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

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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).

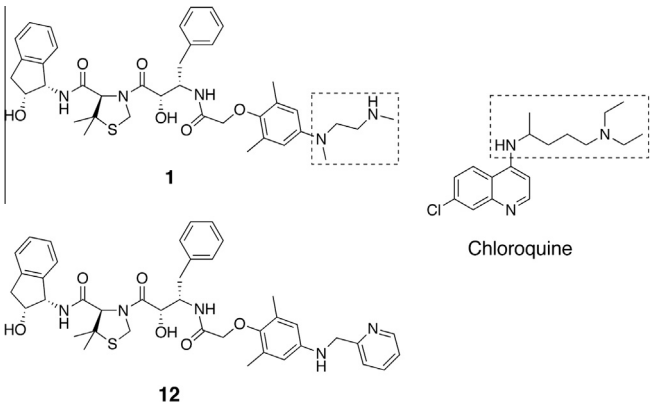

Figure 1. Structures of compounds **1** (KNI-10740), **12** (KNI-10538), and chloroquine. Similar partial structures between **1** and chloroquine are highlighted.

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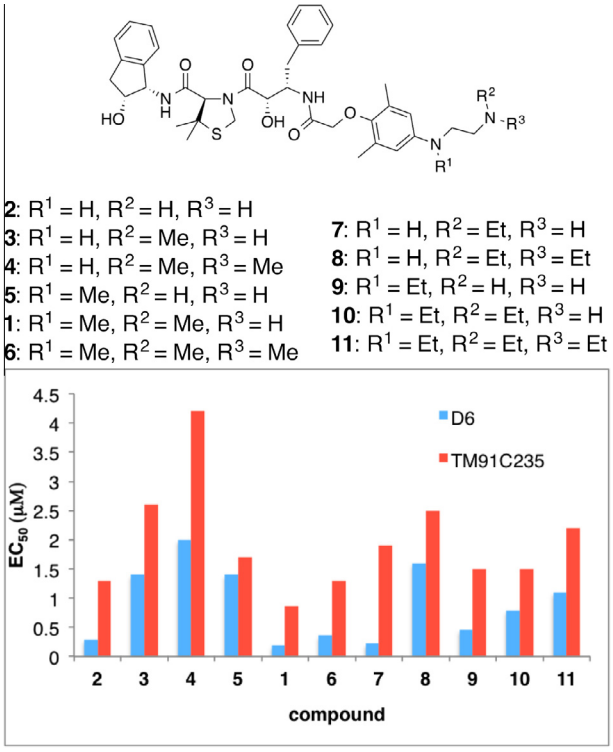


Figure 2. Antimalarial activities of 2-aminoethylamino analogues against D6 and TM91C235.

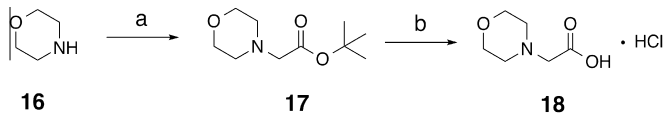
Herein, we report the result of antimalarial activities of 2-aminoethylamino analogues against multidrug-resistant TM91C235 strain, which is Thai origin with chloroquine, mefloquine and pyrimethamine-resistant phenotype, and optimization of the antimalarial activity by avoiding drug-resistant mechanism 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).

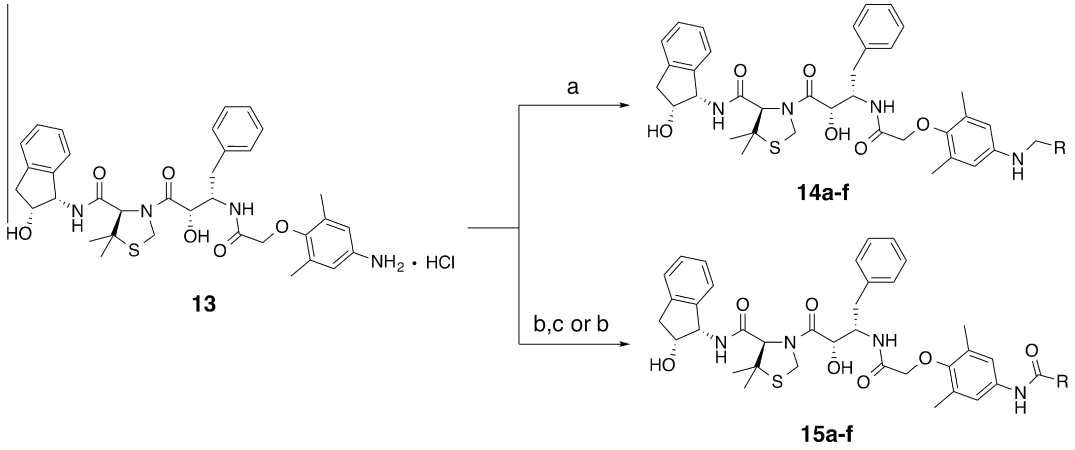
The results were unexpected for us because the inhibition mechanisms of Plm inhibitor and chloroquine are fundamentally different and there was no report about the cross-resistance between Plm inhibitor and chloroquine.[10] Moreover, KNI-10538 (**12**, Fig. 1), our previously reported Plm inhibitor,[7e] 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.

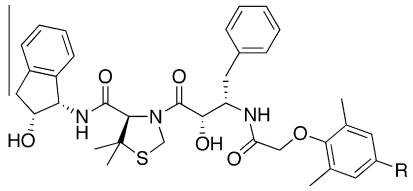


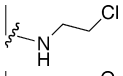
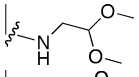
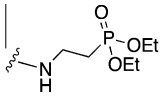
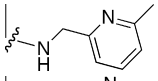
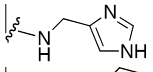
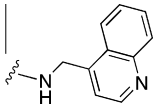
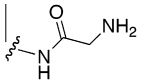
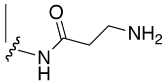
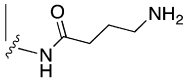
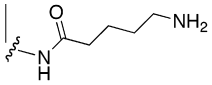
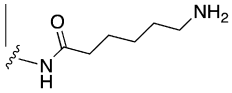
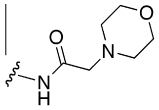
Scheme 1. Reagents and conditions: (a) RCHO, $NaBH_3CN$, 4N-HCl/MeCN, MeOH, rt, over night (for **14a**) or RCHO, $NaBH_3CN$, AcOH, MeOH, rt, 1 h (for **14b–f**); (b) Boc-NH-$(CH_2)_n$-COOH or **18**, BOP, Et_3N, DMF, rt, over night (for **15a–c**, **15f**) or Boc–NH–$(CH_2)_n$–COOH, IBCF, NMM, DMF,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

drug-resistant mechanism of *P. falciparum*, we shifted to modification of 2-aminoethylamino group employing two strategies, changing (i) the terminal amine to other, (ii) whole the 2-aminoethylamino moiety.

Synthesis of new compounds was conducted generally by reductive alkylation or amide condensation reaction as reasonable and efficient medicinal chemistry routes (Scheme 1). Compounds **14a–f** and **15a–f** were synthesized from an inhibitor **13**.[7e] The reductive alkylation using corresponding aldehydes by $NaBH_3CN$ gave **14a–f**. The condensation **13** with Boc-glycine, Boc-β-alanine, or Boc-GABA, by benzotriazol-1-yloxy-tris(dimethylamino)phosphonium hexafluorophosphate (BOP) and additional deprotection provided **15a–c**. In the case of compound **15d** and **15e**, mixed anhydride method using isobutyl chloroformate (IBCF) was chosen to accomplish the amide formation of **13** with Boc-5-aminovaleric acid, and Boc-aminocapronic acid, respectively. 2-Morpholinoacetic acid (**18**) used for condensation reaction with **13** by using BOP to obtain **15f** was prepared from morpholine as shown in Scheme 2. All the synthetic derivatives were purified by reverse-phase HPLC with >95% purity and identified by MALDI-TOF MS.

Table 1
Plm II inhibitory activities of inhibitors with modified substituents



Compound	R	Plm II K_i (nM)
14a		5.5
14b		11
14c		22
14d		4.4
14e		4.8
14f		1.7
15a		1.2
15b		2.3
15c		1.1
15d (KNI-10823)		1.0
15e		5.7
15f		4.3

Structures and Plm II inhibitory activities of newly synthesized compounds are summarized in Table 1. All compounds exhibited potent Plm II inhibitory activities with K_i values of range from 1.0 to 22 nM, and **15d** (KNI-10823) exhibited the most potent Plm II inhibitory activity. For discussion of interactions with Plm II, molecular dynamics (MD) simulations were investigated by Ersmark's group.[14] We performed MD simulation with complex of **15d** with Plm II after energy minimization process of docking model by superpose of the X-ray crystallographic data of KNI-10006 with Plm I[8c] (PDB ID, 3QS1) and rs367 with Plm II[15] (1LEE).[16] As shown in Figure 3, two interactions, salt bridge and hydrogen bond of terminal amine of 4-pentanamide substituent in **15d** with carboxylate of Asp130 and carbonyl group of Ser132 were observed. However, these interactions were not maintained for the long time in the MD simulation. From this simulation, it was considered that cutdown of interactions over time made the Plm II inhibitory activity of **15d** not extremely potent, which **6** and **9** exhibited,[7f] but moderate potent.

On the other hand, the results of antimalarial activities were abundantly different by compounds (Fig. 4). Compounds **14a–f** were designed by changing terminal amine in 2-aminoethylamino moiety to other groups, such as chloride, dimethylacetal, phosphate ester, 3-methyl-2-pyridine, imidazole, or quinoline. In these derivatives, **14b**, **14c**, and **14f** exhibited sub-μM level EC_{50} to D6 strain with $EC_{50\ D6}$, 0.85, 0.60, 0.50 μM, respectively, but other compounds showed antimalarial activities with μM level EC_{50}. However, RI values of **14a–f** were up to 2, indicating these compounds without 2-aminoethylamino moiety possessed almost equal effectivity to both D6 and C235 strains. Transforming 2-aminoetylamino substituent to aminoalkylamide and extending alkyl chain, the interesting result was observed (**15a–e**). The

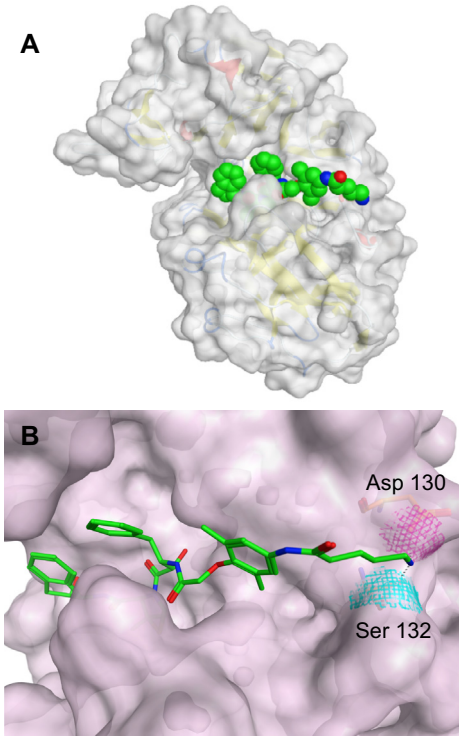


Figure 3. MD simulated pose of compound **15d** (green) bound to Plm II (white surface). (A) Overall of docking model. **15d** was illustrated with space filling model. (B) The interactions between 4-aminopentanamide substituent with Asp130 (magenta mesh area) and Ser132 (cyan mesh area).

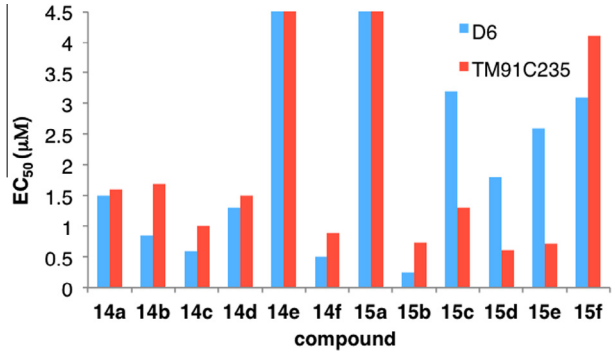


Figure 4. Antimalarial activities of Plm inhibitors with modified substituents against D6 and TM91C235.

antimalarial activity of **15a**, a glycine conjugation derivative, was dramatically low despite potent Plm II inhibitory activity (K_i = 1.2 nM). Extending one methylene from **15a**, the β-alanine derivative **15b** exhibited potent antimalarial activity against both D6 and TM91C235 ($EC_{50\ D6}$ = 0.26 μM, $EC_{50\ TM91C235}$ = 0.73 μM). While we were pleased antimalarial activity of **15b** to TM91C235 is slightly more potent than that of KNI-10740, the RI of **15b** was high value (RI = 2.8). Interestingly, GABA derivative **15c** showed relatively low antimalarial activity ($EC_{50\ D6}$ = 3.2 μM, $EC_{50\ TM91C235}$ = 1.3 μM), but its RI value was substantially up to 1 (RI = 0.41). More with extended chain derived from 5-aminovaleric acid, **15d** exhibited relatively low potency against D6 strain ($EC_{50\ D6}$ = 1.8 μM). On the other hand, fortunately, in the case of TM91C235, **15d** exhibited most potent antimalarial activity against TM91C235 among our Plm inhibitors ($EC_{50\ TM91C235}$ = 0.62 μM) and enhanced RI value (RI = 0.34). The antimalarial activity of **15e** was comparable to that of **15d** ($EC_{50\ D6}$ = 2.6 μM, $EC_{50\ TM91C235}$ = 0.72 μM, RI = 0.28). Morpholine derivative **15f** was not adequate for potent antimalarial activity.

We must refer to the decimal and low RI value of **15c–e**. The result of decimal RI is a proof of that these inhibitor was not effected by drug-resistant mechanism of *P. falciparum* unlike 2-aminoethylamino analogues. We are sure that **15d** (KNI-10823) is new lead compound for discovery of novel effective Plm inhibitor against drug-resistant malaria parasite instead of 2-aminoethylamino analogues, but **15d** lacked a second protonatable amine for double protonated form to accumulate in acidic food vacuole.[7f] Therefore, it is expected further optimization of **15d** with additional weak base substituent would be enable to develop the Plm inhibitor with high potent antimalarial activity against both D6 and TM91C235 strain. We hope our effort provides the direction for development of novel antimalarial agents combating drug-resistant malaria parasites.

Acknowledgments

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References and notes

1. Dondrop, A. M.; Nosten, F.; Yi, P.; Das, D.; Phyo, A. P.; Tarning, J.; Lwin, K. M.; Ariey, F.; Hanpithakpong, W.; Lee, S. J.; Ringwald, P.; Silamut, K.; Imwong, M.; Chotivanich, K.; Lim, P.; Herdman, T.; Sam An, S.; Yeung, S.; Singhasivanon, P.; Day, N. P. J.; Lindegardh, P.; Socheat, D.; White, N. J. *N. Eng. J. Med.* **2009**, *361*, 455.
2. Cooms, G. H.; Goldberg, D. E.; Klemba, M.; Berry, C.; Kay, J.; Mottram, J. C. *Trends Parasitol.* **2001**, *17*, 532.
3. Banerjee, R.; Liu, J.; Beatty, W.; Pelosof, L.; Klemba, M.; Goldberg, D. E. *Proc. Natl. Acad. Sci. U.S.A.* **2002**, *99*, 990.
4. (a) Ruben, A. J.; Kiso, Y.; Freire, E. In *Aspartic Acid Proteases as Therapeutic Targets*; Ghosh, A. K., Ed.; Wiley-VCH GmbH & Co. KGaA, 2010; p 513; (b) Perez, B.; Teixeira, C.; Gomes, J. R. B.; Gomes, P. *Curr. Med. Chem.* **2013**, *20*, 3049.
5. (a) Ersmark, K.; Samuelsson, B.; Hallberg, A. *Med. Res. Rev.* **2006**, *26*, 626; (b) Meyers, M. J.; Goldberg, D. E. *Curr. Top. Med. Chem.* **2012**, *12*, 445.
6. Liu, J.; Gluzman, Y.; Drew, M. E.; Goldberg, E. *J. Biol. Chem.* **2005**, *280*, 1432.
7. (a) Nezami, A.; Luque, I.; Kimura, T.; Kiso, Y.; Freire, E. *Biochemistry* **2002**, *41*, 2273; (b) Nezami, A.; Kimura, T.; Hidaka, K.; Kiso, A.; Liu, J.; Kiso, Y.; Goldberg, D. E.; Freire, E. *Biochemistry* **2003**, *42*, 8459; (c) Kiso, A.; Hidaka, K.; Kimura, T.; Hayashi, Y.; Nezami, A.; Freire, E.; Kiso, Y. *J. Pept. Sci.* **2004**, *10*, 641; (d) Hidaka, K.; Kimura, T.; Tsuchiya, Y.; Kamiya, M.; Ruben, A. J.; Hayashi, Y.; Freire, E.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2007**, *11*, 3048; (e) Hidaka, K.; Kimura, T.; Ruben, A. J.; Uemura, T.; Kamiya, M.; Kiso, A.; Okamoto, T.; Tsuchiya, Y.; Hayashi, Y.; Freire, E.; Kiso, Y. *Bioorg. Med. Chem.* **2008**, *16*, 10049; (f) Miura, T.; Hidaka, K.; Uemura, T.; Kashimoto, K.; Hori, Y.; Kawasaki, Y.; Ruben, A. J.; Freire, E.; Kimura, T.; Kiso, Y. *Bioorg. Med. Chem. Lett.* **2010**, *20*, 4836.
8. For references about X-ray crystallographic analyses of allophenylnorstatine based inhibitors with Plms, see: (a) Clemente, J. C.; Govindasamy, L.; Madabushi, A.; Fisher, S. Z.; Moose, R. E.; Yowell, C. A.; Hidaka, K.; Kimura, T.; Hayashi, Y.; Kiso, Y.; Agbandje-McKenna, M.; Dame, J. B.; Dunn, B. M.; McKenna, R. *Acta Crystallogr., Sect. D* **2006**, *62*, 246; (b) Bhaumik, P.; Xiao, H.; Parr, C. L.; Kiso, Y.; Gustchina, A.; Yada, R. Y.; Wlodawer, A. *J. Mol. Biol.* **2009**, *388*, 520; (c) Bhaumik, P.; Horimoto, Y.; Xiao, H.; Miura, T.; Hidaka, K.; Kiso, Y.; Wlodawer, A.; Yada, R. Y.; Gustchina, A. *J. Struct. Biol.* **2011**, *175*, 73; (d) Bhaumik, P.; Xiao, H.; Hidaka, K.; Gustchina, A.; Kiso, Y.; Yada, R. Y.; Wlodawer, A. *Biochemistry* **2011**, *50*, 8862.
9. Johnson, J. D.; Dennull, R. A.; Gerena, L.; Lopez-Sanchez, M.; Roncal, N. E.; Waters, N. C. *Antimicrob. Agents Chemother.* **2007**, *51*, 1926.
10. For example of comparison in antimalarial activities of Plm inhibitors between chloroquin sensitive strain and resistant strain, see: (a) Francis, S. E.; Gluzman, I. Y.; Oksman, A.; Knickerbocker, A.; Mueller, R.; Brayant, M. L.; Sherman, D. R.; Russell, D. G.; Goldberg, D. E. *EMBO J.* **1994**, *13*, 306; (b) Jiang, S.; Prigge, S. T.; Wei, L.; Gao, Y.; Hundson, T. H.; Gerena, L.; Dame, J. B.; Kyle, D. E. *Antimimicrob. Agents Chemother.* **2001**, *45*, 2577; (c) Moon, R. P.; Tyas, L.; Certa, U.; Rupp, K.; Bur, D.; Jacquet, C.; Matile, H.; Loetscher, H.; Grueninger-Leitch, F.; Kay, J.; Dunn, B. M.; Berry, C.; Ridley, R. G. *Eur. J. Biochem.* **1997**, *244*, 552; (d) Agli, M. D.; Parapini, S.; Galli, G.; Vaiana, N.; Taramelli, D.; Sparatore, A.; Liu, P.; Dunn, B. M.; Bosisio, E.; Romeo, S. Higj. *J. Med. Chem.* **2006**, *49*, 7440; (e) Cunico, W.; Gomes, C. R. B.; Facchinetti, V.; Moreth, M.; Penido, C.; Henriques, M. G. M. O.; Varotti, F. P.; Krettli, L. G.; Krettli, A. U.; da Silva, F. S.; Caffarena, E. R.; de Magalhaes, C. S. *Eur. J. Med. Chem.* **2009**, *44*, 3816.
11. (a) Homewood, C. A.; Warhurst, D. C.; Peters, W.; Baggaley, V. C. *Nature* **1972**, *235*, 50; (b) Krogstad, D. J.; Schlesinger, P. H. *Biochem. Pharmacol.* **1986**, *35*, 547.
12. (a) Bray, P. G.; Martin, R. E.; Tilley, L.; Ward, S. A.; Kirk, K.; Fidock, D. A. *Mol. Microbiol.* **2005**, *56*, 323; (b) Raj, D. K.; Mu, J.; Jiang, H.; Kabat, J.; Singh, S.; Sullivan, M.; Fay, M. P.; McCutchan, T. F.; Su, X. *J. Biol. Chem.* **2009**, *284*, 7687; (c) Chavchich, M.; Gerena, L.; Peters, J.; Chen, N.; Cheng, Q.; Kyle, D. E. *Antimimicrob. Agents Chemother.* **2010**, *54*, 2455.
13. (a) Natarajan, J. K.; Alumasa, J. N.; Yearick, K.; Ekoue-Kovi, K. A.; Casabianca, L. B.; Dios, A. C.; Wolf, C.; Roepe, P. D. *J. Med. Chem.* **2008**, *51*, 3466; (b) Iwaniuk, D. P.; Whetmore, E. D.; Rosa, N.; Ekoue-Kovi, K.; Alumasa, J.; Dios, A. C.; Roepe, P. D.; Wolf, C. *Bioorg. Med. Chem.* **2009**, *17*, 6560; (c) Hocart, S. J.; Liu, H.; Deng, H.; De, D.; Krogstad, F. M.; Krogstad, D. J. *Antimimicrob. Agents Chemother.* **2011**, *55*, 2233.
14. Gutiérrez-de-Terán, H.; Nervall, M.; Ersmark, K.; Liu, P.; Janka, L. K.; Dunn, B.; Hallberg, A.; Åqvist, J. *Biochemistry* **2006**, *45*, 10529.
15. Asojo, O. A.; Afonina, E.; Gulnik, S. V.; Yu, B.; Erickson, J. W.; Randad, R.; Medjahed, D.; Silva, A. M. *Acta Crystallogr., Sect. D* **2001**, *2002*, 58.
16. MD simulation was performed by using MOE 2010.10, Chemical Computing Group Inc., Montreal, Canada with an MMFF94x force field including 20 Å sphere TIP3P water from inhibitor. Asp 214 in catalytic dyad of Plm II and amino group of compound **15d** were protonated assuming the assay condition.