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Original Article

LIGNAN DERIVATIVES POTENTIAL AS *PLASMODIUM FALCIPARUM* LACTATE DEHYDROGENASE INHIBITORS: MOLECULAR DOCKING APPROACH OF ANTIPLASMODIAL DRUG DESIGN

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ABSTRACT

Objectives: To investigate the lignan derivatives potential as *Plasmodium falciparum* Lactate Dehydrogenase (*Pf*LDH) inhibitors by using Computer Aided Drug Design (CADD) and molecular docking approach.

Methods: In finding potential antiplasmodial, *in silico* approach has been utilized. Protein structure of *Pf*LDH has been built through homology modeling. Kobamin has been used to refine the 3D *Pf*LDH structure. Structure validation of *Pf*LDH was done by Ramachandran Plot and ERRAT calculations. The validated *Pf*LDH was ready for molecular docking analysis. lignan derivatives as lead compounds were designed. The pharmacophore of lignan derivatives were assessed by using Molsoft drug likeness. Both protein and Lignan derivatives were docked with Autodock Vina. The best docking score was shown by the lowest affinity energy.

Results: Homology modeling of *Pf*LDH has been built. Moreover, *Pf*LDH refinement and validation were importantly conducted to ensure that *Pf*LDH structure was in good quality. According to Ramachandran Plot and Procheck analysis, *Pf*LDH has good structure quality with 93.39% confidence value. On the other hand, lignan derivatives assessment also has been done by evaluating their physicochemical and pharmacophore properties as lead compounds. From this assessment, it showed that Aristoligol (ARG1), Aristoligone (ARG2), and Ester Asetil Aristoligol (ARG3) showed good compounds to be drug likeness by following Lipinski's rule of five (RO5), while Ester Butiril Aristoligol (ARG4) showed poor RO5 criteria. Bioavailability of four compounds was good in body metabolism, however, ARG3 and ARG4 could not be lead like compounds due to poor lead likeness value. From molecular docking result, the most favorable binding with PfLDH was ARG4 based on its affinity energy value (-8.0 kj/mol), followed by ARG3, ARG2 and ARG1, respectively.

Conclusions: The identification of potential anti plasmodial drugs was successfully accomplished by evaluating synthetic lignan derivatives compound through physicochemical properties and molecular docking analysis. Overall, physicochemical and pharmacophore properties showed good result. Molecular docking interaction has distinct mode interactions of lignan derivatives with *Pf*LDH. We believe that these evaluated compounds could be used as anti plasmodial drugs according to *in silico* evaluation results.

Keywords: *Pf*LDH, Lignan derivatives, Molecular docking, Antiplasmodial, CADD.

INTRODUCTION

Malaria is one of the most invasive tropical diseases in the world especially in most developing countries which affecting mankind and constituting a major public health issue. It affects about 40% of the world's population, or equal approximately 216 million individuals over tropical and subtropical areas of developing countries and causes mainly morbidity and mortality about 800,000 death worldwide [1]. Moreover, approximately one third of the world's population is at risk for exposing the disease. The World Health Organization (WHO) always strikes to actuate campaigns for global malaria eradication [2-4]. Malaria is a global important disease and worldwide burdens due to long-term limitations of vector control and the lack of an effective vaccine, makes therapeutic antimalarial drugs development becomes the serious main strategy to eliminate malaria [5].

The causative agents which responsible to malaria disease are protozoan parasites *Plasmodium sp*. In particular, *Plasmodium falciparum* has been known as the most deadly human malaria parasite, relies on the export of virulence factors to the surface of infected erythrocytes. Over the course of its intraerythrocytic developmental cycle (IDC), the pathogenicity of *Plasmodium falciparum* tightly affects the fluctuation of transcript levels for hundreds of genes [6-8]. *P. falciparum* lactate dehydrogenase (*Pf*LDH) has been considered as a potential molecular drug target due to this parasite's dependence on glycolysis for energy production [9, 10]. Plasmodial lactate dehydrogenase is a terminal enzyme of the glycolytic pathway, has been shown to be biochemically, immunologically and structurally distinct from the mammalian enzyme [11]. Four Plasmodium species have been well known to infect human kind, including *P. falciparum, P. vivax, P. ovale,* and *P. malariae.* Moreover, *P. knowlesi*, has been recently documented to cause human malarial disease in Southeast Asia countries. Most severe diseases and deaths from malaria are caused by *P. falciparum*. It is responsible for about 80% of all malaria cases also responsible for about 90% of deaths caused by malaria [12-15].

By understanding a protein target mechanism in the parasite population is highly important to utilize it effectively in diagnostics and therapeutics [16]. The role of LDH in *P. falciparum* metabolism is a major checkpoint of anaerobic glycolysis, which form lactate from pyruvate reduction. This enzyme has recently become a great deal of attention as a valid therapeutic target for malaria disease. In fact, the *P. falciparum*'s isoform is a key enzyme for energy generation of malarial parasites. These species mostly depend on anaerobic glycolysis for energy production, since they lack a citric acid cycle for ATP formation. Therefore, *Pf*LDH inhibition would potentially cause mortality of *P. falciparum* and it would be a great target to eradicate malaria [17]. Small new bioactive molecules have been recently designed and developed to inhibit target, *Pf*LDH. Hence, inhibition of *Pf*LDH may constitute an efficient way to develop therapeutic potentials [18-22].

Lignan derivatives are potential renewable sources as new anti-malarial drugs, since they contain a quantity of metabolites with a

great variety of structures and pharmacological activities. Traditional preparations such as macerations, extracts, steam baths, concoctions, and decoctions from plant materials, have been the main source of malarial treatment in Indonesia and other developing countries where the disease is endemic [6,7]. *Phoebe declinata Ness* is a plant that has potential anti-malaria due to it contains Aristoligone lignan derivatives which have anti plasmodial activity in *Holostylis reniformis* plant [23]. However, the assessment of lignan derivatives to *Pf*LDH is still unknown. The aim of this research is to investigate the lignan derivatives potential as *Pf*LDH inhibitors by using molecular docking approach through Computer Aided Drug Design (CADD). The novelty of this study is finding the potential lignan derivatives which structurally modified against *Pf*LDH. The main emphasis of this work is to identify the most potential drug candidates against *Pf*LDH. We use high throughput platform such as pharmacophore properties [24, 25] and Lipinski's rule of five [26] as complementary analysis in identifying the best lead compounds of lignan derivatives.

MATERIALS AND METHODS

Protein structure preparation

The amino acid sequences of *Pf* LDH (Entry No. Q27743) was generated from UNIPROT protein database [27, 28]. The 3D structure of protein *Pf*LDH was generated by web based SWISS-MODEL program. All homology-modeling methods consist of the following four steps: (i) template selection; (ii) target template alignment; (iii) model building; and (iv) model evaluation. These steps are iteratively repeated, until a satisfying model structure is determined. The SWISS-MODEL server approach can be described as rigid fragment assembly [29-32].

Protein structure refinement and validation

*Pf*LDH 3D structure was checked by using Procheck [33] to validate its refined structural conformation. Ramachandran plot [34-37] and ERRAT [38] were used to analyze the allowed dihedral phi and psi rotation of amino acids in the protein backbones and the quality of refined 3D structure, respectively.

Lignan derivative structures preparation

All four 3D lignan derivative structures were generated by Chem Draw Ultra 12.0 [39, 40] for the molecular docking experiments and their conformational energy were minimized by using MMFF94 force field. Four molecules of lignan derivatives were designed by substituting the–R positions of lignan. These molecules are: Aristoligol (ARG1), Aristoligone (ARG2), Ester Asetil Aristoligol (ARG3), and Ester Butiril Aristoligol (ARG4), respectively. The molecule structures are depicted in fig. 1. The structures were scored based on their physicochemical properties under Chemicalize (Chem Axon) [41] and Molsoft [42] platforms. These physicochemical properties are important for developing the drug candidate in every stages from design to pre-clinical study.

Molecular docking of Pf LDH-lignan derivative complexes

The preparative protein and ligand coordinates were saved as pdb files. Molecular docking experiment is performed using Autodock Vina program (Vina, The Scripps Institute) [43]. The Autodock Tools is used to add partial charges using Gasteiger method and to arrange the polar hydrogens in the protein. The ligands are set to have flexible torsion angles at all rotatable bonds, while the protein is prepared as a rigid structure. Both protein and ligand are saved as output pdbqt files. For specific docking of ligand lignan derivatives onto the *Pf* LDH protein, the grid box volume was adjusted to 40x40×40 Å in the x, y and z axes, respectively, with grid-sizes have a space up to 1 Å.

The binding energy values were calculated based on the total intermolecular energies (kj/mol) including hydrogen bond energy, Van Der Waals energy, desolvation energy and electrostatic energy. On the other hand, the apropriate torsion angles of ligand are also induced as internal ligand energy. The docking program will evaluate this energy to obtain the best binding mode. The Root-Mean-Square Deviation (RMSD) which less than 2.0 Å was scored during running docking program.

RESULTS AND DISCUSSION

Protein structure of *Pf*LDH has been built from its amino acids sequence. Kobamin was used to refine *Pf*LDH structure, showed better folding structure. *Pf*LDH structure validation from Ramachandran plot (fig. 1) analysis proved that four amino acid residues with non-optimal values phi and psi have been located in alpha-and pi-turns of the LDH polypeptide chains, e. g., Asn1119, Asn75, Asn38 and Asn18.

However, residues in most favored region is 87.8%, indicated a valid 3D model structure (table 1). This validation was also supported by ERRAT analysis (fig.2). On the error axis, two lines are drawn to indicate the confidence of possible reject regions that exceed the error value. The ERRAT calculation is expressed as the percentage of the protein for which the calculated error value falls below the 95% rejection limit. Good high resolution structures generally produce values around 95% or higher. For lower resolution (2.5 to 3 Å) the average overall quality factor is around 91%. In this case, *Pf*LDH structure with 93.39% was acceptable to be used for further *in silico* analysis through docking interaction.

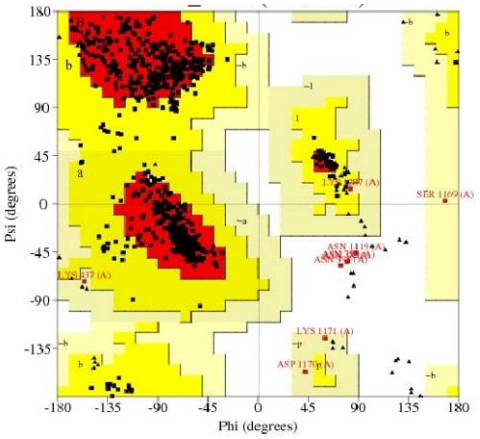

Fig. 1: Ramachandran Plot of *Pf*LDH

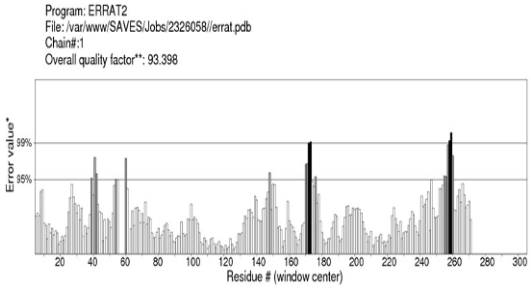

Fig. 2: ERRAT analysis of *Pf*LDH 3D structure

Table 1: Ramachandran plot summary

Plot statistics	Score	Percent
Residues in most favored regions [A,B,L] (red)	815	87.8%
Residues in additional allowed regions [a,b,l,p] (yellow)	104	11.2%
Residues in generously allowed regions [~a,~b,~l,~p] (pale yellow)	5	0.5%
Residues in disallowed regions (white)	4	0.4%
Number of non-glycine and non-proline residues	928	100%

One of lignan derivatives was drawn from natural Aristoligone. The other three derivatives were considered as synthetic structures which one–R group was synthesized to the different side chain (fig. 3). In assessing the physicochemical and pharmacophore of lignan derivatives, we evaluate the lipophilicity of derivative compounds by using Chemicalize platform. This is a crucial parameter in drug development since it impacts both properties and target affinity of drug candidates. In early drug discovery stage, accurate tools for logP prediction are highly desired. LogP value is used to predict octanol/water partition coefficients of chemical compounds. Many calculation methods were developed to assist pharmaceutical areas in drug development. In this study, four compounds have logP values ranging from 4.65 to 6.06 (table 1). In general, an orally active drug has no more than one violation according to Lipinski`s rule of five, as followed criteria: 1. No more than 5 hydrogen bond donors (the total number of nitrogen–hydrogen and oxygen–hydrogen bonds); 2. No more than 10 hydrogen bond acceptors (all nitrogen or oxygen atoms); 3. A molecular mass less than 500 daltons; 4. An octanol-water partition coefficient; 5. logP not greater than 5 [44, 45]. From pharmacopore results showed that ARG1, ARG2 and ARG3 have followed Lipinski`s rule of five, but not ARG4 due to there were found more than two violations of Lipinski criteria.

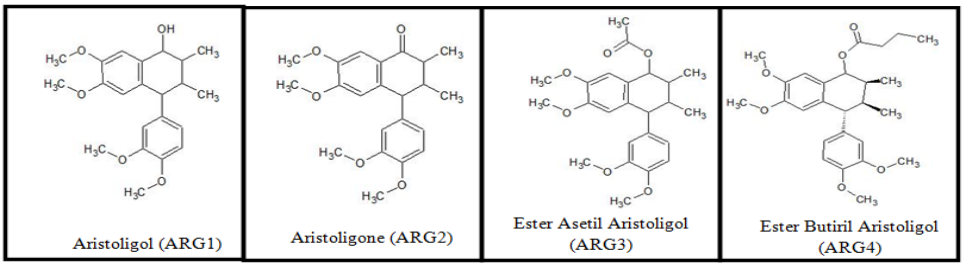

Fig. 3: Four Lignan derivative structures

Table 2: Physicochemical and pharmacopore properties of lignan derivatives

Compound	Molecular formula	Molecular weight	Number of HBA/HBD	pH	LogP	PSA (A²)	Lipinski's RO5
ARG1	$C_{22}H_{28}O_5$	372.45	5/1	7.4	3.64	57.15	Yes
ARG2	$C_{22}H_{26}O_5$	370.18	5/0	7.4	3.90	53.99	Yes
ARG3	$C_{24}H_{30}O_6$	414.20	6/0	7.4	4.08	63.22	Yes
ARG4	$C_{26}H_{34}O_6$	442.24	6/0	7.4	5.22	63.22	No

Table 3: Molecular docking interaction of lignan derivatives with *Pf*LDH summary

Compound	Structure color	Docking Affinity Energy (kj/mol)*	Bioavailability	Lead Likeness	LogSw**
ARG1	Red	-7.4	Yes	Yes	-4.45
ARG2	Blue	-7.7	Yes	Yes	-5.32
ARG3	Cyan	-7.7	Yes	No	-4.82
ARG4	Magenta	-8.0	Yes	No	-5.73

Notes: *Autodock Vina analysis, **Molsoft analysis

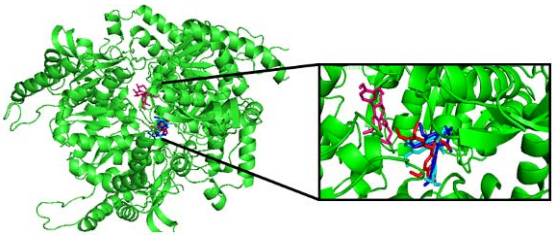

Fig. 4: *Pf*LDH-Lignan derivatives complex through molecular docking analysis, each compounds are colored differently (Green: *Pf*LDH structure in folding ribbon, Red: ARG1, Blue: ARG2, Cyan: ARG3, Magenta: ARG4)

Furthermore, drug likeness is also assessed relied on bioavailability, solubility and lead likeness, respectively (table 2). Drug likeness is a qualitative calculation used in drug design for how similar to drug a substance is with respect to factors like bioavailability. It is estimated from the molecular structure before the substance is even synthesized and tested.

Molecules that violate more than one of Lipinski's rules of five may have problems with bioavailability [46]. According to Veber rule, a good oral bioavailability should have Polar Surface Area (PSA) value less than 140 Å² or less than 12 total hydrogen bonds (acceptors plus donors). The PSA values of lignan derivatives showed within the range of 57 to 64 Å² (table 2). Therefore, all lignan derivatives presented good bioavailability. Moreover, to have a good lead likeness, lead compounds should have LogSw (logarithm of water solubility) less than-5. From LogSw value, we could see that ARG1 and ARG3 have LogSw less than-5, while ARG2 and ARG4 have LogSw more than-5. When synthetic lead compounds are planned to be modified for improving the water solubility or lead compounds permeability purpose, a wide parameter calculation of the structures could be used. It is important to clarify the structural modifications that increase solubility will also decrease drug permeability [47-49].

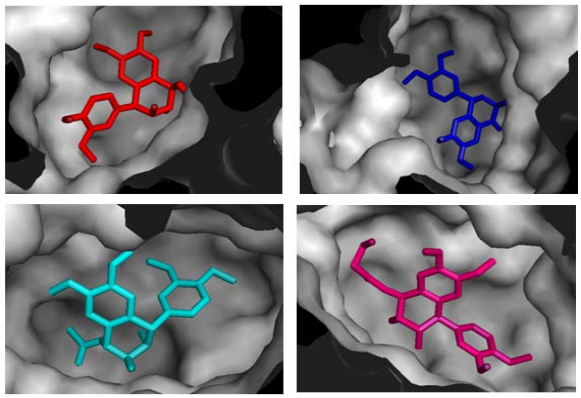

Fig. 5: Lignan derivative modes inside the *Pf*LDH cavity (Grey: *Pf*LDH pocket structure in inner surface form, Red: ARG1, Blue: ARG2, Cyan: ARG3, Magenta: ARG4)

Three dimensional structure of *Pf*LDH were built in SWISS Model web platform which its amino acids sequence was retrieved from UNIPROT, initially. Energy minimization of *Pf*LDH protein structure was employed by using Kobamin refinement platform. included energy minimized and molecular dynamics simulations. The refined *Pf*LDH was then used for docking interaction analysis. Autodock Vina was used for the docking studies. The docked conformation corresponding to the lowest binding energy was selected as the most favorable binding conformation. The total screened four compounds were docked into the active site of *Pf*LDH. The docking energies of all the compounds were represented in kcal/mol. The best mode of protein-ligand complexes are shown on fig.4. Four lignan derivative compounds showed low binding energies and significant affinities with target *Pf*LDH protein (table 3). All ligand conformers were embedded within the active site cavity of protein target. These favored ligand modes were stabilized by hydrogen bonds between the functional group from the ligands with the functional group of side chain residues of *Pf*LDH protein (fig. 5). The best docked compounds were shown with the lowest affinity energy. From docking result, it is shown that ARG4 (-8.0 kj/mol) has the best binding interaction compared to ARG3 (-7.7 kj/mol), ARG2 (-7.7 kj/mol) and ARG1 (-7.4 kj/mol), respectively (table 3). Relied upon this study, lignan derivatives compounds might be used as leads for developing effective antimalarial drugs. However, some poor bioavailability and pharmacopore could be structurally modified in the–R group to improve their bioactivities as lead compounds.

CONCLUSION

We have accomplished our investigation in identifying potential anti plasmodial drug candidates by evaluating synthetic lignan derivatives compound through physicochemical properties and molecular docking analysis. We suggested that all studied lignan derivative compound could be further validated *in vivo* and *in vitro* studies to confirm *in silico* evaluation. We believe that these evaluated compounds could be used as anti plasmodial drugs.

CONFLICT OF INTERESTS

Authors have none to declare

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