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Original Article

MOLECULAR DOCKING STUDY AND PHARMACOPHORE MODELLING OF URSOLIC ACID AS AN ANTIMALARIAL USING STRUCTURE-BASED DRUG DESIGN METHOD

FAIZAL HERMANTO[1,2*], ANAS SUBARNAS[1], AFIFAH BAMBANG SUTJIATMO[2], AFIAT BERBUDI[3]

[1]Department of Pharmacology and Clinical Pharmacy, Faculty of Pharmacy, Universitas Padjadjaran, West Java 45363, Indonesia, [2]Department of Pharmacology and Toxicology, Faculty of Pharmacy, Universitas Jenderal Achmad Yani, West Java 40531, Indonesia, [3]Department of Biomedical Sciences, Parasitology Division, Faculty of Medicine, Universitas Padjadjaran, West Java 45363, Indonesia
*Email: faizal19002@mail.unpad.ac.id

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ABSTRACT

Objective: To investigate the activity of ursolic acid (UA) as antimalarial on various types and classes of *Plasmodium falciparum* (*Pf*) receptors using molecular docking and pharmacophore modeling methods.

Methods: The molecular docking was performed on various classes of the *Pf* receptors, namely Plasmepsin II (Hydroxylase), Enoyl-Acyl Carrier-protein (Oxidoreductase), Triose-Phosphate (Isomerase), and Lactate Dehydrogenase (Oxidoreductase) using Autodock 4.0.1 software.

Results: Three out of four tests (Ursolic Acid on Plasmepsin II, Enoyl-Acyl Carrier, and Lactate Dehydrogenase receptors) indicated a possible effect shown by the lowest free energy binding values obtained, namely-7.76 kcal/mol,-12.15 kcal/mol, and-9.39 kcal/mol, respectively. On Plasmepsin II, Enoyl-Acyl Carrier Protein, Triose-Phosphate Isomerase, and Lactate Dehydrogenase receptors, the UA had lower values of the inhibition constant (2.05 M, 1.25 nm, 1.25 mmol, and 130.79 nM, respectively). The UA also shared similarities with the native ligand according to the critical parameters of amino acid residue interaction (GLY216, SER218, LEU131, TYR77, and VAL78 for 1LF3 receptor; ALA217, LYS285, and TYR267 for 1NWH receptor; ASN233 and ALA234, for 1O5X receptor; and PRO246, ILE31, MET30, and PRO 250 for 1U4O receptor). As for the results of pharmacophore modeling, it was found that the functional groups of hydroxyl and carboxylic acid were the most crucial groups to bond with the key amino acid residues of the receptors.

Conclusion: The UA significantly has potential antimalarial activity against several *Pf* receptors in a competitive manner.

Keywords: Antimalarial, *Plasmodium falciparum*, Ursolic acid

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INTRODUCTION

In 2017, 61% of cases of malaria were suffered by children under five worldwide. Geographically, Africa was the home to nearly 92% (200 million) of malaria cases, which resulted in 404,550 deaths [1]. A significant number of people in Africa die because of this disease each year. The protozoan parasites *Plasmodium falciparum* (*Pf*) and *Plasmodium vivax* (*Pv*) are the primary causes of the prevalent disease. High morbidity and fatality rates are dependent on the parasite that caused the infection [2].

The combination therapy of artemisinin and antibiotics are being used in the treatment and prevention of malaria [3]. However, we are aware that various drug resistance and problem-related side effects of these drugs continue to be the main constraints in the treatment.

A possible option and potential alternative treatment for malaria is herbal medicine. Ursolic Acid (UA) is a pentacyclic triterpenoid substance discovered in the epicuticular waxes of apples in the 1920s. It is extensively present in many different peels of fruits as well as in herbs and other plants that are used as medicines. Some of the pharmacological effects of UA are anti-inflammatory, antioxidant, anticancer, cardioprotector agent, immunomodulator agent, and antimalarial activities [4–6].

Previous research has examined the antimalarial effects of UA as inhibitors of the *Plasmodium falciparum Hypoxanthine-Guanine-Xanthine Phosphoribosyl Transferase* (PfHGXPT) where UA exhibited dose-dependent direct inhibitory action against PfHGXPT. The UA acetate had strong binding affinities for the PfHGXPT and the dissociation constant (KD) reaching 2.8396 µM as reported in the study. The precise contacts and high affinities found in the binding pockets of human and PfHGXPT were demonstrated using molecular docking and dynamics simulations [7].

However, the study only serves as a representative for one kind and class of the receptor target of the *Pf*. Moreover, the explanation of the pharmacophore modeling result is not provided within. Therefore, taken into consideration by the authors, a molecular docking investigation towards the antimalarial activity of UA on various types and classes of receptors was conducted. In addition, we also studied the pharmacophores model to obtain additional information about the molecular mechanisms of the functional group responsible for interacting with the amino acids of the receptors.

MATERIALS AND METHODS

Identification of target receptors and the lead compounds

The targets for this investigation were identified based on the receptors frequently used to evaluate antimalarial effects, particularly from the protein of *Pf.* The lead compounds and receptor targets underwent initial screening based on some parameters, including the method used to extract the receptor, the amino acid content, the source of the organism, and the resolution of each receptor.

Validation using molecular docking method

The validation using molecular docking method was implemented on several structures of *Pf* receptors from different classes [8, 9]. The molecular docking validation method was performed on various classes of the *Pf* receptors, namely Plasmepsin II (Hydroxylase), Enoyl-Acyl Carrier-protein (Oxidoreductase), Triose-Phosphate (Isomerase), and Lactate Dehydrogenase (Oxidoreductase).

The first step taken was using the *protein data bank database* (https://www.rcsb.org/) as the source to download the receptors in (*.pdb*) format [10–13]. Afterwards, the preparation of each receptor was carried out by separating it from the complexed lead compounds using Discovery Studio Visualizer software. To reduce the deviation in the formation results of the hydrogen bonding interaction, the water molecule at the receptor was removed.

Before adding polar hydrogen to the protein molecule and nonpolar merged hydrogen to the ligand molecules to complete the preparation, the Kollman charge and Compute Gasteiger charge were added to the UA ligand and each receptor using Autodock 4.0.1 software. The results of the complete preparation were then saved into (*.pdbqt*) format where the *pdbq* stands for protein data bank partial charge (*q*) and *t* stands for atom type (*t*). The data of the receptor (*.pdbqt*) and the ligand (*.pdbqt*) were then merged and the additional docking parameters were set to generate the Grid Parameter File (*.gpf*) and the Docking Parameter File (*.dpf*) with GA Runs 100 and the energy evaluation 2500000). Using the Command Prompt (CMD) tools, redocking was taken as the last step in order to analyze the data obtained from the validation results of molecular docking.

Virtual screening on test compound

In this study, the tests of antimalarial activities of UA as the test compound was carried out using the Structure-Based Drug Design (SBDD) method. Several structures of *Pf* receptors were used as the test targets, while the Inhibitor EH58, Nicotinamide adenine dinucleotide, 2-phosphoglycerate, and 2,6-naphthalenedicarboxylic acid were used as the lead compounds for the test targets [14]. ChemDraw 2D was used to model the molecular test compound, while ChemDraw 3D was used to perform the energy minimization of the model. After the minimization step was completed, the results of the structures were saved in (*.pdb*) format. The preparation was then carried out using the Autodock 4.0.1 software to add onto both structures and compounds the Compute Gasteiger Charge and non-polar merged hydrogen. The final steps involved pairing the test compound with each target receptor to form a (*.gpf*) file and a (*.dpf*) file [11].

Pharmacophore modelling

The SBDD approach was used to carry out pharmacophore modeling for each complex of UA-receptors that previously docked in molecular docking study. All of the complexes were loaded into the structure-based perspective using Ligandscout 4.4. The interaction was then examined by selecting the yellow box and then followed by the generation of the pharmacophore and its 2D visualization to interpret and assess the results [15, 16].

RESULTS AND DISCUSSION

Table 1: Validation using molecular docking method

PDB ID (Resolution)	Organism	Receptor (Classification)	Complexed ligand	Amino acid interaction	Free energy (ΔG Gibs)	Inhibition constant (CI)	RMSD
1LF3 (2.70 A)	*Plasmodium falciparum*	Plasmepsin II (Hydroxylase)	Inhibitor EH58	GLY216, SER218, LEU131, LEU292, ILE123, ILE300, MET15, TYR77, VAL78	-9.27 kcal/mol	160.60 nM	2.638 A
1NHW (2.35 A)	*Plasmodium falciparum*	Enoyl-Acyl Carrier Protein (Oxidoreduktase)	NAD, TCC	LEU315, SER317, TYR111, ALA217, ASP168, ALA169, LYS285, TYR277, TYR267, ALA312, THR266, GLY110, LEU216, TRP131, SER215.	-12.50 kcal/mol	682.27 pM	0.940 A
1O5X (1.10 A)	*Plasmodium falciparum*	Triose-Phosphate Isomerase (Isomerase)	2-phosphoglycerate	GLY232, GLY173, ASN233, THR172, ALA234, VAL212, SER211, LYS12, GLY171	-5.97 kcal/mol	41.97 µM	1.522 A
1U4O (1.70)	*Plasmodium falciparum*	Lactate Dehydrogenase (Oxidoreduktase)	2,6-naphthalenedicarboxylic acid	PRO246, ILE31, MET30, PRO250	-5.87 kcal/mol	50.19 µM	1.94 A

Table 1 demonstrates the results of the evaluation methods based on the Root Mean Standard Deviation (RMSD) results, where the population cluster is the parameter key. RMSD is a number that quantifies the difference between the position of the native ligands before docking and after redocking, where the maximum prerequisite number is 2 Armstrong [17]. The data from the test results showed that three of four receptors satisfied the criteria, because the RMSD values for the receptors of Enoyl-acyl-carrier protein, Triose-phosphate isomerase, and Lactate dehydrogenase are below 2A, namely 0.940 A, 1.522 A, and 1.940 A, respectively. As for the receptor of *Plasmepsin II,* the RMSD value was above the prerequisite, namely 2.638 A. Those clusters of receptors are referred to as the best clusters and the best molecular docking since they presented the distribution of data from 100 docking conformations [18]. The RMS tolerance defined by "rmstol" in the docking parameter file determined the clustering results of docked conformations (dpf). Based on the possibility that the preferred conformation will be docking to the protein target, the more clusters, the better [19]. According to the validation results from the docking method, the free energy binding values of the Inhibitor EH85, nicotinamide adenine dinucleotide, 2-phosphoglycerate, and 2,6-naphthalenedicarboxylic acid are-9.27 kcal/mol,-12.50 kcal/mol,-5.97 kcal/mol, and-5.87 kcal/mol, respectively and corresponding to an inhibitory constant of 160.60 nM, 682.27 pM, 41.97 µM, and 50.19 µM.

Table 2: Virtual screening results

PDB ID	Amino acid residue		Free energy (ΔG Gibs)		Inhibition constant	
	Ursolic acid	Native ligand	Ursolic acid	Native ligand	Ursolic acid	Native ligand
1LF3	TYR192, GLY36, GLY216, TYR77, VAL78, ILE290, ASP34, THR217, SER218, SER79, SER37, ASN76, LEU131	GLY216, SER218, LEU131, LEU292, ILE123, ILE300, MET15, TYR77, VAL78	-7.76 kcal/mol	-9.27 kcal/mol	2.05 µM	160.60 nM
1NHW	GLY106, ASP107, LYS285, ARG318, ALA319, ALA320, TYR267, ALA217, ILE105	LEU315, SER317, TYR111, ALA217, ASP168, ALA169, LYS285, TYR277, TYR267, ALA312, THR266, GLY110, LEU216, TRP131, SER215.	-12.15 kcal/mol	-12.50 kcal/mol	1.25 nM	682.27 pM
1O5X	SER73, ALA234, LYS237, GLY72, ASN233, ASN213, THR214, ILE243	GLY232, GLY173, ASN233, THR172, ALA234, VAL212, SER211, LYS12, GLY171	-3.96 kcal/mol	-5.97 kcal/mol	1.25 mmol	41.97 µM
1U4O	HIS195, ILE31, THR97, GLY32, LEU163, MET30, VAL138, PRO250, ASN140, THR139, THR101, ALA98, GLY27, LEU167, PRO246, TYR247	PRO246, ILE31, MET30, PRO250	-9.39 kcal/mol	-5.87 kcal/mol	130.79 nM	50.19 µM

As presented in table 2, the virtual screening result of the test compound on *Pf* receptors being compared to the native ligands of each receptor using the SBDD method with GA runs 100 and medium energy 250.000. The data includes the residues of amino acids (a parameter used to compare the activity of the test compound and the lead compounds based on the type of interaction and amino acids), the free energy (a parameter used to assess the strength of the interaction formed where the lower the energy, the stronger the bond and the spontaneous bond formed) [20], and the inhibition constant values (an estimation of the drug potencial based on the value of the inhibition constant, in which a lower value indicates a greater biological activity) [21].

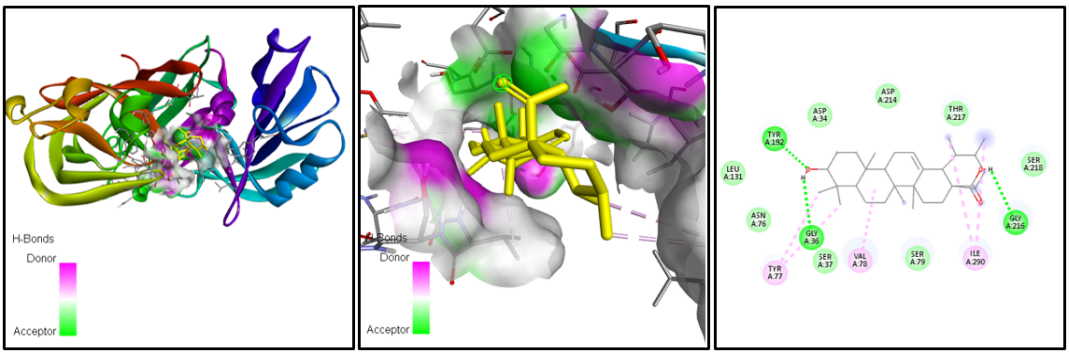

Fig. 1A: 2D and 3D Visualization between Ursolic acid and Plasmepsin II

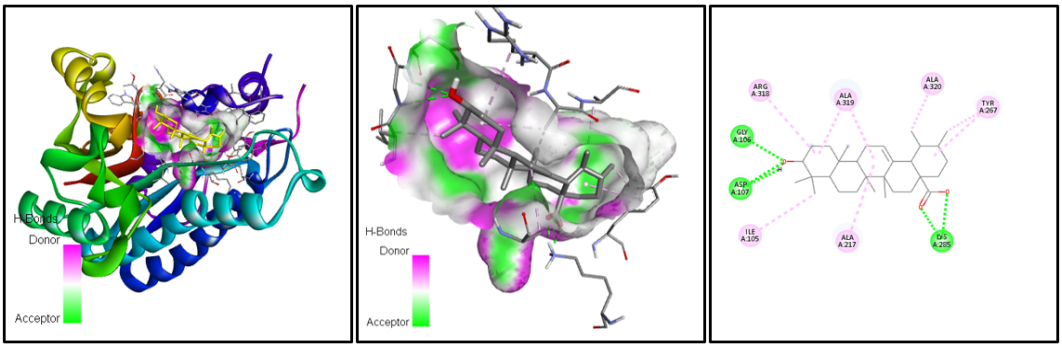

Fig. 1B: 2D and 3D visualization between ursolic acid and enoyl-acyl carrier protein

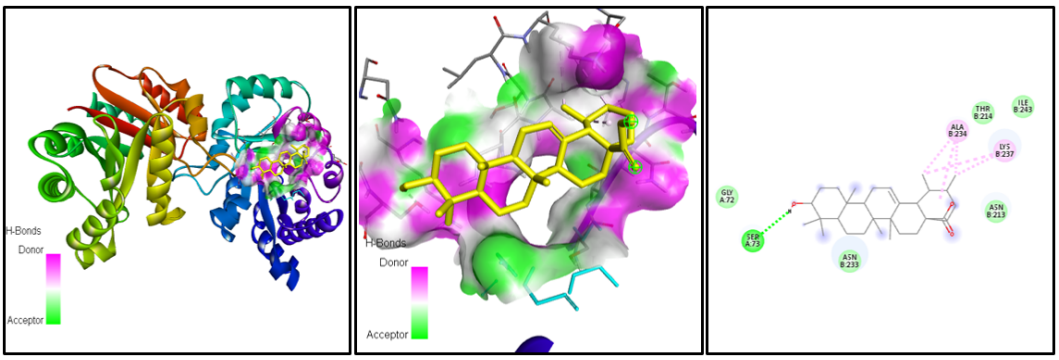

Fig. 1C: 2D and 3D Visualization between ursolic acid and triose-phosphate isomerase

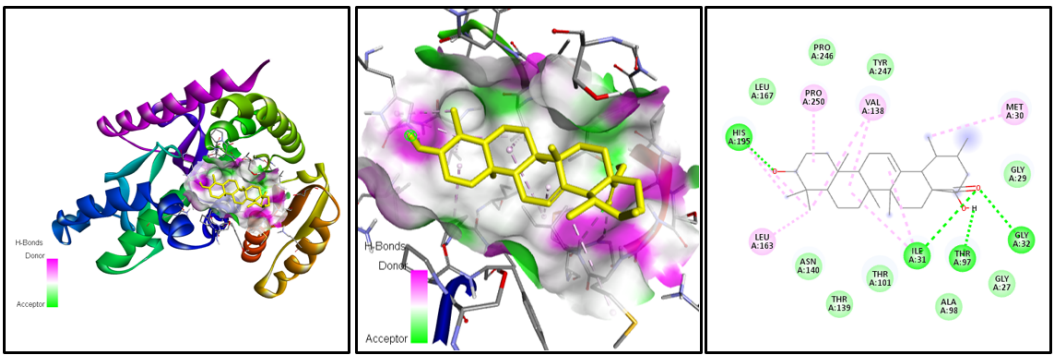

Fig. 1D: 2D and 3D visualization between ursolic acid and lactate dehydrogenase

Fig. 1A-D 3D visualization shows the binding site of UA on the *Pf* receptors, which can be used to assess whether the drug has a competitive or non-competitive inhibitory activity. Based on these findings, for all testing of UA binding with the *Pf* receptors had the same active site with the lead compounds. We found that the lead compounds and the amino acid residues of UA had interacted in the same manner (GLY216, SER218, LEU131, TYR77, and VAL78 for 1LF3 receptor; ALA217, LYS285, and TYR267 for 1NWH receptor; ASN233 and ALA234, for 1O5X receptor; and PRO246, ILE31, MET30, and PRO 250 for 1U4O receptor). This essential amino acid can be used to demonstrate how similar the active lead compounds and the UA compound are to each receptor [19, 21].

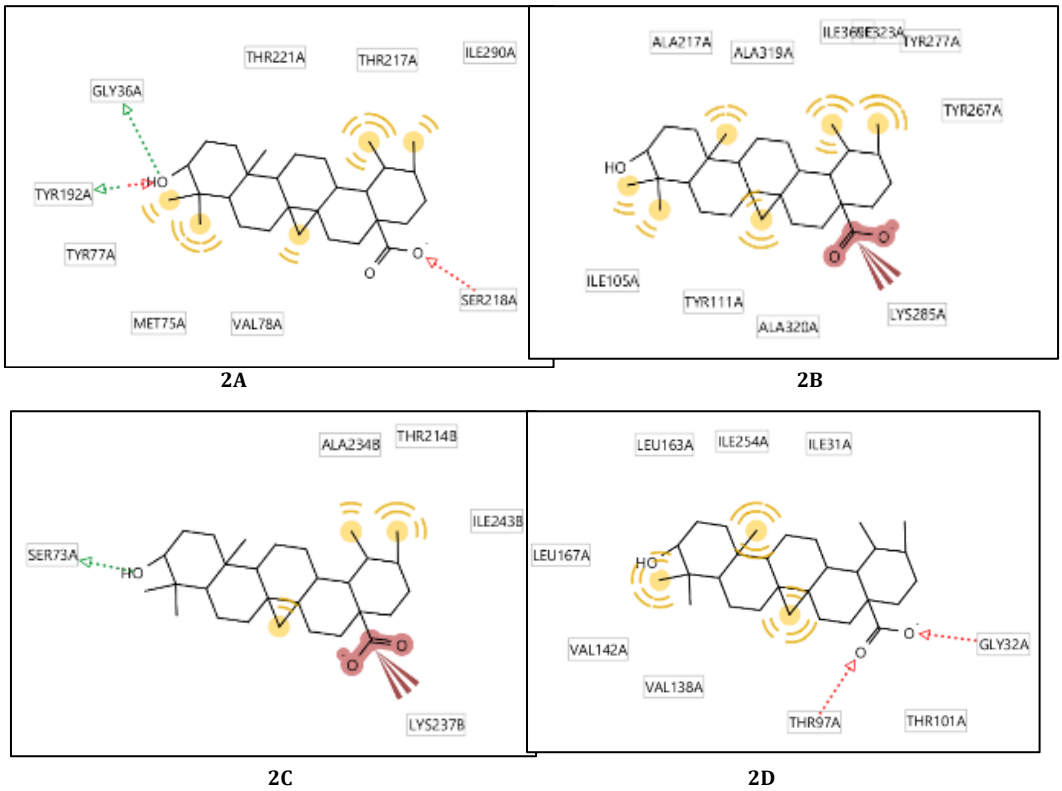

Fig. 2: A-D 2D Visualization of pharmacophore modeling between ursolic acid with A). Plasmepsin II; B). Enoyl-acyl carrier protein; C). Triose-phosphate isomerase; D). Lactate dehydrogenase

Fig. 2 shows the UA pharmacophore model on *Pf* receptors. Pharmacophore modeling was used to study the functional groups that interact with the targets, as well as the possible structural modifications that may be developed to increase effectiveness and/or to address the limits of the physicochemical properties of the UA [15, 22]. The results showed that carbocyclic acid and hydroxyl were the main functional groups responsible to bond with the significant amino acid residues on each receptor, while hydrocarbon emerges as the potential component that can be modified in future drug development due to its unintended interaction with the amino acid residues. After doing a literature review about the effects of UA compounds on various targets from various diseases, we found that hydroxyl and carboxyl groups play a crucial role in the interaction of amino acids at the receptors; hence, we are in the same conclusions [7, 23, 24].

Based on the analysis findings from the Protein Data Bank database, some approaches used to determine the structure of the receptors were electron microscopy, X-ray crystallography, NMR spectroscopy, and electron microscopy. Each approach offers a unique set of advantages and disadvantages. The structures of the molecules were in the X-ray diffraction pattern in X-ray crystallography, which contains information on the conformation and distance between atoms that are close to one another for NMR spectroscopy. For each approach, numerous pieces of information were used to develop the final atomic model. The X-ray method was employed to obtain all of the *Pf* receptors used in this study. In addition, the receptors originating from *Pf* proteins served as an excellent model for malaria. Due to its resolution value having close to 2 Armstrong, the receptor with a resolution value of 3A was considered as the most fulfilling receptor for the standard [25]. The resolution value indicates how close the derived structure resembles the initial receptor structure. The validation results of the molecular docking method on the *Pf* receptors emphasized the significance of the RMSD values. The difference between the positions of native ligands after docking and redocking were represented by the RMSD value, which must be less than 2 Armstrong [17]. According to the findings, all four receptors—Plasmepsin II, Enoyl-Acyl Carrier, Triose-Phosphate, and Lactate Dehydrogenase had RMSD values of 2.638 A, 0.940 A, 1.522 A, and 1.94 A, respectively; the free energy binding values were-9.27 kcal/mol,-12.50 kcal/mol,-5.97 kcal/mol, and-5.87 kcal/mol, respectively; and the constant inhibition value was 160.60 µM. Furthermore, based on the values of energy binding, the receptors had interaction with both hydrogen bonds and non-hydrogen bonds on a number of amino acid residues (table 1). From molecular docking screening results, it can be seen that these values satisfied the evaluation standards of UA as antimalarial [26]. According to the findings, three of the four tests (UA on Plasmepsin II, Enoyl-Acyl Carrier, and Lactate Dehydrogenase) indicated a possible effect due to the lowest free energy binding, which is-7.76 kcal/mol,-12.15 kcal/mol, and-9.39 kcal/mol, respectively. A lower free energy binding corresponds to lower energy activation. As a result, there is a chance that the UA compound and the receptors will interact and trigger a spontaneous reaction [27]. The UA compound has a lower value of the inhibition constant determined by the constant inhibition results (2.05 µM, 1.25 nM, 1.25 mmol, and 130.79 nM on Plasmepsin II, Enoyl-Acyl Carrier Protein, Triose-Phosphate, and Lactate Dehydrogenase, respectively). A comparative low value is believed to have considerable power since the molecule has a large inhibitory capacity at low doses, which symbolizes the ability of the drug to inhibit the receptors or enzymes [28]. Considering the energy and inhibition constants, one of the factors

affecting the activities of the compound is determined by how its structure interacts with the amino acids at its receptors. The UA compound interacts similarly with the native ligands through similar amino acid residues (GLY216, SER218, LEU131, TYR77, and VAL78 for 1LF3 receptor; ALA217, LYS285, and TYR267 for 1NWH receptor; ASN233 and ALA234, for 1O5X receptor; and PRO246, ILE31, MET30, and PRO 250 for 1U4O receptor) (fig. 1.) Based on the results; we also highlighted the hydrogen bonding interaction of the UA (table 2) since we are aware that this contact is reversible and significantly stronger than other forms of interaction [29, 30]. The test conducted to the comparable interactions between the UA compound and the lead compounds demonstrates the same activity in binding to the receptor. Competitively, the binding of the ursolic acids in the active pocket of receptors can inhibit this *Pf* from activation [31]. This mechanism of action inhibits the growth of *Pf*.

In pharmacophore modeling studies, the hydroxyl and the ether functional groups of the UA compound work as hydrogen bonding donors and acceptors that interact with amino acids of the receptors like TYR192, GLY36, SER218, LYS285, SER73, LYS237, THR97, and GLY32 (fig. 2). Hydroxyl and carboxylic acid are the primary components that are responsible to bond with important amino acid residues of the receptors. While hydrocarbon groups, which can be seen from the results of pharmacophore modeling, may play a role in changing the structure of this molecule in the course of drug development in the future because of their potential interaction with important amino acid residues.

This suggests that UA might be a viable option for drug development in the future to achieve maximum efficacy, good physicochemical properties, and low side effects.

CONCLUSION

The ursolic acid has antimalarial effects by competitively inhibiting the *Plasmodium falciparum* receptors. It shows a significant amount of hydrogen bonding interaction, a low value of inhibitory constants, and a comparative type of amino acid interaction when compared to the native ligands of the receptors. From the pharmacophore modeling, the major functional groups responsible for bonding with the key amino acid residues of the receptors are hydroxyl and carboxylic acid, while the hydrocarbon emerges as a possible subject that can be researched and altered in subsequent drug development.

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Nil

AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTERESTS

Declared none

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