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Original Research Article

In Silico Investigation of Tropical Natural Product for Wild-Type and Quadrupole Mutant *Pf*DHFR Inhibitors as Antimalarial Candidates

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ABSTRACT

Plasmodium falciparum dihydrofolate reductase (*Pf*DHFR) is an essential enzyme in the development of parasitic DNA and its inhibition often leads to the impediment of parasite growth. Several studies have also shown that a genetic mutation in this enzyme can cause reduced receptor responsiveness and efficacy of antimalarial drugs. Therefore, this study aimed to examine 100 compounds derived from Indonesian medicinal plants as potential antimalarial candidates using a structure-based virtual screening approach. The PASS online was used to screen 100 compounds, and those with Pa values higher than 0.3 were docked with the wild-type (PDB code:1j3i) and quadrupole mutant *Pf*DHFR (PDB code:1j3k). The stability of the chemical complex was then examined using molecular dynamics simulations, followed by an assessment of the pharmacokinetic profile and drug-likeness parameters. The top 5 compounds were then identified with binding energies ranging from -9.7 to -10.0 kcal/mol for the wild-type *Pf*DHFR-TS and from -9.2 to -10.0 kcal/mol for the quadrupole mutant *Pf*DHFR. The results showed that compound C90 exhibited the most stable and impressive score in its pharmacokinetic and drug-likeness assessment.

Keywords: Antimalarial, *In silico*, Medicinal plant, *Plasmodium falciparum*, Virtual screening

Introduction

Malaria is a severe disease affecting various countries around the world and requires comprehensive treatment. In addition, it is caused by the pathogenesis of *Plasmodium* spp protozoa, particularly *Plasmodium falciparum* and *Plasmodium vivax*,[1] which are responsible for the initiation of erythrocyte breakdown. According to the 2020 World Health Organization (WHO) report, there were 409 fatalities among the 229 million malaria cases.[2] Several countries have also been reported to have high endemicity of the disease, including Africa, the Eastern Mediterranean, and Southeast Asia.[2] In Southeast Asia, Indonesia had the highest number of cases, with 94,610 in 2021.[3] Previous reports have identified Papua, West Papua, Maluku, and East Nusa Tenggara as the largest contributors in Indonesia.[4] The high number of cases in these areas necessitates a significant effort to reduce malaria prevalence, such as the discovery of new antimalarial drugs.

In the development of antimalarial drugs, folate is an important cofactor for the synthesis of purines and pyrimidines, which are essential components for DNA biosynthesis.[5] Consequently, it is a metabolic byproduct that influences the survival of malaria parasites. *Plasmodium falciparum* dihydrofolate reductase (*Pf*DHFR) is widely known as an enzyme that converts dihydrofolate (DHF) to tetrahydrofolate (THF), which is required for DNA synthesis.[6] These findings show that *Pf*DHFR is a key target receptor in studies related to the development of antimalarial drugs.

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Cycloguanil and pyrimethamine are antimalarial drugs known for their ability to inhibit the function of the DHFR enzyme. However, a genetic mutation in the DHFR gene alters the amino acid residues within the binding pocket, specifically Ser108, Cys59, Ile51, Ile164, and Ala16.[7] This, in turn, causes changes in the conformation of the amino acid residues, thereby reducing the effectiveness of antifolate drugs, and posing a challenge in the discovery of new medicines.

In the context of medicinal intervention, traditional herbs have become increasingly prominent for treating various ailments.[8] Local cultures often harness the therapeutic potential of plant extracts in addressing a diverse range of health issues. This empirical utilization serves as evidence to evaluate the efficacy of active chemicals. Taek et al. conducted an ethnopharmacological investigation on the usage of medicinal herbs for malaria treatment,[9] revealing that the Timorese Island Society used 41 plants to treat the disease. Some of these plants include *Strychnos ligustrina*, *Calotropis gigantea*, *Cleome rutidosperma*, *Physalis angulata*, and *Alstonia spectabilis*. This scientific knowledge provides significant empirical data for the development of antimalarial drugs. However, literature reviews show that 41 therapeutic plants have not yet been examined for their biological activity.

In silico studies comprise the analysis of biological activity through a structural bioinformatics approach, offering an efficient, effective, and environmentally friendly method of identifying drug candidates, including antimalarials.[10] The prediction of the biological activity of drug candidates can be optimized before being used as a reference for laboratory testing, making *in silico* studies essential in the search for novel medications.[11]

In this current study, datasets comprising 100 compounds found in 41 traditional medicine plants were employed using a literature study approach. Subsequently, structure-based virtual screening was carried out on the datasets. The binding energy and chemical interactions of the hit compounds with the receptors were investigated. Molecular dynamics simulations were conducted on the hit compounds to gain a more comprehensive understanding of their temporal stability, which

was a key strategy for discovering new medicines. Therefore, this study aimed to identify compounds derived from traditional medicinal plants on Timor Island, Indonesia, with the potential to act as a DHFR inhibitor. For comprehensive data, the target receptors used were the wild-type and the quadrupole mutant *Pf*DHFR. In subsequent studies, the obtained compounds were used as the leading components to be isolated and studied for antimalarial activity in wet laboratories.

Materials and Methods

Materials

All stages of the analysis used various software applications, such as ChemDraw Professional 2017 version 16.0.1.4, Chem 3D 2017 version 16.0.1.4, Gaussian version 9, Chimera 2018 version 1.13.1, AutoDock Vina 2011 version 1.1.2, AutoDock Tools 2017 version 1.5.6, PyMol version 2.5.2, and Discovery Studio Client 2021 version 21.1.0.20298. Furthermore, web tools, including pkCSM, SwissAdme, and Molsoft tools, were employed for analyzing pharmacokinetic properties included.

Validation of Docking

Enzyme crystal structures with wild-type *Pf*DHFR-TS and quadrupole mutant *Pf*DHFR were obtained from the RSCB database (http://www.pdb.org/) with XRD resolutions of 2.33 Å and 2.10 Å, respectively. The native ligand of the 2 crystal structures, WR99210, was separated and prepared by adding Gasteiger charges and hydrogen atoms. Meanwhile, the protein structure was prepared using AutoDock Tools (ADT) version 1.5.6, which included polar hydrogen atoms and Kollman charges. The natural ligand was docked into the wild-type *Pf*DHFR-TS enzyme with a grid box (18 Å x 18 Å x 18 Å) center at (27.697, 6.953, 58.369) Å and the quadrupole mutant *Pf*DHFR with a grid box (18 Å x 18 Å x 18 Å) center at (28.849, 6.560, 58.886) Å. The root-mean-square deviation (RMSD) value of <2 Å showed that the docking procedure was valid.[12]

Preparation of Ligand Test

A dataset of active compounds from Indonesian medicinal plants was obtained using a literature study approach. The 2D structure of active compounds was sketched and converted into a 3D structure using ChemDraw Professional 16. Furthermore, geometry optimization of active compounds was carried out using Gaussian 9 software with the Austin Model 1 (AM1) method. Gasteiger charges and hydrogen atoms were added to the ligand and saved in the pdbqt extension using AutoDock Tools (ADT) 1.5.6.

Antimalarial Activity Prediction

The antimalarial profile of 100 active compounds (Table S1) was predicted using PASS online (http://www.way2drug.com/passonline/). Compounds with a Pa value above 0.3 were subjected to molecular docking. A Pa value above 0.3 indicated that the compound had potential as an *in silico* antimalarial agent.[13]

Molecular Docking

The ligand with a Pa value greater than 0.3 was subsequently subjected to molecular docking with both the wild-type *Pf*DHFR-TS and the quadrupole mutant *Pf*DHFR. Molecular docking using AutoDock Vina was performed based on a validated docking procedure. Binding energy and chemical interaction were evaluated to investigate the compound's potential as antimalarial.

Molecular Dynamics (MD) Simulation

The stability of the ligand in the binding pocket for wild-type and quadrupole mutant *Pf*DHFR was evaluated through molecular dynamics simulation using the Gromacs 2023 package.[14] The ligand's topology was generated using SwissParam (https://www.swissparam.ch/) in line with the CHARMM all-atom force field.[15] Meanwhile, the protein's topology was prepared with the force field of the CHARMM27 all-atom force field using gmx pdb2gmx tools.[16] Subsequently, the protein and ligand topologies were merged, and water molecules were introduced into the system using the TIP3P model.[17] The system was neutralized by adding sodium ions (Na^+) and chloride ions (Cl^-).

The equilibration of the system was conducted under NVT (constant number of particles, volume, and temperature) and NPT (constant number of particles, pressure, and temperature) conditions for a duration of 10 ns at a temperature of 300 K. The MD production for 50 ns was executed using gmx grompp tools. The trajectory of MD was used to create the root-mean-square deviation (RMSD) for the backbone and ligand, root-mean-square fluctuation (RMSF) for the fluctuation analysis, radius of gyration (Rg), and solvent accessible surface area (SASA).

Pharmacokinetic Properties Analysis

The pharmacokinetic properties of a drug candidate were crucial for the process of drug development. In addition, the pharmacokinetic features of active substances comprised adsorption, distribution, metabolism, excretion, and toxicity (ADMET). This feature was determined using the pkCSM internet tools (https://biosig.lab.uq.edu.au/pkcsm/prediction). The Lipinski rule of the top 5 compounds was determined using SwisAdme (http://www.swissadme.ch/). The drug-likeness score was calculated using the Molsoft web tools (https://molsoft.com/mprop/).

Result and Discussion

Validation of docking

Docking protocol validation was an essential first step in determining whether the docking procedure was valid and reliable. The docking protocol was valid when the re-docking compound's RMSD value was 2. Figure 1 shows the results of superimposing the re-docking ligand (green color) on the native ligand (blue color). The RMSD value for native ligand in wild-type *Pf*DHFR-TS was 0.232, and 0.746 in quadrupole mutant *Pf*DHFR.

Antimalarial Activity of Prediction

A set of 100 active compounds (Table S1) was assessed for their antimalarial activity using PASS online. The analysis provided Pa values, indicating the probability of a compound acting as an antimalarial agent, which was visually presented in Figure 2. Compounds with a Pa value >0.3 were subjected to molecular docking. The study of new drug discovery using *in silico* analysis has advanced rapidly. *In silico* studies were developed to search for new compounds that were effective as drugs due to their rapid, cost-effective, and efficient procedures.[18] Preliminary screening of the antimalarial activity of active compounds in several medicinal plants in East Nusa Tenggara, Indonesia, had been carried out (Figure 2). Among the 100 compounds used in the screening process, 51 had a Pa score above 0.3. In addition, there were also active compounds found in 2 types of plants (*Brucea javanica* and *Quassia indica*), namely C17 and C76, with a Pa score of 0.9. Compounds with a Pa score greater than 0.9 had potential and a high probability of being tested as antimalarial agents.[19] The results showed that those with a value between 0.3 and 0.7 had potential as *in silico* antimalarial agents but had a low probability experimentally. The 2 groups of compounds with Pa values > 0.3 were used for the virtual screening process based on molecular docking.

Molecular Docking

Compounds with a Pa value >0.3 were docked to the wild-type and quadrupole mutant *Pf*DHFR enzymes. The molecular docking results showed the top 5 antimalarial candidates in Table 1. The 5 compounds in Table 1 were selected based on the lowest binding energy values obtained from the molecular docking process against the wild-type *Pf*DHFR-TS enzyme and the quadrupole mutant *Pf*DHFR, as shown in Figure 3.

The Molecular docking of 51 compounds was performed on 2 key enzymes for drug discovery, namely the wild-type *Pf*DHFR-TS (1j3i) and the quadrupole mutant *Pf*DHFR (1j3k). The study found 5 plant species that could be used as antimalarial agents. These included *Acacia farnesiana*, *Dendrophthoe pentandra*, *Syzygium aqueum*, and *Paramignya trimera* (Table 1).

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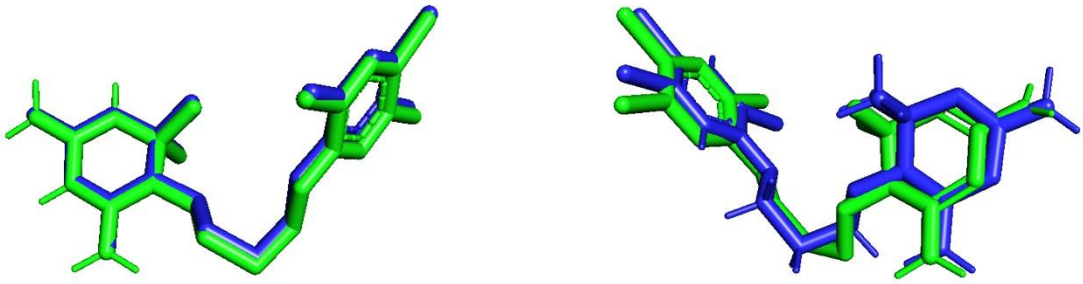

a) b)

Figure 1: Superimpose ligand redocking against native ligand WR99210 in (a) wild-type and (b) quadrupole mutant *Pf*DHFR.

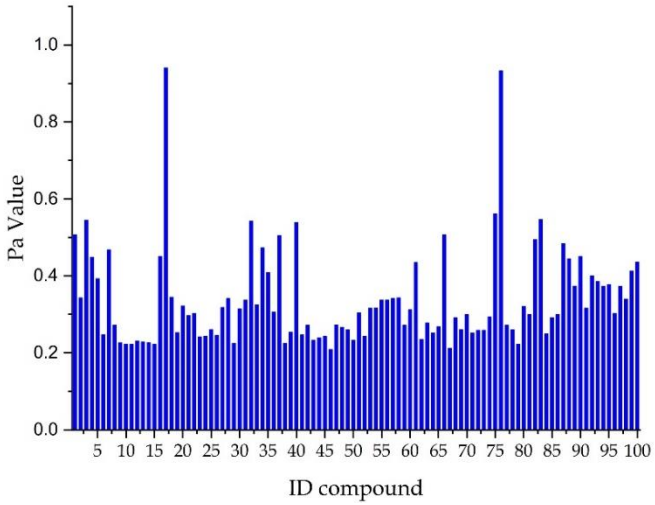

Figure 2: Antimalarial activity prediction graph

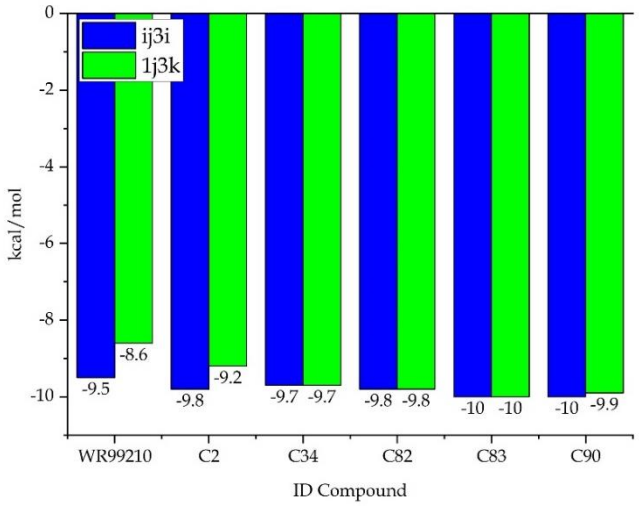

Figure 3: Binding energy of the 5 best compounds against wild-type and quadrupole *Pf*DHFR

The binding energies of each active compound from medicinal plants were lower than those of WR99210, a natural ligand (Figure 3). In addition, Figure 3 showed that the binding energy for the 5 candidate antimalarial compounds (C2, C34, C82, C83, and C90) ranged from -9.7 to -10.0 kcal/mol. For the wild-type *Pf*DHFR-TS enzyme, compounds C83 and C90 had the lowest binding values, indicating that these compounds had the most stable binding to the receptor. Compound C83 (-10.0 kcal/mol) had a more stable bond with the quadrupole mutant *Pf*DHFR compared to C90 (-9.9 kcal/mol). The data obtained were consistent with activity prediction results using PASS online. Compound C83 was the active component from the *Syzygium aqueum* plant,[22] having the highest Pa score of 0.546. As shown in Figure 4, the suggested antimalarial agent candidate compounds had precise chemical interactions with the wild-type *Pf*DHFR-TS. The top 5 compounds (C2, C34, C82, C83, and C90) showed a binding mode similar to the native ligand, namely WR99210. Ile14, Cys15, Asp54, Ile164, and Tyr170 were amino acid residues that served as active sites and had been discovered to bind to WR99210 through hydrogen bonds (Table S2).[24–26] The WR99210 complex with wild-type *Pf*DHFR-TS was kept stable by interactions between pi-sigma and alkyl groups in amino acid residues Met55 and Phe58. The chemical interactions of compounds C83 and C90 with the lowest binding energy values showed an interaction mode similar to compound WR99210.

The compound C83, a flavonoid glycoside, appeared to form hydrogen bonds with Ile14, Ala16, and Tyr170 at the hydroxyl position of the glucose substituent. Alkyl interactions were found at Phe58 for hydrophobic interactions. Compound C82 did not bind to Ile14 but formed hydrogen bonds with Asp54, Ile164, and Tyr170. The results showed that only Ile164 and Tyr170 formed hydrogen bonds with the compound C90. Although only 2 hydrogen bonds were engaged in this interaction, the bond distance observed on Tyr170 (1.73 Å) was smaller than that shown by WR99210 (2.51 Å). Therefore, compound C90 could be considered to have a binding energy of -10.0 kcal/mol. The hydrophobic interactions of Met55, Phe58, and Ile112 found in WR99210 facilitated the complex stability of compound C90 with the receptor.

The interaction between the top 5 antimalarial candidates (C2, C34, C82, C83, and C90) and the quadrupole mutant *Pf*DHFR was also worth discussing (Figure 5). Compound C83, which had the lowest binding energy, interacted with key amino acids in the receptor binding pocket. The glucose substituent continued to play a role in hydrogen bonding with Ile14. The Phe58 amino acid residue formed a bond with C83 through pi-alkyl interactions (Table S3). Meanwhile, compound C82 stabilized the complex through hydrogen bonding interactions with Phe58 and Leu164. The results showed that compound C90 formed hydrogen bonds with the amino acid Leu164, as found in C83. Hydrophobic interactions with several amino acids, such as Leu46, Met55, and Phe58, strongly supported the stability of the complex between compound C90 and the quadrupole mutant *Pf*DHFR.[27]

Molecular Dynamics Simulation

The complex stability between hit compounds (C82, C83, and C90) and wild-type *Pf*DHFR-TS was confirmed by molecular dynamics (MD) simulation over 50 ns. The RMSD backbone was computed for 3 complexes, as shown in Figure 6a. The wild-type *Pf*DHFR-TS backbone of these complexes showed a significant increase of up to 10 ns. This was due to the effects of protein backbone adaptation observed in the first simulation. The investigation showed that the protein backbone of complex C90 had a mean RMSD value of 0.20 nm, slightly lower compared to complexes C82 and C83.

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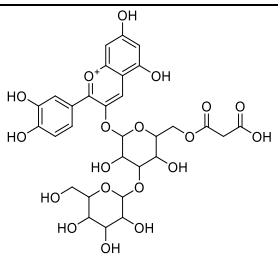
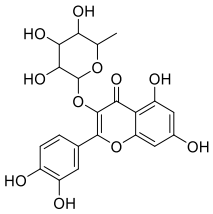
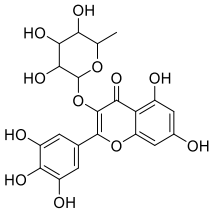
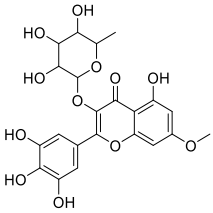
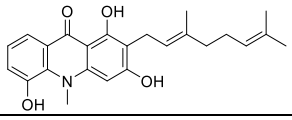
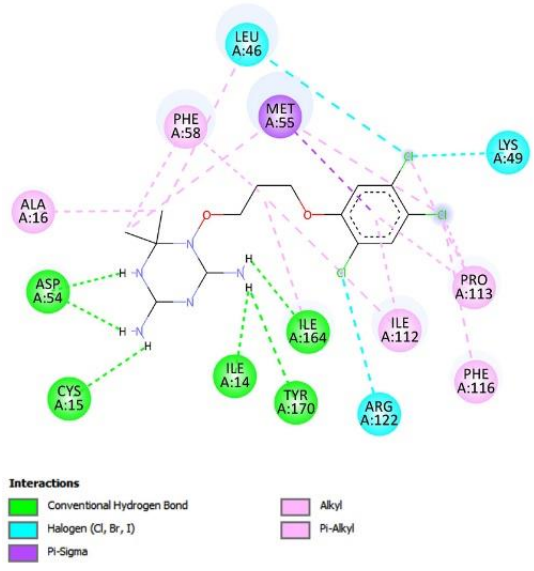
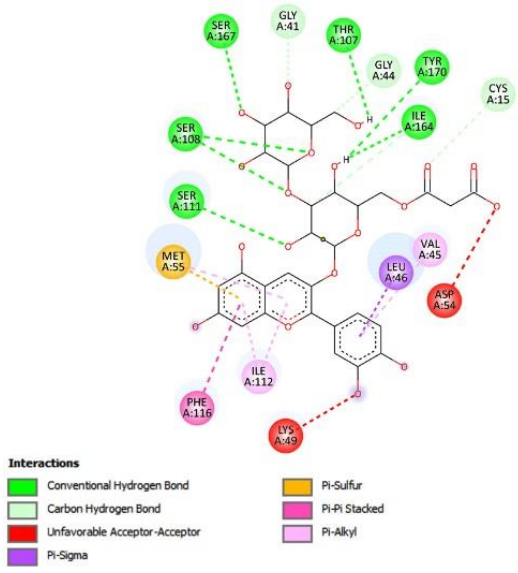
Table 1: Antimalaria *in silico* pharmacology results of the 5 best compounds subjected to molecular docking method

ID Compound	Chemical structure	PubChem ID	Plant source	Pa Value	Ref
C2		74976941	*Acacia farnesiana*	0.343	20
C34		5353915	*Dendrophthoe pentandra*	0.473	21
C82		5352000	*Syzygium aqueum*	0.495	22
C83		74978406	*Syzygium aqueum*	0.546	22
C90		10596746	*Paramignya trimera*	0.451	23



a)



b)

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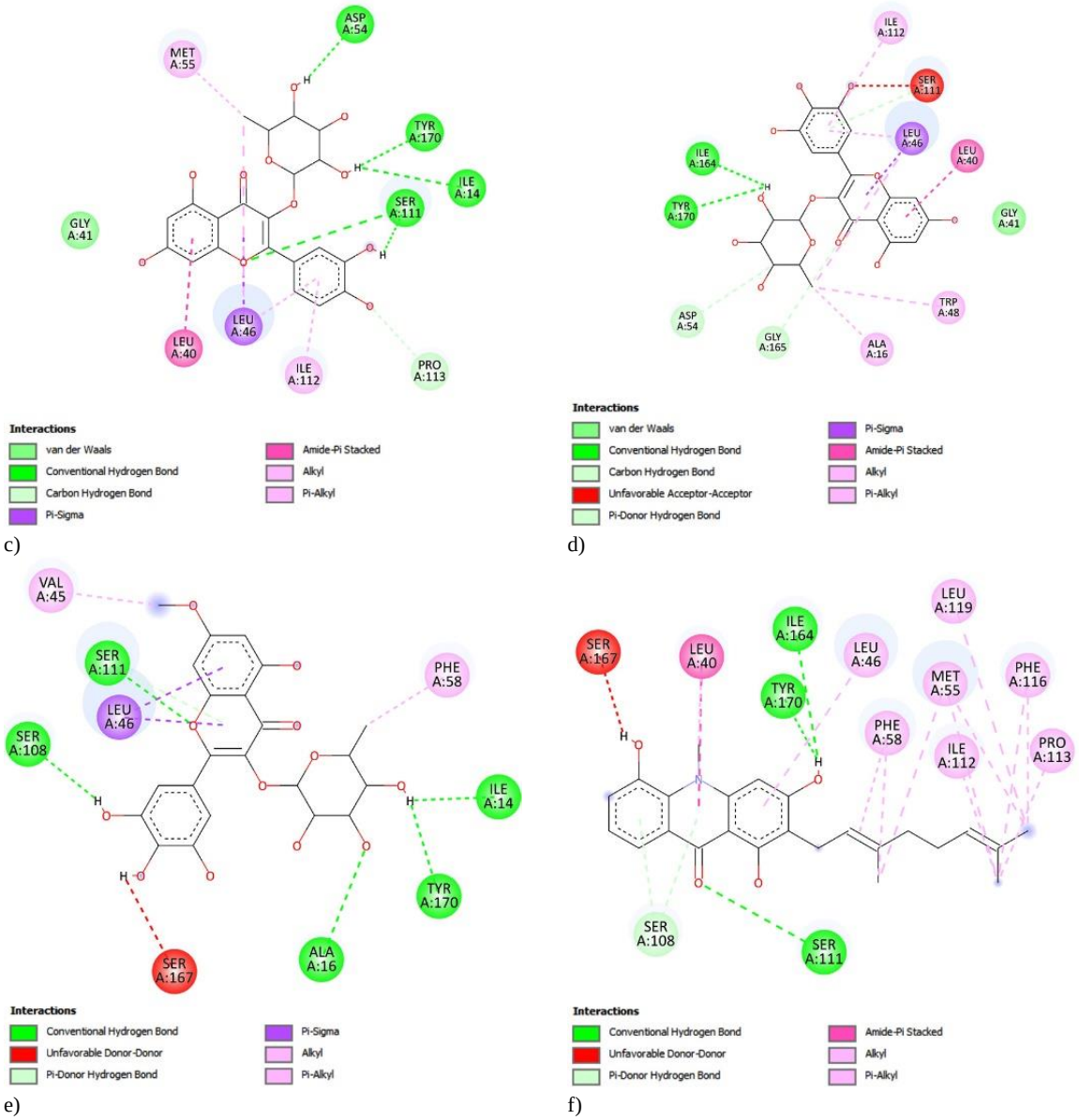

Figure 4: 2D interaction of best compound against the wild-type *Pf*DHFR-TS binding pocket: (a) WR99210, (b) C2, (c) C34, (d) C82, (e) C83, and (f) C90.

The ligand's stability within the binding site of wild-type *Pf*DHFR-TS was evaluated. The RMSD plot was used to determine how well the ligand suited the protein (Figure 6b). In the early phase, which lasted up to 30 ns, compound C83 exhibited a mean RMSD of 0.15 nm. However, as the simulation exceeded 30 ns, its mean RMSD increased, eventually reaching 0.42 nm. Based on these findings, compound C83 did not possess the capacity to produce enduring interactions at the binding site. Compound C90 (blue) exhibited consistent behavior throughout the experiment, as evidenced by the RMSD value of 0.29 nm.

The fluctuation of each amino acid residue on the wild-type *Pf*DHFR-TS enzyme throughout the simulation was assessed and presented as an RMSF plot (Figure 6c). The higher value of RMSF suggested the great flexibility of amino acid residues.[28] Consequently, ligand interaction at the binding site weakened. The RMSF plot of complex compound C90 was found to be lower compared to compounds C82 and C83.

An Rg plot (Figure 6d) was used to determine the tendency of folded proteins by studying the compactness of proteins in each complex.[29] When simulating for 15 ns, the protein in complex C82 showed a declining Rg value until the simulation finished with a mean Rg value of approximately 1.80 nm. Although the Rg of protein in complex C90 was slightly greater (1.82 nm) at the end of the simulation than in complex C82 (1.78 nm), proteins in complex C90 exhibited steady behavior during the over-time simulation. This study suggested that compound C90 in complex proteins was maintained.

The solvent-accessible surface area (SASA) of each complex was investigated to determine the tenacity of ligand interactions with water molecules.[28] The mean SASA values for compounds C82, C83, and C90, which are 125.95, 126.87, and 124.88 nm^2, were presented in Figure 6e. These findings suggested that compound C90 in complexes was less likely to interact with water molecules, making it more stable compared to others.

In the quadrupole mutant *Pf*DHFR-TS enzyme, the complex stability of compounds C82, C83, and C90 was also investigated and was shown in Figure 7. The RMSD plot of the protein backbone in 3 complexes showed a fluctuating trend. However, it was clear that the protein backbone in complex C90 (shown in blue) was likely to decrease over the simulation compared to complexes C82 and C83. For complexes C82, C83, and C90, the mean RMSD value of the protein backbone was 0.21 nm, 0.20 nm, and 0.19 nm, respectively.

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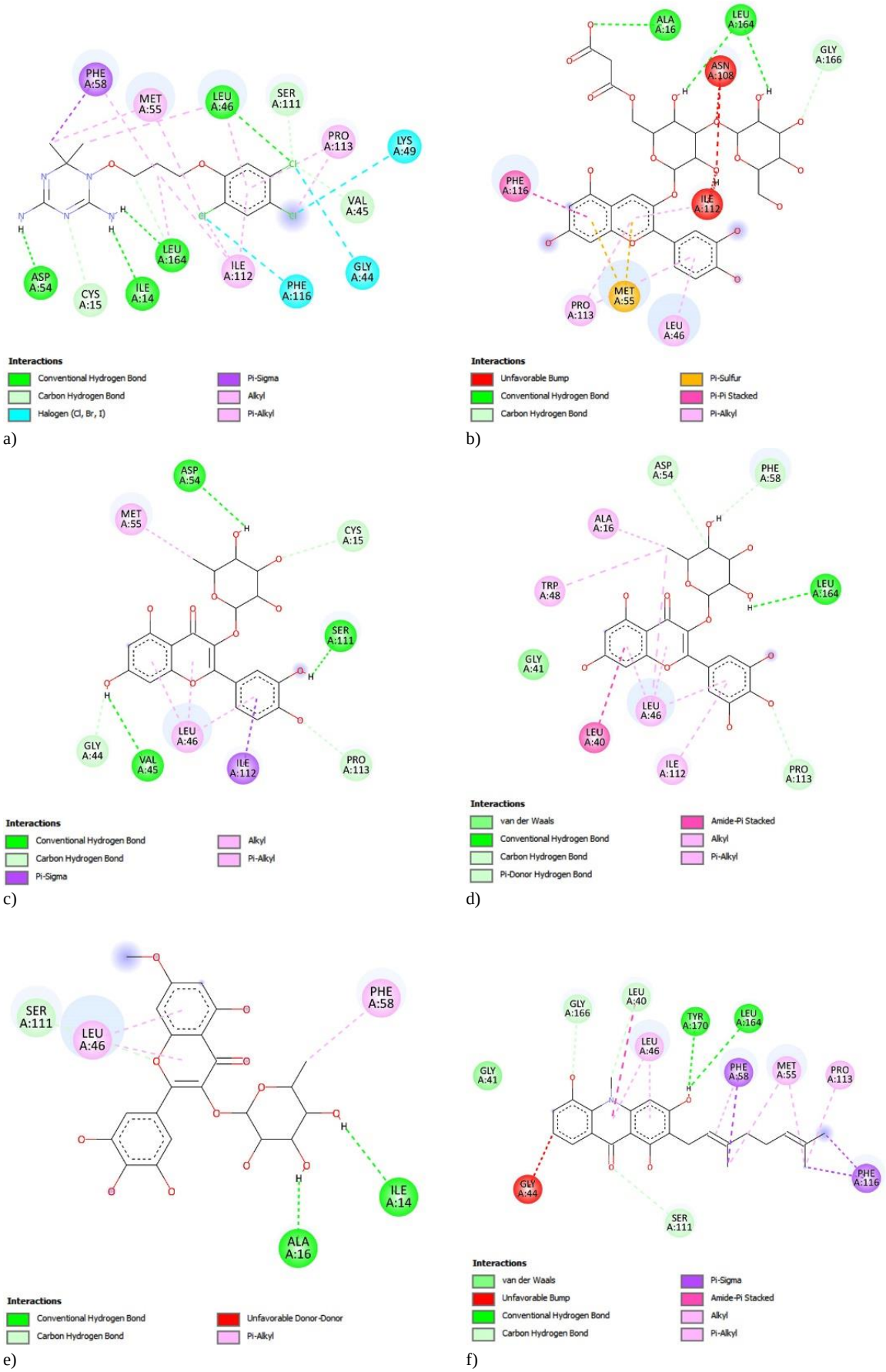

Figure 5: 2D interaction of the compound hits against the quadrupole mutant *Pf*DHFR binding pocket: (a) WR99210, (b) C2, (c) C34, (d) C82, (e) C83, and (f) C90.

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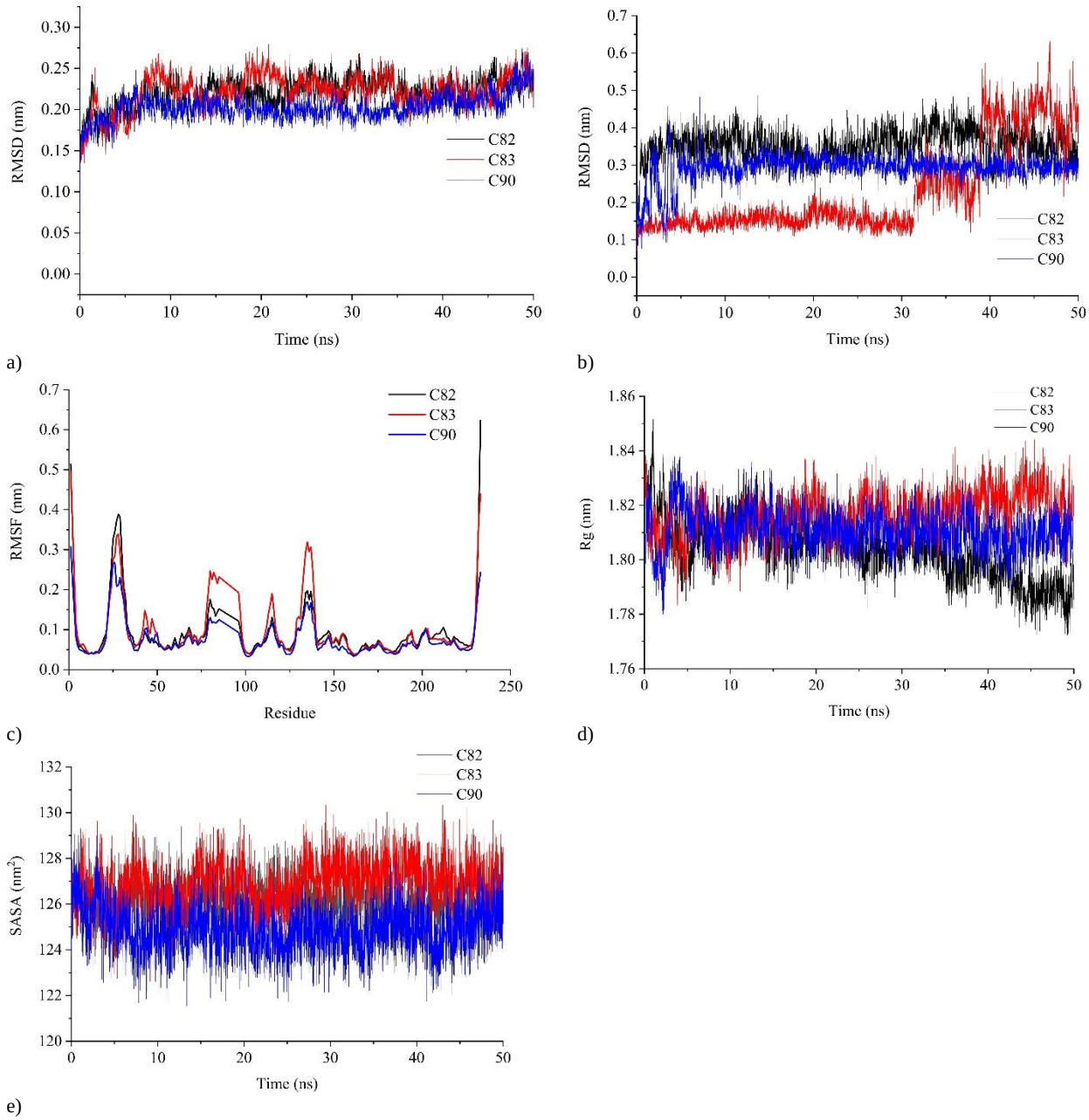

Figure 6: MD simulation results on wild-type *Pf*DHFR-TS enzyme: a) RMSD backbone, b) RMSD ligand, c) RMSF, d) Radius of gyration, e) SASA

The stability of the ligand at the binding site of the quadrupole mutant *Pf*DHFR was also investigated, as shown in Figure 7b. Compound C90 had a low RMSD (0.17 nm) up to a 5 ns time simulation. The RMSD value of compound C90 was continuously increased until a time simulation of 10 ns, where the equilibrium point was established with a mean RMSD of 0.37 nm. Although the RMSD pattern of compound C83 remained consistent across simulations, its mean RMSD was 0.46 nm. This result was still higher compared to the value obtained for compound C90.

A similar trend was seen in the fluctuating pattern of amino acid residues in proteins of 3 complexes (Figure 7c). Some important amino acid residues on binding sites, such as Ile14, Cys15, Asp54, Ile164, and Tyr170, had a low RMSF value. Due to the relative rigidity of amino acid residues, the binding site of the quadrupole mutant *Pf*DHFR-TS enzyme could be effectively occupied by 3 compound hits.

The Rg plot (Figure 7d) showed the protein compactness in the 3 complexes being evaluated. In the over-time simulation, complex C82 displayed a fluctuating pattern. During time simulation, complex C83 had a higher Rg value. Meanwhile, complex C90 fluctuated from early simulation to 35 ns of simulation and remained generally steady until the process was completed. The ligand was likely to remain at the protein's binding site when Rg remained constant. The SASA plot (Figure 7e) was another metric investigated to assess the stability of the tested compound. Compounds C82, C83, and C90 had mean SASA values of 125.92, 124.71, and 125.31 nm^2, respectively. These findings showed that compounds C83 and C90 interacted with water molecules compared to C82.

Pharmacokinetics Properties Analysis

The pharmacokinetic properties of the top 5 antimalarial candidates were evaluated. The resulting data contained information about their absorption, distribution, metabolism, and excretion. As an essential aspect of drug development, toxicity prediction was crucial to ensure the safety and efficacy of potential treatments. The toxicity assessment,

performed using the AMES evaluation, examined whether these compounds could serve as natural medicines without inducing harmful side effects and promoting overall health. The pharmacokinetic properties and toxicity predictions of the 5 candidate compounds for antimalarial agents were presented in Table 2.

In this study, Table 2 also presented the AMDET properties of the top 5 antimalarial candidates. In terms of absorption, compound C90 scored > 90 and 0.8 for the intestinal absorption and Caco2 permeability categories, respectively. This indicated that it had effective absorption properties and was suitable for oral use.[30] For the distribution profile, compounds C2, C34, C82, and C83 had low volume of distribution (VDss) values (<-0.15), while C90 was in the medium (0.16) category. Based on this finding, compound C90 tended to be unstable when bound to blood plasma, enabling it to penetrate the membrane to display treatment effects and not induce side effects.[31,32] All compounds in this study showed low BB and PS log values.

CYP2D6 and CYP3A4 were isoforms used in determining the metabolic ability of the top 5 antimalarial candidates (C2, C34, C82, C83, and C90). These isoforms were responsible for drug metabolism and detoxification processes.[33] According to the prediction results, none of the recommended compounds acted as substrates or inhibitors in CYP2D6 and CYP3A4. Apart from C2 (0.16 log mL min^{-1} Kg^{-1}) in the excretion data, all recommended compounds had good total clearance. The AMES toxicity study revealed that 2 of the top 5 antimalarial candidates (C2, C34, C82, C83, and C90) were toxic (C82 and C83). These findings were consistent with the VDss data, showing that these 2 candidates had low VDss values. Compounds C82 and C83 had a strong affinity for blood plasma, making them difficult to distribute and toxic.[32]

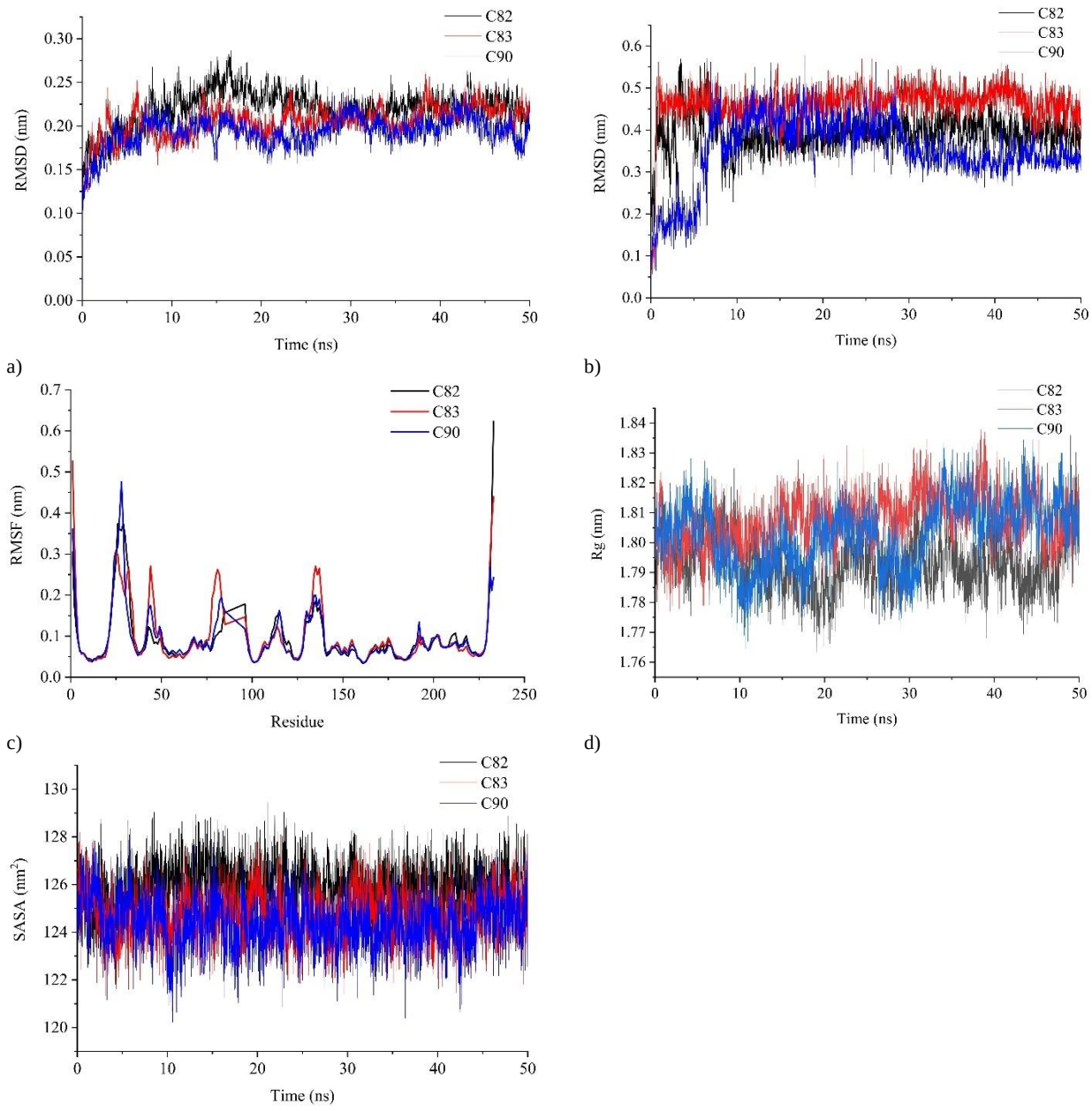

Figure 7: MD simulation results on Quadrupole mutant *Pf*DHFR-TS enzyme: a) RMSD backbone, b) RMSD ligand, c) RMSF, d) Radius of gyration, e) SASA

The drug-likeness properties of the top 5 antimalarial candidates were also examined. The drug-likeness properties measured several Lipinski rules, including molecular weight (MW), log P, number of hydrogen bond acceptors, number of hydrogen bond donors, and PSA. Table 3 displays the outcomes of drug-likeness predictions. Furthermore, a drug-likeness score was a number that described a compound's potential to be developed as a drug and was calculated using Molsoft web tools.

Based on the Lipinski rule, the top 5 antimalarial candidates (C2, C34, C82, C83, and C90) were also evaluated for drug-likeness. Table 3 showed that compound C90 did not violate this rule. The drug-likeness score was calculated using Molsoft web tools, and compound C90 had the highest score with the same properties as a drug. Based on the findings of the overall analysis, it was believed to have high potential for development as an antimalarial agent. In wild-type *Pf*DHFR-TS and quadrupole mutant *Pf*DHFR, compound C82 had the highest Pa value and the lowest binding energy. However, molecular dynamics simulations suggested that compound C90 had superior complex stability. The ADME properties also suggest that it was non-toxic, but confirmation of toxicity properties *in vitro* in the laboratory was still needed.

Conclusion

In conclusion, a database of active compounds derived from medicinal plants was subjected to virtual screening. After a series of *in silico* studies on 100 active compounds, compound C90, originating from the *Paramignya trimera* plant, emerged as a promising candidate for potential antimalarial agents. The study by molecular docking of compound C90 generated binding energies of -10.0 kcal/mol and -9.9 kcal/mol for wild-type *Pf*DHFR-TS and quadrupole mutant *Pf*DHFR, respectively. The molecular dynamics simulation showed stable interactions with binding sites for both the wild-type and quadrupole mutant *Pf*DHFR. Furthermore, it was non-toxic and had favorable pharmacokinetic properties. The results showed that compound C90 had the highest drug-likeness score of 1.29 and did not violate the Lipinski rule. These findings provided scientific guidance for future studies isolating compound C90 and confirming antimalarial tests in wet laboratories.

Conflict of Interest

The authors declare no conflict of interest.

Authors' Declaration

The authors hereby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them.

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Table 2: Pharmacokinetic properties and toxicity of candidate antimalarial compounds

	C2	C34	C82	C83	C90
Absorption					
Intestinal absorption (%)	8.09	60.01	58.76	63.04	96.86
Caco2 permeability (nm sec^{-1})	8.87	-0.61	-0.80	-0.62	0.813
Distribution					
Volume of distribution (log L Kg^{-1})	-0.25	-0.24	-0.27	-0.35	0.16
Blood-brain barrier (log BB)	-3.14	-2.11	-2.42	-2.31	-0.99
Central nervous system (log PS)	-6.7	-4.83	-5.21	-5.08	-2.02
Metabolism					
CYP2D6 substrate (Yes/No)	No	No	No	No	No
CYP3A4 substrate (Yes/No)	No	No	No	No	No
CYP2D6 inhibitor (Yes/No)	No	No	No	No	No
CYP3A4 inhibitor (Yes/No)	No	No	No	No	No
Excretion					
Total clearance (log mL min^{-1} Kg^{-1})	0.16	0.64	0.61	0.68	0.43
Toxicity					
AMES toxicity (Yes/No)	No	No	Yes	Yes	No

Table 3: Drug-likeness properties of the top five candidate compounds

Compound	[a]MW	[a]Log P	[a]Number of HBA	[a]Number of HBD	[a]PSA (A^2)	Violation of Lipinski rule	[b]Drug-likeness score
C2	697.57	-2.09	19	11	315.96	3	0.14
C34	448.38	1.27	11	7	190.28	2	0.82
C82	464.38	1.71	12	8	210.51	2	0.67
C83	478.40	2.44	12	7	199.51	2	0.57
C90	393.48	3.22	4	3	82.69	-	1.29

[a]calculated by SwissAdme, [b]calculated by Molsoft web tools

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