ISSN 0974-3618 (Print)
0974-360X (Online)

www.rjptonline.org

RESEARCH ARTICLE

Search for Lead Compounds and *In Vitro* Assay of Potential Inhibitors of *Plasmodium falciparum* Prolyl-tRNA Synthetase from Natural Compound Database

Rosita Handayani[1]*, Tegar Achsendo Yuniarta[2], Sukardiman[1], Aiyi Asnawi[3]
[1]Pharmaceutical Science Department, Faculty of Pharmacy,
Universitas Airlangga, Surabaya, East Java, 60115, Indonesia.
[2]Faculty of Pharmacy, Universitas Surabaya, Surabaya, East Java, 60293, Indonesia.
[3]Department of Pharmacochemistry, Faculty of Pharmacy,
Universitas Bhakti Kencana, Jl. Soekarno Hatta No.754, Bandung, West Java, 40617, Indonesia.
*Corresponding Author E-mail: **rosita.handayani@ff.unair.ac.id**

ABSTRACT:

Background: Malaria continues to be a serious problem in several countries, marked by an increase in the number of cases and a high morbidity rate. One of the commonly adopted strategies in drug discovery is by performing compound screening using computational tools, known as virtual screening. This technique allows one to screen multitudes of chemical compounds *in silico*, thus saving cost and time by reducing the amount of tested compound *in vitro*. Recently, *P. falciparum* prolyl-tRNA synthetase (PfPRS) is one of the top priority targets to be explored of potent inhibitors. This enzyme plays an important role in attaching L-proline into tRNA, which then will be incorporated into protein sequence. Its inhibition would halt the protein synthesis and kill the parasite. **Methods:** Hierarchical virtual screening was performed against PfPRS enzyme using 2D followed by 3D similarity method implemented in Infinisee 3.2.0 and SeeSAR 12.1.0, respectively. *1-(pyridin-4-yl) pyrrolidin-2-one* based analog, which was previously discovered as potent antimalarial agent, was used as template to screen potential hits from Molport Database of Purchasable Natural Product Compounds. Compounds with high similarity value were evaluated by molecular docking using SeeSAR 12.1.0 approach. The best scoring compounds were subjected into ADMET prediction, molecular dynamics simulation, and *in vitro* assay against *P. falciparum*. **Results:** Two compounds were obtained from virtual screening and molecular docking process, with predicted IC_{50} value lies on micromolar and nanomolar range. These compounds also satisfy ADMET characteristics in general as well as showing stability during 100 ns molecular dynamics simulation. Bioassay study showed that both compounds yielded < 10 µg/mL inhibitory concentration. **Conclusion:** This study has discovered two novel compounds using *in silico* approach, which can be further developed as potential antimalarial agents.

KEYWORDS: Antimalarial, 3D7, Prolyl-tRNA synthetase, *Plasmodium falciparum*, Virtual screening.

Received on 07.01.2024 Revised on 13.07.2024
Accepted on 21.10.2024 Published on 28.01.2025
Available online from February 27, 2025
Research J. Pharmacy and Technology. 2025;18(2):529-536.
DOI: 10.52711/0974-360X.2025.00079

INTRODUCTION:

Malaria continues to be a serious problem in several countries, marked by an increase in the number of cases and a high morbidity rate. This situation has been exacerbated by disruptions in healthcare services due to the COVID-19 pandemic[1]. Until the year 2020, malaria was estimated to have caused 627,000 deaths and 241 million cases, with 77% of the deaths in children under 5 years old[2]. Malaria could potentially experience a resurgence under conducive circumstances, and recurrence may manifest at any juncture in an individual's lifespan subsequent to infection with the

parasite, even after successful treatment and clearance of parasitemia through the administration of antimalarial medication[3]. The World Health Organization (WHO) has set strategic goals to create a malaria vaccine with an efficacy of over 75% by the year 2030. However, this goal remains a significant challenge as the vaccines developed so far have not achieved the expected efficacy[1]. In addition to vector control, artemisinin-based combination therapy (ACTs) has become the gold standard for treatment and has played a crucial role in malaria control for the past 20 years[4]. However, new issues have arisen as the *Plasmodium falciparum* parasite has become resistant to artemisinin in the Southeast Asia region[5,6], leading to a decrease in the efficacy of ACTs in malaria treatment[7,8]. The heightened mortality and morbidity rates in malaria are principally attributed to the resistance exhibited by *P. falciparum* towards conventional antimalarial medications[9,10].

One of the commonly adopted strategies in drug discovery is by performing compound screening using computational tools, known as virtual screening. This technique has become an integral part of the process of designing and developing new drugs due to its advantages in terms of time and cost efficiency compared to conventional biological activity screening, thus conserving available resources[11]. Protein target selection is crucial in virtual screening to ensure the compatibility of the results with in vitro and in vivo testing. MalDA (Malaria Drug Accelerator), an international consortium of 17 malaria research groups, has mapped out proteins important for malaria treatment across different priority levels[12]. The highest level in this mapping is referred to as "high priority protein target," which signifies proteins with the highest validation level and great potential to become effective drug targets. Consequently, proteins at this level are worthy of prioritization as targets for testing in order to develop effective drugs. The criteria for suitability are supported by data indicating that these proteins play essential roles in the development or pathophysiology of malaria, affecting the survival and reproduction of Plasmodium parasites, and being selective for *Plasmodium* species.

One protein included in the high priority protein category according to the consortium is Prolyl-tRNA Synthetase (PRS), a type of enzyme belonging to the Aminoacyl-tRNA synthetase (aaRS) family. The PRS enzyme plays a crucial role in the protein translation process of Plasmodium falciparum by catalyzing the activation of proline via aminoacylation reaction with tRNA. This step is necessary to transfer the said amino acid to growing protein. Inhibiting the PRS enzyme would halt the replication and metabolic cycle of Plasmodium in the blood, thereby interrupting the progression of malaria[13]. One of the interesting inhibitors is the pyridine-pyrrolidinone group (Figure 1). This class of compounds has recently been found to exhibit promising inhibitory activity against the PRS enzyme of *Plasmodium falciparum.* These compounds are believed to occupy the active site of ATP, a coenzyme that aids in the aminoacylation reaction between proline and tRNA[14].

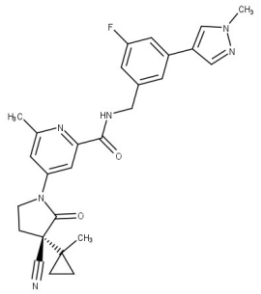


Figure 1. Structure of pyridine-pirrolidinone based compound (Compound A) with high potency against PRS enzyme in *Plasmodium falciparum*.

This research is conducted to identify potential new drug compounds from natural sources that could inhibit the PRS enzyme. The process of selecting active compounds is carried out through virtual screening of hundreds of thousands of existing compounds, resulting in the identification of two lead compounds that exhibit high binding affinity with the PRS enzyme. Activity confirmation is performed through molecular dynamic simulations and in vitro tests on Plasmodium-infected blood cells. In addition, evaluation of various pharmacokinetics aspect was performed *in silico* to assess the feasibility of the compounds to be developed as oral drugs.

MATERIAL AND METHODS:

2D Similarity-based virtual screening:

Compound A in SMILES format was used as a template for 2D similarity-based virtual screening in infiniSee 3.2.0. This software applied Feature Trees algorithm, which represents molecules as a graph-like system. The alignment of screened molecule towards template provides a pattern of corresponding substructure, which then further evaluated based on their property to yield similarity score[15]. MolPort Purchasable Natural Compounds was used as a source for screened compounds. The number of compounds obtained from this step was limited to 500, which satisfied similarity threshold of 0.8 and total scaffold diversity to each other (*i.e.* Total Diversity = 1).

3D Similarity-based virtual screening:

Compound A (Figure 1), which was previously built in SMILES format, was converted into 3D structure and used as a template for 3D similarity-based virtual screening in SeeSAR 12.1.0 (BioSolveIT, 2022). This software implemented FlexS algorithm, which aligns 3D structure of template molecule with screened molecule

in an incremental fashion. The molecules were first decomposed into smaller fragments, then the first fragment of two molecules were aligned. These so-called anchor fragments were then added by the remaining respective fragments, and the alignment result was evaluated according to various parameters (e.g. intramolecular interaction, physicochemical properties, etc.)[16]. This approach was used to filter 500 compounds obtained from the previous step, with similarity score of 0.6 as minimum acceptable value.

Molecular Docking:

Molecular docking step was performed against prolyl-tRNA synthetase *P. falciparum* (PDB ID: 4YDQ)[17] using SeeSAR version 12.1.0; BioSolveIT GmbH, Sankt Augustin, Germany, 2023, www.biosolveit.de/SeeSAR. Previously, the protein was prepared using PDB Reader module[18] in CHARMM-GUI webserver[19]. This step was to ensure the completeness of amino acid residues in the protein. ATP binding site was defined as docking target, since it is postulated that compound A worked as enzyme inhibitor in the particular site[14]. The compounds obtained from the previous step were docked using FlexX algorithm. This algorithm works in an identical way with FlexS, in which the docked compound is partitioned into smaller fragments and slowly built back to its original structure. The evaluation of ligand fitness in binding site takes place at each fragment addition[20]. In this study, each docked compound was expected to generate 20 binding poses which were then evaluated using HYDE scoring function. HYDE scoring function is able to not only estimate ligand binding affinity, but also visualize score contribution from each individual atom. In addition, this scoring function considers only hydrogen bond formation and desolvation energy between ligand-protein, without any specific weighting nor calibrated into specific target[21]. Ultimately, ligand-receptor interaction was evaluated using Discovery Studio Visualizer 2021.

Molecular Dynamics and MM-GBSA:

Molecular dynamics simulation was performed for best-scoring ligand-protein complex. The step was done in Gromacs 2021.3 for 100 ns. Ligand topology was constructed using General AMBER Force-Field (GAFF)[22] with the aid of ACPYPE[23], whereas the protein topology was built using AMBER99SB-ILDN[24]. Protein-ligand complexes were built inside triclinic space and solvated with TIP3P water[25]. Neutralization of the systems was achieved with the introduction of Na^+ or Cl^- ions. NVT and NPT equilibrations were performed for 100 ps, prior to the production of molecular dynamics simulation. In addition, free energy binding calculation was also performed using MM-GBSA approach implemented in gmx_MMPBSA[26]. Complete simulation trajectories were used for the calculation, where it consists of the molecular mechanics and solvation energy.

ADMET Evaluation:

ADMET parameters of best compounds were evaluated *in silico* using ADMETLab 2.0 webserver[27]. Several aspects were checked such as drug-likeness using Lipinski rule of five[28] and Veber rule[29], absorption, blood-brain barrier penetration, cytochrome P_{450} 3A4 interaction, half-life excretion, and hepatotoxicity parameters (human hepatotoxicity and drug induced liver injury).

Antimalarial Bioassay:

In this study, *Plasmodium falciparum* strain 3D7 (known for its sensitivity to chloroquine) was cultivated employing the Trager and Jensen method[30]. The cultures were cultivated in human O+ red blood cells with 5% hematocrit in RPMI 1640 (Gibco BRL, USA) supplemented with 22.3mM HEPES (Sigma), hypoxanthine, sodium bicarbonate, and 10% human O+ plasma. As a positive control, chloroquine diphosphate was used. Antimalarial assay was conducted in a 24-well microplate. Each well was seeded with 1% initial and experimental parasitemia (1mL/well of suspension). The plates were then incubated in a multi gas incubator at 37°C for 48 hours. Following incubation, samples were gathered and used to create a thin smear on a glass slide, which was subsequently fixed with methanol and stained using Giemsa. Parasite count was observed under a microscope and juxtaposed with the negative control to determine the percentage of parasite growth inhibition. The IC_{50} value was computed utilizing Probit Analysis [31].

RESULT AND DISCUSSION:

Similarity-based virtual screening:

Similarity score is often used as an approach in virtual screening for its speed in producing result, particularly against large chemical database[32]. This approach works according to similar property principle, which stated that two similar molecules should possess identical bioactivity[33]. Here, 2D and 3D similarity-based screening was implemented in hierarchical manner to filter the database into lesser number of compounds prior to further evaluation using a more rigorous method. These sequential approaches successfully yielded 14 hits, which satisfies the above-mentioned criteria (Table 1). Upon analyzing the result, it can be seen that majority of the hits possess spirocyclic moiety which are commonly found in natural product[34]. In addition, two other motifs were obtained from this step, which is coumarin[35] and quinazolinone[36]

Molecular Docking :

Molecular docking is a part of structure-based drug design method, which can be used to estimate the interaction between ligand and specific binding site [37]. This method was implemented in our subsequent step, where 14 compounds obtained were docked against ATP-binding site of *P. falciparum* prolyl-tRNA synthetase. [14,. 17] FlexX software had generated 280 docking poses, which then evaluated using HYDE algorithm. This method has yielded two most potential compounds which could possess antimalarial activity, based on their predicted bioactivity range (Compound 7 and Compound 9). HYDE algorithm provided estimated range of Ki value, from which Compound 7 yields micromolar activity and Compound 9 yields picomolar activity (Figure 1). It also provides mapping on each atom contribution to the final score, where green-colored atom indicating positive contribution, and red for negative one (Figure 2). Further analysis of overall molecular torsion shows that both docking pose were found to be energetically favorable (Figure 2), indicating conformer stability in docking process [38]. It was also found that both compounds were affected by both intra and intermolecular clash (Figure 3, yellow arrow). Upon visual inspection, steric clash was observed between carbonyl backbone of Ala476 and methyl moiety in methoxy group of two compounds. It is argued that replacement of methoxy group to hydroxyl could potentially eliminate this steric clash. Meanwhile, intramolecular clash was observed as shown in Figure 2 and functional group modification could also be performed to avoid the clash. HYDE algorithm also evaluated ligand-lipophilicity efficiency (LLE) value, where only Compound 9 yielded best result (Figure 2). This approach estimates the efficiency of ligand binding with respect to lipophilicity value. High LLE value means a ligand has low IC_{50} without possessing exorbitant lipophilicity [39]

Table 1. Hit compounds obtained from 2D and 3D similarity-based virtual screening.

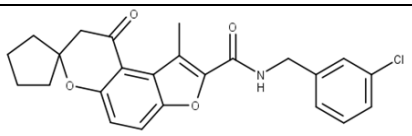
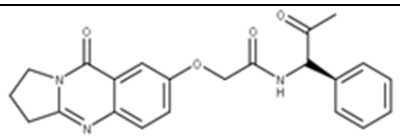
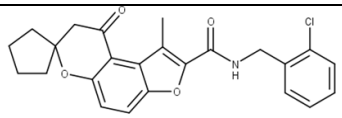
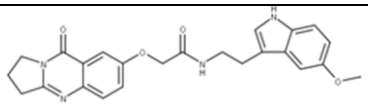
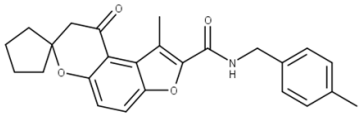
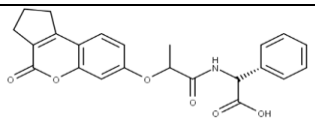
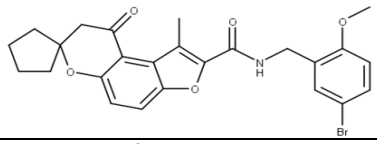
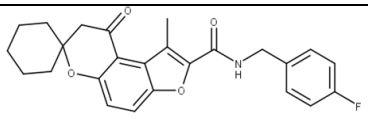
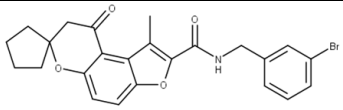
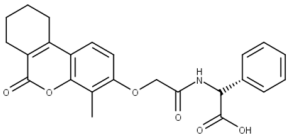
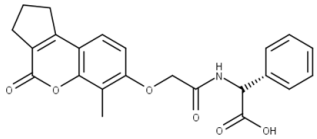
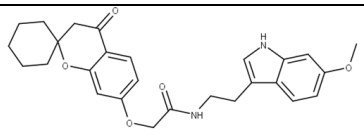
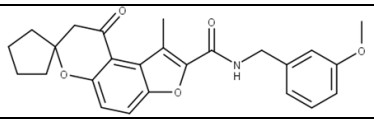
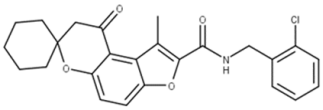
No	Hit Compounds		
1		8	
2		9	
3		10	
4		11	
5		12	
6		13	
7		14	

Figure 2. Molecular docking results using FlexX and HYDE approaches.

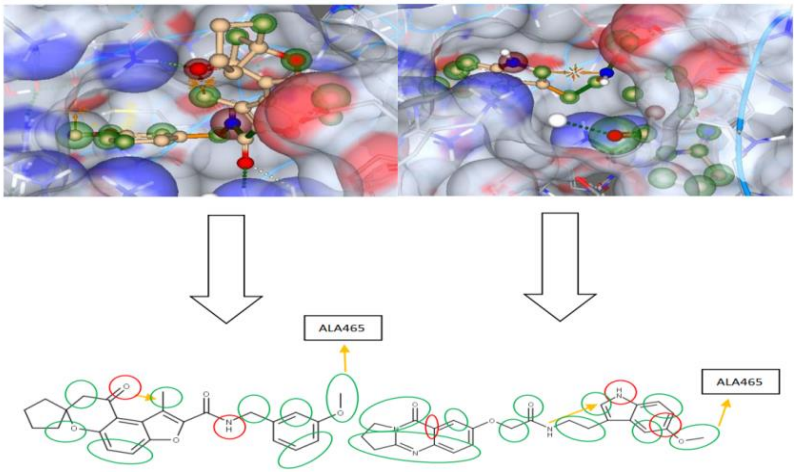


Figure 3. Docking pose and atomic contribution analysis in 3D and 2D of Compound 7 (left) and Compound 9 (right)

Table 2. Ligand-Receptor interaction observed in Discovery Studio Visualizer

Interaction	Compound 7	Compound 9
Hydrogen Bond	Arg390, Gln475, Thr478, Thr512	Arg390, Gln475, Thr478, His480, Thr512
C-H---O Hydrogen Bond	Ala476, Cys511	Glu338, Ala476, Cys511, Thr512
Metal Interaction (Mg^{2+})	-	C=O quinazolinone
π-Alkyl Interaction	Arg514	Phe335, Arg514
Alkyl-Alkyl Interaction	Arg514	Phe335, Pro358, Cys511, Arg514
π-Cation Interaction	-	Arg390
π- π Stacked Interaction	-	Phe335
Unfavorable Interaction	-	Glu452

Ultimately, specific ligand-protein interactions showed in Table 3. It can be seen that several amino acid residues play important role in ligand interaction, such as Arg390, Gln475, Thr478, His480, and Thr512 which makes hydrogen bond. It can also be seen that Compound 9 made a higher number of non-bonded interactions than Compound 7, which contributed to better docking score estimate. It is argued that tricyclic quinazolinone scaffold contributes to various hydrophobic and aromatic interactions with the binding site. However, Compound 9 also made metal interaction with magnesium cofactor, which was less observed in Compound X previously *via* molecular docking simulation[40]. In addition, different results were obtained in terms of steric clash interpretation. Previously, FlexX-HYDE algorithm interpreted Ala476 interaction with methoxy moiety as intramolecular clash (Figure 3). On the contrary, Discovery Studio Visualizer indicated it as carbon-hydrogen bond interaction (C-H---O) (Table 2). Further studies are needed to verify the correctness of this finding, since this interaction can play a significant role in determining ligand-protein binding, even if it is weaker than any other interaction[41].

Molecular Dynamics & MM-GBSA

Molecular dynamics simulation is often used as a tool to evaluate stability of ligand-protein binding[42]. Here, the two best docked complex were simulated for 100 ns in 298 K. The results indicated stability of both ligands during the simulation period, as can be seen from the ligand RMSD plot (Figure 4). Meanwhile RMSF plot showed high peaks in catalytic domain, anti-codon binding domain, and C-terminal domain, which indicate high flexibility in those area[17] (Figure 5). Subsequently, MM-GBSA calculation was performed in order to evaluate the ligand binding energy more accurately. As explained previously, MM-GBSA energy consists of the summation of molecular mechanics energy and solvation free energy (Generalized Born-Solvent Accessible Surface Area), which then subtracted by the change of conformational entropy [43]. The results showed that free-binding energy value of both compounds are in accordance with their respective docking score, where Compound 9 scored lower than Compound 7.

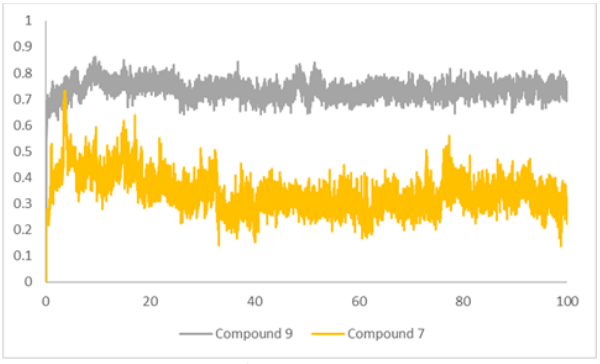


Figure 4. RMSD plot of compound 7 and 9 during 100 ns simulation (x-axis depicts simulation time (ns); y-axis depicts ligand RMSD (Å))

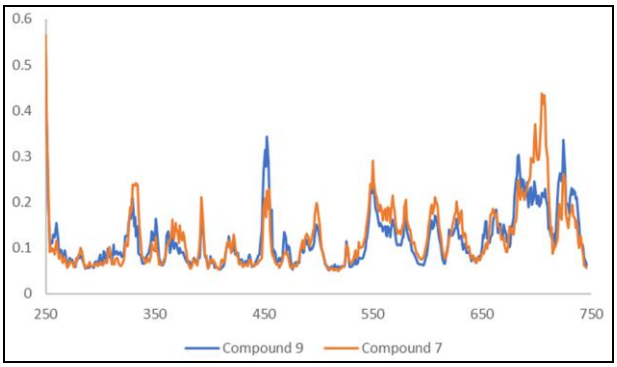


Figure 5. RMSF plot of compound 7 and 9 during 100 ns simulation (x-axis depicts amino acid residue number; y-axis depicts amino acid RMSD (Å))

Table 3. Free energy binding evaluation of compound 7 and 9

Delta Energy Component	Compound 7	Compound 9
Δ G Molecular-Mechanics	-57.54	-72.52
Δ G Solvation	23.58	19.94
Δ TOTAL	-33.97	-52.57

ADMET Evaluation:

ADMET evaluation of a hit compound from screening is a necessary step to avoid the late-stage attrition in drug discovery, since the failure of developing an effective compound often stems from inadequate ADMET characteristics. This approach is mostly used *in vitro* and *in vivo* assay and has been generally implemented in many pharmaceutical industries[44]. *In silico* approach can also be employed in the preliminary study[45]. In this study, both compound 7 and 9 were subjected to *in silico* drug-likeness and ADMET characterization. The majority of ADMETLab 2.0 results are presented in classification model using symbols (+) and (-) to indicate positive and negative probability, respectively. In addition, responses are categorized based on their probability score, where a more positive or negative symbol signifies a higher the probability of the corresponding result will take place[27].

Drug-likeness is a 'rule-of-thumb' concept to define chemical compounds that have sufficiently acceptable ADMET properties (Kerns & Di, 2007). Two widely used drug-likeness rules are Lipinski rule of five and Veber rule. Here, it can be seen that both compound 7 and 9 adhere to these rules. Further examination of ADMET results showed that both compounds exhibit acceptable absorption parameters based on CaCo-2 permeability and do not penetrate the blood-brain barrier. However, both compounds are found to be potential inhibitors of cytochrome P450 3A4. Considering the pivotal role of 3A4 isoform in metabolism of approximately half of the drugs in use[46], additional confirmation through *in vitro* and *in vivo* assay is needed to evaluate the effect they might induce. Half-life value ($T_{1/2}$) presented in ADMETLab 2.0 is a probability score derived from the combination of clearance and volume of distribution, ranging from 0 (short half-life) to 1 (long half-life)[27]. The results indicate that Compound 7 is predicted to possess shorter half-life than Compound 9, implying faster elimination rate. However, validation through pharmacokinetics assays is essential, given its impact on the dosing regimen. A drug with shorter half-life is more suitable for endemic areas, while the longer one is more adequate for prophylactic treatment[44]. From hepatotoxicity prediction, it can be seen that all compound could be hepatotoxic. This potential issue needs to be addressed, as it could impact drug candidate development, especially in clinical trial and post-marketing surveillance phases.

Antimalarial Bioassay:

The Trager and Jensen culture method involved using candle jars or cell flasks containing human red blood cells in a culture solution along with human serum and a specific gas mix high in CO_2 and low in O_2. Creating an environment that mimics physiological conditions, including temperature change, immune reactions, and gas composition, is crucial for in vitro[30,47]. In our *in vitro* study both compound 7 and compound 9 exhibited growth inhibition of the parasite in a concentration-dependent manner. The percentage inhibition data was further processed using Probit Analysis. From the testing, the IC_{50} values of compound 7 and compound 9 were found to be 5.78±0.8µg/mL and 9.38±0.5µg/mL, respectively. This inhibition potential is still lower compared to chloroquine, the standard drug, which showed an IC_{50} of 0.21±0.1µg/mL. However, both IC_{50} values of the tested compounds indicate values <10 µg/mL, considered active and potentially useful in inhibiting the growth of *Plasmodium falciparum*[48].

Table 4. ADMET Parameters of Compound 7 and 9

Compound	**Drug-Likeness**		**Absorption**	**Distribution**	**Metabolism**		**Excretion**	**Toxicity**	
	Lipinski Rule of Five	Veber Rule	CaCo-2 Permeability	Blood-Brain Barrier Penetration	CYP 3A4 Substrate	CYP 3A4 Inhibitor	$T_{1/2}$	Human Hepatotoxicity	Drug Induced Liver Injury
Compound 7	Pass	Pass	-4.808	No (--)	No (--)	Yes (+++)	0.060	Yes (++)	Yes (++)
Compound 9	Pass	Pass	-5.130	No (--)	No (--)	Yes (+++)	0.459	Yes (+++)	Yes (++)

Table 5. IC_{50} values of tested compounds against *P. falciparum* strain 3D7.

Compound name	% Inhibition at each concentration (µg/mL)						
	0 (negative control)	10	1	0.1	0.01	0.001	IC_{50}
Compound 7	0	55.6±0.3	37.0±1.6	29.2±0.6	23.7±0.6	12.6±0.9	5.78±0.8
Compound 9	0	52.1±1.8	32.6±0.6	22.3±0.3	12.9±1.8	2.8±0.6	9.38±0.5
Chloroquine	0	111.5±1.7	66.8±0.8	42.4±0.3	24.6±0.1	10.8±0.1	0.21±0.1

CONCLUSION:

Two compounds were obtained from virtual screening and molecular docking process, with predicted IC_{50} value lies on micromolar and nanomolar range. These compounds also satisfy ADMET characteristics in general as well as showing stability during 50 ns molecular dynamics simulation. Bioassay study showed that both compounds yielded <10 µg/mL inhibitory concentration, which can be further developed as potential antimalarial agents.

ACKNOWLEDGEMENT:

We would like to thank Airlangga University for funding and supporting this research trough Airlangga Research Fund Program 2023 with Grant Number 758/UN3.1.5/PT/2023.

REFERENCES:

1. Jagannathan P., Kakuru A. Malaria in 2022: Increasing challenges, cautious optimism. Nat Commun. 2022; 13(1): 2678, https://doi.org/10.1038/s41467-022-30133-w.
2. WHO. The 2020 edition of the World Malaria Report. vol. 73. 2020.
3. Agarwal AK., Yadav CP., Kuity P., Mishra J. Development of antimalarial pharmacotherapy and its importance in malaria treatment/public health program. Asian J Pharm Anal. 2022; 12(4): 233–42.
4. Arute JE., Agbamu E., Agare G., Achi CJ., Odili VU., Omomulere A. Physicians' Antimalarial Utilization Practices in Children below 5 years in a Secondary and Primary Health Facilities in Delta State. Res J Pharm Technol. 2022; 15(6): 2756–60.
5. Ashley EA., Dhorda M., Fairhurst RM., Amaratunga C., Lim P., Suon S., et al. Spread of artemisinin resistance in Plasmodium falciparum malaria. N Engl J Med. 2014; 371(5): 411–23, https://doi.org/10.1056/NEJMoa1314981.
6. Mutiah R., Badiah R., Hayati EK., Widyawaruyanti A. Activity of Antimalarial Compounds from Ethyl Acetate Fraction of Sunflower Leaves (Helianthus annuus L.) against Plasmodium falciparum Parasites 3D7 Strain. Asian J Pharm Technol. 2017; 7(2): 86–90.
7. Sharma R., Kumawat MK., Sharma GK. In-silico design and molecular docking studies of some novel 4-aminoquinoline-monastrol hybrids for their antimalarial activity. Res J Pharm Technol. 2022; 15(10): 4589–93.
8. Dubey S., Bhardwaj S., Parbhakaran P., Singh E. In silico Prediction of Pyrazoline Derivatives as Antimalarial agents. Asian J Pharm Res. 2022; 12(2): 119–24.
9. Suruse PB., Duragkar NJ., Shivhare UD., Raut SY., Warokar AS. Contribution of Traditional Medicines to the Development of Modern Medicine for Malaria. Res J Pharmacol Pharmacodyn. 2011; 3(1): 1–4.
10. Math AAK., Kaushik M., Krishnamoorthy E. Preliminary screening for cytotoxicity and antiplasmodial activity of fish protein hydrolysates on erythrocytes infected with Plasmodium falciparum 3D7. Res J Pharm Technol. 2023; 16(4): 1721–6.
11. Svensson F., Karlén A., Sköld C. Virtual screening data fusion using both structure- and ligand-based methods. J Chem Inf Model. 2012; 52(1): 225–32, https://doi.org/10.1021/ci2004835.
12. Forte B., Ottilie S., Plater A., Campo B., Dechering KJ., Gamo FJ., et al. Prioritization of Molecular Targets for Antimalarial Drug Discovery. ACS Infect Dis. 2021; 7(10): 2764–76, https://doi.org/10.1021/acsinfecdis.1c00322.
13. Manickam Y., Chaturvedi R., Babbar P., Malhotra N., Jain V., Sharma A. Drug targeting of one or more aminoacyl-tRNA synthetase in the malaria parasite Plasmodium falciparum. Drug Discov Today. 2018; 23(6): 1233–40, https://doi.org/10.1016/j.drudis.2018.01.050.
14. Okaniwa M., Shibata A., Ochida A., Akao Y., White KL., Shackleford DM., et al. Repositioning and Characterization of 1-(Pyridin-4-yl)pyrrolidin-2-one Derivatives as Plasmodium Cytoplasmic Prolyl-tRNA Synthetase Inhibitors. ACS Infect Dis. 2021; 7(6): 1680–9, https://doi.org/10.1021/acsinfecdis.1c00020.
15. Lessel U., Wellenzohn B., Lilienthal M., Claussen H. Searching Fragment Spaces with Feature Trees. J Chem Inf Model. 2009; 49(2): 270–9, https://doi.org/10.1021/ci800272a.
16. Lemmen C., Lengauer T. Time-efficient flexible superposition of medium-sized molecules. J Comput Aided Mol Des. 1997; 11(4): 357–68. https://doi.org/10.1023/A:1007959729800.
17. Jain V., Yogavel M., Oshima Y., Kikuchi H., Touquet B., Hakimi MA., et al. Structure of prolyl-tRNA synthetase-halofuginone complex provides basis for development of drugs against malaria and toxoplasmosis. Structure. 2015; 23(5): 819–29. https://doi.org/10.1016/j.str.2015.02.011.
18. Jo S., Cheng X., Lee J., Kim S., Park S-J., Patel DS., et al. CHARMM-GUI 10 years for biomolecular modeling and simulation. J Comput Chem. 2017; 38(15): 1114–24, https://doi.org/https://doi.org/10.1002/jcc.24660.
19. Jo S., Kim T., Iyer VG., Im W. CHARMM-GUI: A web-based graphical user interface for CHARMM. J Comput Chem. 2008; 29(11): 1859–65, https://doi.org/https://doi.org/10.1002/jcc.20945.
20. Rarey M., Kramer B., Lengauer T., Klebe G. A fast flexible docking method using an incremental construction algorithm. J Mol Biol. 1996; 261(3): 470–89, https://doi.org/10.1006/jmbi.1996.0477.
21. Schneider N., Lange G., Hindle S., Klein R., Rarey M. A consistent description of HYdrogen bond and DEhydration energies in protein-ligand complexes: methods behind the HYDE scoring function. J Comput Aided Mol Des. 2013; 27(1): 15–29, https://doi.org/10.1007/s10822-012-9626-2.
22. Wang J., Wolf RM., Caldwell JW., Kollman PA., Case DA. Development and testing of a general amber force field. J Comput Chem. 2004; 25(9): 1157–74, https://doi.org/https://doi.org/10.1002/jcc.20035.
23. Sousa da Silva AW., Vranken WF. ACPYPE - AnteChamber PYthon Parser interfacE. BMC Res Notes. 2012; 5: 367. https://doi.org/10.1186/1756-0500-5-367.
24. Lindorff-Larsen K., Piana S., Palmo K., Maragakis P., Klepeis JL., Dror RO., et al. Improved side-chain torsion potentials for the Amber ff99SB protein force field. Proteins. 2010; 78(8): 1950–8, https://doi.org/10.1002/prot.22711.
25. Jorgensen WL., Chandrasekhar J., Madura JD., Impey RW., Klein ML. Comparison of simple potential functions for simulating liquid water. \jcp. 1983; 79(2): 926–35, https://doi.org/10.1063/1.445869.
26. Valdés-Tresanco MS., Valdés-Tresanco ME., Valiente PA., Moreno E. gmx_MMPBSA: A New Tool to Perform End-State Free Energy Calculations with GROMACS. J Chem Theory Comput. 2021; 17(10): 6281–91. https://doi.org/10.1021/acs.jctc.1c00645.
27. Xiong G., Wu Z., Yi J., Fu L., Yang Z., Hsieh C., et al.

ADMETlab 2.0: an integrated online platform for accurate and comprehensive predictions of ADMET properties. Nucleic Acids Res. 2021; 49(W1): W5–14, https://doi.org/10.1093/nar/gkab255.
28. Lipinski CA., Lombardo F., Dominy BW., Feeney PJ. Experimental and computational approaches to estimate solubility and permeability in drug discovery and development settings. Adv Drug Deliv Rev. 1997; 23(1): 3–25. https://doi.org/https://doi.org/10.1016/S0169-409X(96)00423-1.
29. Veber DF., Johnson SR., Cheng H-Y., Smith BR., Ward KW., Kopple KD. Molecular properties that influence the oral bioavailability of drug candidates. J Med Chem. 2002; 45(12): 2615–23. https://doi.org/10.1021/jm020017n.
30. Trager W., Jensen JB. Human malaria parasites in continuous culture. Science. 1976; 193(4254): 673–5. https://doi.org/10.1126/science.781840.
31. Ekasari W., Widya Pratiwi D., Amanda Z., Suciati., Widyawaruyanti A., Arwati H. Various Parts of Helianthus annuus Plants as New Sources of Antimalarial Drugs. Evid Based Complement Alternat Med. 2019; 2019: 7390385, https://doi.org/10.1155/2019/7390385.
32. Hu G., Kuang G., Xiao W., Li W., Liu G., Tang Y. Performance Evaluation of 2D Fingerprint and 3D Shape Similarity Methods in Virtual Screening. J Chem Inf Model. 2012; 52(5): 1103–13. https://doi.org/10.1021/ci300030u.
33. Eckert H., Bajorath J. Molecular similarity analysis in virtual screening: foundations, limitations and novel approaches. Drug Discov Today. 2007; 12(5–6): 225–33. https://doi.org/10.1016/j.drudis.2007.01.011.
34. Chupakhin E., Babich O., Prosekov A., Asyakina L., Krasavin M. Spirocyclic motifs in natural products. Molecules. 2019; 24(22). https://doi.org/10.3390/molecules24224165.
35. Stefanachi A., Leonetti F., Pisani L., Catto M., Carotti A. Coumarin: A Natural, Privileged and Versatile Scaffold for Bioactive Compounds. Molecules. 2018; 23(2). https://doi.org/10.3390/molecules23020250.
36. Bhat M., Belagali SL., Mamatha S V., Sagar BK., Sekhar EV. Chapter 7 - Importance of quinazoline and quinazolinone derivatives in medicinal chemistry. In: Atta-ur-Rahman BT-S in NPC, editor. vol. 71. Elsevier; 2021. p. 185–219.
37. Ferreira LG., Dos Santos RN., Oliva G., Andricopulo AD. Molecular docking and structure-based drug design strategies. Molecules. 2015; 20(7): 13384–421, https://doi.org/10.3390/molecules200713384.
38. Kuhn B., Guba W., Hert J ér. ôm., Banner D., Bissantz C., Ceccarelli S., et al. A Real-World Perspective on Molecular Design. J Med Chem. 2016; 59(9): 4087–102, https://doi.org/10.1021/acs.jmedchem.5b01875.
39. Leeson PD., Springthorpe B. The influence of drug-like concepts on decision-making in medicinal chemistry. Nat Rev Drug Discov. 2007:881–90, https://doi.org/10.1038/nrd2445.
40. Yuniarta TA., Wawo JE., Kesuma D. Molecular Docking Study of 1- (Pyridin-4-YL)Pyrrolidine-2-One Derivate Against PROLYL-tRNA Synthetase in Plasmodium Falciparum. Pharmacoscript. 2022;5(2):157–71.
41. Bulusu G., Desiraju GR. Strong and Weak Hydrogen Bonds in Protein-Ligand Recognition. J Indian Inst Sci. 2020;100(1):31–41, https://doi.org/10.1007/s41745-019-00141-9.
42. Liu K., Watanabe E., Kokubo H. Exploring the stability of ligand binding modes to proteins by molecular dynamics simulations. J Comput Aided Mol Des. 2017; 31(2): 201–11, https://doi.org/10.1007/s10822-016-0005-2.
43. Tuccinardi T. What is the current value of MM/PBSA and MM/GBSA methods in drug discovery? Expert Opin Drug Discov. 2021;16(11):1233–7, https://doi.org/10.1080/17460441.2021.1942836.
44. Shearer TW., Smith KS., Diaz D., Asher C., Ramirez J. The Role of In Vitro ADME Assays in Antimalarial Drug Discovery and Development. Comb Chem High Throughput Screen. 2005;8(1).
45. Kar S., Roy K., Leszczynski J. In Silico Tools and Software to Predict ADMET of New Drug Candidates BT - In Silico Methods for Predicting Drug Toxicity. In: Benfenati E, editor. New York, NY: Springer US; 2022. p. 85–115.
46. Guengerich FP., Bartleson CJ. Principles and methods of toxicology. 1994.
47. Crispim M., Verdaguer IB., Silva SF., Katzin AM. Suitability of methods for Plasmodium falciparum cultivation in atmospheric air. Mem Inst Oswaldo Cruz. 2022;117(13):1–8, https://doi.org/10.1590/0074-02760210331.
48. O'Neill D H; Boardman, P; Phillipson, J D; Warhurst, D C MJB. Plants as Sources of Antimalarial Drugs Part. 1. In vitro Test Method for the Evaluation of Crude Extracts from Plants. Planta Med. 1985;51(05):394–8, https://doi.org/10.1055/s-2007-969529.