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Article

In Silico Evaluation of Quinoline Derivatives as PfLDH Inhibitors through Molecular Docking, Lipinski's Rule, and ADMET Profiling

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Abstract. Malaria is a major global health problem caused by *Plasmodium falciparum*, with increasing resistance to existing antimalarial drugs highlighting the urgent need for new therapeutic agents. This study aimed to evaluate quinoline-spiro derivatives as potential inhibitors of *P. falciparum lactate dehydrogenase* (PfLDH), a key enzyme involved in parasite energy metabolism, using an in silico approach. Molecular docking was performed to assess ligand-protein interactions, followed by drug-likeness evaluation based on Lipinski's Rule of Five and pharmacokinetic-toxicity prediction using ADMET analysis. The results showed that all tested compounds exhibited favorable binding interactions with PfLDH, thus demonstrating potential as enzyme inhibitors. Several compounds exhibited stronger binding affinity than reference ligands, suggesting that structural modifications with the spiro framework enhance interaction with the target protein. Most compounds also met drug-likeness criteria, although there were minor deviations. Among the compounds evaluated, one candidate, (Z)-2-((2-(7- chloroquinolin-4 -yl) hydrazinyl) methyl) -4-(3- methylbenzylidene)-2-azaspiro[4.5] decan-3-one showed the most balanced profile, based on results obtained by combining strong binding interactions with favorable pharmacokinetic properties and predicted low toxicity. Quinoline-spiro derivatives may be promising candidates for the development of antimalarial drugs targeting PfLDH. This study describes the integrated in silico evaluation of quinoline-spiro derivatives as PfLDH inhibitors, as drug candidates for further development.

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1. Introduction

Malaria is a deadly disease caused by Plasmodium falciparum, producing complex and complex symptoms and associated with high parasitemia and severe hemolysis due to parasite division and replication after red blood cell invasion, which can lead to death if left untreated [1]. This disease remains a global health problem, with more than half the world's population potentially exposed to infection. Currently, more than 100 countries are reported to be affected, with an estimated 200 million clinical cases and more than 500,000 deaths occurring annually [2-3]. The emergence of resistance to widely used conventional drugs, such as chloroquine and artemisinin [4], and insecticides, has resulted in an increase in the number of cases and deaths from malaria [5-6]. This situation has driven the need to develop new drug candidates with higher biological efficacy and improved toxicity profiles [7].

Quinoline derivatives are heterocyclic compounds that have diverse biological activities, including as antimalarial agents. Furthermore, this compound is also reported to possess antibacterial, antifungal, antiviral, anti-inflammatory, anticancer, and antiprotozoal activity. Quinoline derivatives are also utilized in photodynamic therapy and show potential in inhibiting enzymes such as α-glucosidase and α-amylase, thus offering broad prospects for drug development [8]. The spiro skeleton structure, when combined with the quinoline structure, has been reported to enhance the compound's properties as an effective biological inhibitor, warranting further investigation [9]. Quinoline and its analogs are reported to have substantial biological applications, and this group attracts chemical modifications that produce their pharmacological effects. In recent years, the synthesis of the quinoline-spiro skeleton under various reaction conditions has been reported [9-10].

The enzyme Plasmodium falciparum lactate dehydrogenase (PfLDH) was chosen as the target protein. The structural differences between PfLDH and human LDH provide the basis for the designed compound's potential for high selectivity against the parasite while minimizing effects on host cells [11]. Plasmodium in the living body is influenced by an enzyme, lactate dehydrogenase, which plays a role in energy production in P. falciparum [12]. Selective inhibition of Lactate Dehydrogenase (LDH) activity will disrupt the parasite's energy metabolism, thereby inhibiting the growth and survival of Plasmodium falciparum [13-14].

Conventional drug development for deadly diseases is generally time-consuming, expensive, labor-intensive, and produces significant waste [15]. Therefore, an alternative drug development method is needed that has the potential to find new drugs that are more efficient and cost-effective in silico using the molecular docking method [16]. Research on quinoline derivatives combined with a spiro framework as inhibitors of the Plasmodium falciparum lactate dehydrogenase (PfLDH) enzyme with PDB code 1CET is still limited. Previous studies only reported on hybrid antimalarials by combining two pharmacophoric compounds that have the potential to reduce resistance, such as aminoquinoline-based hybrids and other quinoline conjugates [17-18]. In addition, studies that evaluate the molecular interactions and pharmacokinetic profiles of these compounds through in silico approaches such as molecular docking, ADMET analysis, and Lipinski's rule have not been widely reported. Therefore, further research is needed to evaluate the potential of quinoline derivatives with a spiro framework as selective antimalarial drug candidates for the PfLDH target.

2. Experimental Section

2.1. Materials

The tools used in this in silico study were a computer with an Intel Core i5 processor, running Windows 11 (64-bit). The software utilized included MOE 2022.02, the Protein Data Bank (PDB), PubChem, and pkCSM. MOE 2022.02 was employed for molecular docking simulations, including ligand preparation, energy minimization, and analysis of ligand–protein interactions.

The Protein Data Bank (PDB) was used to obtain the three-dimensional structure of the target protein (PfLDH). PubChem served as a database for retrieving and verifying the chemical structures and properties of the ligands. Meanwhile, pkCSM was used to predict the pharmacokinetic and toxicity profiles (ADMET) of the compounds. The materials used in this study were synthesized quinoline derivative compounds, which were utilized as test ligands in the molecular docking study.

2.2. Protein and Ligand Preparation

A total of fifteen quinoline derivative compounds with the code 4a-4o that have been synthesized and reported to have phytochemical profiles were used as test ligands [19]. These derivative compounds were then evaluated through computational analysis and compared with Dihydroartemisinin-piperaquine as a control ligand to assess the validation of molecular docking. The chemical structure of each compound was reconstructed, made in three dimensions using KingDraw software, while the chemical structure of the control ligand was obtained in the form of SMILES code from the PubChem data limit. Each ligand was treated at physiological pH conditions through a protonation process, then structure optimization was carried out using the energy minimization method using the AMBER10 force field to maintain parameter conformity with the previously prepared protein structure [20].

Ligands that have gone through the optimization stage are then stored in the Molecular Operating Environment (MOE) database format (.mdb) for use in the docking simulation stage. The three-dimensional structure of the P. Falciparum lactate dehydrogenase protein in three dimensions was obtained from the Protein Data Bank (PDB) database with the PDB code: 1CET. Protein preparation was performed using QuickPrep, available in the MOE2022.02 software. Next, the protein structure was further processed by removing water molecules, correcting structural imperfections, removing native ligands, and determining the protein's active site as the basis for determining the grid center, which represents the coordinates of the ligand binding site on the target protein.

2.3 Molecular Docking

Molecular docking simulations were performed using the Dock feature in the MOE2022.02 software. The docking process begins with the placement of the ligand into the active site of a predetermined protein, with the help of dummy atoms as markers for the binding center. The interaction search area was determined using a grid box centered on the protein's active site coordinates (grid center: x, y, z), with a grid size that encompasses all important residues around the active pocket. The Triangle Matcher algorithm with the London dG scoring function was used as the placement method. The initial pose was set 100 times with the best pose generated using the Rigid Receptor feature with the GBVI/WSA dG scoring function. The docking results were saved in .mdb format, analyzed using the MOE Database Viewer. The parameters analyzed were the binding affinity value or docking score (S) and the Root Mean Square Deviation (RMSD) value. The interaction of the ligand and the target protein with the best parameters was visualized to see the best molecular interaction.

2.4 Lipinski's Rule of Five Prediction

Evaluation of the suitability of compounds to Lipinski's rules was carried out using pkCSM software (https://biosig.lab.uq.edu.au/pkcsm/), the parameters analyzed included molecular weight (<500

g/mol), number of hydrogen bond donors (<5), number of hydrogen bond acceptors (<10) and log P value (<5) [21].

2.5 Pharmacokinetic Study

Pharmacokinetic properties analysis through ADMET was predicted by inputting SMILES notation of the ligand into pkCSM software (https://biosig.lab.uq.edu.au/pkcsm/) [22]

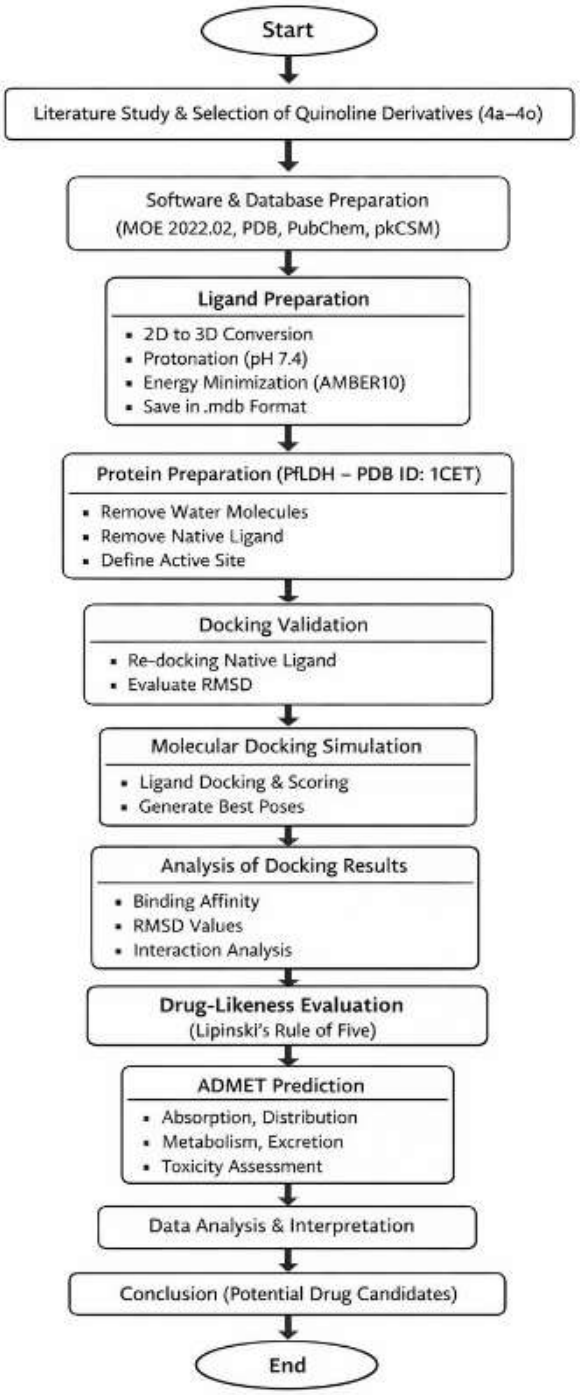

Figure 1. Flowchart of research

3. Results and Discussion

Docking validation is performed using the re-docking method or re-molecular docking of the native ligand bound to the receptor with the same parameters as the test ligand docking process. This validation process aims to verify the active binding site on the receptor by evaluating the RMSD value [23]. The lower the RMSD value, the better the match between the structure and protein [24]. RMSD values are classified into four categories, namely values less than 1 Å are considered excellent, values of 1–2 Å are categorized as good, values of 2–3 Å are moderate, while values greater than 3 Å are considered inaccurate or incorrect [25].

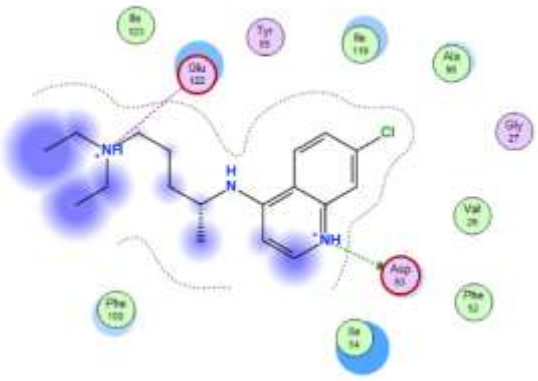

Figure 2. PfLDH protein interaction with native ligand

The re-docking results show that the ligand forms an ionic bond with the main residue Glu122, and forms a hydrogen bond as a donor with the residue Asp53. The RMSD value obtained is 1.1448 Å indicating that the ligand pose resulting from the re-docking has a good match with the ligand position in the crystal structure. This indicates that the docking method used has a good level of accuracy in predicting the orientation of the ligand on the active site of the receptor [26], so that the docking protocol used can be considered valid and suitable for use in analyzing the interaction of test compounds with target receptors.

Molecular docking studies were conducted to evaluate the ability of quinoline derivative compounds to bind to the target protein Plasmodium falciparum lactate dehydrogenase which plays an important role in the energy metabolism of malaria parasites. The docking score indicates how strongly and optimally the ligand binds to the protein, thus helping to determine the best conformation of the ligand and protein [27]. RMSD is used to assess the level of match between the results of the docking model and the native structure of the target protein. A smaller RMSD value indicates that the docking results are closer to the reference structure [28]. Docking results are categorized as good if the RMSD is $\leqslant$ 2.0 Å, sufficient in the range of 2.0 – 3.0 Å, and poor if the RMSD is $\geqslant$ 3.0 Å [29].

Based on the docking results, all compounds exhibited negative binding affinity values, indicating that the interaction between the ligand and the receptor occurs spontaneously. The range of binding energies obtained (-6.8642 to -8.0300 kcal/mol) indicates that the compounds have sufficient affinity for the active site of the protein. Compound 2 exhibited the lowest binding affinity value (-8.0300 kcal/mol), indicating the highest stability of the ligand-receptor complex compared to the other compounds and the control ligand. Compared to the control ligand, dihydroartemisinin-piperaquine (-7.5251 kcal/mol), several test compounds, such as compounds 1, 2, and 11, exhibited higher affinity. This indicates that modifying the quinoline structure to a spiro framework has the potential to enhance binding ability to the target protein.

Analysis of amino acid residue interactions showed that the ligand binds to the same active site as the control ligand, indicating a competitive inhibition mechanism. Key residues such as Gly196, Arg109, His195, and Ala318 are frequently involved in interactions, indicating that these regions are ligand binding hotspots. Furthermore, the RMSD parameter was used to evaluate the accuracy of the docking poses. Compounds with RMSDs <2 Å (e.g., compounds 1, 3, 11, and 12) indicated stable conformations and valid predictions. Conversely, RMSD values >3 Å for some compounds indicated a possible suboptimal ligand orientation within the active site.

Molecular docking results showed that all quinoline-spiro derivative compounds had negative binding affinities, indicating spontaneous interactions with the target protein PfLDH. Among the tested compounds, compound 2 showed the highest binding affinity, followed by compounds 1 and 11, and was able to interact with key residues such as Gly196, Arg109, His195, and Ala318, which are part of the protein's active site. These findings indicate that modification of the spiro structure of the quinoline backbone plays a role in increasing the stability of ligand-protein interactions. These results align with the QSAR/SAR approach in previous studies, which showed that the biological activity of quinoline derivatives is influenced by the presence of certain substituents, such as electron-donating groups and halogen substituents at the para position, which can enhance reactivity and molecular interactions.

Furthermore, DFT-based analysis and molecular dynamics simulations in the study confirmed that the stability of ligand-protein complexes is also influenced by electron distribution and hydrogen bonding ability [19]. Docking results not only indicate the strength of the interaction but also provide insight into the stability and orientation of the ligand in inhibiting enzyme activity. Molecular docking results can predict the most favorable orientation by observing the minimum energy generated between the ligand and receptor after forming a stable complex [30].

Table 1. Molecular Docking Results of Quinoline-Spiro Derivatives as Inhibitors of PfLDH

No.	Compounds	Binding Energy (kcal/mol)	RMSD (A)	Residue involved	Type of Bond
1	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(4-hydroxybenzylidene)-2-azaspiro[4.5]decan-3-one	-7.9619	1.4647	Gly196, Gly 196	pi-H pi-H
2	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(4-methoxybenzylidene)-2-azaspiro[4.5]decan-3-one	-8.0300	3.1085	Gly196, Gly 196	pi-H pi-H
3	(Z)-4-(4-chlorobenzylidene)-2-((2-(7-chloroquinolin-4-yl) hydrazinyl)methyl)-2-azaspiro[4.5]decan-3-one	-7.7558	1.9409	Asn140, Ala318, Lys101, Arg109	H-donor pi-H pi-cation pi-H
4	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(4-fluorobenzylidene)-2-azaspiro[4.5]decan-3-one	-7.1770	2.5462	Met325	H-donor
5	(Z)-4-(2-bromobenzylidene)-2-((2-(7-chloroquinolin-4-yl) hydrazinyl)methyl)-2-azaspiro[4.5]decan-3-one	-7.3350	3.2809	Gly196	pi-H

6	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(furan-2-ylmethylene)-2-azaspiro[4.5]decan-3-one	-7.3545	4.4141	His195 Gly196	pi-cation pi-H
7	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(thiophen- 2-ylmethylene)-2-azaspiro[4.5]decan-3-one	-6.8642	3.1024	Met325	pi-H
8	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(pyridin-3-ylmethylene)-2-azaspiro[4.5]decan-3-one	-7.1893	2.6538	His195 Gly196 Asn197	pi-cation pi-H pi-H
9	(Z)-4-benzylidene-2-((2-(7-chloroquinolin-4-yl)hydrazinyl) methyl)-2-azaspiro[4.5]decan-3-one	-7.2734	2.4200	Arg109	H-akseptor
10	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(3-methylbenzylidene)-2-azaspiro[4.5]decan-3-one	-7.5832	2.6250	Thr101 Gly196	H-donor pi-H
11	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(2,4-dichlorobenzylidene)-2-azaspiro[4.5]decan-3-one	-7.7229	1.9659	Ala318 Arg109	H-donor pi-cation
12	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(2-hydroxybenzylidene)-2-azaspiro[4.5]decan-3-one	-7.3313	1.5794	Asp143	H-donor
13	(Z)-4-benzylidene-2-((2-(7-chloroquinolin-4-yl)hydrazinyl) methyl)-2-azaspiro[4.5]decan-3-one	-7.1796	1.7900	-	-
14	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(3-methylbenzylidene)-2-azaspiro[4.5]decan-3-one	-7.4743	3.2166	Lys101 Arg109	pi-H pi-cation
15	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(2,4-dichlorobenzylidene)-2-azaspiro[4.5]decan-3-one	-7.3573	2.1615	Met325	pi-H
16	Dihydroartemisinin-piperaquine (control)	-7.5251	2.1682	Ala318 His195 Lys101 Gly196 Asn197 Val240	H-donor H-pi pi-H pi-H pi-H pi-H

Lipinski's rule analysis aims to predict whether a compound has adequate properties to be an effective drug when taken orally. This rule serves as an initial guideline in assessing the similarity of a candidate compound to a particular drug by looking at the physicochemical properties of the substance and its pharmacological effects [31]. These assessed parameters are important for seeing the oral bioavailability of a compound [32-33]. If the molecular weight is too large, it will reduce its biological

effectiveness so that it takes a long time to be absorbed by the body, and it is difficult to penetrate through membranes, both on the skin and in the digestive tract [34]. The parameters of donor hydrogen bonds and acceptor hydrogen bonds indicate how much a compound is able to form hydrogen bonds during the absorption process in the body. If the number of hydrogen bond donors reaches 10 or more, and the number of acceptors is 5 or more, then the energy required to absorb the compound will be greater [35-36]. The LogP value indicates how easily a compound dissolves in fat or non-aqueous solvents, such as oil. The higher the LogP value, the more easily the compound dissolves in fat. This is important because fat-soluble compounds more easily penetrate cell membranes in the body.

Table 2. Results of Lipinski's Rule of Five Prediction

No	Compounds	Molecular Weight	Hydrogen Bond Donors	Hydrogen Bond Acceptors	logP
		(<500g/mol)	**(<5)**	**(<10)**	**(<5)**
1	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(4-hydroxybenzylidene)-2-azaspiro[4.5]decan-3-one	477.008	3	5	5.5901
2	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(4-methoxybenzylidene)-2-azaspiro[4.5]decan-3-one	491.035	2	5	5.8931
3	(Z)-4-(4-chlorobenzylidene)-2-((2-(7-chloroquinolin-4-yl) hydrazinyl)methyl)-2-azaspiro[4.5]decan-3-one	495.454	2	4	6.5379
4	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(4-fluorobenzylidene)-2-azaspiro[4.5]decan-3-one	478.999	2	4	6.0236
5	(Z)-4-(2-bromobenzylidene)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-2-azaspiro[4.5]decan-3-one	495.454	2	4	6.5379
6	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(furan-2-ylmethylene)-2-azaspiro[4.5]decan-3-one	539.905	2	4	6.647
7	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(thiophen- 2-ylmethylene)-2-azaspiro[4.5]decan-3-one	450.97	2	5	5.4775
8	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(pyridin-3-ylmethylene)-2-azaspiro[4.5]decan-3-one	478.999	2	4	6.0236
9	(Z)-4-benzylidene-2-((2-(7-chloroquinolin-4-yl)hydrazinyl) methyl)-2-azaspiro[4.5]decan-3-one	467.038	2	5	5.946
10	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(3methylbenzylidene)-2-azaspiro[4.5]decan-3-one	433.943	2	5	4.991

No	Compounds	Molecular Weight	Hydrogen Bond Donors	Hydrogen Bond Acceptors	logP
		(<500g/mol)	(<5)	(<10)	(<5)
11	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(2,4-dichlorobenzylidene)-2-azaspiro[4.5]decan-3-one	539.905	2	4	6.647
12	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(2-hydroxybenzylidene)-2-azaspiro[4.5]decan-3-one	462.981	3	5	5.3441
13	(Z)-4-benzylidene-2-((2-(7-chloroquinolin-4-yl)hydrazinyl) methyl)-2-azaspiro[4.5]decan-3-one	432.995	2	4	5.596
14	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(3-methylbenzylidene)-2-azaspiro[4.5]decan-3-one	446.97	2	3	5.11
15	(Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(2,4-dichlorobenzylidene)-2-azaspiro[4.5]decan-3-one	501.845	2	4	6.9028
16	Dihydroartemisinin-piperaquine	819.875	1	11	7.6108

Based on the Lipinski's rule analysis, it was found that most of the quinoline derivative compounds investigated satisfied several key parameters, particularly the number of hydrogen bond donors and acceptors, which remained within the acceptable limits. This indicates that these compounds have good potential to form interactions with biological targets without compromising membrane permeability. However, violations were still observed in the lipophilicity parameter (logP) and, in some compounds, also in molecular weight exceeding 500 Da.

Compound 10 was the only compound that fulfilled all Lipinski's rule criteria without any violations, suggesting that it is likely to possess the most optimal oral bioavailability. Meanwhile, compounds 1, 2, 3, and 12 can still be considered to relatively comply with Lipinski's rule, as they exhibited only a single violation, generally associated with high logP values. This single violation is typically considered acceptable in the early stages of drug development. Compounds 6 and 11 exhibited more than one violation, particularly in the logP and molecular weight parameters, which may potentially affect their absorption and distribution within the body. These results indicate that although not all compounds fully comply with Lipinski's rule, the majority still possess favorable characteristics to be developed as drug candidates. Compounds with the fewest violations, particularly compound 10, have greater potential for further development due to their more balanced physicochemical properties compared to the other compounds.

An ADMET study is an approach to evaluating the pharmacokinetics of a drug compound, encompassing aspects of absorption, distribution, metabolism, excretion, and toxicity [37]. This study predicts how a drug compound and its effects will occur in the body [38]. Predictions for a drug include how much of the drug is absorbed orally and through the gastrointestinal tract. If absorption is poor, distribution and metabolism will lead to neurotoxicity and nephrotoxicity [39].

The results of the ADMET analysis showed that all test compounds showed good absorption values based on the CaCo-2 permeability parameter and the Human Intestinal Absorption (HIA) value. The Caco-2 permeability value in the moderate to high range indicates that the compound is able to pass through the intestinal epithelial membrane effectively. The Caco-2 model itself has been recognized as a standard method in predicting oral absorption because it has similarities to human intestinal enterocytes and correlates with the fraction of drugs absorbed in vivo [40].

Table 3. Result of Pharmacokinetic Study

Parameters		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
Absorption	Caco2 permeability (log Papp in 10-6 cm/s)	0.972	0.969	1.257	1.053	1.257	1.254	0.785	1.257	1.104	0.931	1.092	0,782	1.421	0.903	1.465	0.81
	Pgp substrate	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
	Intestinal absorption (% Absorbed)	85.552	88.433	86.775	87.274	86.775	86.708	90.961	87.678	90.431	91.586	90.514	85.554	91.22	84.228	86.011	100
Distribution	VDss (log L/kg)	-0.179	-0.123	-0.023	-0.007	-0.023	-0.012	0.489	-0.0148	0.087	-0.267	0.052	-0.187	0.297	0.011	0.262	1.444
	BBB permeability (log BB)	-0.712	-0.06	0.068	-0.09	0.068	0.067	-0.889	0.0681	-0.04	-0.47	-0.051	-0.953	0.035	-0.138	0.009	-1.45
Metabolism	CYP1A2 inhibitior	No	No	No	No	No	No	No	No	Yes	No	Yes	Yes	Yes	Yes	No	No
	CYP2C9 inhibitior	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No
	CYP3A4 inhibitior	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	Yes
Excretion	Renal OCT2 substrate	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No	No
	Total Clearance (log ml/min/kg)	0.054	0.097	-0.667	-0.095	-0.063	-0.085	0.086	-0.08	0	-0.063	-0.095	0.1	-0.059	-77.692	-0.213	0.306
Toxicity	AMES toxicity	No	No	No	Yes	No	No	No	No	No	No	No	Yes	No	Yes	Yes	No
	Hepatotoxicity	Yes	Yes	Yes	Yes	Yes	Yes	Yes	Yes	No	Yes	No	Yes	Yes	No	No	Yes
	Oral Rat Acute Toxicity (LD50) (mol/kg)	2.837	2.803	2.949	2.649	2.949	2.943	2.526	2.872	2.557	2.976	1.127	2.329	2.187	2.482	2.731	3.214

This is supported by the high HIA value (85–100%), which indicates that the compound has the potential for excellent oral bioavailability. However, the majority of compounds were identified as P-glycoprotein (P-gp) substrates, which can cause the efflux transporter mechanism to occur, thereby reducing the amount of drug absorbed. Parameters such as P-gp and Caco-2 permeability are important indicators in ADMET evaluation because they directly influence the success of drug absorption in the body [41-43].

In terms of distribution, a relatively low volume of distribution (VDss) value indicates that the compound tends to remain in the plasma and is not widely distributed to body tissues [43]. Furthermore, pharmacokinetic parameters such as volume of distribution are important factors in determining how a drug is distributed in the body and influence its plasma concentration [44]. Furthermore, a low Blood-Brain Barrier (BBB) permeability value (indicated by a negative logBB value) indicates that the compound has limited ability to penetrate the blood-brain barrier [45]. This condition can be advantageous if the therapeutic target is not in the central nervous system, as it can minimize the potential for side effects on the brain [46]. Recent studies have also confirmed that BBB parameters are crucial indicators in determining whether a drug will act on the central nervous system or only on peripheral tissues [45].

In terms of metabolism, most compounds have shown potential as inhibitors of cytochrome P450 enzymes, especially CYP2C9 and CYP3A4, which are key isoenzymes in drug metabolism. Cytochrome P450 enzymes are known to play a crucial role in phase I drug metabolism through oxidation reactions that facilitate the elimination of xenobiotic compounds from the body. Inhibition of these enzymes can lead to increased drug concentrations in the systemic circulation due to reduced metabolic rates. This condition has the potential to lead to drug-drug interactions, which can increase the risk of toxicity or reduce therapeutic efficacy. Furthermore, CYP3A4 and CYP2C9 isoenzymes are known to be involved in the metabolism of most circulating drugs, making them important targets for pharmacokinetic evaluation. Recent research also confirms that predicting interactions with CYP450 isoenzymes is a key parameter in modern ADMET studies because it directly relates to drug safety and efficacy [40], [47-50].

Regarding excretion, a relatively low total clearance value indicates that the compound is eliminated slowly from the body, potentially having a longer half-life. This can provide the advantage of a longer duration of drug action, but also has the potential to lead to compound accumulation if not properly regulated. Clearance evaluation is a crucial component of ADMET studies as it relates to determining the dose and frequency of drug administration. A thorough understanding of cytochrome P450 (CYP450)-mediated metabolism is key to predicting ADMET profiles. Graph-based models such as Graph Neural Networks (GNN) and various in silico prediction tools have been developed to improve the precision of drug interaction identification and the effectiveness of molecular elimination in the body [51-52].

Regarding toxicity parameters, several compounds showed positive results in the AMES test, indicating mutagenic potential, and hepatotoxicity was predicted for most compounds. This indicates that despite compounds having favorable pharmacokinetic profiles, safety remains a major concern. Recent studies have reported that toxicity evaluation, such as mutagenicity (AMES) and hepatotoxicity, is a crucial step in drug candidate screening because it is associated with the risk of long-term side effects [53]. Validation using data from failed drug candidates in clinical development has shown that this computational approach is highly effective in identifying hepatotoxicity risks early [54]. The LD50 value in the moderate range indicates that the acute toxicity of the compound is still within tolerable limits, although it still requires further experimental validation. In general, modern computational approaches have enabled faster and more efficient toxicity predictions to help prioritize compounds with the most optimal safety profiles [55].

Overall, the ADMET prediction results indicate that the test compound 10 (Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(3-methylbenzylidene)-2-azaspiro[4.5]decan-3-one has good potential as an oral drug candidate because it is supported by high absorption capacity and suitable distribution to non-central nervous system targets. However, the potential interaction with CYP450 enzymes, its properties as a P-gp substrate, and indications of toxicity such as hepatotoxicity and mutagenicity are limiting factors that need further optimization. Therefore, modification of the molecular structure is still needed to improve the safety profile without reducing its biological activity.

4. Conclusion

Based on the results of the conducted study, it can be concluded that quinoline derivative compounds have potential as antimalarial drug candidates based on an in silico approach. Molecular docking studies revealed that several compounds exhibit good affinity toward the target protein and are capable of forming stable interactions with key amino acid residues. Lipinski's rule of five analysis indicated that most compounds meet the druglikeness criteria, although some violations were observed, particularly in the lipophilicity parameter. Meanwhile, ADMET analysis demonstrated that the majority of the compounds possess relatively favorable pharmacokinetic profiles with low toxicity levels. Among all the tested compounds, compound (Z)-2-((2-(7-chloroquinolin-4-yl)hydrazinyl)methyl)-4-(3methylbenzylidene)-2-azaspiro [4.5]decan-3-one number 10 showed the most optimal performance based on the combined results of molecular docking, compliance with Lipinski's rule, and a well-balanced ADMET profile. Therefore, this compound has strong potential to be further developed as an antimalarial drug candidate. However, further experimental studies are required to validate the in silico predictions obtained in this research.

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