

Case Report

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The active compound of the sponge *Niphates olemda* against *Plasmodium falciparum* phosphoethanolamine methyltransferase (pfpmt) based on in silico study

Frederick¹, Prawesty Diah Utami¹, Olivia Mahardani Adam¹, Fitri Handajani¹

Abstract

Antimalarial drug resistance to *P. falciparum* has become a global problem in recent decades. This encourages the need for exploration to find alternative treatments, which come from marine product like Sponge *Niphates olemda* that contain active substrat. This research was conducted to determine the activity sponge *Niphates olemda* on *P. falciparum* phosphoethanolamine methyltransferase (PFPMT) through an in silico study. This research is a pure experimental research using the One Shot Experimental Study research design method. Observations were only made once between the variables studied through three analyzes, namely molecular docking analysis, ADME prediction analysis, and active compound toxicity analysis. The results showed that there were 4 active compounds (Niphateolide A, Kapakahine A, Kapakahine B, and Kapakahine F) that had a better binding affinity than artemisinin so that they had antimalarial potential. However, only Niphateolide A met Lipinski's criteria in ADME analysis, but was more toxic than the other three active compounds and also artemisinin. Based on the results of molecular docking analysis, it was found that the active compound Niphateolide A from the sea sponge *Niphates olemda* has antimalarial potential on target protein *Plasmodium falciparum* phosphoethanolamine methyltransferase.

Keywords: niphates olemda; active compounds; pfpmt; in silico

Correspondence:

Prawesty Diah Utami
prawesty.diah@hangtuah.ac.id

1. Faculty of Medicine, Universitas Hang Tuah Surabaya, Surabaya, Indonesia

Introduction

Malaria is a continuous health problem occurring worldwide, especially in developing countries like Indonesia. In 2020, it was estimated that there were 241 million malaria cases worldwide. Indonesia holds the second highest ranking (after India) in Southeast Asia for the number of malaria cases, according to the World Health Organization (WHO) in the World Malaria Report 2020. Although there was a decline between 2010 and 2014, malaria cases in Indonesia tended to stabilize from 2014 to 2019.¹ The cause of the transmission of the *Plasmodium* parasite in humans is through the bite of an infected female *Anopheles* mosquito.²

The trend of malaria cases in Indonesia has generally been declining since 2018. However, cases increased again in 2019, reaching 250,628 cases. Then, the number of cases decreased in 2020

and continued to decline in 2021.³ In 2010, the number of positive malaria cases in Indonesia reached 465.7 thousand; in 2020, the number of positive instances decreased to 235.7 thousand. The cause of malaria is 86.4% due to *Plasmodium falciparum* and 6.9% due to *Plasmodium vivax*.⁴ Infections caused by *P. falciparum* can lead to more severe clinical manifestations, such as severe anaemia and organ dysfunction, which can result in death.⁵ The mortality of the mother and fetus during pregnancy can also occur due to *P. falciparum* parasitic infection, as the parasite can infect the fetus through the placenta of the infected mother.⁶ Malaria is even one of the contributing factors to the occurrence of dementia in people in developing countries.⁷ The treatment for malaria-infected patients with *P. falciparum* and *P. vivax* is essentially the same, involving the administration of artemisinin-based combination therapies (ACT). This is the standard therapy for all malaria cases, as per WHO guidelines. However, the discovery of

ACT resistance in malaria patients over time has escalated into a global issue. The resistance of antimalarial drugs to *P. falciparum*, especially in Southeast Asia, has become a significant concern in recent decades.

With the emergence of artemisinin resistance, there is a need for an alternative to malaria therapy so that malaria management can be more effective. The alternative could utilize various natural resources, especially marine ones, which are abundant in Indonesia. Indonesia has many natural resources, especially aquatic biota, that have yet to be explored. Indonesia's aquatic biota is abundant in freshwater and the sea. One of them is the sponge *Niphates olemda*.

Method

This research is an experimental study using the One Shot Experimental Study design method. Observations were conducted only once between the variables studied through three analyses: molecular docking analysis, ADME prediction analysis, and toxicity analysis of the active compounds. The research, conducted at the esteemed Bioinformatics Laboratory, CV Delta Science, Malang, East Java, Indonesia from May to August 2022, holds significant promise. The subject of this research is the active compounds found in *Niphates olemda*, namely Niphateolide A, Kapakahine A, Kapakahine B, and Kapakahine F. They were then analyzed using molecular docking methods, ADME prediction, and toxicity prediction.

Results and Discussion

In this research, the data on the active compound Niphateolide A from the sponge *Niphates olemda* and the structure of *Plasmodium falciparum* phosphoethanolamine methyltransferase (PfPMT) were obtained from (<https://pubchem.ncbi.nlm.nih.gov/>). Molecular docking methods are conducted to observe the binding capability of active compounds to the target protein. The prediction of the interaction strength between receptors and ligands can be observed from the binding affinity value. The more negative the value, the stronger the interaction that occurs between the receptor and the ligand. If the tested active compound has a score close to the control, it can be predicted that the active compound may act as an antagonist to the target protein. The docking conducted on PfPMT shows that all active compounds have a greater binding affinity compared to artemisinin used as a control.

PfPMT is an enzyme in *P. falciparum* that plays a role in maintaining the survival of *P. falciparum* by synthesizing PC. This process occurs through SDPM, where PC is formed to quickly produce new membranes, thereby supporting the rapid multiplication of the parasite not only during the intraerythrocytic cycle but also

during the development of gametocytes.⁸ The molecular docking results for the active compound *Niphates olemda* are shown in Figures 1 to 5 and Table 1. In addition to binding affinity, it can also be observed from Figures 1 to 5 that in the binding site of the control and the compound Niphateolide A, there are similarities in the residues TYR160, TYR27, and ILE36, which have been identified as residues bound by artemisinin. However, for the binding site of kapakahine F, the similarity is only found in the residue TYR27. Meanwhile, no common inhibited residues were identified in the compounds kapakahine A and kapakahine B.

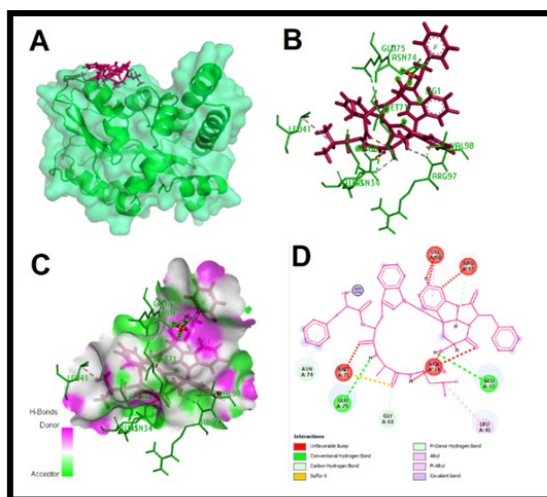


Figure 1. Interaction between artemisinin and *PfPMT*,

A – B. 3D complex display, C. Complex hydrogen bond, D. 2D complex display, green indicates the *P. falciparum* phosphoethanolamine methyltransferase protein, pink indicates the compound.

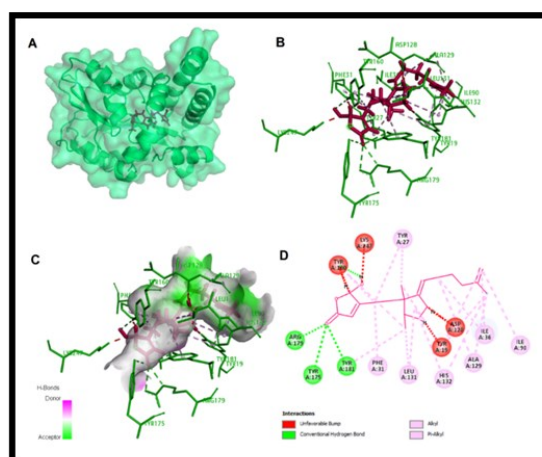


Figure 2. Interaction between *Niphateolide A* and *PfPMT*

A – B. 3D complex display, C. Complex hydrogen bond, D. 2D complex display, green indicates the *P. falciparum* phosphoethanolamine methyltransferase protein, pink indicates the compound.

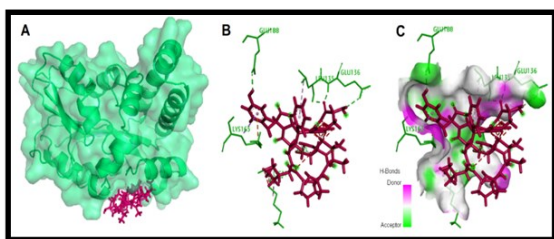


Figure 3. Interaction between *Kapakahine A* and *PfPMT*

A – B. Complex 3D Display, C. Complex Hydrogen Bonds, green indicates the protein *P. falciparum* phosphoethanolamine methyltransferase, pink indicates the compound.

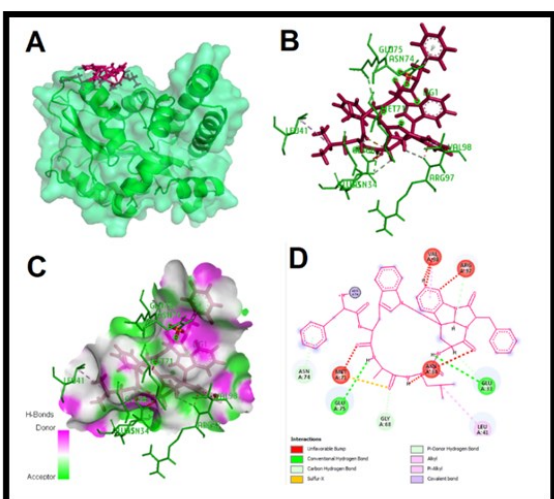


Figure 4. Interaction between *Kapakahine B* and *PfPMT*

A – B. 3D complex display, C. Complex hydrogen bond, D. 2D complex display, green indicates the *P. falciparum* phosphoethanolamine methyltransferase protein, pink indicates the compound.

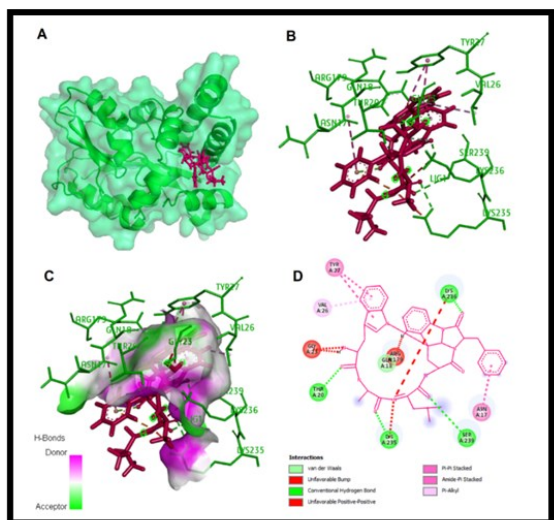


Figure 5. Interaction between *Kapakahine F* and *PfPMT*

A – B. 3D Complex View, C. Complex Hydrogen Bonding, D. 2D Complex View, green

indicates the *P. falciparum* phosphoethanolamine methyl transferase protein, pink indicates the compound.

Table 1. Molecular docking between PfPMT and ligand

No	Componen	Skor binding affinity (kJ/mol)
1	Artemisin	-148
2	Niphateolide A	-252.5
3	Kapakahine A	-281.5
4	Kapakahine B	-276
5	Kapakahine F	-265.67

The ADME prediction results for the active compound Niphates olema, obtained using the SWISS ADME webserver (<http://www.swissadme.ch/index.php>), provide crucial insights into the physicochemical and pharmacokinetic properties of potentially medicated compounds. This knowledge is instrumental in understanding the compound's absorption, distribution, metabolism, and excretion, thereby enhancing our understanding of its potential therapeutic applications.

By adhering to Lipinski's rule of five,⁹ we can confidently determine the potential of a compound for absorption. The criteria, which include not more than 5 hydrogen bond donors, no more than ten hydrogen bond acceptors, a Molecular Weight less than 500 g/mol, and a Log P (CLogP) not greater than 5 (or MlogP no more than 4.15), guided us to the discovery that Niphateolide A is the active compound that meets these stringent standards.

Table 2. ADME prediction

Compound	Niphateolide A	Kapakahine A	Kapakahine B	Kapakahine F	Artemisin
Weight (g/mol)	318.45	1053.25	848.99	70.81	262.30
Hydrogen Bond Acceptors	3	10	7	6	4
Hydrogen Bond Donors	1	6	5	4	1
Atom number	23	77 (arom. 21)	63 (arom. 27)	52 (arom. 21)	19
Water solubility	soluble	Non soluble	Non soluble	Non soluble	soluble
GI Absorption	High	Low	Low	Low	High
BBB permeant	+	-	-	-	+
Bioavailability	0.55	0.17	0.17	0.17	0.55
Log P	4.43	2.44	2.99	2.00	1.53

Lipinski rule of 5:
 No more than 5 hydrogen bond donors
 No more than 10 hydrogen bond acceptors
 Molecular weight less than 500 g/mol
 Log P (CLogP) not more than 5 (or MlogP not more than 4.15)

To predict the toxicity of active compounds, we utilized the Pro-Tox webserver (http://tox.charite.de/protox_II/), a widely recognized tool in the field of toxicology. This webserver allows us to input a toxic dose parameter or Lethal Dose 50 (LD50) in mg/kg of weight and a toxicity class according to the chemical labelling system globally harmonized system (GHS)¹⁰. The classification of toxicity of the compound itself is divided into six

classes: class one fatal when swallowed ($LD50 \leq 5$), class two fatal if swelled ($5 < LD50 \leq 50$), class three toxic when swallowed ($50 < LD50 \leq 300$), class four hazardous when swollen ($300 < LD50 \leq 2000$), class fifth can be dangerous if swollen ($2000 < LD50, \leq 5000$), class six non-toxic ($LD50, > 5000$). (ProTox-II - Prediction of TOXicity of Chemicals, n.d.). The toxicity prediction results can be seen in Table 3.

Table 3. Toxicity analysis of the active compound

Compound	Niphateolide A	Kapakahine A	Kapakahine B	Kapakahine F	Artemisin
Prediction LD50 (mg/kgBW)	34	200	200	200	900
Toxicity prediction (class)	2	3	3	3	4

The study explored the potential for inhibition of Niphateolide A active compound from *Niphates olemda* sea sponge against PfPMT receptors from *Plasmodium falciparum* in silico study methods. The results of the analysis predicted the potential of the active composite of *Niphates olemda* as an antimalaria using the molecular method of docking the active Compound against PFPMT, the analysis of absorption, distribution, metabolism, expression (ADME) as well as the toxicity test of active marine sponge *Niphates olemda*.

The active compound found in *Niphates olemda* is known to this day to be Niphateolide A, Kapakahine A, kapakahine B, and Kapahine F. Niphathene A is a molecular composition filled with the structure of C₂₀H₃₀O₃ obtained from the extract of sea sponge, namely *Niphates olemda*. For its therapeutic effect, what is now known is that this compound acts as an anti-cancer agent.¹² Meanwhile, kapakahine is a cyclic peptide isolated from the *Niphates olemda* sponge and obtained

through the partition between ethyl acetate and water through the Kupchan procedure. Kapakahine has more than one type and has a unique structural feature; namely, the two tryptophan residues (Trp-1 and Trp-2) are not connected by amide bonds but by N-C bonds of the Trp-1 indole nitrogen to the trp-2 indole beta carbon.¹¹

Molecular docking is the most popular method of computational drug design, based on the concept of "wall and key" created by Emil Fischer (1894), and enables large-scale prediction of whether and how small molecules bind macromolecular targets. The binding force produced by these two molecules is called binding affinity.¹³ There will be three kinds of bonds on docking: hydrogen bonds, hydrophobic interactions, and unfavorable bonds. Hydrogen bonds play a role in the strength of the protein-ligand bond, whereas hydrophobic interactions play a part in stabilizing the bonds that occur. So, both hydrogen binding and hydrophobic interfaces

play a part in the value of the binding affinity.¹⁴ An unfavorable bump can be interpreted as a less well-binding area as an inhibitor because there are some other effects of residues that are less or cannot be taken into account by the docking application. Binding affinity is generally expressed in the dissociation constant (Kd), inhibition constant (Ki) or maximum half-inhibition concentration (IC50). The IC50 value depends on the target and ligand concentration, and a low IC50 means a high binding affinity. Similarly, a low inhibitory constant means a higher binding Affinity. Generally speaking, the value of the dissociation constant and the inhibition constant, respectively, are expressed as negative logarithms. Therefore, the more negative the binding value of affinity, the stronger the bond.¹⁵

The molecular docking tests conducted on PfPMT target proteins obtained docking strength values of each compound: Niphateolide A (-252.5), Kapakahine A (-281.5), Kapahine B (-276), and Kapahine F (-265.7) with artemisinin control (-184). These values are based on complex hydrogen bonds through the Docker virtual Molegro program 5.0 with the Molecular surface van der Waals parameter maximum 5.¹³

The molecular docking done on the active compounds Niphateolide A, Kapakahine A, Kapakahine B, and Kapahine F aims to see the active substance's ability to inhibit PfPMT so that no PC is formed, which will indirectly inhibit the parasite's multiplication. If the PC is not synthesized, it will cause a significant reduction in multiplication, which can lead to total inhibition of SDPM.¹⁶

The ADME prediction must meet Lipinski's rule of five.⁹ So that a compound can be determined to be well absorbed or not, it must first meet all the criteria. Lipinski criteria not more than five hydrogen bond donors, no more than ten hydrogen bond acceptors, Molecular Weight less than 500 g/mol, Log P (CLogP) not greater than 5 (or MlogP not more than 4.15). Niphateolide A is also water-soluble and has a high GI absorption and bioavailability. Kapakahine A does not meet the Lipinski criteria because of its molecular weight greater than 500 g/mol, Hydrogen Bond Donors more than 5. It is not water-soluble, absorptive rate in GI is low, does not penetrate the Blood Brain Barrier and low bioavailability, so it is less suitable for use as a drug. The Kapakahine B compound does not meet the Lipinski criteria because the molecular weight exceeds 500 g/mol.

The Kapakahine F compound does not meet the Lipinski criteria because its molecular weight is greater than 500 g/mol.

Finally, there is a study for toxicity testing. In this trial, the compound Niphateolide A has the lowest LD50 value of 34 mg/kgBB, so it falls into class 2 and is fatal when swallowed. The compounds Kapakahine A, Kapkahine B, and Kapahine F have equally low LD50 of 200 mg/

kgBB in class 3. Compared to artemisinin, which has an LD50 of 900 mg/kgBB, the most toxic compound is Niphateolide A.

Conclusion

Based on the test of the active Compound found in the Niphates olema sea sponge against the PfPMT receptor on Plasmodium falciparum based on in silico studies, it can be concluded that the active CompoundCompound in the Sea sponge has an inhibitory effect on the Plasmodium falciparum protein of the PfPMT Receptor based on the molecular docking analysis done. Molecular analysis of the docking showed that all the active components of the sea sponges have a higher binding affinity than artemisinin, thus showing a higher inhibition potential for PfPMT. The predictive analysis of ADME of the Seas Sponge Niphate olemda protein demonstrated that Niphateolide A has the best ADME capabilities, almost identical to artemisinin, because it has sufficient water solubility as well as a molecule weight of less than 500 g/mol, so that the compounds are quickly suppressed from the body, high GI absorption, and can be used for Brain malaria, which is highly cerebral.

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