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Original Article

INTERACTIONS OF GANODERIOL-F WITH ASPARTIC PROTEASES OF HIV AND PLASMEPSIN FOR ANTI-HIV AND ANTI-MALARIA DISCOVERY

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ABSTRACT

Objective: HIV-1 has been a killer disease since two decades ago, while a potential cure has not yet discovered due to the fast mutations of the HIV-1 enzymes,i.e reverse transcriptase, integrase, and protease. Apart of HIV-1, malaria has been the biggest cause of death in human and it is mostly found in the East part of Indonesia. There are some enzymes in the food vacuole of *Plasmodium falciparum* which are the targets of anti-malaria discovery, e.g. plasmepsin I, II, IV, and histo-aspartic protease (HAP). Both plasmepsin and HIV-1 protease are aspartic proteases, therefore a single drug could be designed to inhibit both enzymes. Ganoderiol-F is a triterpenoid isolated from the stem of *Ganodermasinense*which shows inhibition on HIV-1 protease with IC_{50} 20-40 µM.

This project was aimed to study and visualize the interaction of ganoderiol-F with HIV-1 protease and plasmepsin I for anti-HIV and anti-malaria discovery.

Methods: Preparation of the ligand comprises of geometry optimization using MM+ method and Polak-Ribiere (conjugate gradient) algorithm. Molecular docking using AutoDock 4.2 was used to put the ligand into the binding site of both aspartic proteases. Pepstatin-A was used as comparison. The amino acid residues near the drug-target interactions and affinity of the drugs were identified.

Results: The affinity of ganoderiol-F is higher towards HIV-1 protease (binding energy= -11.40kcal/mol and K_i= 4.68nM) than to plasmepsin I (binding energy= -9.96 kcal/mol and K_i= 50.94 nM), meanwhile pepstatin-A has better affinity towards HIV-1 protease (binding energy= -4.52 kcal/mol and K_i= 496.13 µM) than to plasmepsin I (binding energy= -3.07 kcal/mol and K_i= 5.98 mM).

Conclusions: According to the values of binding energy and inhibition constant, ganoderiol-F could be developed further as both anti-HIV and anti-malaria.

Keywords: AIDS, Ganoderiol, HIV-1 protease, Malaria, Molecular docking, Plasmepsin I, *Plasmodium falciparum.*

INTRODUCTION

HIV-1 protease is one of a promising new chemotherapeutics target. HIV-protease inhibitors restrain the viral maturation by preventing the formation of structural and functional proteins and form immature, non-infectious virus. Structurally, HIV-1 protease is a homodimer protein, containing 99 amino acids in each chain, with an active site located in the dimer interface [9]. 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 [6].

HIV-1 protease and Plasmepsin I enzymes are both aspartic proteases, which catalytic sites contain two aspartic acid residues. They are usually located at the bottom of a cleft in the enzyme surface [11]. Aspartic proteases are inhibited by pepstatin-A, a naturally occurring peptide containing two statins, which replace amino acids [10,13]. The hydroxyl group of the statine binds tightly to the catalytically-active aspartic acid residues in the active site of protease, thereby mimicking the transition state of the peptide cleavage [14]. Plasmepsin Iwhich is found in the food vacuole of *Plasmodiumfalciparum* degrades the hemoglobin directly [1]. The active site of Plasmepsin I contains two important aspartic acid residues, Asp32, and Asp125 [2], both plays a role in the degradation of hemoglobin in the food vacuole of *Plasmodium falciparum.*

Ganoderiol-F is a triterpenoid isolated from the stem of *Ganodermasinense*which inhibits HIV-1 protease with IC_{50} 20-40 µM[12]. Based on this *in vitro* result, an *in silico* approach by molecular docking was interesting to be carried out to study and visualize the interaction of ganoderiol-F with HIV-1 protease and plasmepsin I for anti-HIV and anti-malaria discovery.

MATERIALS AND METHODS

Molecular Modeling Preparation

ASUS U45J operated by Windows 7 Home Premium, Intel® Core™ i5 CPU M450 @ 2.40GHz, 64-bit, harddisk 444 GB, and RAM memory 4.00 GB was used to run molecular docking processes. Softwares installed were (1) ChemBioDraw® Ultra 13.0 free trial supported by Cambridge Soft Corporation (downloaded from www.cambridgesoft.com), to draw the ligand structures in 2D and 3D which are bound to HIV-1 protease and Plasmepsin I (2) Hyperchem Professional 8.0 (10 days usage), with verification code: 0-34733, supported by Hypercube Incorporation (downloaded from www.hyper.com), for geometry optimization and analysis of molecules properties. (3) Swiss-pdbViewer version 4.1 (downloaded from http://spdbv.vital-it.ch), to repair the incomplete crystallized structures and to remove unnecessary receptor chain. (4) Ligand Explorer (available at http://www.pdb.org/pdb/explore), to visualizes 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 molecules format throughout the research. Three dimensions enzyme structures used in this research were HIV-1 protease (PDB code: 1HXW, resolution: 1.8 Å) and Plasmepsin I(PDB code: 3QS1, resolution: 3.1 Å). Three dimensions crystallized ligandwaspepstatin-A (PDB code: 1HDH, resolution: 1.7 Å). The ligand was downloaded from Protein Data Bank (www.pdb.org) database online. Two and three dimensions of ganoderiol-F were drawn using ChemBioDraw® Ultra 13.0 free trial.

Preparation of macromolecules

a. HIV-1 protease (PDB code: 1HXW) and Plasmepsin I(PDB code: 3QS1) were downloaded from Protein Data Bank (www.pdb.org).

b. HIV-1 protease dimer andPlasmepsin I monomer were separated andfixed using Swiss-pdbViewer v.4.1.
c. The coordinates and volume of active site of HIV-1 protease and Plasmepsin Iwere calculated using Q-SiteFinder.

Preparation of ligands

a. Two and three dimensions of pepstatin-A and ganoderiol-F were produced by ChemBioDraw Ultra 13.0 free trial.
b. Geometry optimizations were done by using Hyperchem Professional 8.0with force field molecular mechanics MM+ method, Polak-Ribiere (conjugate gradient) algorithm.
c. Each molecule was analyzed with QSAR parameters and the electrostatic potential properties using Hyperchem Professional 8.0

Docking of Ligands on HIV-1 protease and Plasmepsin I

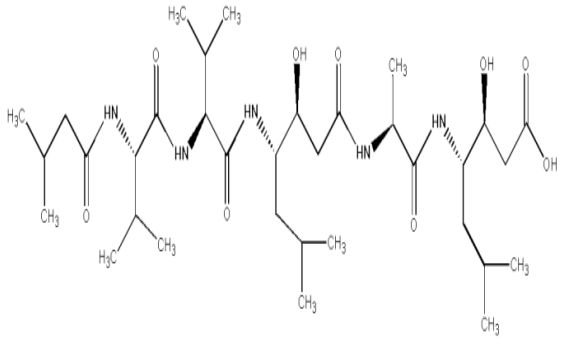
a. Pepstatin-A and ganoderiol-F were docked on the active sites of HIV-1 protease and Plasmepsin I. Docking was repeated 8 times for each target. Number of docking runs was set to 20, while other parameters were set to default.
b. Results of docking were interpreted.

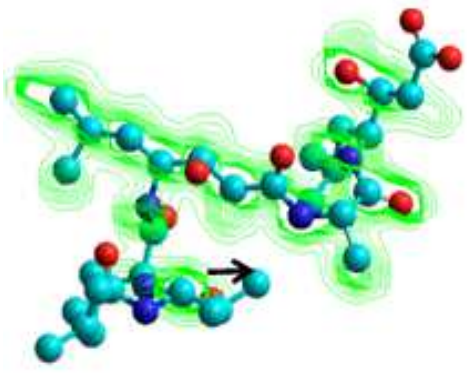
RESULTS AND DISCUSSION

Three dimension structures of pepstatin-A and ganoderiol-F (Figure 1) were analyzed using Hyperchem Professional 8.0. The results obtained are shown in Table 1. Ganoderiol-F is more lipophilic than pepstatin-A which is shown by its higher value of LogP compared to pepstatin-A.

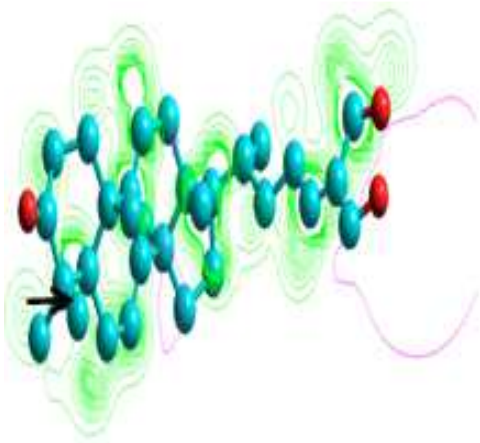
Table 1: Analysis of Ligands

Ligands	Optimization Energy Values [kcal/mol]	Mass [atomic mass unit]	Volume [Å]	LogP
Pepstatin-A	-28.03	685.90	2049.2	1.99
Ganoderiol-F	-7853.22	454.69	1320.7	6.16





(a)



(b)

Fig. 1: 2D and 3D structures of pepstatin-A (a) and ganoderiol-F (b)

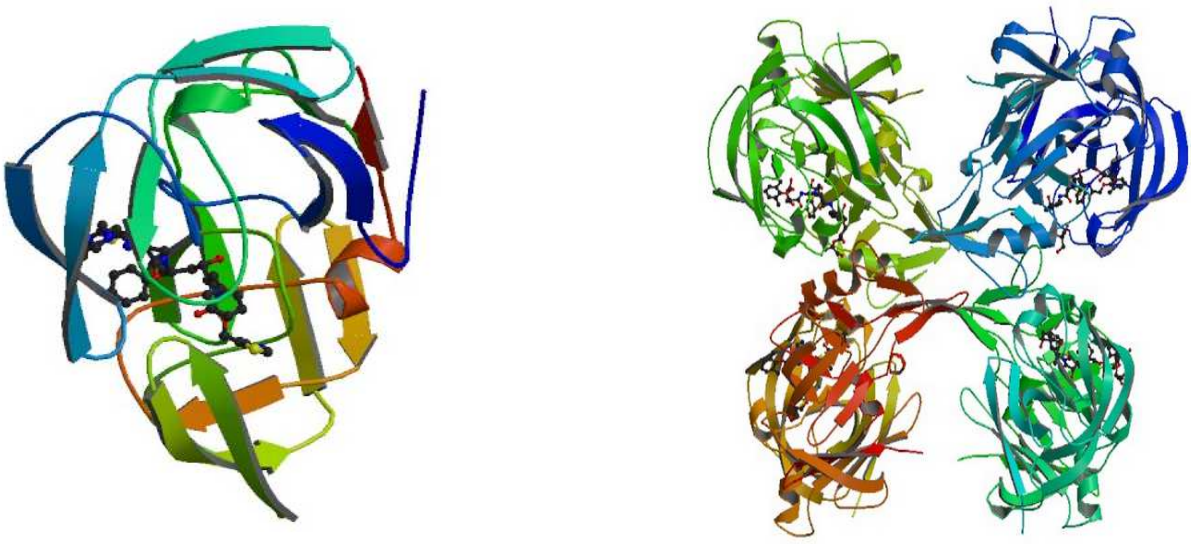
Potential electrostatic maps of both compounds are showed on the right side.

Based on the potential electrostatic map (Figure 1), both pepstatin-A and ganoderiol-F are electropositive molecules (showed by green contour on the molecules), which mean that both compounds could interact with electronegative environment in the correct sterical fits.

HIV-1 protease (PDB code: 1HXW) enzyme (Figure 2a), a homodimer which volume is 18,523 Å^3, was isolated from *Homo sapiens* with resolution 1.80 Å [8]. Active binding site volume is around 482 Å^3. The position of this site lies between 1, 9, -4 (x, y, z) and -25, 34, 16 (x, y, z). Plasmepsin I (PDB code: 3QS1) enzyme (Figure 2b), a homo tetramer consists of four chains, A, B, C, and D, was isolated from *Plasmodium falciparum* with resolution 3.10 Å^3 [3]. Chain A was obtained using Swiss-pdbViewer v.4.2 so that the site of docking could be more specific. The volume of Plasmepsin I chain A is 30,681 Å.

The result of predicted Ki and binding energy values of dockings of pepstatin-A and ganoderiol-F on active site HIV-1 protease are shown in Table 2, and Table 3 respectively.

Docking of pepstatin-A into the catalytic site of HIV-1 protease was repeated eight times (Table 2) with grid dimension 38x38x38 Å^3. Total Kollman charge calculated forHIV-1 protease enzyme was 5.998, while total Gasteiger charge calculated forpepstatin-A was -1.0006.



(a) (b)

Fig. 2: 3D structures of HIV-1 protease PDB code 1HXW (a) and plasmepsin I PDB code 3QS1 (b) downloaded from www.pdb.org

Table 2: Docking of Pepstatin-A into the catalytic site of HIV-1 protease

Docking	Binding energy (kcal/mol)	Predicted Ki (µM)	Amino acid residues
1	-4.35	652.68	Arg8, Leu23, Asp25, Gly27, Ala28, Asp29, Asp30, Val32, Ile47, Gly48, Gly49, Ile50, Pro81, Val82, Ile84
2	-4.45	548.60	
3	-4.68	372.96	
4	-4.54	469.94	
5	-4.47	525.09	
6	-4.61	420.92	
7	-4.52	482.28	
8	-4.51	496.55	
Mean	**-4.516**	**496.13**	
SD	**0.100**	**84.35**	
CV	**-0.022**	**0.17**	

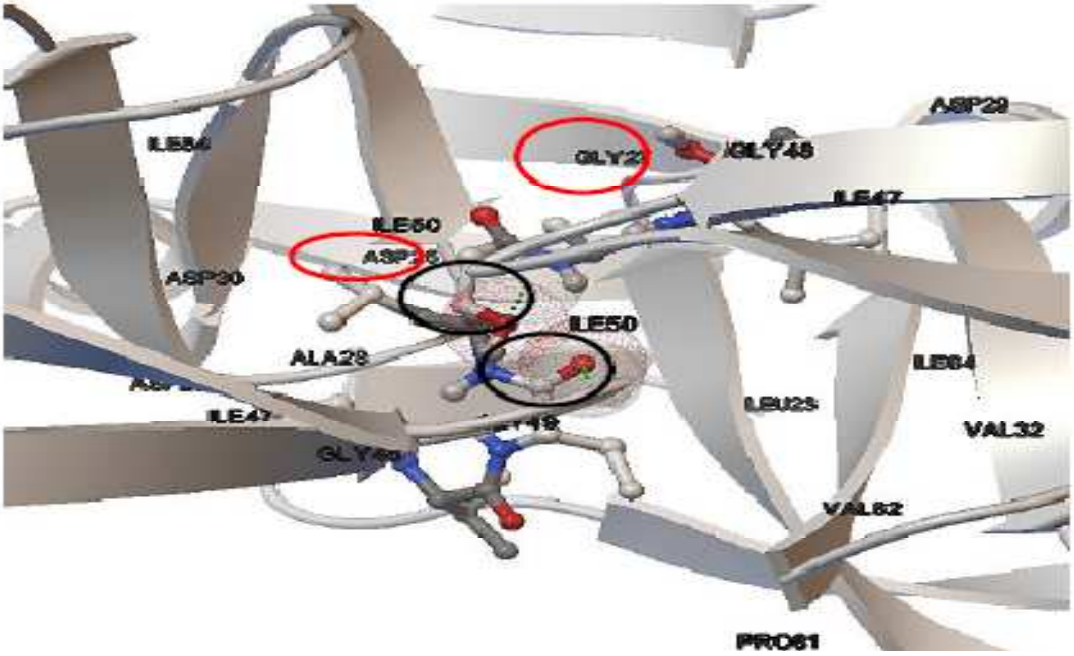


Fig. 3: Docking results of pepstatin-A into the catalytic site of HIV-1 protease (PDB code: 1HXW) Explanation

The similarity of amino acid residues compared to those of the references was 92.3%. The docking results showed that pepstatin-A interacted with the binding site of HIV-1 protease through the formationof two hydrogen bonds (Figure 3) toIle50 of chain A and B. The results also showed that the ligand was situated around the amino acid catalytic triad, Asp25-Thr26-Gly27, as mentioned by Jaskolski*et a*l.[7], however Thr26 amino acid was absent at the catalytic site of the receptor. The mean value of predicted Ki was 496.13 µM. This results supported previous experiments by Wiley and Rich who concluded that the hydroxyl group of the pepstatin-A binds tightly to the catalytically-active aspartic acid residues in the active site of protease, thereby mimicking the transition state of the peptide cleavage [14].Aspartic proteases are inhibited by pepstatin-A, a naturally occurring peptide containing two statins [10,13].

a. Receptor is shown by gray ribbons.
b. Ligands are shown by sticks.
c. DDE triad amino acids are shown by red circle.
d. Hydrogen bonds are shown by black circle.

Docking of ganoderiol-F into the catalytic site of HIV-1 protease was repeated eight times (Table 3) with grid dimension 38x38x38 Å^3. Total Kollman charge calculated forHIV-1 protease enzyme was 5.998, while total Gasteiger charge calculated forganoderiol-F was -0.0001.

It could be observed thatthree hydrogen bonds were formed from this docking (Figure 4). The position of ganoderiol-F was located between the catalytic triad (Asp25/Asp25' and Gly27/Gly27'; Thr26 was not detected, while Gly27' (from chain B) was present. The mean value of predicted Ki was 4.68 nM, which meantthat the affinity of ganoderiol-F towards HIV-1 protease is better than pepstatin-A. This result supported in vitro experiments of Sato, Zhang and their colleagues, who concluded that ganoderiol-F, an active compound of *Ganodermasinense,*inhibits HIV-1 protease with IC_{50} 20-40 µM[12].

Table 3: Docking of Ganoderiol-F into the catalytic site of HIV-1 protease

Docking	Binding energy (kcal/mol)	Predicted Ki (nM)	Amino acid residues
1	-11.38	4.59	Asp25, Gly27, Ala28, Asp29,
2	-11.65	2.87	Asp30, Val32, Ile47, Gly48,
3	-11.56	3.35	Gly49, Ile50, Ile84
4	-11.01	8.56	
5	-11.56	3.36	
6	-11.36	4.75	
7	-11.21	6.05	
8	-11.47	3.91	
Mean	**-11.40**	**4.68**	
SD	**0.2100**	**1.8941**	
KV	**-0.0184**	**0.3983**	

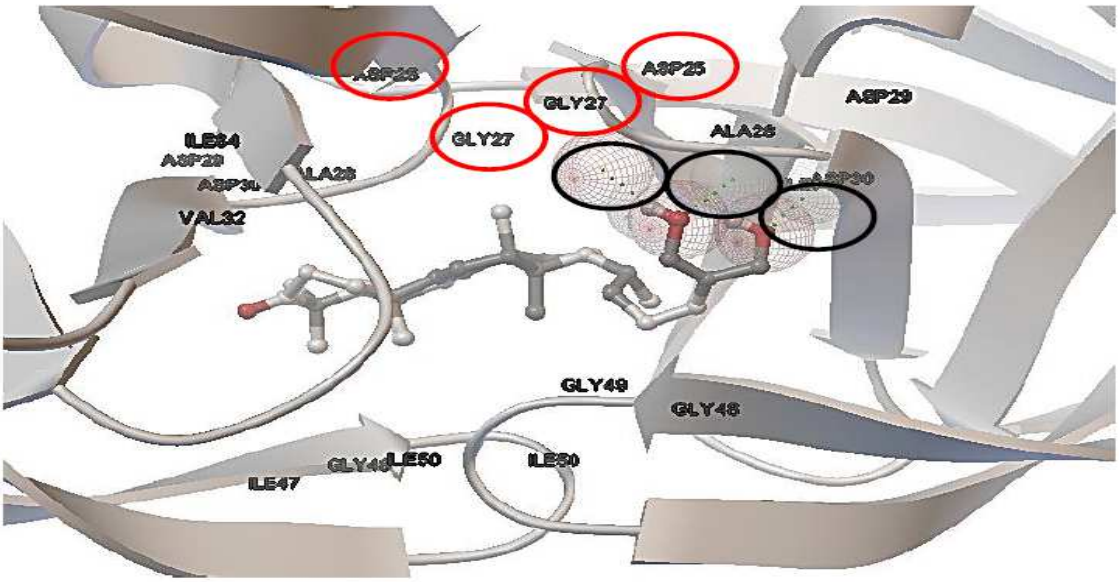


Fig. 4: Docking results of ganoderiol-F into the catalytic site of HIV-1 protease (PDB code: 1HXW) Explanation

a. Receptor is shown by gray ribbons.
b. Ligands are shown by sticks.
c. DDE triad amino acids are shown by red circle.
d. Hydrogen bonds are shown by black circle.

The result of predicted Ki and binding energy values of dockings of pepstatin-A and ganoderiol-F into the catalytic site of Plasmepsin I are shown in Table 4, and Table 5 respectively. Docking of pepstatin-A into the catalytic site of Plasmepsin I was repeated eight times (Table 4) with grid dimension 38x38x38 Å^3. Total Kollman charge calculated forPlasmepsin Ienzyme was -7.0, while total Gasteiger charge calculated forpepstatin-A was -1.0006. Docking of ganoderiol-F into the catalytic site of Plasmepsin I was repeated eight times (Table 5) with grid dimension 38x38x38 Å^3. Total Kollman charge calculated for the enzyme was -7.0, while total Gasteiger charge for ganoderiol-F was -0.0001.

Table 4: Docking of Pepstatin-A into the catalytic site of Plasmepsin I

Docking	Binding energy (kcal/mol)	Predicted Ki (mM)	Amino acid residues
1	-2.81	8.70	
2	-3.30	3.81	Asp32, Gly34, Ser35, Tyr75, Val76, Ser77, Ile120, Tyr189, Asp215, Gly217, Thr218, Ser219, Thr222, Leu291, Ile300
3	-2.98	6.56	
4	-2.90	7.46	
5	-3.09	5.39	
6	-2.81	8.67	
7	-3.29	3.88	
8	-3.38	3.35	
Mean	**-3.07**	**5.98**	
SD	**0.230**	**2.189**	
CV	**-0.0749**	**0.3662**	

The docking results of pepstatin-A on Plasmepsin I showed that two hydrogen bonds were formed during their interaction(Figure 5), with Ki and binding energy values 5.98 mM and -3.07 kcal/mol, respectively.

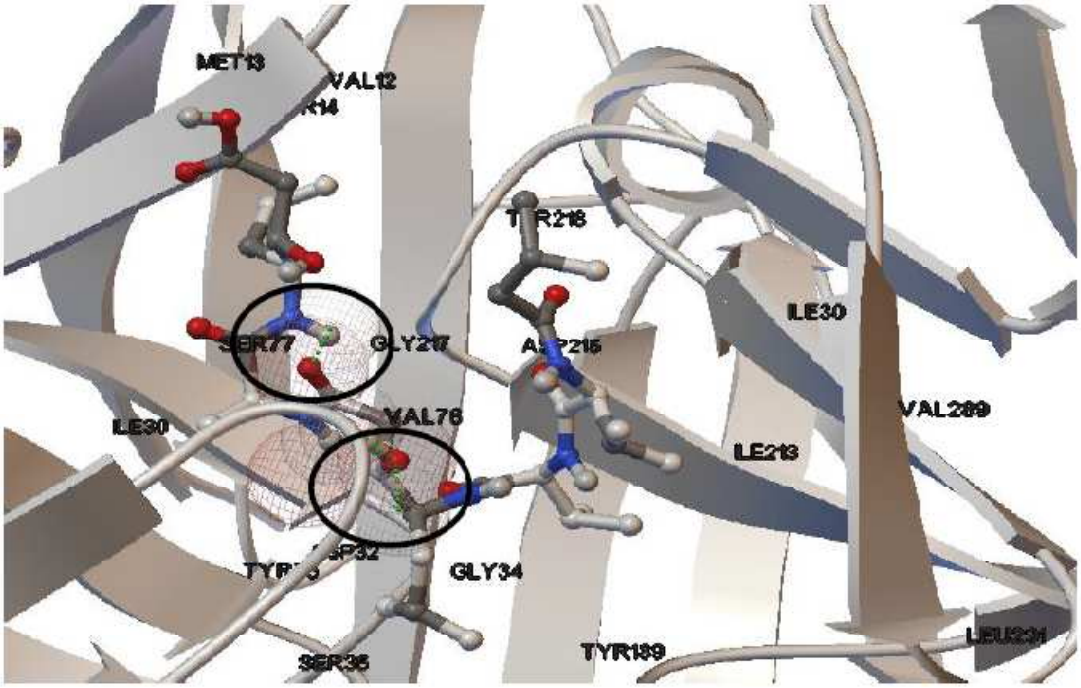


Fig. 5: Docking results of pepstatin-A on active site of Plasmepsin 1 (PDB code: 3QS1)

Table 5: Docking of Ganoderiol-F into the catalytic site of Plasmepsin I

Docking	Binding energy (kcal/mol)	Predicted Ki (nM)	Amino acid residues
1	-10.10	39.42	Val12, Met13, Ile30, Asp32, Gly34, Tyr75, Val76, Ser77, Phe109, Phe117, Ile120, Asp215, Gly217, Thr218, Ser219
2	-9.97	49.09	
3	-9.96	49.88	
4	-9.95	51.22	
5	-9.83	62.15	
6	-9.87	58.65	
7	-9.96	49.70	
8	-9.99	47.43	
Mean	**-9.96**	**50.94**	
SD	**0.0805**	**6.9131**	
CV	**-0.0081**	**0.1361**	

The docking results of ganoderiol-F with Plasmepsin I showed that three bonds were formed (Figure 6), with Ki and binding energy values 50.94 nM and -9.96 kcal/mol, respectively.

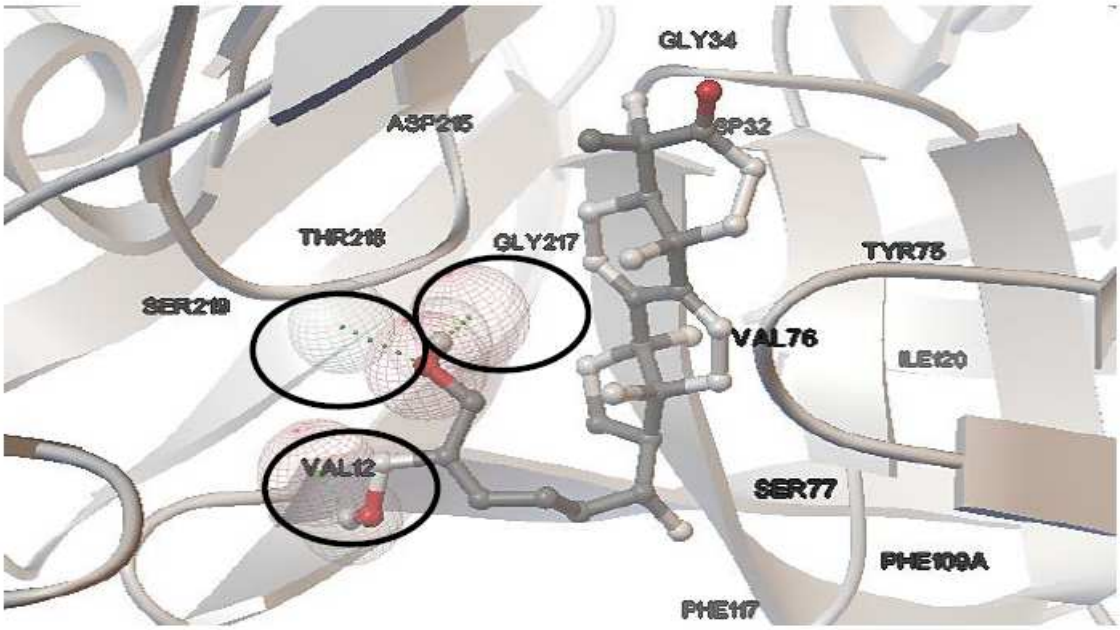


Fig. 6: Docking results of ganoderiol-F on active site of Plasmepsin 1 (PDB code: 3QS1)

According to these results, ganoderiol-F showed better affinity towards HIV-1 protease and Plasmepsin I compared to pepstatin-A. These data indicated that ganoderiol-F could be developed as both anti-HIV and anti-malaria.

CONCLUSION

Ganoderiol-F a triterpenoid compound of *Ganodermasinense*showed a good affinity towards HIV-1 Protease and plasmepsin I, therefore ganoderiol-F could be developed further as both anti-HIV and anti-malaria.

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