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In Silico Study of Andrographolide and Its Derivatives as HIV-1 Protease Inhibitors for Anti-HIV/AIDS Drug Discovery

Sandra Megantara[1,2*], Daryono Hadi Tjahjono[1], Rahmana Emran Kartasasmita[1], Maria Immaculata Iwo[1], Jutti Levita[2], Slamet Ibrahim[1]

[1]School of Pharmacy, Bandung Institute of Technology, Jl. Ganesha 10 Bandung
[2]Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, University Padjadjaran, Indonesia.

ABSTRACT

HIV (Human Immunodeficiency Virus) is a retrovirus that attacks and damage cells CD4+ lymphocytes. For the prevention of infection and replication of HIV, is currently used antiretroviral. However, antiretroviral widely used today has the disadvantage of many unpleasant side effects, drug interactions and drug resistance. Studies *in silico* states that andrographolide has antimalarial activity which works by inhibiting aspartic proteases, and obtained the best three generations that have potential as aspartic protease inhibitors. If a antiretroviral protease inhibitors (ARPIs) that is used as an anti-HIV/AIDS can work as antimalarial, a hypothesis was proposed an aspartate protease inhibitor can be used as an anti-HIV/AIDS. The objective of this paper was to investigate the interaction between andrographolide and its derivatives, with the ligand binding domain of HIV-1 protease to find the most favorable binding site as well as to predict the binding mode. Pepstatin, a protease inhibitor, was used as the standard, lopinavir and ritonavir (ARPIs drugs) ware used as comparison. LigandScout Software was used to conduct pharmacophore modelling, virtual screening and molecular docking. From pharmacophore modelling showed that there were three hydrophobic interactions and two hydrogen bond between andrographolide and amino acid residues in binding site of HIV-1 protease enzyme. Result from virtual screening showed that all andrographolide derivatives hit the pharmacophore with pharmacophore-fit score were 72.92, 72.13, 71.22 for derivates i, ii and iii respectively. Docking studies showed that pepstatin, lopinavir and ritonavir still gave better binding interactions to HIV-1 protease than andrographolide and its derivatives, binding affinity and inhibition constant values were Ei = -10.4 kcal/mol; Ki = 0.01 uM (pepstatin), Ei = -10.3 kcal/mol; Ki = 0.02 uM (lopinavir), Ei = -10.3 kcal/mol; Ki = 0.02 uM (ritonavir), Ei = -8.2 kcal/mol; Ki = 0.98 uM (andrographolide), Ei = -9.80 kcal/mol; Ki = 0.07 uM (i), Ei = -9.80 kcal/mol; Ki = 0.07 uM (ii), Ei = -10.0 kcal/mol; Ki = 0.05 uM (iii). According to the result, we concluded that andrographolide and its derivatives still potential and could be developed as protease inhibitor for anti-HIV/AIDS drugs.

Keywords: Andrographolide, andrographolide derivatives, anti-HIV/AIDS, HIV-1 protease inhibitor, *in silico*

Corresponding author

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INTRODUCTION

HIV (Human Immunodeficiency Virus) is a retrovirus that can attack and damage cells CD4 + lymphocytes (T-helper) [1]. To multiply the amount of virus, the HIV virus requires transcriptase enzymes, protease and integrase. Protease cleavage of the precursor required for virus Gag polyprotein and GagPol so as adults will be able to transmit the virus particles [2]. Antiretroviral drugs are used for the prevention of infection and replication of HIV [3].

The mechanism of antiretroviral drugs assortment, one of which is a protease inhibitor (ARPIs) [4]. With the protease inhibitor, the breakdown of protein can be prevented so that the production of new virus particles can be slowed [5]. Inhibition of HIV-1 protease is an alternative strategy of inhibition of reverse transcriptase in the treatment of HIV-1 infection [6].

In a previous study found that andrographolide can interact with plasmepsin an aspartate protease enzyme. Pharmacophore of andrographolide are hydroxyl group at C-14, C-19 and oxygen atoms in the lactone ring. Pharmacophore identification was done through structured based drug design method with plasmepsin I, II and IV as the target biomacromolecules [7].

A bioinformatics analysis showed that plasmepsin II in P. falciparum is similar to secretion of aspartic proteases of *Candida albicans* is the first nonretroviral microorganisms that produce most resembles the eukaryotic protease HIV-1 protease. Research conducted at the National Center for Biotechnology Information (NCBI) by using the program vector alignment search tool (VAST), data reveal a very significant that there are similarities between the structure of the HIV-1 protease with plasmepsin II, including the amino acid sequence of the active site is almost identical [8]. Because of the similarities between the structure of the HIV-1 protease with plasmepsin, has been done the research effectiveness of some antiretroviral drugs protease inhibitors (ARPIs) which is used for the treatment of HIV/AIDS is used as an antimalarial drug. From the results of research show that saquinavir, ritonavir and lopinavir can inhibit plasmepsin II and IV of parasite *P. falciparum* [9].

If a ARPIs commonly used as an anti-HIV/AIDS can work against plasmepsins and have the effect of antimalarial, it appears the hypothesis may also apply the opposite that an aspartic protease inhibitor which works on plasmepsin can be used as an anti-HIV/AIDS.

The objective of this research was to investigate the interaction between andrographolide and its derivatives, with the ligand binding domain of HIV-1 protease to find the most favorable binding site as well as to predict the binding mode. Pepstatin, a protease inhibitor, was used as the standard, lopinavir and ritonavir (ARPIs drugs) ware used as comparison. LigandScout Software was used to conduct pharmacophore modelling, virtual screening and molecular docking.

MATERIALS AND METHODS

Hardware and software:

Docking calculations were carried out on branded Sony Vaio PC Linux Ubuntu 14.04 LTS as the operating system, with Intel 2.30 GHz Core i5 and 4 GB memory hardware. The software used for pharmacophore modelling, virtual screening and molecular docking was LigandScout 4.0. Meanwhile, PyMOL and AutoDock Vina 1.1 were used for RMSD calculation and binding algorithms.

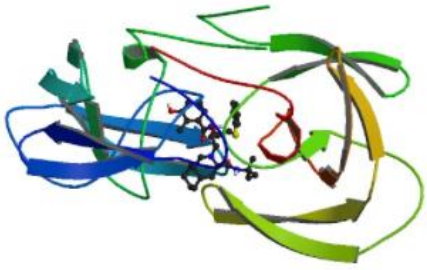

Figure 1: Crystal Structure of HIV-1 Protease (1OHR)

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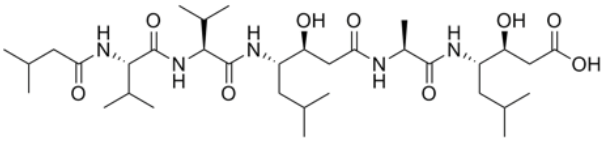
Validation of docking protocols was carried out by redocking native ligand to receptor. The RMSD values calculations were performed using *rms_cur* module in PyMol.

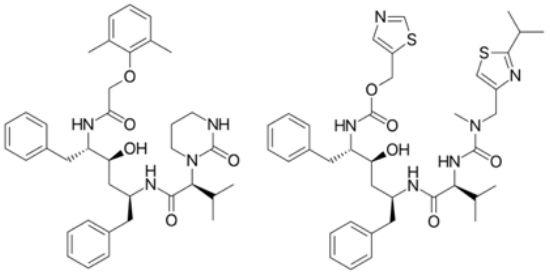
Pharmacophore modelling obtained by docked andrographolide into the active site of HIV-1 protease first, and then the pharmacophore was generated by using LigandScout software.

Virtual screening process begins with validating pharmacophore features with retrospective validation method, which were tested against 536 compounds of active ligands (actives) and 35.750 compounds of inactive ligand (decoys) ware obtained from DUD-E (http: //dude.docking.org /).

The docking process was performed to pepstatin as standard ligand, lopinavir and ritonavir as ARPIs drugs (Fig. 2), Test compounds were andrographolide and derivatives i, ii, and iii (Fig. 3).

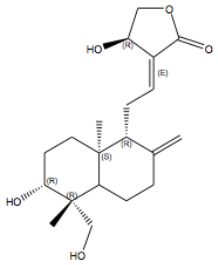


Pepstatin

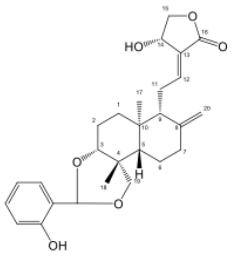


Lopinavir Ritonavir

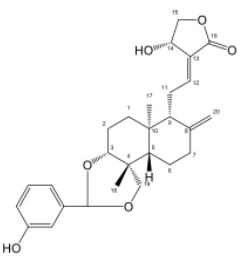
Figure 2: Standard Ligand and ARPIs Drugs



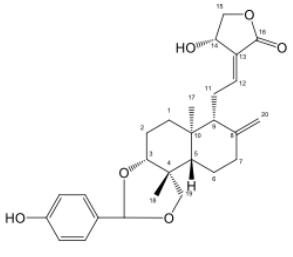
Andrographolide



3,19-2-hidroxybenzilidene andrographolide (i)



3,19-3-hidroxybenzilidene andrographolide (ii)



3,19-4-hidroxybenzilidene andrographolide (iii)

Figure 3: Test Compounds

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RESULTS AND DISCUSSION

Results and Discussion

The internal validation was aimed to examine whether the docking protocols can reproduce the pose of the co-crystal ligand. The objective function used in the internal validation was the root mean square distance (RMSD) value between the heavy atoms of the docked pose and the crystal structure pose. The protocol is acceptable if the RMSD value is less than 2.0 [10]. The best RMSD value of 100 repetitions were 1.225 Å. The result of RMSD values from redocking process it can be concluded that the docking protocols were valid.

From pharmacophore modelling showed that there were three hydrophobic interactions and two hydrogen bond between andrographolide and amino acid residues in binding site of HIV-1 protease enzyme. From that interaction pharmacophore features ware generated by LigandScout software (Fig. 4).

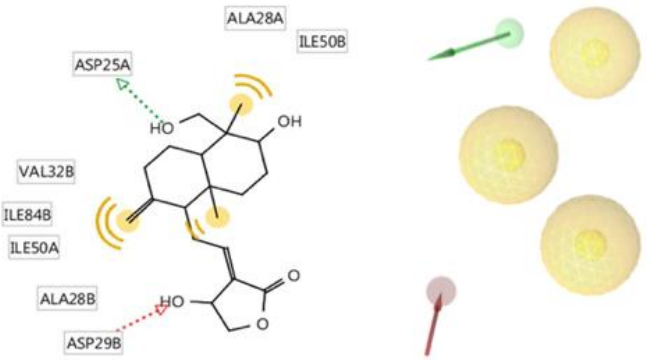

Figure 4: Pharmacophore of Andrographolide in The Binding Site of HIV-1 Protese Enzyme

Retrospective validation for generated pharmacophore results showed that the pharmacophore features ware valid because AUC value above 50% (Fig. 5). The ideal value of the AUC is 100% which indicates that all known ligands are ranked higher than their decoys. In random sampling, the AUC value is 50%. The $EF_{1\%}$ value represents the early enrichment of the protocols, while the AUC value represents the global enrichment [11].

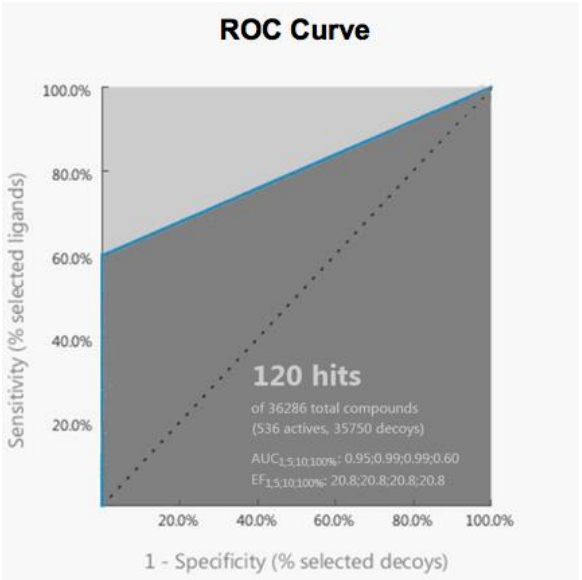

Figure 5: ROC Curve of Retrospective Validation of Pharmacophore Features

The virtual screening and molecular docking results of reference ligand and test compounds are presented in Table 1.

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Table 1: Virtual screening and docking score results

Test Compounds	Pharmacophore-Fit Score		Docking Score	
	Hit Status	Score	Ei	Ki
Pepstatin	-	-	-10.4	0.01
Lopinavir	-	-	-10.3	0.02
Ritonavir	-	-	-10.3	0.02
Andrographolide	-	-	-8.2	0.98
i	Hit	72.92	-9.8	0.07
ii	Hit	72.13	-9.8	0.07
iii	Hit	71.22	-10.0	0.07

Ei = Binding Affinity (Kcal/Mol)
Ki = Inhibition Constant (µM)

Result from virtual screening showed that all andrographolide derivatives hit the pharmacophore. This predict that all andrographolide derivatives can interact with HIV-1 protease receptor. Docking studies showed that pepstatin, lopinavir and ritonavir still gave better binding interactions to HIV-1 protease than andrographolide and its derivatives, but according the value, andrographolide and its derivatives still potential and could be developed as protease inhibitor for anti-HIV/AIDS drugs.

The binding poses of test compounds based on the best docking result with HIV-1 Protease showed in (Fig. 6, 7, 8 and 9).

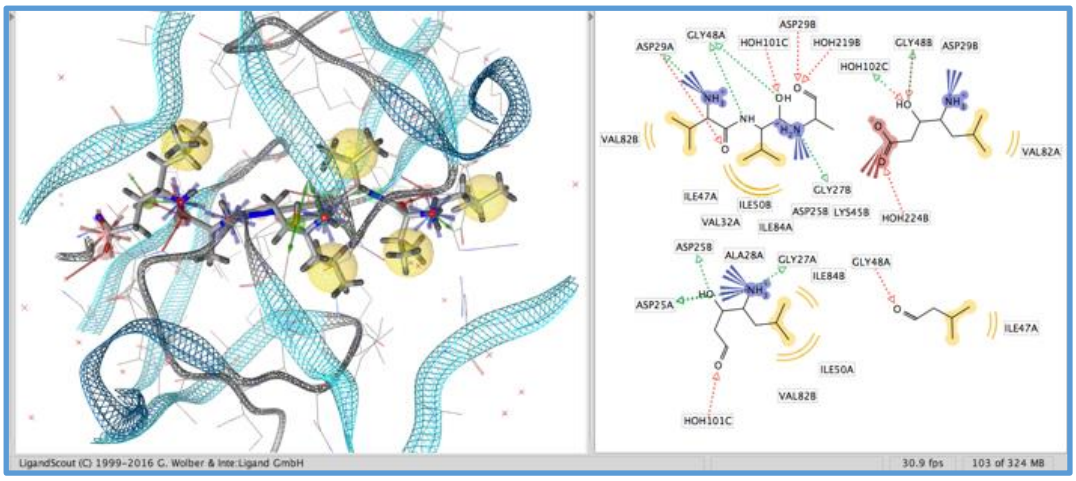

Figure 6: Interaction of Pepstatin in The Binding Pocket of HIV-1 Protease

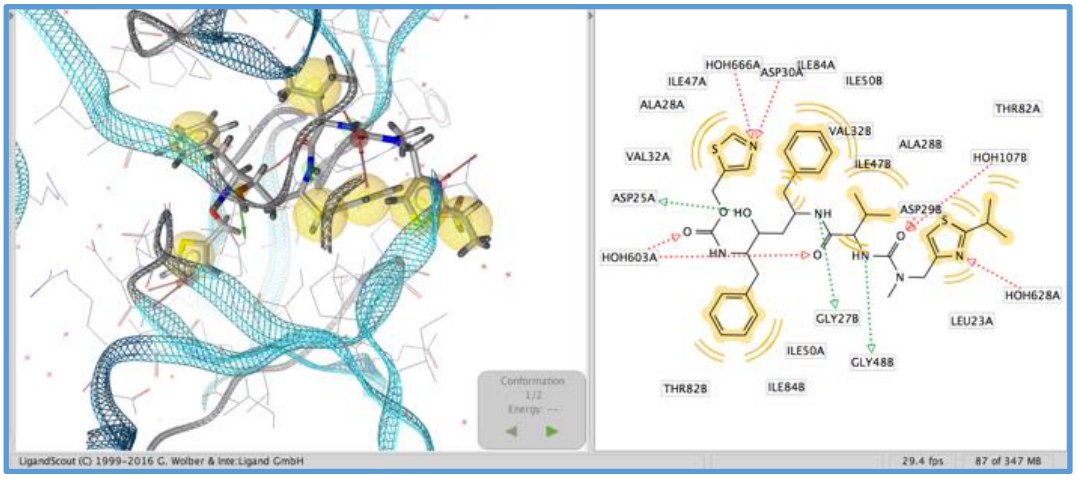

Figure 7: Interaction of Lopinavir in The Binding Pocket of HIV-1 Protease

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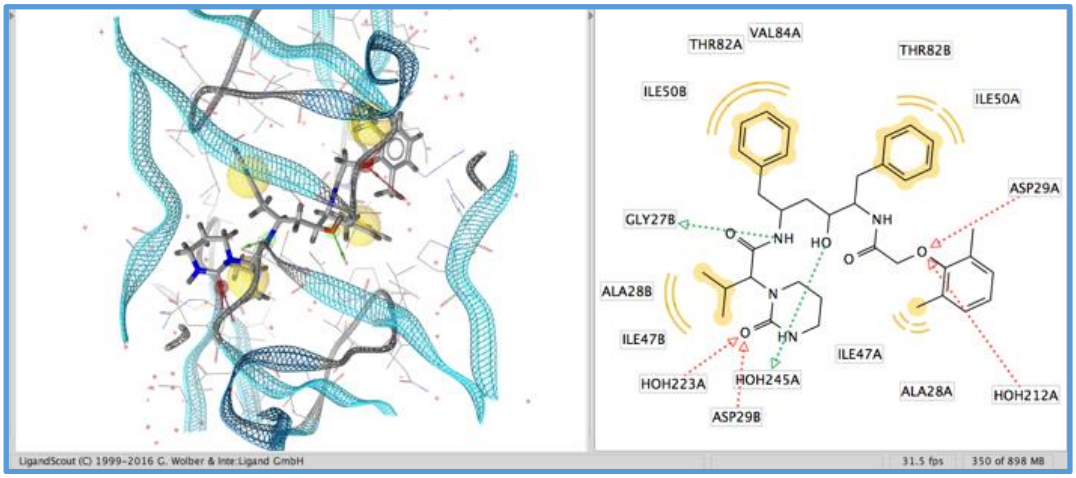

Figure 8: Interaction of Ritonavir in The Binding Pocket of HIV-1 Protease

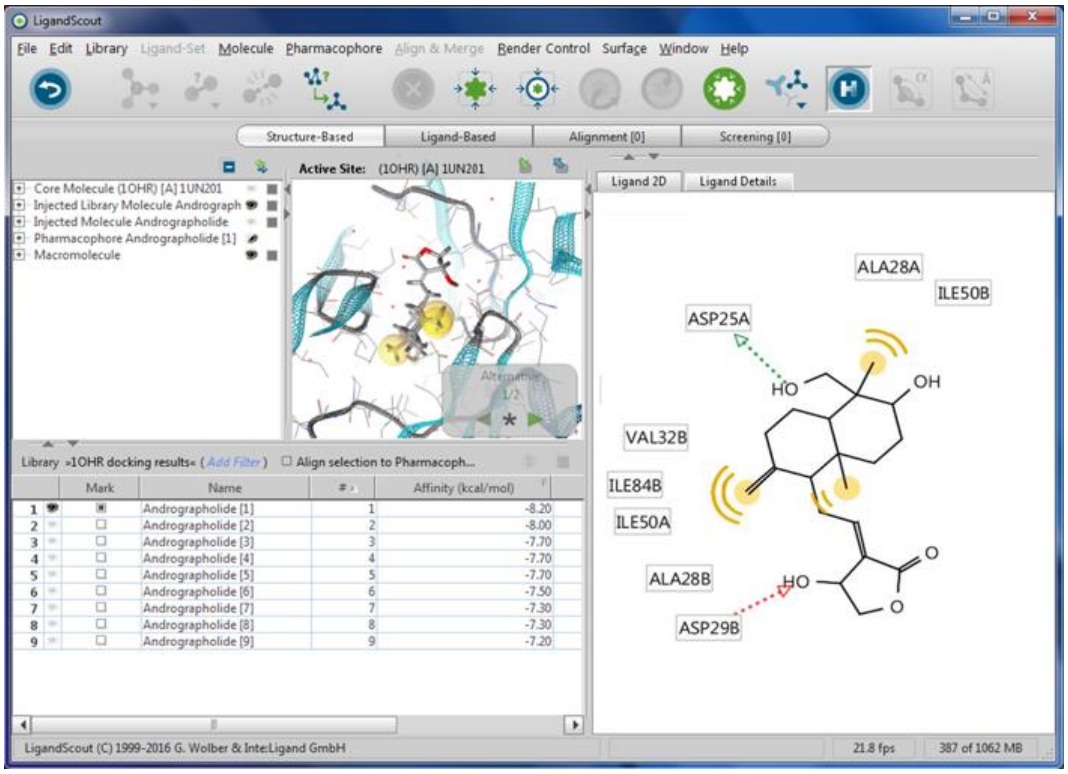

Figure 9: Interaction of Ritonavir in The Binding Pocket of HIV-1 Protease

Based on that interactions, andrographolide was able to bind with two important aspartic acid residues in the binding pocket HIV-1 Protease Inhibitor [12], same as references pestatin, lopinavir and ritonavir.

CONCLUSIONS

Andrographolide and its derivatives potential and could be developed as protease inhibitor for anti-HIV/AIDS drugs.

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