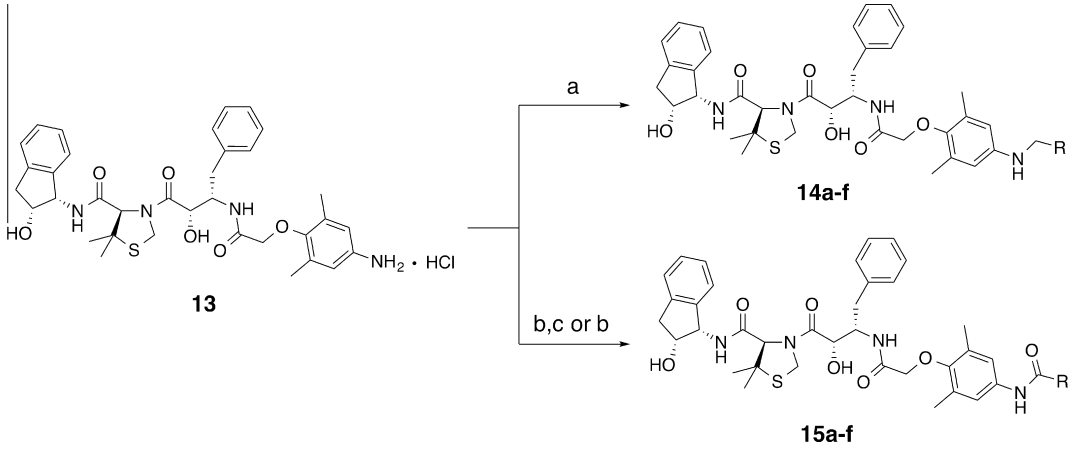
The results were unexpected for us because the inhibition mechanisms of Plm inhibitor and chloroquine are fundamentally different and there was no report about the cross-resistance between Plm inhibitor and chloroquine.[10] Moreover, KNI-10538 (**12**, Fig. 1), our previously reported Plm inhibitor,[7e] did not exhibit such significant activity reduction against W2 strain, which is Vietnamese origin and resistant to both chloroquine and pyrimethamine and sensitive to mefloquine ($EC_{50\ D6} = 0.56\ \mu M$, $EC_{50\ W2} = 0.73\ \mu M$, RI = 1.3). Recently, it was uncovered that allophenylnorstatine-containing inhibitors without 2-aminoethylamino moiety showed potent antimalarial activity against both D6 and W2 strains.[4a] These results suggested that the attachment of 2-aminoethylamino substituent to Plm inhibitor caused the reduction of activity against chloroquine-resistant *P. falciparum*. However, the basic substituent is important for our Plm inhibitors with respect to accumulation in acidic food vacuole where Plms exist by protonation trapping.[7f,11]

The drug-resistance mechanism of *P. falciparum* is believed to cause from mutation or amplification of the genes coding for proteins involved in drug transport such as multidrug transporter 1 (PfMDR1), chloroquine resistance transporter (PfCRT) and Pgh1, a homologue of mammalian P-glycoprotein.[12] Simply, we thought that in the case of TM91C235, these transport proteins could recognize 2-aminoethylamino analogues and trigger the inhibitor efflux from acidic food vacuole to result the reduction of antimalarial activities. In other word, the problem of 2-aminoethylamino analogues would be responsible for their similar structural feature with chloroquine, that is, the substituted alkanediamine group (Fig. 1). It was noteworthy that modification of alkanediamine moiety in chloroquine is a promising strategy to improve RI value and efficacy against chloroquine-resistant parasite.[13]

On the purpose for enhancement of antimalarial activity against TM91C235 strain and identification of compounds avoiding



Scheme 2. Reagents and conditions: (a) *tert*-butyl bromoacetate, K_2CO_3, DMF, rt, over night; (b) 4 N-HCl/dioxane, rt, over night.



Scheme 1. Reagents and conditions: (a) RCHO, $NaBH_3CN$, 4N-HCl/MeCN, MeOH, rt, over night (for **14a**) or RCHO, $NaBH_3CN$, AcOH, MeOH, rt, 1 h (for **14b–f**); (b) Boc-NH-$(CH_2)_n$-COOH or **18**, BOP, Et_3N, DMF, rt, over night (for **15a–c**, **15f**) or Boc–NH–$(CH_2)_n$–COOH, IBCF, NMM, DMF, −15 °C to rt, over night (for **15d**, **15e**); (c) 4N-HCl/dioxane, anisole, rt, 1 h.