ISSN: 0975 -8542

Journal of Global Pharma Technology

Available Online at: www.jgpt.co.in

RESEARCH ARTICLE

Pharmacophore Screening and Molecular Docking of Andrographolide and Its Derivatives on Plasmepsin as Anti-Malarial Drug

S. Megantara[1*], M. Akmal Fauzan[1], F. Amelia Saputri[1], F. Ferdiansyah Sofian[2]

[1.] *Department of Pharmaceutical Analysis and Medicinal Chemistry, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.*

[2.] *Department of Biological Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, Indonesia.*

***Corresponding Author:** *S. Megantara*

Abstract

The study was conducted to find out andrographolide and its derivatives which could interact with plasmepsin, an aspartic protease enzyme that usually used as target for antimalaria. The study began with pharmacophore modeling using LigandScout software. Pharmacophores were searched for plasmepsin I, II, and IV, with pdb codes were 3QS1, 1SME, and 1LS5 respectively. Pharmacophores were generated using structure-based and ligand-based pharmacophore methods and retrospective validation was used to validate them. Pharmacophore screening was carried out for andrographolide and its derivatives, then for the hit compounds, continued by molecular docking using AutoDock Vina module in LigandScout software. Pharmacophores for plasmepsin I, II, and IV consist of hydrogen bond donors, hydrogen bond acceptors and hydrophobic interactions, with the best validated AUC values for each model were 0.73, 1.00, and 1.00 respectively. Andrographolide derivatives (AND10 and AND15) had high fit scores for plasmepsin I, II, and IV pharmacophore. The results of molecular docking showed that andrographolide and its derivatives (AND10 and AND15) interacted well with the plasmepsin by binding to important aspartic amino acid residues in the active site. From the binding affinity, AND15 was the best compound interacted with plasmepsin I, II, and IV with ΔG values were 1, 2, and 3kcal/mol respectively. Andrographolide derivative (AND15) was the best compound interacted with plasmepsin, so it was potential to be developed into new antimalarial drug.

Keywords: *Andrographolide, Molecular docking, Pharmacophore screening, Plasmepsin.*

Introduction

Malaria is still an influential cause of death in the world. The number of deaths in the world caused by malaria reaches 435 thousand [1]. There is 53% of malaria cases caused by *Plasmodium falciparum* and continues to increase until 2017 including in Indonesia [2]. Indonesia is in the second place with the most malaria cases after India in the South-East Asia Region [1].

Anti-malarial drugs grow after stopping the use of chloroquine in 1969 due to an increase in mortality and morbidity especially in Africa, and in 1976 it was known that the spread of resistance in Papua New Guinea [3][4]. The spread of *P. falciparum* resistance to drugs is increasingly widespread in tropical countries including Indonesia [5].

Therefore, the new alternative medicine for anti-malarial is needed. *P. falciparum* attacks erythrocytes and destroys most of the host cell hemoglobin because the metabolism of hemoglobin is one of the key metabolic processes for survival in parasites that are in human blood.

There are several protease enzymes involved in this process in parasitic food vacuoles. One of them is plasmepsin which is the aspartic protease and is responsible for the initial cleavage of hemoglobin until followed by other protease enzymes [6].

P. falciparum is identified as having ten types of aspartic protease protein and three of them are plasmepsin I, II, and IV which play a role in the initial cleavage of hemoglobin [7]. Andrografolid can inhibit *P.bergei*, a type of malaria, with 39-46% [8] and in *P. falciparum* malaria [9].

The combination of andrographolide with chloroquine can reduce the resistance value from 48% to 12.5%, but this does not increase its effectiveness in antimalarial activity. Andrographolide as an antimalarial is believed to have a short duration of action due to poor andrographolide activity when at low doses [10]. In this study, we did pharmacophore screening to get the andrographolide derivates that has the best antimalarial activity. After that, molecular docking was done to obtain the interaction between andrographolide derivates and plasmepsin.

Materials and Method

The plasmepsin I, II, and IV crystal structure complexed with pepstatin (PDB code: 3QS1, 1SME, and 1LS5) were obtained from the Protein Data Bank (http://www.rcsb.org/pdb), can be seen in fig. 1. Database of active compounds and decoys for aspartic protease inhibitor was obtained from The Binding Database (https://www.bindingdb.org) and Database of Useful Decoys Enhanced (DUD-E) (http://dude.docking.org). A total of 50 test compounds of andrographolide derivatives namely (AND1-AND50) were obtained from several research journals whose structure can be seen in Fig. 2 [11-16].

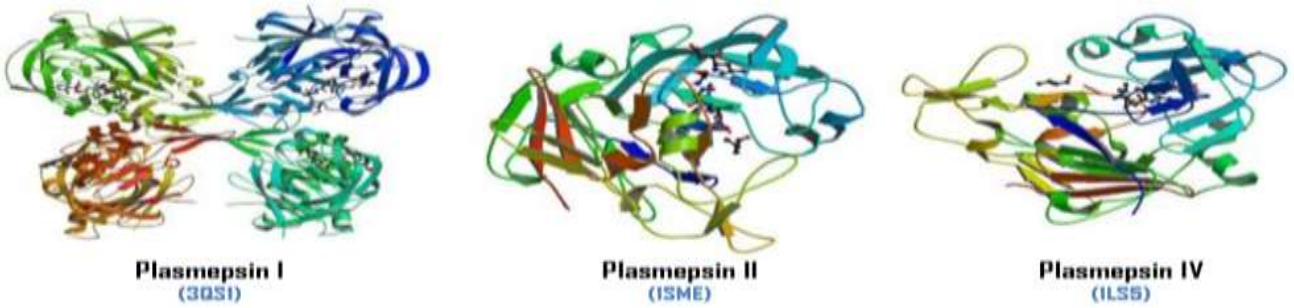


Fig. 1: Crystal structure of plasmepsin I, II, and IV

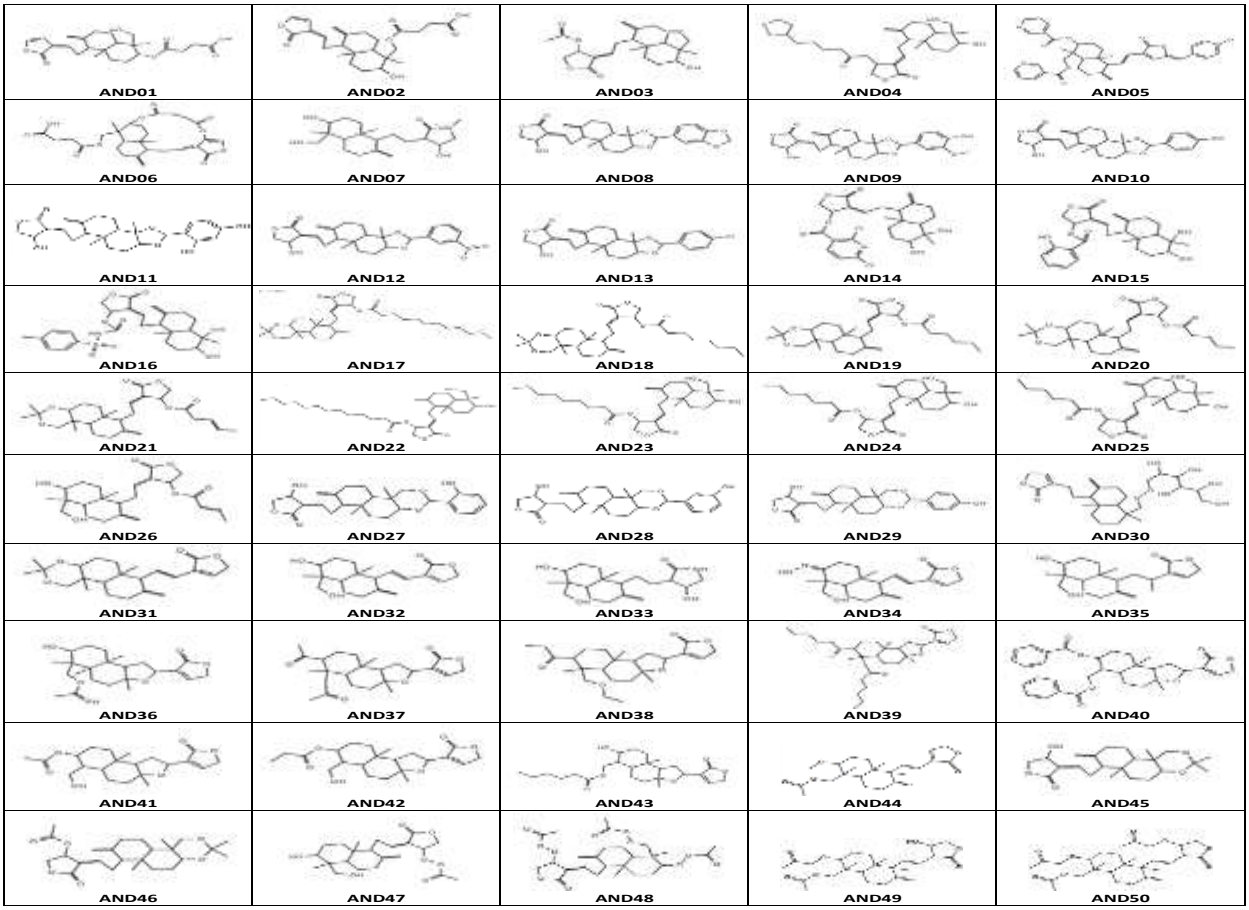


Fig. 2: Andrographolide derivatives test compounds

Pharmacophore Modeling

LigandScout 4.1 was used for pharmacophore modeling [17]. The methods were structure - based and ligand - based pharmacophore modeling. In the structure - based method, pharmacophore was based on the complex crystal structure of pepstatin bound to the Plasmepsin I, II, and IV receptors. Each crystal structure was analyzed for its interaction with the natural ligands inside.

Ligand Scout automatically generated the pharmacophore for each complex. In the ligand - based method, pharmacophore modeling was based on a database of ligands that are known to be active against plasmepsin I, II, and IV. The database was taken from The Binding Database website (https://www.bindingdb.org) with the number of active compounds for plasmepsin I, II, and IV 203, 587, and 11 respectively. LigandScout would align the active compound, and automatically generate several pharmacophore options for each target.

Pharmacophore Validation

The method used was retrospective validation by creating an active ligand database obtained from The Binding Database website (https://www.bindingdb.org) and decoys that were automatically generated by the engine on the DUD-E website (http://dude.docking.org). The active database used was a collection of ligands that were known and proven to have activity against plasmepsin, especially plasmepsin I, II, and IV.

The decoys database was a ligand that had a structure similar to an active ligand but had no activity or did not cause interactions on the receptors. The database functions as a ligand filter to be tested whether it had a similar pharmacophore structure that was assessed by the Receiver Operating Characteristic Curve (ROC Curve) parameter such as Area under Curve (AUC) and Enrichment Factor (EF).

Pharmacophore Screening

After obtaining the best and valid pharmacophore model, the model was chosen for used in screening test compounds. A collection of test compounds optimized by MMFF94 energy minimization than converted to screening database [18].Ligand Scout would process it to produce some of the best compounds by looking at the level of similarity or fit score. Test compounds that had high fit scores would be selected as compounds that were allegedly having the same activities and interactions based on the similarity of the pharmacophores.

Molecular Docking

Molecular docking was carried out for andrografolid and its derivatives which have the best results from pharmacophore screening process before. The docking program used was Autodock Vina [19] which was integrated in the LigandScout 4.0 program. The molecular docking protocol was validated by redocking natural ligands (pepstatin) into plasmepsin I, II, and IV receptors on each crystal structure. Test compounds were optimized using the MMFF94 method, and predicted Lipinski's Rule of Five parameters using the LigandScout 4.0 program. Bonding modes, binding affinity and inhibition constants were examined for each test compound.

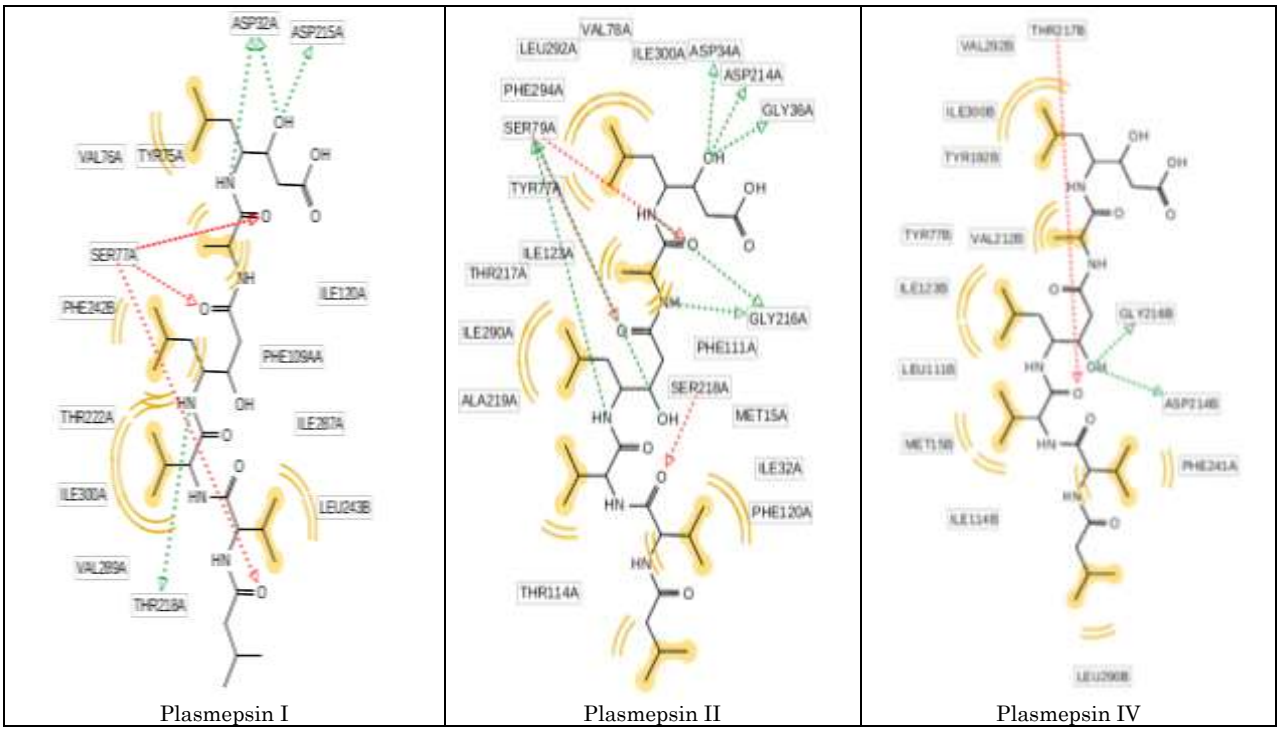
Results and Discussion

Structure - Based Pharmacophore Modeling

The interaction of pepstatin with plasmepsin I, II, and IV consist of acceptor or donor hydrogen bonds, and several hydrophobic interactions with amino acid residues in the receptors binding site (Fig. 3).

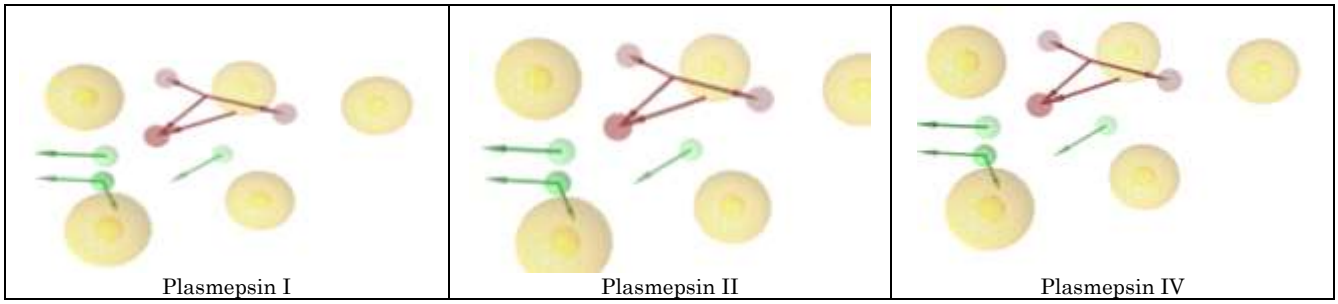
Pharmacophores models for each plasmepsin varies can be seen in Fig. 4. Plasmepsin I pharmacophore consists of five hydrophobic interactions, three hydrogen donor bonds, and three hydrogen acceptor bonds. Plasmepsin II pharmacophore consists of six hydrophobic interactions, five donor hydrogen bonds, and three hydrogen acceptor bonds.

Finally, in Plasmepsin IV there are six hydrophobic interactions, two hydrogen donor bonds, and one hydrogen acceptor bond. Differences in pharmacophoric sites in each plasmepsin certainly occur because of differences in the macromolecules of each plasmepsin that affect which groups are bound to the amino acids that make up the macromolecules.



Plasmepsin I | Plasmepsin II | Plasmepsin IV

Fig. 3: Interaction of pepstatin with plasmepsin I, II and IV

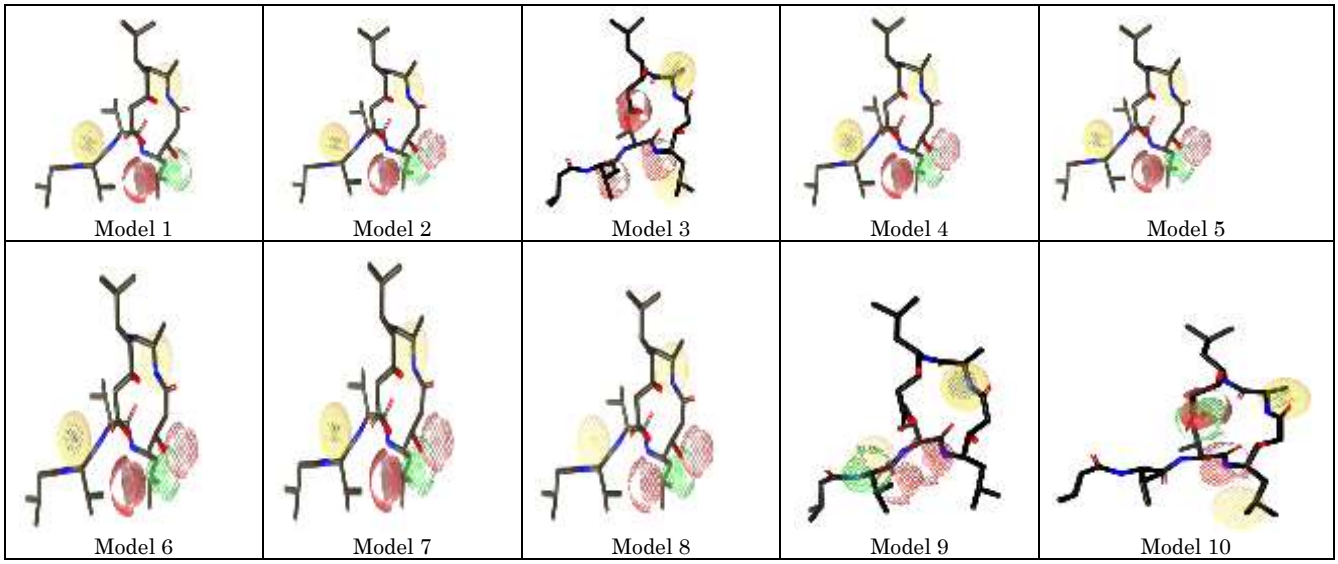


Plasmepsin I | Plasmepsin II | Plasmepsin IV

Fig. 4: Structure - Based Pharmacophore Models of Plasmepsin I, II, and IV (Green arrows: hydrogen bond donors, red arrows: hydrogen bond acceptors, yellow spheres: hydrophobic interactions)

Ligand - Based Pharmacophore Modeling

Database of active ligand for plasmepsin I produced 10 pharmacophore models (Fig. 5), for plasmepsin II produced 1 pharmacophore model (Fig. 6), and for plasmepsin IV produced 10 pharmacophore models (Fig.7).



Model 1 | Model 2 | Model 3 | Model 4 | Model 5

Model 6 | Model 7 | Model 8 | Model 9 | Model 10

Fig. 5: Ligand - Based Pharmacophore Models of Plasmepsin I

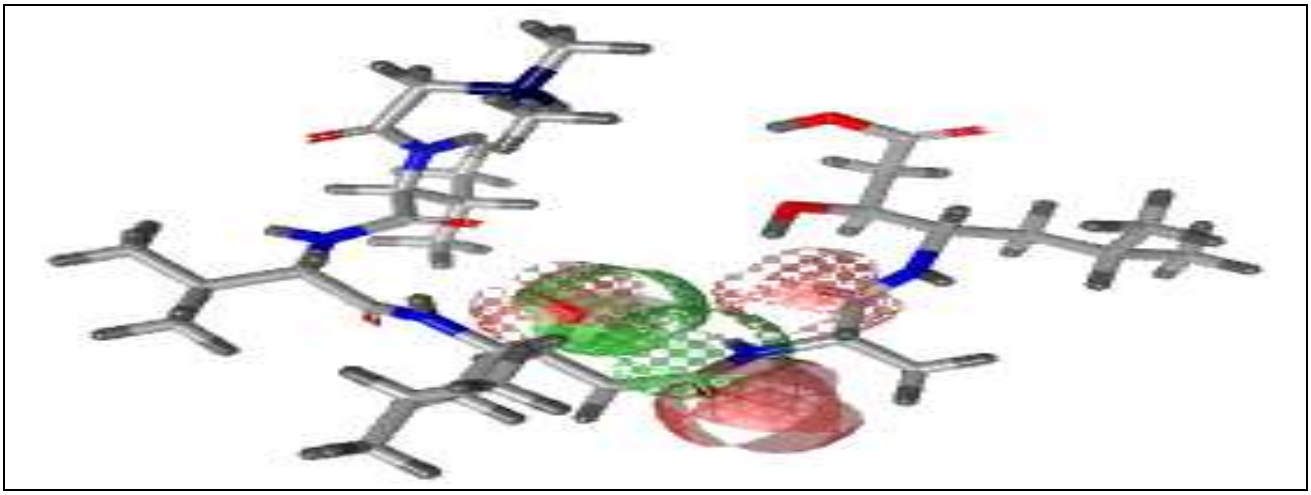


Fig. 6: Ligand - Based Pharmacophore Models of Plasmepsin II

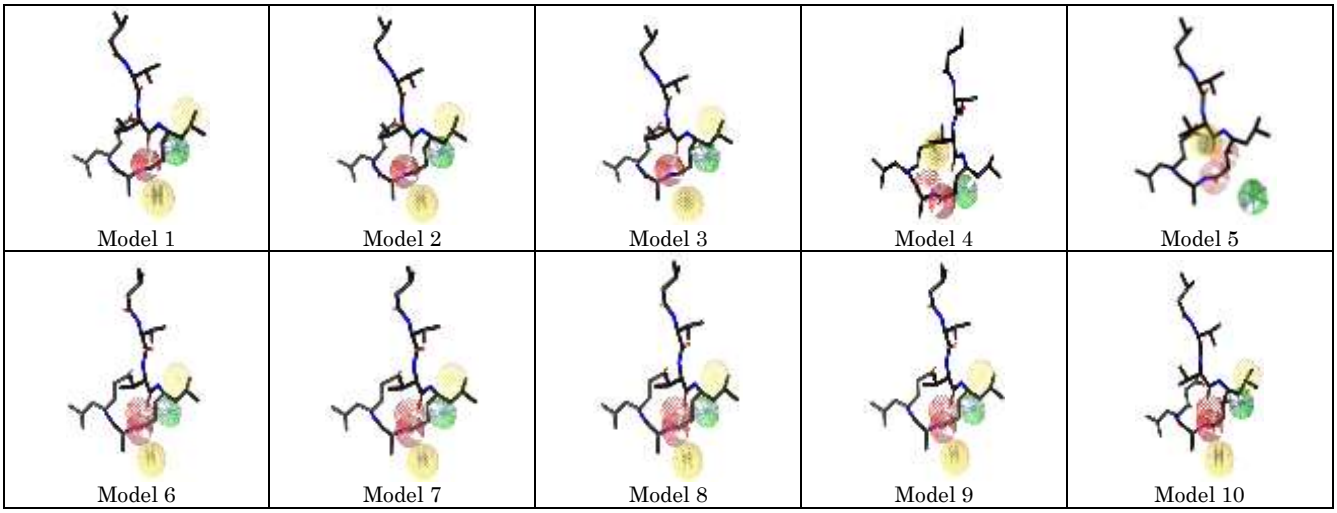


Fig. 7: Ligand - Based Pharmacophore Models of Plasmepsin IV

The yellow circle is a hydrophobic interaction pharmacophore group, the red circle is the hydrogen acceptor pharmacophore, and the green circle is the hydrogen donor pharmacophore. The difference of each model in these plasmepsin lies in the number of pharmacophore groups and the types of pharmacophores that exist.

Plasmepsin II does not have a large variety of pharmacophore choices; there is only one model of pharmacophore features that is predicted to have good affinity. This is due to the fact that ligand clusters do not have an effect on variations in the pharmacophore feature model.

Pharmacophore Validation

The results obtained from the structure - based pharmacophore modeling validation process, none of the compounds from the database were hit with the pharmacophore model either the active ligand database or decoys. Efforts were made such as eliminating some pharmacophore features to ease the work of Ligand Scout if indeed the effect was too much pharmacophores of pepstatin and differences in position, number, to the type of pharmacophore, but the results remained unchanged.

This means that there are no compounds that are really similar to the pharmacophore model, so the pharmacophore model produced from this structure - based method is declared invalid and cannot be used to screen the test compound.

From the ten pharmacophore models obtained from the ligand - based pharmacophore modeling in plasmepsin I, model 1 is the best model because from ROC curve in Fig. 8, the highest AUC value of 0.73 and EF is 1.1. Pharmacophore is valid because AUC value more than 0.7 [20], so screening of test compounds for plasmepsin I can be carried out using the model 1 pharmacophore.

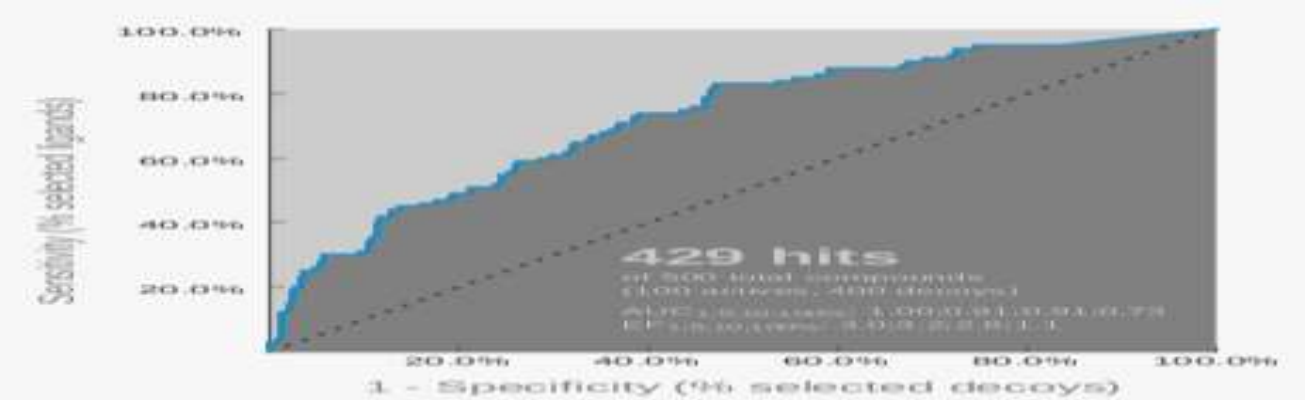


Fig. 8: ROC Curve Pharmacophore Model 1 on Plasmepsin I

From the only one available pharmacophore model obtained for plasmepsin II, ROC curve in Fig. 9 show AUC 1.00 and EF 2.3 is obtained; pharmacophore is valid so screening test compounds can be carried out using that particular model for plasmepsin II.

Fig. 9: ROC Curve Pharmacophore Model 1 on Plasmepsin II

From the ten pharmacophore models obtained from the ligand - based pharmacophore modeling in plasmepsin IV, model 2 is the best model because from ROC curve in Fig. 10, the highest AUC value of 1.00 and EF is 5.0. Pharmacophore is valid, so screening test compounds could be carried out using model 2 for plasmepsin IV.

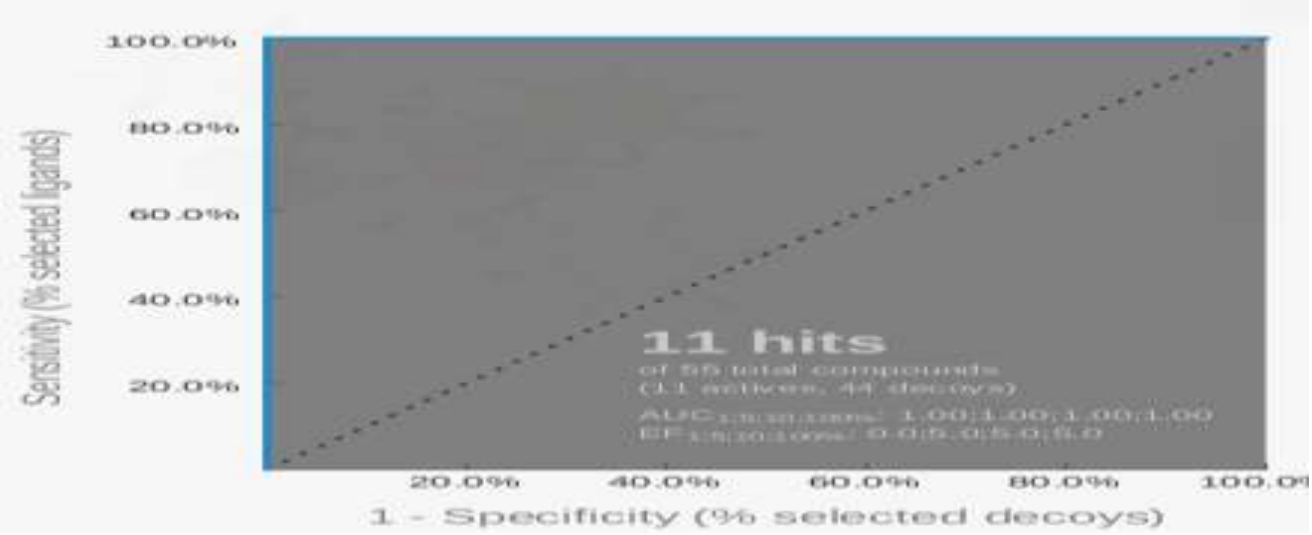


Fig. 10: ROC Curve Pharmacophore Model 2 on Plasmepsin IV

Pharmacophore Screening

The results of pharmacophore screening of test compounds can be seen in table 1. For plasmepsin I, II, and IV total hit compounds obtained are 28, 32, 17 respectively. From the overall results of the test compound with the highest hit there is a difference in each plasmepsin, but at the relationship of compounds in the three plasmepsin tested, there are several test compounds related to the three plasmepsin. Looking at the 10 best sequences of test compounds, there are two compounds namely AND10 and AND15 that were hit with plasmepsin I, II, and IV. Therefore, AND10 and AND15 are predicted to have pharmacophores suitable for the three plasmepsins and can be candidated for multi-target drugs.

Table 1: The pharmacophore fit score of test compounds

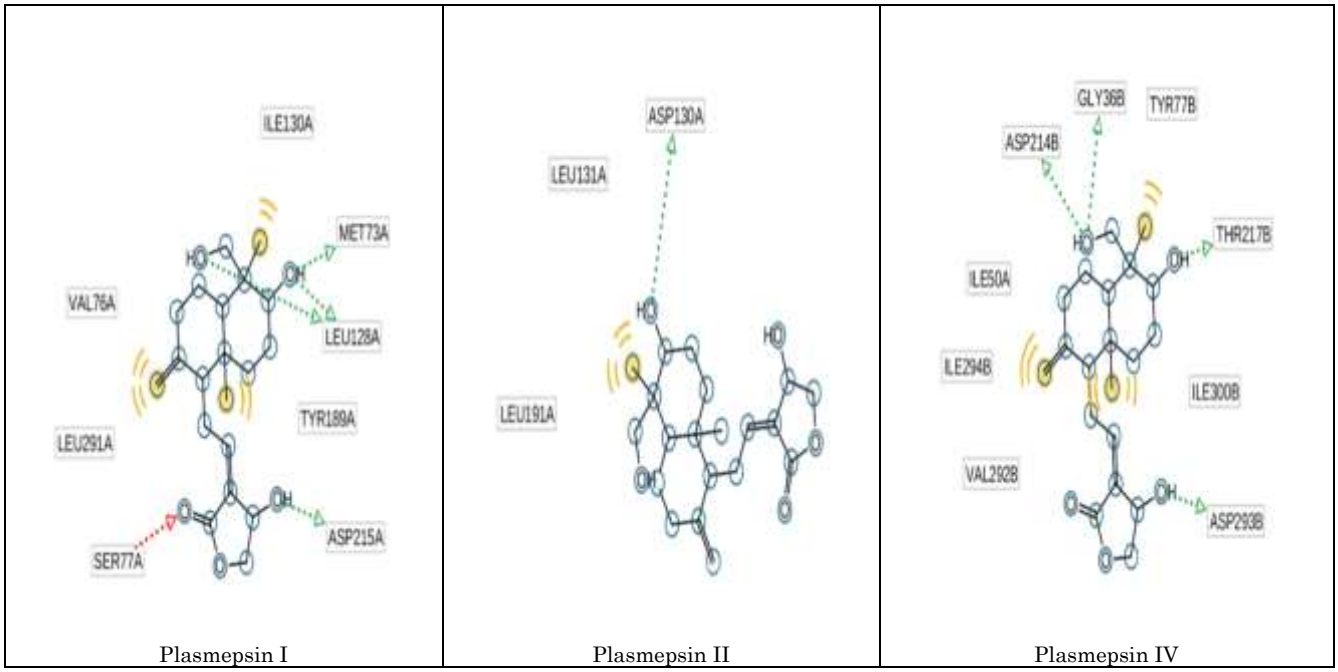
No.	Plasmepsin					
	I		II		IV	
	Compound	Fit Score	Compound	Fit Score	Compound	Fit Score
1	AND05	40.59	AND30	51.15	AND10	43.44
2	AND15	39.44	AND10	50.57	AND28	43.34
3	AND16	39.01	AND36	50.34	AND36	43.27
4	AND13	35.67	AND01	44.11	AND27	37.85
5	AND43	35.55	AND11	44.01	AND30	37.79
6	AND10	34.96	AND15	43.71	AND15	37.59
7	AND03	34.94	AND07	43.27	AND43	37.36
8	AND23	34.80	AND27	43.15	AND47	37.30
9	AND24	34.56	AND33	43.13	AND45	36.91
10	AND20	34.50	AND09	43.13	AND08	36.47
11	AND39	34.46	AND04	42.95	AND33	36.34
12	AND36	34.26	AND32	42.53	AND09	35.99
13	AND09	34.23	AND03	42.52	AND12	35.97
14	AND45	34.08	AND14	42.47	AND03	35.97
15	AND33	33.85	AND16	42.46	AND16	35.81
16	AND34	33.73	AND26	42.39	AND13	35.53
17	AND12	33.71	AND13	42.34	AND11	35.16
18	AND26	33.57	AND25	42.27		
19	AND27	33.54	AND22	42.27		
20	AND38	33.40	AND24	42.25		
21	AND07	33.30	AND23	42.25		
22	AND06	33.17	AND47	42.19		
23	AND25	32.94	AND35	42.18		
24	AND28	32.93	AND42	36.34		
25	AND40	32.76	AND34	36.34		
26	AND02	32.65	AND43	36.26		
27	AND11	32.53	AND12	26.19		
28	AND31	32.28	AND45	36.17		
29			AND28	36.17		
30			AND29	36.16		
31			AND08	36.16		
32			AND49	36.15		

Molecular Docking

Docking result can be seen in table 2. Interaction of andrographolide with plasmepsin I, II, and IV show bonds with essential amino acids aspartate in binding site (Fig. 11). ASP215A and ASP293 are bound by the C-14 hydroxyl group in the lactone ring, ASP214 is bound by the C-19 hydroxyl group, and ASP130 is bound by the C-3 hydroxyl group. Some aspartates have similarities with previous pepstatin in pharmacophore modeling. As with plasmepsin I, ASP215 and plasmepsin IV, ASP214.

Table 2: Docking result of test compounds with plasmepsin I, II, and IV

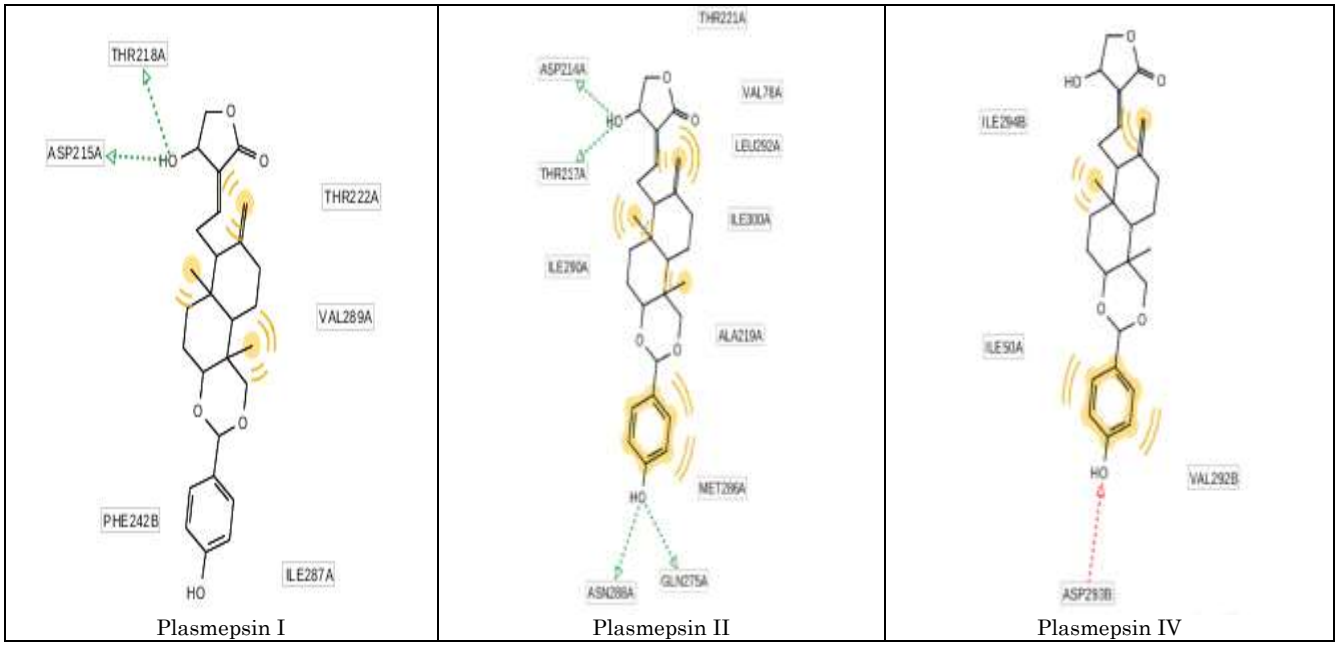
Test Compounds	Binding Affinity (kcal/mol)			Inhibition Constant (μM)		
	Plasmepsin			Plasmepsin		
	I	II	IV	I	II	IV
Andrografolid	-7.70	-7.70	-6.30	2.28	2.28	24.21
AND10	-8.50	-8.50	-7.60	0.59	0.59	2.70
AND15	-8.80	-8.80	-8.30	0.36	0.36	0.83



Plasmepsin I | Plasmepsin II | Plasmepsin IV

Fig. 11: Interaction of Andrographolide with Plasmepsin I, II, and IV

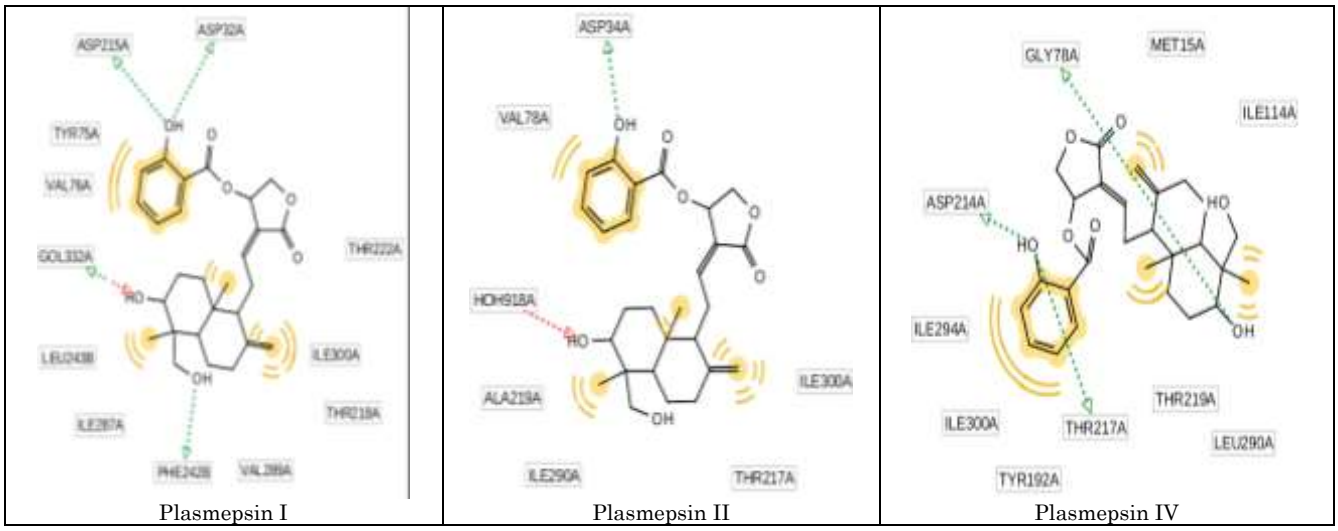
AND10 docking result in to plasmepsin I, II, and IV show bonds with essential amino acids aspartate in binding site (Fig. 12). AND10 has better binding affinity than andrographolide in plasmepsin I, II, and IV.



Plasmepsin I | Plasmepsin II | Plasmepsin IV

Fig. 12: Interaction of AND10 with Plasmepsin I, II, and IV

AND15 docking result in to plasmepsin I, II, and IV also show bonds with essential amino acids aspartate which is the same as andrographolide and pepstatin before, that are ASP34, ASP214, and ASP215 (Fig. 13). AND15 has a lowest binding affinity if compare to andrographolide and AND10, which means AND15 interacts better with plasmepsin I, II, and IV.



Plasmepsin I | Plasmepsin II | Plasmepsin IV

Fig. 13: Interaction of AND10 with Plasmepsin I, II, and IV

According to Lipinski rules, a good drug molecule requires for consumption via the oral route with log P value ≤ 5, molecular weight ≤ 500, donor hydrogen group ≤ 5, and hydrogen acceptor group ≤ 10 [10]. Properties of standard compounds and test compounds based on Lipinski's Rule of Five can be seen in Table 3.

Table 3: Physicochemical predictions based on Lipinski's Rule of Five

Compound	Molecular Weight	Log P	Hydrogen Bond Acceptor	Hydrogen Bond Donor
Pepstatin	685.904	1.467	9	7
Andrographolide	368.385	3.370	1	1
AND 10	440.536	3.609	5	2
AND 15	456.535	3.054	5	2

Andrographolide, AND15, and AND10 all of them meet the Lipinski's Rule of Five requirements which means all three can be made as oral drugs. Log P values indicate the lipophilicity of a compound. The greater the values of Log P, the more hydrophobic compounds are and the easier it will be to penetrate lipid bilayers. Large mass molecules will find it more difficult to penetrate lipid bilayers and tend to experience a first pass effect, i.e. metabolic breakdown before it reaches the systemic blood circulation which can cause the drug to lose its effectiveness. Hydrogen bonding will affect the pharmacophore group which will give biological activity to a compound

Conclusion

Ligand - based pharmacophore method is better than structure - based pharmacophore in modeling a valid pharmacophore that used to screen test compound in plasmepsin. Pharmacophore in plasmepsin I consist of 2 hydrophobic groups, 1 hydrogen bond donor and 1 hydrogen bond acceptor group. Pharmacophore in plasmepsin II consist of 2 hydrogen bond donor and 3 hydrogen bond acceptor groups. Pharmacophore in plasmepsin IV consist of 2 hydrophobic groups, 1 hydrogen bond donor and 1 hydrogen bond acceptor group. From pharmacophore screening and molecular docking results, andrographolide derivative (AND15) was the best compound interacted with plasmepsin, so it was potential to be developed into new antimalarial drug.

Acknowledgment

This research was funded by the Ministry of Research, Technology and Higher Education Republic of Indonesia grant in 2019.

References

1. WHO (2018) World Malaria Report. World Health Organization.
2. WHO (2017) Annex 6A - Reported malaria cases and deaths. World Malaria Report 2017. Geneva, Switzerland: WHO, 222-225.
3. Singh Sidhu AB, Verdier-Pinard D, Fidock DA (2002) Chloroquine resistance in

Plasmodium falciparum malaria parasites conferred by pfcrt mutations. Sci., 298(5591):210–3.

4. Aweeka FT, German PI (2008) Clinical pharmacology of artemisinin-based combination therapies. Clin Pharmacokinet., 47(2):91–102.
5. WHO. Guidelines for the Treatment of Malaria. World Health Organization. 2015.
6. Francis SE, Sullivan Jr. DJ, Goldberg DE (1997) Hemoglobin metabolism in the malaria parasite Plasmodium falciparum. Annu Rev Microbiol., 51:97–123.
7. Coombs GH, Goldberg DE, Klemba M, Berry C, Kay J, Mottram JC (2001) Aspartic proteases of Plasmodium falciparum and other parasitic protozoa as drug targets. Trends Parasitol., 17(11):532–7.
8. Misra P, Pal NL, Guru PY, Katiyar JC, Tandon JS (1991) Antimalarial activity of traditional plants against erythrocytic stages of plasmodium berghei. Pharm Biol., 29(1):19–23.
9. Najib NARN, Furuta T, Kojima S, Takane K, Ali Mohd M (1999) Antimalarial activity of extracts of Malaysian medicinal plants. J Ethnopharmacol., 64(3):249–54.
10. Zein U, Fitri LE, Saragih A (2013) Comparative study of antimalarial effect of sambiloto (Andrographis paniculata) extract, chloroquine and artemisinin and their combination against plasmodium falciparum in-vitro. Acta Med Indones., 45(1):38-43.
11. Chang RS, Ding L, Gai-Qing C, Qi-Choa P, Ze-Lin Z, Smith KM (1991) Dehydroandrographolide Succinic Acid Monoester as an Inhibitor against the Human Immunodeficiency Virus. Proc Soc Exp Biol Med., 197(1): 59-66.
12. Aromdee C (2014) Andrographolide: Progression in its modifications and applications-a patent review (2012-2014). Expert Opin Ther Pat., 24(10):1129-38.
13. Uttekar MM, Das T, Pawar RS, Bhandari B, Menon V, Nutan, et al (2012) Anti-HIV activity of semisynthetic derivatives of andrographolide and computational study of HIV-1 gp120 protein binding. Eur J Med Chem., 56: 368-74.
14. Wang Z, Yu P, Zhang G, Xu L, Wang D, Wang L, et al (2010) Design, synthesis and antibacterial activity of novel andrographolide derivatives. Bioorg Med Chem., 18(12):4269-74.
15. Megantara S, Halimah H, Putrianty A, Tjahjono DH, Kartasasmita RE, Iwo MI, et al (2018) Microwave-assisted synthesis of hydroxybenzylidene-andrographolides and its inhibitory activity against HIV-1 protease. J Appl Pharm Sci., 8(3):009-13.
16. Zhou B, Zhang D, Wu X (2013) Biological Activities and Corresponding SARs of Andrographolide and Its Derivatives. Mini-Reviews Med Chem., 13(2):298-309.
17. Wolber G, Langer T (2005) LigandScout: 3-D pharmacophores derived from protein-bound ligands and their use as virtual screening filters. J Chem Inf Model., 45(1):160-9.
18. Halgren TA (1999) MMFF VI. MMFF94s option for energy minimization studies. J. Comput. Chem., 20(7):720-9.
19. Trott O, Olson AJ (2010) AutoDock Vina: improving the speed and accuracy of docking with a new scoring function, efficient optimization, and multithreading. J Comput Chem., 31(2):455-61.
20. Niu MM, Qin JY, Tian CP, Yan XF, Dong FG, Cheng ZQ, et al (2014) Tubulin inhibitors: Pharmacophore modeling, virtual screening and molecular docking. Acta Pharmacol Sin., 35(7):967-79.

