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DFT and molecular docking study of *Vladinol F* as pF-DHFR-TS inhibitors

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Abstract: Molecular screening was a primer method used for drug discovery and to find the lead compounds. A high failure rate could be gotten when identifying some drug variables which did not meet the requirements so that it needed an alternative method development to resolve these deficiencies. In this research, NMR data correlation was used to encouraging the accuracy and precision when designing a potential compound; furthermore, molecular docking approach was used as the molecular screening to learn and inspect the drug mechanism to targeted enzyme. This research was conducted to study the potency of Vladinol F as antimalarial inhibitor. From the Mean Absolute Percentage Error (MAPE) data, it was obtained 8.80% and 12.23% for its proton and carbon respectively. Geometry structure and electronic properties was evaluated. Moreover, the observed activities, in vitro and in silico, were compared by docking study on *P. falciparum* dihydrofolate reductase-thymidylate synthase (*pf*-DHFR-TS).

Keywords: Antimalarial, DFT, Docking, *Vladinol F*.

Introduction

Malaria is an infectious disease caused by a protozoan parasite that infects human red blood cells. There are 207 million cases annually, of which 627,000 die, mainly in Africa (Sonin et al., 2013). In 2015, he had 429,000 deaths from her 212 million medical cases in pregnant women and children (World Health Organization, 2016). Malaria is characterized by fever, fatigue, vomiting and headache. In severe cases, malaria can cause convulsions, coma, and even death (Malau & Azzahra, 2020). The rapid and widespread spread of malaria parasites in nearly all endemic areas. It has prompted researchers to develop new antimalarial drugs (Vial & Ancelin, 1998). There are several reports of parasite resistance to artemisinin combination therapy (ACT), the drug of choice for treating tropical malaria (Nsanzabana, 2019: Phyo et al., 2012; Zhu et al., 2022). The search and development of new chemical entities (active ingredients) for antimalarial drugs is critical to solving emerging resistance problems (Tople et al., 2023).

Dryobalanops oblongifolia is a kind of the genus *Dryobalanops* of the Dipterocarpaceae family. *Dryobalanops* is called basswood in local communities (Purwaningsih, 1970) and it is known to be a source of oligostilbene (Siti Aminah et al., 2006; Syah et al., 2003). A previously isolated component from *D. oblongifolia* reported that Vladinol F has potential antiplasmodium activity (Indriani et al., 2017). That study conducted successfully isolated Vladinol F from *D. oblongifolia*. Vladinol F has potential as an antimalarial parasite. Vladiol F antiprotozoal activity test against *Plasmodium falciparum* showed an IC_{50} value of 3.51 μg/mL. In the study conducted by (Indrani et al., 2021), only in vitro tests were performed, so in this study, researchers wanted to observe the correlation between Vladinol F ligands and receptors in silico used Density Functional Theory (DFT) and Molecular Docking method.

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The molecular structure of Vladinol F was optimized using the DFT-Becke model three-parameter hybrid functional Lee-Yang-Parr method (B3LYP) based on the molecular structure calculation set 6-31++g(d,p). It was done. Data analysis calculations were performed using Gaussian 09W software. Data analysis results are graphically visualized in Gauss View 6.0. The charge distribution is analyzed by the molecular electrostatic potential (MEP). From the energy gap, the electron density distribution is calculated in highest occupied molecular orbital (HOMO) - lowest unoccupied molecular orbital (LUMO) form. DFT methods are used to calculate reactivity parameters such as electronegativity (x), chemical potential (μ), global hardness (η), global softness (s), and electrophilic index (ω). In addition to the DFT method, a molecular docking analysis was also performed to predict the interaction between the Vladinol F ligand and the PDB ID.1j3i receptor based on binding properties.

Materials and Methods

Study area

This study was conducted to determine the efficacy of Vladinol F as an antimalarial agent. The area of this research was DFT analysis and molecular docking to investigate how Vladinol F interacted with malarial proteins. The materials used in this study were Vladinol F (Figure 1) and resetor of *plasmodium falciparum* wild-type dihydrofolate reductase thymodylate synthase (pF-DFHR-TS) (PDB ID: 1j3i). Software used in this study are Gaussian 09W, GaussView6, Dock6, BIOVIA Discovery Studio, and Chimera.

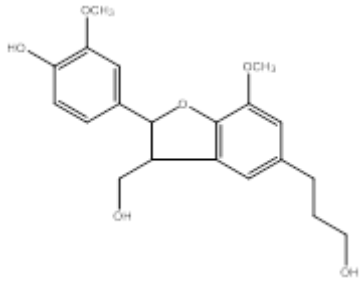

Fig 1. Structure of Vladinol F (Indriani, et.al.,2017)

Procedures

DFT Study

The DFT expressed in B3LYP/6-31++g (d,p) model was used to calculated the ground-state molecular structures. All of the calculations were done with the Gaussian 09W program. The graphical interface GaussView 6 was used to visualize the optimized geometry, global reactivity, chemical shift and molecular electrostatic potential (MEP).

Molecular docking

The DOCK6 package performed all steps in molecular docking. Receptor of this study was pF-DHFR-TS with PDB ID: 1j3i. The redocking stage aimed to determine the binding site of the Vladinol F based on the WRA coordinates. Several parameters were used in the redocking process, such as grid-spacing: 0.3 Å, center (X: 28.017, Y: 8.509 Z: 57.986), and dimensions (X: 26.738, Y: 23.785, Z: 27.784). The interaction energy in the molecular docking process was calculated using a grid score functional approach with the *anchor-and-grow* algorithm.

Data analysis

Design of experiment was a technique to screening molecule as antimalarial by studying the effects on the structure. For this study, experiment was designed by using DFT and Molecular docking method. The data were collected and analysis from the calculation was chemical shift (proton and carbon), global reactivity and molecular docking. The data from chemical shift was calculated and evaluated correlation and accuracy between experimental and theoretical through statistical analysis, such as coefficient of determination (R^2 and R_0^2), mean absolute error/MAE (Eqn. 1), root mean square error/RMSE (Eqn. 2) and mean absolute percentage error/MAPE (Eqn. 3).

$$MAE = \frac{1}{n}\sum_{i-1}^{n}\left|\delta_i^{theo} - \delta_i^{exp}\right| \quad (1)$$

$$RMSE = \sqrt{\frac{1}{n}\sum_{i-1}^{n}\left(\delta_i^{theo} - \delta_i^{exp}\right)^2} \quad (2)$$

$$MAPE = \frac{\sum_{i-1}^{n}\left|\frac{\left|\delta_i^{theo} - \delta_i^{exp}\right|}{\delta_i^{exp}}\right|}{n} \text{ x } 100 \quad (3)$$

The charge distribution was visualized through molecular electrostatic potential (MEP) analysis. Electron density distribution in the form of HOMO-LUMO was studied through energy gap analysis (Eqn. 7). The distribution of electrons in the HOMO and LUMO regions were the primary references in studying molecule reactivity. Several global reactivity parameters were calculated, including electronegativity/χ (Eqn. 8), chemical potential/μ (Eqn. 9), global hardness/η (Eqn. 10), global softness/S (Eqn. 11) and electrophilicity index/ω (Eqn. 12).

$$\Delta E_{gap} = E_{HOMO} - E_{LUMO} \quad (7)$$

$$\chi = -\frac{1}{2}(E_{LUMO} - E_{HOMO}) \quad (8)$$

$$\mu = -\chi \quad (9)$$

$$\eta = \frac{1}{2}(E_{LUMO} - E_{HOMO}) \quad (10)$$

$$S = \frac{1}{2\eta} \quad (11)$$

$$\omega = \frac{\mu^2}{2\eta} \quad (12)$$

Results and Discussion

Chemical Shift

Chemical shift modeling was performed on gas phases using B3LYP/6-311++ (d,p) basis set as a reference approach. Chemical shift modeling in this study is proton (^{1}H-NMR) and carbon (^{13}C-NMR). The chemical shift reflects the molecular structure and it can therefore be used to obtain structural information. In our work, we compared between experimental and theoretical of Vladinol F to know the accuration and correlation of the analyzed structure and analysis the result using statistical analysis. The results of the statistical analysis of chemical shift which can be seen in (Table 1) and the graphical of regression can be seen in (Figure 2).

Table 1. Statistical analysis: Accuration and correlation of chemical shift between experimental and theoritical for *Vladinol F*

Parameters	Gas
Proton Chemical Shift	
MAE	0.49
RMSE	0.83
MAPE	8.81
R^2	0.80
R_0^2	0.80
Carbon Chemical Shift	
MAE	11.19
RMSE	11.75
MAPE	12.23
R^2	0.99
R_0^2	0.99

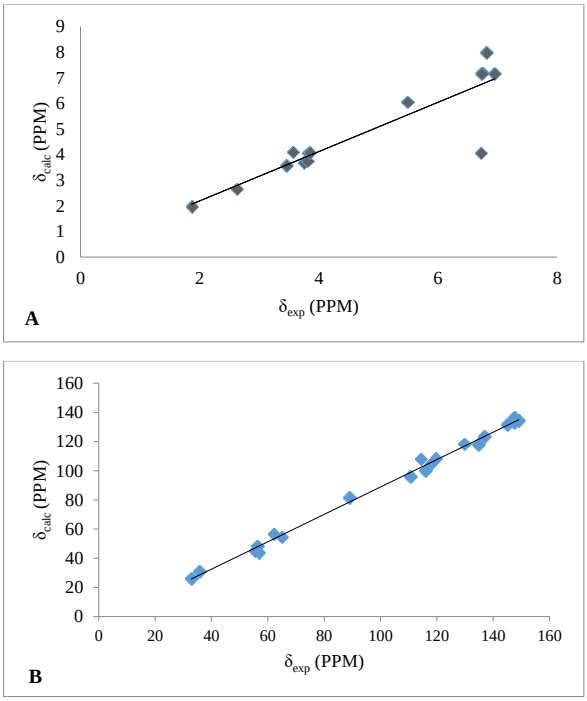

Fig 2. Chemical shift between experimental (δ_{exp}) and theoretical (δ_{calc}): (A) Proton Chemical Shift (^{1}H NMR), (B) Carbon Chemical Shift (^{13}C NMR)

Electronic Structure Properties

The optimized structure study aims to understand several electronic structure properties of Vladinol F compound, including molecular electrostatic potential (MEP) and global reactivity. The result visualization of molecular electrostatic potential (MEP) can be seen (Figure 3), the global reactivity

can be seen (Table 3) and the visualitation of HOMO (Highest order molecular orbitals) and LUMO (Lowest order molecular orbitals) can be seen (Figure 4).

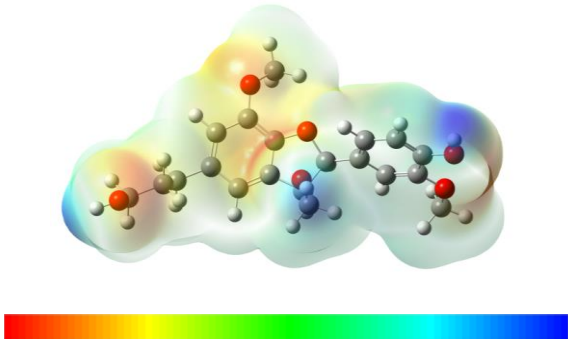

Fig 3. Molecular electrostatic potential was calculated using DFT/B3LYP/6-311++G(d,p) method with isoval: 0.004. The red color represents negative charges, while the blue represents positive charges

Table 2. Calculation of global reactivity was calculated using DFT/B3LYP-6-311++G (d,p) method in the gas state

Molecular Property	FF
E_{HOMO} (eV)	-0.206
E_{LUMO} (eV)	-0.020
E_{gap}	-0.186
Electronegativity	-0.093
Chemical potential	0.093
Hardness	0.093
Softness	0.046
Electrophilicity Index	0.0004

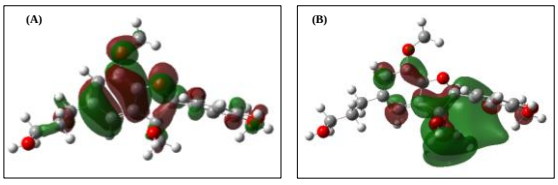

Fig 4. Visualisation of HOMO (A) and LUMO (B) Surface calculated using DFT/B3LYP/6-31++G(d,p) with isovalue 0.02

Molecular Docking Studies

Target structure information and data were collected from the Protein Data Bank (PDB). The target structure of this study is the wild-type Plasmodium falciparum dihydrofolate reductase thymidylate synthase (PDB ID: 1J3I). The design and result of docking can be seen (Figure 5).

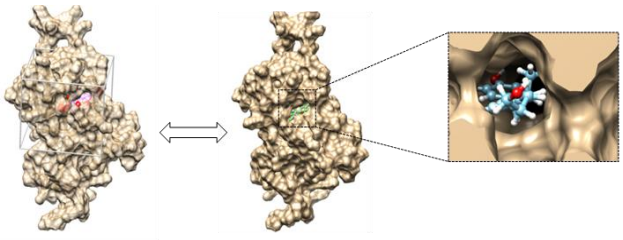

Fig 5. Molecular docking analysis. (A) Grid-box preparation based on the coordinates of selected spheres, (B) Inhibitor- Vladinol F docking based on functional grid score, (C) The Vladinol F conformation on the WR99210 binding site

In this study, molecular docking was performed with Vladinol F as the compound showed antimalarial activity. Molecular docking was performed to predict the interaction between the secondary methoxy group of Vladinol F and the amino acids of the PfDHFR TS protein. Figure 2 shows the results of molecular docking of Vladinol F to protein 1J3I.

Table 3. Summary of results molecular docking was calculated using Dock6 Package

Compound	Grid Score	E_{vdw}	E_{ele}	No. of H-bonds interaction	Key residues interacted with ligand
Vladinol F	-64.926	-59.696	-5.229	4	Ala16, Asp54, Ile164, Ile112, Met55, Phe58, Ser108, Leu40
WR99210	-65.361	-62.980	-2.381	4	Ala16, Asp54, Ile164, Ile112, Met55, Phe58, Cys15, Ile14, Leu48, Pro113, Phe116

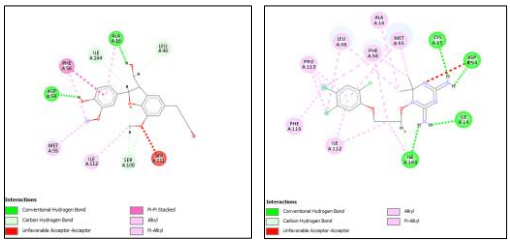

Fig 6. Residues interacted with ligand **(A)** *Vladinol F* and **(B)** *WR99210* was analysis using BIOVIA Discovery Studio

Discussion

Briefly, a molecular screening of Vladinol F as an antimalarial agent was performed by DFT and molecular docking studies. For DFT calculations, the researchers wanted to know the stability and reactivity of the compound Vladinol F before docking to the malaria receptor. In addition to stability and reactivity, DFT is also used to determine chemical shifts in silico. The results of DFT calculations were compared with those of chemical shift experiments (Indriani et al., 2021). Data were subjected to statistical analysis tests MAE (mean absolute error), RMSE (mean squared error), MAPE (mean absolute percentage error),

and regression tests to determine the accuracy and structural correlation of Vladinol F connections between experiments and in silico.

Based on table 1, the result of our work indicated that the accuracy of model chemical shift has excellent and good criteria so can deserves further analysis. Calculations of the chemical proton shifts of the compounds showed good correlation with the experimental results (Figure 2). Details of the proton chemical shifts of Vladinol F are given in Table S1. Correlation results by linear regression analysis showed values of R^2 and $R_0^2 \geq$ 80% for proton chemical shift and ≥ 90% for carbon chemical shift. On the other hand, the accuracy analysis between experiment and theory is represented by the MAE, RMSE and MAPE parameters as shown in Table 1. Our results showed that the MAPE of the model was less than 20%. Smaller parameter values generally result in better accuracy (Abdjan et al., 2022). The results of our work showed that the model accuracy met a good standard and deserves further analysis.

The result global reactivity of Vladinol F compound show in the table 2. Orbital-level energy analysis provides information for considering the electrochemical behavior of molecules. HOMO (E_{HOMO}), LUMO (E_{LUMO}) and gap energy (ΔE_{gap}) were estimated using DFT/B3LYP/6-311++G (d.p) in gas state. HOMO orbitals act as electron donors and LUMO orbitals act as electron acceptors. The HOMO-LUMO energy parameter has been important in understanding molecular reactivity. The results showed that the ΔEgap of Vladinol F was −0.186 eV (table 2). Based on figure 3, the most negative region of the molekul is detected around the methoxy group then a positive electrostatic potential is found in the area of the hydroxyl group. If the biological activity analysis is carried out, it is most likely that the methoxy group in the area plays an important role in the formation of hydrogen bonds at the target.

The mechanism of inhibition of Vladinol F compounds was studied by molecular docking simulations. Vladinol F is available from the Brookhaven PDB (ID: 1J3I). Indeed, the dihydrofolate reductase of *Plasmodium falciparum*, thymidylate synthase, is an important target for antimalarial drugs. Inhibition of this enzyme can interfere with parasite deoxythymidine monophosphate generation and DNA synthesis. This is because it is involved in catalyzing a series of reactions in the thymidylate cycle. From the docking results (Table 3), Vladinol F is favored against *Plasmodium falciparum* dihydrofolate reductase-thymidylate synthase with a grid score of -64.926 kcal/mol, corresponding to a grid score of -65.361 kcal/mol for the co-crystallized ligand. The value of grid score is closeted. At the active site of P. falcifarum dihydrofolate reductase-thymidylate synthase, the compound Vladinol F interacts with the Ala16, Asp54, Ile164, Ile112, Met55, and Phe58. This is the key amino acid for antimalarial activity by the protein-ligand co-crystal structure of WR99210. Vladinol F, on the other hand, is in the *P. falcifarum* dihydrofolate reductase thymidylate synthase active site comprises residues of Ala16, Asp54, Ile164, Ile112, Met55, Phe58, Ser108, and Leu40. Because the Ala16, Ser108, and Ile164 residues are located in the active site of the mutated protein, Vladinol F was expected to exhibit malarial activity also against chloroquine-resistant strains. Residues involved in the mutant active site were Ala16, Cys50, Asn51, Cys59, Ser108, and Ile164 (Syahri et al., 2017).

Conclusions

A DFT and molecular docking were used to study correlation structure and the antimalarial activity of Vladinol F compound. Accuration and Correlation of the structure Vladinol F showed that good value with the Mean Absolute Percentage Error (MAPE) data, it was obtained 8.80% and 12.23% for its proton and carbon respectively. The molecular docking showed that Vladinol F has antimalarial activity result by indicating the interaction of the methoxy group in the Vladinol F compound to Ala16, Asp54, Ile164, Ile112, Met55, Phe58, Ser108, Leu40 amino residues from the *Pf*DHFR-TS protein and the grid score of Vladinol F was -64.926 Kcal/mol.

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