

Computational Study of Triterpenoids of *Ganoderma lucidum* with Aspartic Protease Enzymes for Discovering HIV-1 and Plasmepsin Inhibitors

Devinna Kang¹, Mutakin Mutakin¹ & Jutti Levita¹

¹Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Jl. Raya Bandung-Sumedang Km.21 Jatinangor Sumedang, West Java 45363, Indonesia

Correspondence: Jutti Levita, Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Padjadjaran University, Jl. Raya Bandung-Sumedang Km.21 Jatinangor Sumedang, West Java 45363, Indonesia. Tel: 622-2779-6200. E-mail: jutti.levita@unpad.ac.id

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Abstract

Rapid resistance development of HIV-1 and *Plasmodium falciparum* parasite requires discovery of more potent new drugs. Aspartic protease enzymes expressed by HIV-1 and *P. falciparum* could be used as important drug targets. The catalytic site is located at the bottom of a cleft in the enzyme surface and consists of triad Asp25, Thr26, Gly27. Important aspartic acids are Asp32 and Asp215. Aspartic proteases are inhibited by pepstatin-A, a naturally occurring peptide containing two statins, which replace the amino acids. The hydroxyl group of the statin binds tightly to the catalytically-active aspartic acid residues in the active site of protease, thereby mimicking the transition state of the peptide cleavage. Previous study proved that ganoderiol-F, a triterpenoid isolated from the stem of *Ganoderma sinense* showed higher affinity towards HIV-1 protease (binding energy = -11.40 kcal/mol and K_i = 4.68 nM) than to plasmepsin I (binding energy = -9.96 kcal/mol and K_i = 50.94 nM). In this paper, computational studies of *G. lucidum* triterpenoids with aspartic protease enzymes of HIV-1 and plasmepsin I, were performed using AutoDock 4.2. Nelfinavir and KNI-10006 were used as the standards for HIV-1 protease and plasmepsin I, respectively. The four triterpenoids are able to interact with both enzymes. Ganoderat acid-B showed the best affinity to HIV-1 protease (binding energy = -7.49 kcal/mol and K_i = 0.001 mM) which is better than nelfinavir. Furthermore, the best affinity to Plasmepsin I is showed by ganodermanondiol (binding energy = -7.14 kcal/mol and K_i = 0.005 mM which is better than KNI-10006. According to the values of binding energy and inhibition constant, triterpenoids of *G. lucidum* could be developed further as both anti-HIV and anti-malaria.

Keywords: Aspartic proteases; HIV-1; Malaria; *Ganoderma lucidum*; Plasmepsin I

1. Introduction

AIDS and malaria are health problems that occur in many parts of the world. According to WHO and UNAIDS, 35 million people are globally living with HIV at the end of 2013. That same year, 2.1 million people became newly infected, and 1.5 million died of AIDS-related causes. Furthermore, in 2012 malaria caused an estimated 627000 deaths (WHO, 2014).

Aspartic proteases play key roles in the biology of malaria parasites and human immunodeficiency virus type 1 (HIV-1). Parikh and colleagues tested the activity of seven HIV-1 protease inhibitors against cultured *P. falciparum*. All compounds inhibited the development of parasites at pharmacologically relevant concentrations. These findings suggest that use of HIV-1 protease inhibitors may offer clinically relevant antimalarial activity (Parikh et al, 2005). This inhibition may occur due to aspartic proteases, e.g. Plasmepsin I (PM I), present in the food vacuole of *P. falciparum*. PM I, II, and IV and histo-aspartic protease encode hemoglobin-degrading food vacuole proteases. Despite having a histidine in place of one of the catalytic aspartic acids conserved in other aspartic proteases, histo-aspartic protease is an active hydrolase. A bioinformatic analysis has demonstrated that *P. falciparum* PM II, which is similar to the secretory aspartic protease 2 of *Candida albicans* (the first nonretroviral microorganism proven to be susceptible to PMs is one of the eukaryotic proteases that most resemble the HIV-1 protease (Banerjee et al, 2002; Savarino et al, 2005).

The protein is composed of three regions, the catalytic core domain (Asp25, Gly27, Ala28, Asp29, and Asp30), flap (Ile47, Gly48, Gly49, and Ile50), and the C-terminal region (Pro81 and Ile84). The amino acid residues of catalytic core are known to be highly conserved residues to which a potent inhibitor may bind strongly (Hou, 2008).

Previous study proved that ganoderiol-F, a triterpenoid isolated from the stem of *Ganoderma sinense* showed higher affinity towards HIV-1 protease (binding energy= -11.40 kcal/mol and K_i = 4.68 nM) than to plasmepsin I (binding energy= -9.96 kcal/mol and K_i = 50.94 nM) (Levita, 2014). *In vitro* studies showed that triterpenoid compounds from *G. lucidum*, e.g. ganolucidic acid-A, ganoderat acid-B, ganoderat acid- β and ganodermanondiol, exerted anti-HIV activity with IC_{50} values of 70, 170, 20 and 90 μ M, respectively (Northrop, 2001). To examine the interaction and activity of the compounds, molecular docking was performed. Docking results then were compared with the standards, that are Nelfinavir and KNI-10006.

2. Materials and methods

2.1 Materials

Personal computer operated by Windows Vista, Intel® Core™ Intel® Core™ 2 Duo P8400 @ 2.26 GHz, 32-bit, harddisk 222 GB, and RAM memory 4.00 GB.

Softwares used were:

- (1) ChemBioDraw® Ultra 13.0 free trial supported by Cambridge Soft Corporation (www.cambridgesoft.com), to draw the 2D structure of the ligands
- (2) Hyperchem Professional 8.0 (10 days usage, verification code: 0-36279), supported by Hypercube Incorporation (www.hyper.com), for geometry optimization and analysis of ligand properties.
- (3) Swiss-PdbViewer 4.1 (<http://spdbv.vital-it.ch>), to repair the incomplete crystallized structures and to separate receptor monomer.
- (4) Ligand Explorer (available at <http://www.pdb.org/pdb/explore>), to visualize the interactions of bound ligands in protein structures.
- (5) AutoDock 4.2 (downloaded from <http://autodock.scrips.edu>) for molecular docking process.
- (6) OpenBabel GUI (downloaded from <http://openbabel.org>), to convert the file format throughout the research.

2.2 Methods

2.2.1 Preparation of HIV-1 PR (PDB code: 3EKX) and PM I (PDB code: 3QS1)

- Three dimensions enzyme structures used in this research, HIV-1 PR (PDB code: 3EKX, resolution: 1.97 Å), and PM I (PDB code: 3QS1, resolution: 3.1 Å), were downloaded from Protein Data Bank (www.pdb.org) database online.
- HIV-1 PR and PM I monomers were separated from the protein using Swiss-Pdb Viewer 4.1.
- Active sites of HIV-1 PR and PM I were analyzed by using Q-SiteFinder and Discovery Studio v.3.5

2.2.2 Preparation of the Ligands

Ganolucidic acid-A, ganoderat acid-B, ganoderat acid- β and ganodermanondiol were produced by ChemBioDraw Ultra 13.0 *free trial*.

- Geometry optimizations were done using HyperChem v.8.0 with force field semi empirical PM3 method, Polak-Ribiere (conjugate gradient) algorithm.
- Each molecule was analyzed with QSAR (quantitative structure-activity relationship) parameters and the electrostatic potential properties.

2.2.3 Validation

- Nelfinavir structure (produced by ChemBioDraw Ultra 13.0) was superimposed with crystallized nelfinavir structure (obtained from PDB) using HyperChem Professional 8.0.
- Both nelfinavir and KNI-10006 (produced by ChemBioDraw Ultra 13.0) were redocked to HIV-1 PR and PM I with 20 times iteration using AutoDock 4.2.

2.2.4 Docking of Ligands to HIV-1 PR and PM I

- Ligands (ganolucidic acid-A, ganoderat acid-B, ganoderat acid- β and ganodermanondiol) were docked in the active sites of HIV-1 PR and PM I.

b. Results were analyzed using association coefficient (Cramer's V), agreement and inter-rater reliability (Cohen's Kappa).

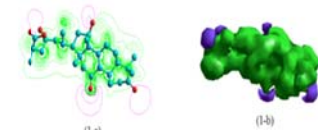
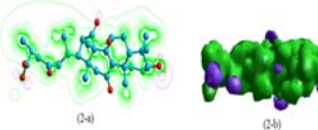
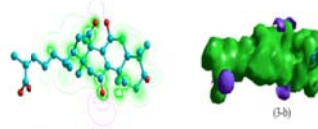
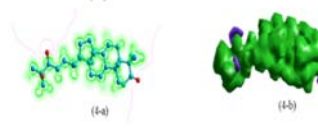
3. Results and Discussion

HIV-1 PR (PDB code: 3EKX) enzyme, a homodimer, was isolated from *Homo sapiens* with resolution 1.97 Å (King, 2012). Active binding site volume is 341 Å³. The position of this site lies between 9, 19, 4 (x, y, z) and 25, 43, 25 (x, y, z).

PM I (PDB code: 3QS1) enzyme, a homotetramers that consists of chain A, B, C, and D, was isolated from *P. falciparum* with resolution 3.10 Å³ (Bhaumik, 2011). Active binding site volume is 249 Å. The position of this site lies between 20, -17, -3 (x, y, z) and 41, -1, 12 (x, y, z).

The ligands were analyzed by employing HyperChem Professional 8.0. The results are shown in Table 1. The most lipophylic compound is ganodermanondiol, which is shown by the highest Log P value (6.45). It is obvious that the lipophylicity is caused by its less HB donors and acceptors when compared to the others. Ganodermanondiol has two hydroxyls and one carbonyl.

Table 1. Ligand Properties

Ligand	Energy (kcal/mol)	Log P	Volume (Å ³)	Massa (amu)	Electrostatic potential map [calculated using HyperChem]
Ganolucidic acid-A	-8070.4512	5.72	1335.27	500.68	
Ganoderat acid-B	-8158.5932	4.80	1364.70	516.67	
Ganoderat acid-β	-8060.0440	5.45	1348.03	500.68	
Ganodermanondiol	-7981.6760	6.45	1322.31	456.71	

Validation was performed by overlaying structures resulted RMS error value 0,3840 Å, it means programs that used for drawing and geometry optimizing (ChemBioDraw Ultra 13.0 free trial and HyperChem v.8.0) are valid.

All ligands are located in the catalytic site of HIV-1 PR. The binding modes is similar with nelfinavir, a known inhibitor of HIV-1 PR. Ganolucidic acid A (Fig.1a), ganoderat acid B (Fig.1b) and ganodermanondiol (Fig.1d) interact via three hydrogen bonds, while ganoderat acid β (Fig.1c) and nelfinavir (Fig.1e) showed two hydrogen bonds. According to the predicted Ki of the ligands, ganoderat acid B showed better affinity towards HIV-1 PR than nelfinavir (Table 2). It could be predicted that ganolucidic acid A, ganoderat acid B, ganoderat acid β, and ganodermanondiol inhibit the substrates of protease competitively (Fig.1).

These results are suitable with the statement Jaskolski *et al* (1991) that the catalytic site consists of triad Asp25,Thr26,Gly27 and protease substrates interact directly with the aspartic acid residues of each monomer to catalyze the reaction (Jaskolski, 1991).

Table 2. Docking of Ligands on HIV-1 PR

Ligand	Ei (kcal/mol)	Ki (mM)	Hydrogen Interaction
Ganolucidic acid A	-5.55	0.086	HIV N (H) → H (O) Arg8 (1.739 Å) HIV N (H) → H (O) Arg8 (1.752 Å) GA O (H) → H (O) Gly27 (1.939 Å)
Ganoderat acid B	-7.49	0.001	GB O (H) → H (O) Asp25 (1.703 Å) HIV N (H) → H (O) Ile50 (1.706 Å) HIV O (H) → H (O) Thr80 (2.143 Å)
Ganoderat acid β	-4.22	0.810	HIV N (H) → H (O) Thr26 (1.836 Å) HIV N (H) → H (O) Ile50 (1.955 Å)
Ganodermanondiol	-4.57	0.444	GD O (H) → H (O) Asp25 (2.165 Å) GD O (H) → H (O) Asp25 (1.718 Å) HIV O (H) → H (O) Gly27 (1.739 Å)
Nelfinavir (re-docking)	-4.33	0.672	NEL O (H) → H (O) Asp25 (1.985 Å) NEL O (H) → H (N) Asp25 (2.24 Å)

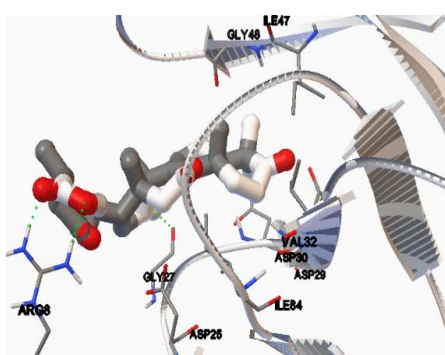
Table 3. Association Coefficient, Agreement and Inter-Rater Reliability Amino Acid of the Ligands with Nelfinavir

Ligand	Correlation*		Proximity**
	Cramer's V	P	Kappa
Ganolucidic acid A	0.55	0.008	0.55(95%CI:[0.20,0.90])
Ganoderat acid B	0.18	0.390	0.18(95%CI:[-0.23,0.59])
Ganoderat acid β	0.28	0.190	0.27(95%CI:[-0.13,0.68])
Ganodermanondiol	0.46	0.027	0.45(95%CI:[0.08,0.83])

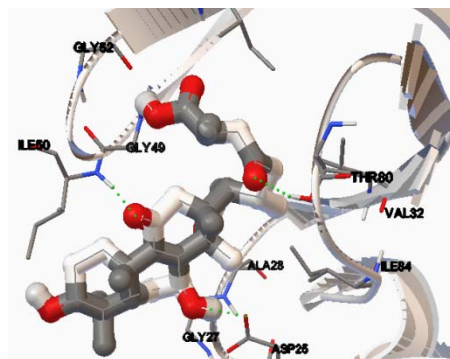
* Relation coefficient was calculated based on Cramer's V association compared with the amino acid residues from re-docking of nelfinavir.

** Proximity is calculated based on Cohen's Kappa method compared with the amino acid residues from re-docking of nelfinavir.

Based on the statistical results, the compounds which have similar amino acid residues closest to nelfinavir are ganolucidic acid A and ganodermanondiol, results of p-value less than 0.05 indicates significant results. Cohen's Cappa measurement results for ganolucidic acid A and ganodermanondiol showed affinity with moderate levels of agreement. While the compound that least like is ganoderat acid β and ganoderat acid B, the resulting p-value of more than 0.05 indicates that the result is not significant. Cohen's Cappa measurement results ganoderat acid β showed affinity with fair level agreement and ganoderat acid B showed affinity with small level agreement.



(a)



(b)

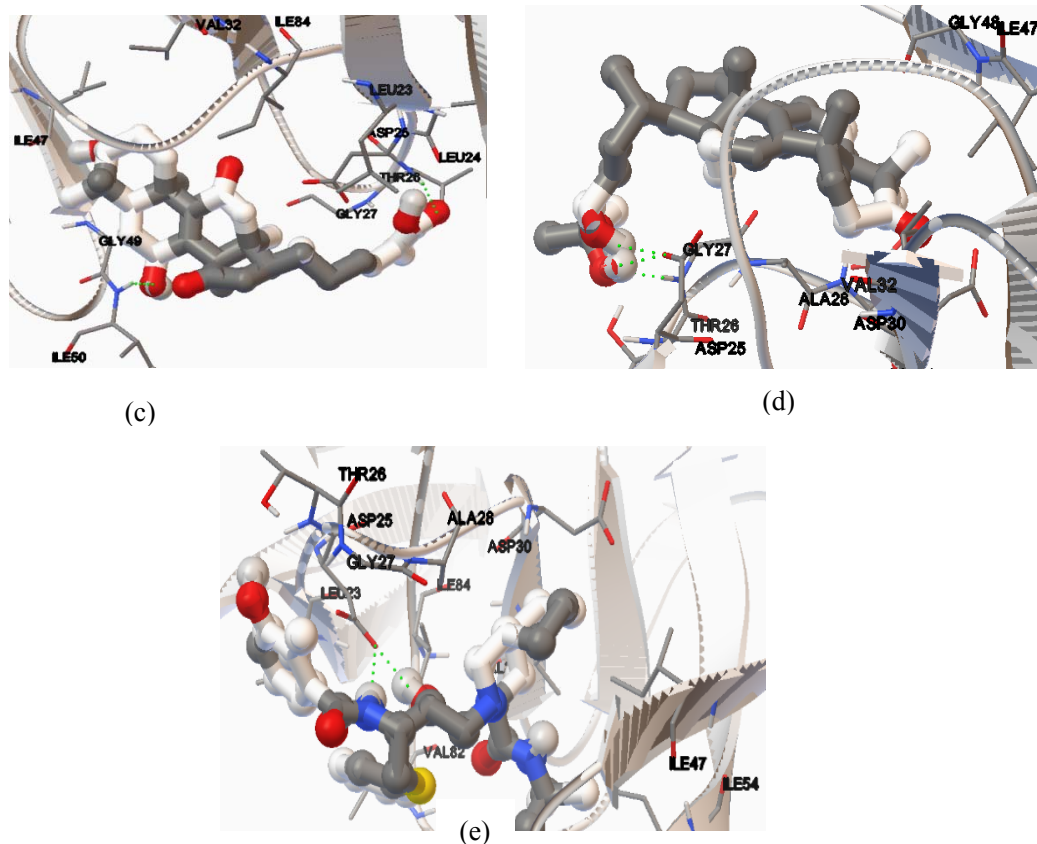
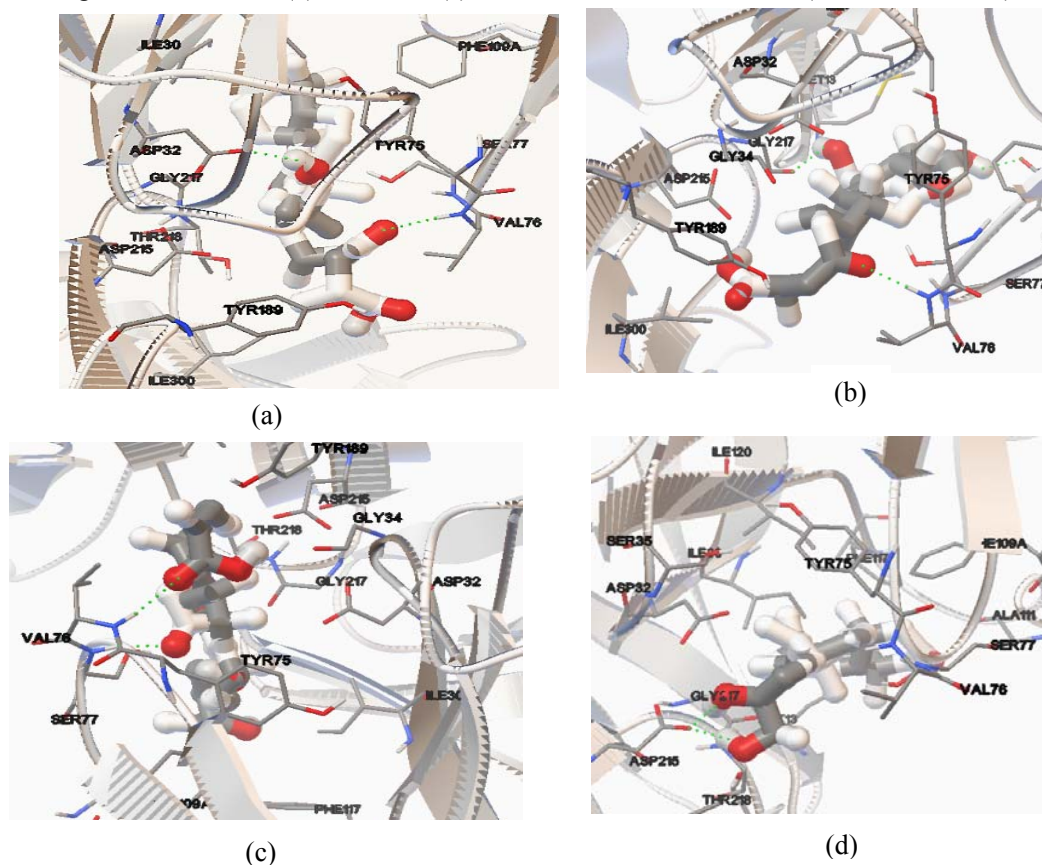


Figure 1. Docking results of ganolucidic acid A (a), ganoderat acid B (b), ganoderat acid β (c), and ganodermanondiol (d), nelfinavir (e) on the active site of HIV-1 PR (PDB code: 3EKX).



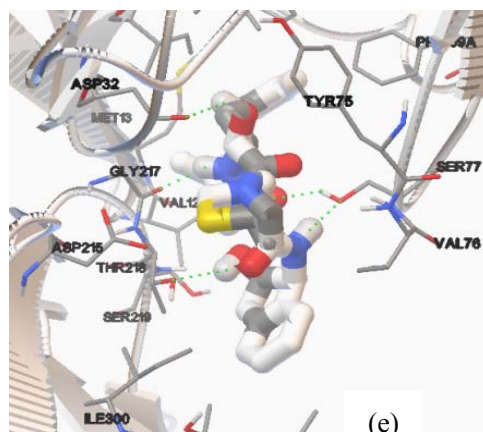


Figure 2. Docking results of ganolucidic acid (a), ganoderat acid B (b), ganoderat acid β (c), and ganodermanondiol (d), KNI-10006 (e) on the active site of PM I (PDB code: 3QS1)

All ligands are located in the catalytic site of PM I, as showed by the existence of two important aspartic acids, Asp32 and Asp215 (Fig.2). The binding modes is similar with KNI-10006, a known inhibitor of PM I. Protease substrates interact directly with aspartic acid residues of each monomer to catalyze the reaction, therefore, according to docking results, it could be predicted that ganolucidic acid A, ganoderat acid B, ganoderat acid β , and ganodermanondiol inhibit the substrates of protease competitively (Fig.2).

Ganoderat acid B interaction with PM I showed that it formed three hydrogen bonds (Fig. 2b Table 4), two bonds were formed between the ganolucidic acid A (Fig. 2a Table 4), ganoderat acid β (Fig. 2c Table 4), ganodermanondiol with PM I (Fig. 2d Table 4), and five bonds were formed between the KNI-10006 with PM I (Fig. 2e Table 4). According to the predicted K_i of the ligands, ganodermanondiol showed better affinity towards PM I than KNI-10006 (Table 4).

Table 4. Docking of Ligands on PM I

Ligand	Ei (kcal/mol)	K_i (mM)	Hydrogen Interaction
Ganolucidic acid A	-6.89	0.008	GA O (H) \rightarrow H (O) Asp32 (1.924 Å) PM1 N (H) \rightarrow H (O) Val76 (2.13 Å)
Ganoderat acid B	-5.42	0.105	PM1 N (H) \rightarrow H (O) Val76 (2.061 Å) GB O (H) \rightarrow H (O) Ala111 (1.823 Å) GB O (H) \rightarrow H (O) Gly217 (1.849 Å)
Ganoderat acid β	-5.73	0.063	PM1 N (H) \rightarrow H (O) Val76 (2.217 Å) PM1 N (H) \rightarrow H (O) Ser77 (2.243 Å)
Ganodermanondiol	-7.14	0.005	GD O (H) \rightarrow H (O) Asp215 (1.879 Å) GD O (H) \rightarrow H (O) Asp215 (1.838 Å)
KNI-10006 (re-docking)	-5.46	0.099	KNI O (H) \rightarrow H (O) Asp32 (2.018 Å) KNI O (H) \rightarrow H (N) Ser77 (2.072 Å) PM1 O (H) \rightarrow H (O) Ser77 (1.876 Å) KNI O (H) \rightarrow H (O) Gly217 (2.085 Å) KNI O (H) \rightarrow H (O) Thr218 (2.132 Å)

Based on the statistical results, the compounds which have similar amino acid residues closest to KNI-10006 are ganodermanondiol and ganolucidic acid A, results of p-value less than 0.05 indicates significant results. Cohen's Cappa measurement results ganodermanondiol showed affinity with substantial level of agreement whereas for ganolucidic acid A showed affinity with moderate levels of agreement. The compounds that least like are ganoderat acid B and ganoderat acid β , results of p-value more than 0.05 indicates that the results are not significant. Cohen's Cappa measurement results two compounds showed affinity with a fair level of agreement.

Table 5. Association Coefficient, Agreement and Inter-Rater Reliability Amino Acid of the Ligands with KNI-10006

Ligand	Correlation*		Proximity**
	Cramer's V	P	Kappa
Ganolucidic acid A	0.57	0.002	0.54(95%CI:[0.21,0.86])
Ganoderat acid B	0.30	0.125	0.29(95%CI:[-0.08,0.67])
Ganoderat acid β	0.30	0.125	0.29(95%CI:[-0.08,0.67])
Ganodermanondiol	0.62	0.0009	0.61(95%CI:[0.30,0.92])

* Relation coefficient was calculated based on Cramer's V association compared with the amino acid residues from re-docking of KNI-10006.

** Proximity is calculated based on Cohen's Kappa method compared with the amino acid residues from re-docking of KNI-10006.

4. Conclusion

The binding modes of ganolucidic acid A, ganoderat acid B, ganoderat acid β and ganodermanondiol, triterpenoids of *G. lucidum*, resemble that of KNI-00006, a known inhibitor of PM I, while according to the predicted K_i of the ligands, ganoderat acid B showed better affinity towards HIV-1 PR than nelfinavir. These compounds could be developed further as both anti-HIV and anti-malaria.

References

- Banerjee, R., Liu, J., Beatty, W., Klemba, M., & Goldberg, D. E. (2002). Four plasmepsins are active in the *Plasmodium falciparum* food vacuole, including a protease with an active-site histidine, *Proc Natl Acad Sci USA*, 99(2), 990-995. <http://dx.doi.org/10.1073/pnas.022630099>
- Bhaumik, P., Gusthina, A., & Wlodawer, A. (2011). Crystal structure of KNI-10006 complex of Plasmepsin I (PMI) from *Plasmodium falciparum*. *J.Struct.Biol.*, 175, 73-84. <http://dx.doi.org/10.1016/j.jsb.2011.04.009>
- Colin, B., Humphreys, M. J., Matharu, P., Granger, R., Horrocks, P., & Moon, R. P. (1999). A distinct member of the aspartic proteinase gene family from the human malaria parasite *Plasmodium falciparum*. *FEBS Lett*, 447,149-154.
- Hou, T., McLaughlin, W. A., & Wang, W. (2008). Evaluating the Potency of HIV-1 Protease Drugs to Combat Resistance. *Proteins*, 71(3), 1163-1174. <http://dx.doi.org/10.1002/prot.21808>
- Jaskólski, M., Tomasselli, A. G., Sawyer, T. K., Staples, D. G., Heinrikson, R. L., Schneider, J., Kent, S. B., *et al.* (1991). Structure at 2.5-Å resolution of chemically synthesized human immunodeficiency virus type 1 protease complexed with a hydroxyethylene-based inhibitor, *Biochemistry*, 30(6), 1600-1609.
- King, N. M., Prabu-Jeyabalan, M., Bandaranayake, R. M., Nalam, M. N., Nalivaika, E. A., Ozen, A., Yilmaz, N. K., & Schiffer, C. A. (2012). Extreme entropy-enthalpy compensation in a drug-resistant variant of HIV-1 protease. *Acs Chem Biol*, 7, 1536-1546. <http://dx.doi.org/10.1021/cb300191k>
- Levita, J., Heng, C., & Mutakin, K. (2014). Interactions of ganoderiol-f with aspartic proteases of HIV and plasmepsin for anti-HIV and anti-malaria discovery. *International Journal of Pharmacy and Pharmaceutical Science*, 6(5), 561-566. <http://www.ijppsjournal.com/Vol6Issue5/9474.pdf>
- Northrop, D. B. (2001). Follow the protons: a low-barrier hydrogen bond unifies the mechanisms of the aspartic proteases. *Acc Chem Res*, 34(10), 790-797.
- Parikh, S., Gut, J., Istvan, E., Goldberg, D. E., Havlir, D. V., & Rosenthal, P. J. (2005). Antimalarial activity of human immunodeficiency virus type 1 protease inhibitors. *Antimicrob Agents Chemother*, 49, 2983-2985. <http://dx.doi.org/10.1128/AAC.49.7.2983-2985.2005>
- Savarino, A., Cauda, R., & Cassone, A. (2005). Aspartic proteases of *Plasmodium falciparum* as the target of HIV-1 protease inhibitors, *J Infect Dis*, 191, 1381-1383. <http://dx.doi.org/10.1086/428781>
- WHO. (2014). MDG 6: combat HIV/AIDS, malaria and other diseases. Tersedia di: http://www.who.int/topics/millennium_development_goals/diseases/en/ [Accessed on 18th June 2014]

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