Current Trends in Biotechnology and Pharmacy
Vol. 17(3) 929 - 936, JUly 2023, ISSN 0973-8916 (Print), 2230-7303 (Online)
DOI: 10.5530/ctbp.2023.3.30

In Silico Molecular Docking of the Antimalarial Flavonoid Compound Macaranga (*Macaranga tanarius*) Against the PfDHFR Enzyme

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ABSTRACT

Malaria is an acute febrile disease caused by the parasite Plasmodium spp. It is a global health problem, especially in Indonesia. Drug resistance to antimalarial drugs is one of the health challenges faced. The discovery and development of new antimalarial drugs to overcome the current antimalarial drug resistance are highly needed in the future. Natural substances, especially from plants, are widely used in antimalarial treatment. One of them is the Macaranga plant (*Macaranga tanarius*), which is used as an antimalarial remedy in Kalimantan. The aim of this research was to conduct in-silico molecular docking of potential antimalarial chemical compounds found in the Macaranga plant. The analysis method used in this study was in-silico molecular docking using AutoDock v.4.2.6 software, and the data was visually analyzed in two dimensions using LigPlus v 1.4.5. The research results showed that the compound Tanariflavanone D had a lower binding free energy (ΔG) of -9.44 kcal/mol compared to the native compound WRA609 (ΔG = -8.44 kcal/mol) and the positive control compound Pyrimethamine (ΔG = -7.06 kcal/mol). Tanariflavanone D is suspected to have activity in inhibiting the PfDHFR enzyme, thus potentially serving as an antimalarial drug.

Keywords: malaria, antimalarial, macaranga

Introduction

Malaria is an acute febrile disease caused by the *Plasmodium* spp. parasite, transmitted to humans through the bite of infected female *Anopheles mosquitoes*. There are five species of parasites that cause the largest burden of malaria in humans, and two of them, *P. falciparum* and *P. vivax*, pose significant threats. *Plasmodium falciparum* is the most deadly and common malaria parasite in Africa, while *Plasmodium vivax* is dominant in most countries outside of sub-Saharan Africa. Globally, it is estimated that there were 241 million malaria cases in 2020 across 85 malaria-endemic countries (including the French Guiana region), an increase from 227 million cases in 2019, with most of this increase coming from countries in the African region. The WHO Southeast Asia region accounts for approximately 2% of the global malaria burden. Malaria cases have decreased by 78%, from 23 million in 2000 to around 5 million in 2020. Incidence of malaria in this region has decreased by 83%, from approximately 18 cases per 1000 at-risk population in 2000 to around three cases in 2020. (1)

Resistance to antimalarial drugs is a major cause of morbidity and mortality in tropical countries. Resistance has complicated

malaria treatment and poses a threat to disease control and eradication efforts. Antifolate derivative compounds are a group of drugs that competitively inhibit the enzyme dihydrofolate reductase (DHFR), a key enzyme in the folate pathway, thus disrupting the parasite's nucleotide metabolism. (2) The dihydrofolate reductase domain of the bifunctional enzyme *P. falciparum*, known as dihydrofolate reductase-thymidylate synthase (DHFR-TS), is one of the validated targets in malaria chemotherapy. (3) This enzyme catalyzes the conversion of dihydrofolate (DHF) to tetrahydrofolate (THF) by utilizing nicotinamide adenine dinucleotide phosphate (NADPH), which is essential for DNA synthesis. Inhibiting the DHFR enzyme effectively disrupts DNA synthesis, ultimately leading to the death of the parasite's cells. (4)

The combination of drug design and in-silico processing is a suitable approach for discovering new inhibitors against *Plasmodium* spp. This combination can have a significant impact due to its cost-effectiveness, speed, and applicability to various receptor target structures. The in-silico docking and molecular dynamics approaches can be employed to gain a better understanding of the mechanism of action of a chemical compound or macromolecule, such as proteins or peptides, on a molecular scale. This enables the design of structure-based drugs. (5)

The receptor protein target, DHFR, is utilized for in-silico molecular docking and molecular dynamics studies to explore the molecular interactions of secondary metabolite compounds derived from natural sources, particularly from plants, as potential antimalarial candidates. In this study, the enzyme structure DHFR-TS from *Plasmodium falciparum* is employed as the target. The test compounds are sourced from secondary metabolites of the plant species Macaranga (*Macaranga tanarius*), a member of the Euphorbiaceae genus. Macaranga plants have been traditionally used in Kalimantan communities for malaria treatment. These plants contain a combination of terpenoid compounds along with flavonoids and stilbenoids, including Nymphaeol A, Nymphaeol B, Nymphaeol C, Tanariflavanone A, and Tanariflavanone D. (6)

This study aims to determine the binding free energy and hydrogen bond interactions of the amino acid residues of the PfDHFR enzyme with the test compounds, which are flavonoid derivatives (Nymphaeol A, Nymphaeol B, Nymphaeol C, Tanariflavanone A, and Tanariflavanone D). Several stability parameters in molecular docking will be examined, including the binding free energy (ΔG), the types of chemical interactions formed, the hydrogen bond distances between the amino acid residues and the test compounds, and the binding regions of the test compounds on the PfDHFR-TS amino acid residues.

Materials and Methods

Preparation of the Protein structure

The receptor protein used in this simulation is the Wild-type *Plasmodium falciparum* Dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) with PDB ID: 1J3I, downloaded from the RSCB Protein Data Bank (https://www.rcsb.org/). The receptor protein used has a complete number of amino acid residues. Subsequently, the receptor protein preparation involved removing water molecules, other atoms, and ligands present in the receptor protein complex (7,8) A check was performed on the receptor protein for any missing residues or chain breaks, and if found, they were then modeled using the Modeller v9.23 application in UCSF Chimera v1.15. The resulting structure of the receptor protein was optimized using Dock Prep in UCSF Chimera v1.15 with the standard parameters for the forcefield = AMBER ff14SB and other remaining parameters = AM1-BCC. The prepared structure of the receptor protein was saved in the "filename.pdb" format. (9)

Preparation of the ligands

The natural ligand structure was obtained from the complex structure of the protein-ligand on the Wild-type *Plasmodium falciparum* Dihydrofolate reductase-thymidylate synthase (PfDHFR-TS) receptor protein. The structure of the natural ligand, namely 6,6-Dimethyl-1-[3-(2,4,5-Trichlorophenoxy)Propoxy]-1,6-

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Dihydro-1,3,5-Triazine-2,4-Diamine (WRA609), was separated using the UCSF Chimera v1.15 application, and hydrogen atoms were then added. The optimized ligand structure was saved in either the “filename.pdb” or “filename.mol2” format. (9) The test compounds used in this study, which have the potential as antimalarial agents, are Nymphaeol A, Nymphaeol B, Nymphaeol C, Tanariflavone A, and Tanariflavone D. (6) The structures of these compounds were obtained from PubChem (https://pubchem.ncbi.nlm.nih.gov/). To prepare the ligand structures, UCSF Chimera v1.15 was utilized with the following parameters: hydrogen atoms were added, and charges were assigned using the Gasteiger method. (10,11)Subsequently, the ligand structures were minimized for energy optimization using the following parameters: maximum number of steps = 10000; steepest descent step size = 0.02 Å; conjugate gradient steps = 1000; conjugate gradient step size = 0.02 Å; and update interval = 10. The minimized ligand structures were then saved in either the “filename.pdb” or “filename.mol2” format.

Validation molecular docking method

The validation process was conducted by re-docking the natural ligands to the PfDHFR-TS enzyme. This step was performed using the AutoDock MGL Tools v1.5.6 application, where the prepared protein and ligand files in “filename.pdb” format were converted to “filename.pdbqt” format. (7) Next, the Parameter Grid was determined, and the position of the Center Grid Box was set at x = 27.665 Å; y = 6.654 Å; z = 58.206 Å, with a spacing of 0.375 Å and Grid Box dimensions of x = 40 Å; y = 40 Å; and z = 40 Å.

Molecular docking was conducted using AutoDock v4.2.6 with the following parameters: Number of Genetic Algorithm (GA) runs = 100; Population Size = 300; and Maximum Number of Evaluations = 2500000 (medium). (12,13) The validity of the re-docking results is confirmed if the Root Mean Square Deviation (RMSD) value is less than 2 Angstroms (RMSD ≤ 2 Å). (14–17)

Molecular docking of ligands in PfDHFR enzymes

The molecular docking simulation of the test compounds to the PfDHFR-TS enzyme was conducted using the same protocol as the validated re-docking of the natural ligands to the PfDHFR-TS enzyme. The analysis results revealed the hydrogen bond conformations formed between the enzyme and the test ligands, along with the values of the free energy of hydrogen bonds that were formed between the amino acid residues and the test ligands.

Data analysis

The results of molecular docking include the free energy (ΔG) of binding and the hydrogen bond interactions formed between the amino acid residues of the protein and the ligands of the test compounds. The binding energy is used to indicate the strength of the interaction between the ligands and the protein. A lower value of the free energy of binding (ΔG) indicates a stronger and more stable binding. The types of hydrogen bonds formed are used to analyze the mechanisms of the interactions that occur between the ligands and the protein. Hydrogen bonds are critical for stabilizing the ligand-protein complex and play a crucial role in determining the specificity and affinity of the ligands for the protein’s active site. The two-dimensional visualization of the molecular docking results is performed using LigPlus v1.4.5. This visualization allows for a clearer understanding of how the ligands interact with the protein’s active site and the specific amino acid residues involved in the binding. (18) .

Results and Discussion

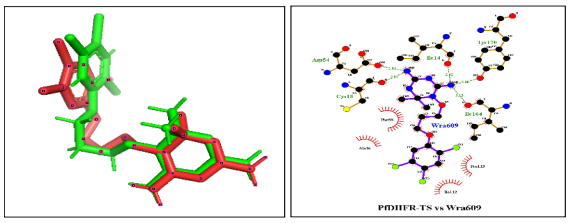
The molecular docking process begins with validation by performing re-docking of the natural ligand present in the protein-ligand complex structure. The natural ligand used as a reference is one that has potential as an enzyme inhibitor. In the case of the PfDHFR-TS enzyme (PDB ID: 1J3I), the natural ligand is 6,6-Dimethyl-1-[3-(2,4,5-Trichlorophenoxy) Propoxy]-1,6-Dihydro-1,3,5-Triazin-2,4-Diamin (WRA609). The results of the re-docking sim-

ulation show an RMSD value of 1.09 Å, which is less than 2 Å, indicating that the molecular docking protocol used is valid (Figure 1a). (14,16) The calculated free energy of binding (ΔG) is -8.44 kcal/mol, and the simulation yields an Inhibition Constant (KI) value of 655.42 nM. A more negative value of the free energy of binding (ΔG) indicates a stronger and more stable interaction between the receptor protein and the ligand. (19) The analysis of hydrogen bond interactions and hydrophobic interactions can be seen in Table 1, and the two-dimensional visualization using LigPlus v1.4.5 is shown in Figure 1b. The hydrogen bond interactions formed after the molecular docking simulation correspond to the interactions present in the RSCB Protein Data Bank for the receptor protein structure with PDB ID: 1J3I. These hydrogen bond

Table 1. Molecular validation results of the PfDHFR-WRA609 docking complex

Ligand	Bond free energy (ΔG) (kcal/mol)	The constant inhibits (Ki).	Hydrogen bond interactions	hydrophobic interactions	RMSD(Å)
WRA	-8.44	655,42 nM	Ile14 (2,82 Å); Cys15 (2,92 Å); Asp54 (2,93 Å); Ile164 (3,12 Å); Tyr170 (3,00 Å)	Phe58; Ala16; Pro113; Ile112	1.09

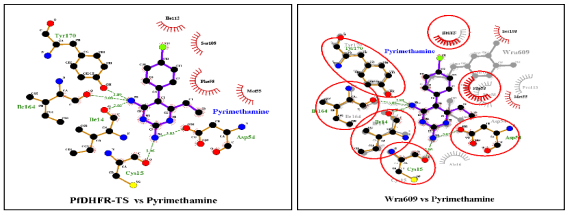
interactions occur at amino acid residues that constitute the binding site, which is the target amino acid residue for the molecular docking simulation of the test ligands. Therefore, it is expected that the test ligands will have similar interactions with the target amino acid residues as the natural ligand on a molecular level.



(a) (b)

Figure 1. a) Results of re-docking the natural ligand WRA609 on PfDHFR-TS enzyme, with an RMSD value of 1.09 Å. The green ligand represents the reference ligand, while the red ligand represents the re-docking result. b) Two-dimensional visualization of molecular interactions in the docking complex between PfDHFR-TS enzyme and the natural ligand WRA609 (6,6-Dimethyl-1-[3-(2,4,5-Trichlorophenoxy)Propoxy]-1,6-Dihydro-1,3,5-Triazine-2,4-diamine).

The results of molecular docking simulation using the test ligand on PfDHFR-TS enzyme were compared with the positive control, the antimalarial compound Pyrimethamine, which acts as an inhibitor on PfDHFR-TS enzyme. The simulation yielded a free energy of binding (ΔG) of -7.06 kcal/mol and an inhibition constant (Ki) of 6.72 μM for Pyrimethamine. These results are relatively higher compared to the free energy of binding (ΔG) of the natural ligand WRA609. The hydrogen bond interactions and hydrophobic amino acid residues of PfDHFR-TS with the Pyrimethamine test ligand can be observed in Table 2, and the visualization of interactions between PfDHFR-TS enzyme amino acid residues and Pyrimethamine ligand is shown in Figure 2a.



(a) (b)

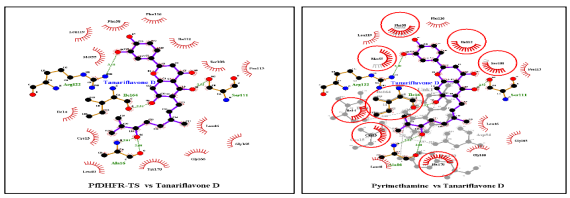
Figure 2. (a) Two-dimensional visualization of molecular docking interactions between Pyrimethamine and PfDHFR-TS enzyme. (b) Two-dimensional comparison visualization of molecular docking interactions between WRA609 and Pyrimethamine with PfDHFR-TS enzyme.

The two-dimensional visualization comparison of natural ligand WRA609 and the positive control Pyrimethamine (Figure 2b) shows that both compounds have the same hydrogen bonding interactions with amino acid residues Ile14, Cys15, Asp54, Ile164, and Tyr170 (residues marked in red circles). Thus, Pyrimethamine, used as the positive control, exhibits inhibitory activity against the PfDHFR-TS enzyme.

Table 2. Ligand molecular docking test results on the PfDHFR enzyme.

Ligands	CID PubChem	Bond free energy (ΔG) (kcal/mol)	The constant inhibits (Ki).	Hydrogen bond interactions	hydrophobic interactions
Pyrimethamine	4993	-7.06	6,72 µM	Ile14 (2,60 Å); Cys15 (2,96 Å); Asp54 (3,03 Å); Ile164 (3,08 Å); Tyr170 (2,89 Å)	Ile112; Ser108; Phe58; Met55
Nymphaeol A	639465	-9.04	235,83 nM	Arg122 (2,91 Å); Ser111 (2,74 Å)	Cys59; Leu119; Phe58; Phe116; Met55; Ile112; Pro113; Ile164; Gly 165; Ser108; Leu40; Leu46; Trp48; Cys15; Ala16; Tyr170
Nymphaeol B	10387631	-8.82	343,07 nM	Asp54 (3,09 Å)	Pro113; Met55; Ile112; Cys15; Ile14; Ser111; Leu46; Phe58; Ile164; Ala16; Val95; Gly41; Tyr170; Leu40 ; Val45
Nymphaeol C	10323393	-10.54	18,72 nM	Ser108 (2,86 Å); Ser108 (2,79 Å); Ile164 (2,74 Å)	Tyr170; Gly166; Cys15; Gly165; Ile14; Leu46; Ser111; Thr185; Phe58; Ile112; Asp54; Cys59; Met55; Pro113; Arg122; Leu19; Phe16
Tanariflavanon A	11730903	-10.16	35,89 nM	Ile164 (2,60 Å); Ile164 (2,81 Å); Leu46 (2,85 Å)	Cys50; Met55; Lys49; Phe116; Cys15; Ala16; Tyr170; Ser111; Asp54; Ile112; Phe58; Ile14; Leu40; Val45; Ser108; Gly166; Gly165; Met104
Tanariflavanon D	11247668	-9.44	120,39 nM	Arg122 (3,10 Å); Ile164 (2,51 Å); Ser111 (2,52 Å); Ala16 (2,81 Å); Ala16 (2.88)	Phe116; Phe58; Leu119; Met55; Ile112; Ser108; Pro113; Ile14; Cys15; Leu46; Gly165; Gly166; Tyr170; Leu40

The results of molecular docking analysis using potential antimalarial compounds from the Macaranga plant (*Macaranga tanarius*), including Nymphaeol A, Nymphaeol B, Nymphaeol C, Tanariflavanone A, and Tanariflavanone D, can be seen in Table 2. The results indicate that the test compound Tanariflavanone D has a free binding energy (ΔG) of -9.44 kcal/mol and an inhibition constant (Ki) of 120.39 nM. Although the test compounds Nymphaeol A, Nymphaeol B, Nymphaeol C, and Tanariflavanone A have lower free binding energies (ΔG), they form more hydrogen bond interactions with the PfDHFR-TS enzyme [(Arg122 (3.10 Å); Ile164 (2.51 Å); Ser111 (2.52 Å); Ala16 (2.81 Å); Ala16 (2.88)], supporting the stability of the protein-ligand complex. This is further supported by the hydrogen bond distances between PfDHFR amino acid residues and Tanariflavanone D, which range from 2.51 to 3.10 Å (Figure 4). (15) The stability of the interactions between PfDHFR-TS amino acid residues and Tanariflavanone D is also supported by hydrophobic interactions. (20) A comparison of Tanariflavanone D with the positive control Pyrimethamine shows that Tanariflavanone D forms only one hydrogen bond with the Ile164 amino acid residue (Figure 3b). However, the test ligand is capable of forming hydrogen bonds with other amino acid residues to achieve stability and a low free binding energy (ΔG) with the PfDHFR-TS enzyme residues.



(a) (b)

Figure 3. (a) Two-dimensional visualization of molecular interactions in the docking complex of Tanariflavanone D with PfDHFR-TR enzyme. (b) Two-dimensional visualization of molecular interactions in the docking complex of Pyrimethamine and Tanariflavanone D with PfDHFR enzyme.

Conclution

The molecular docking results of potential antimalarial compounds from the Macaranga plant revealed the compound Tanariflavanone D, which has a binding free energy (ΔG) of -9.44 kcal/mol. This energy is lower than the natural compound WRA609 with a ΔG of -8.44 kcal/mol and the positive control compound Pyrimethamine with a ΔG of -7.04 kcal/mol. Tanariflavanone D showed hydrogen bond interactions with amino acid residues of the PfDHFR-TS enzyme, including Arg122 (3.10 Å), Ile164 (2.51 Å), Ser111 (2.52 Å), Ala16 (2.81 Å), and Ala16 (2.88 Å).

Conflict Of Interest: Authors Declares No Conflict of Interest

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