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Original Article

Blockade of Multiple Pathways of *P. falciparum* by Quinoxaline from Curry Fish (*S. hermanni*) Using an *in silico* Approach

Prawesty Diah Utami[1,*], Varidianto Yudo[2], Retno Budiarti[2]

[1]Department of Parasitology, Faculty of Medicine, Universitas Hang Tuah, Surabaya, INDONESIA.
[2]Department of Microbiology, Faculty of Medicine, Universitas Hang Tuah, Surabaya, INDONESIA.

ABSTRACT

Introduction: The escalating mortality and morbidity rates due to malaria present an unsolved global health problem. Previous *in vivo* research has revealed the antimalarial effect of *S. hermanni*. However, the mechanism of quinoxaline inhibition from curry fish against *P. falciparum* remains unknown, prompting this *in silico* investigation to identify inhibition pathways. **Objectives:** This study aims to uncover the inhibitory mechanism pathways of quinoxaline from *S. hermanni* against numerous proteins in *P. falciparum* using an *in silico* approach. **Materials and Methods:** The PDB, UniProt, and PubChem databases were utilized to obtain target protein and ligand structures. The Molegro molecular docking tool was employed to assess the interactions between the target protein and ligand and evaluate the protein target and ligand (control or active compound). 3D visualization of the target protein-ligand interaction was conducted using Discovery Studio. Pharmacokinetic and toxicity prediction analysis of quinoxaline was performed using PkCMS. **Results:** Quinoxaline can bind to *P. falciparum* proteins through similar amino acid residues or different pathways compared to the controls via inhibitor, active, substrate, and cofactor sites, exhibiting various binding affinities. Pharmacokinetic assays revealed that quinoxaline possesses good water solubility, intestinal absorption, and the ability to penetrate the BBB/CNS. However, it exhibits poor skin permeability and limited distribution properties. It can interfere with the P450 function and demonstrates excellent excretion properties. Toxicity analysis indicated that quinoxaline has no toxic effects but can induce skin sensitization. **Conclusion:** Quinoxaline from curry fish can effectively block multiple metabolic pathways of *P. falciparum* and has no toxic effect. However, it still exhibits moderate pharmacokinetic properties.

Keywords: Quinoxaline, Curry fish, *In silico*, *P. falciparum*, Antimalarial.

Correspondence:
Dr. Prawesty Diah Utami
Department of Parasitology, Faculty of Medicine, Universitas Hang Tuah, Surabaya, INDONESIA.
Email: prawesty.diah@hangtuah.ac.id
ORCID ID: 0000-0002-8527-4953

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INTRODUCTION

The aquatic ecosystem poses challenges as it harbors various diverse biotas and holds potential as a therapeutic resource. Over the past few years, marine organisms such as *Sargassum*, sea cucumbers, sponges, and corals have yielded numerous bioactive compounds. Numerous bioactive substances have recently been extracted from marine organisms, including sponges, corals, *Sargassum*, corals, and sea cucumber. These bioactive substances have demonstrated antibiotic, antiviral, antiparasitic, anti-inflammatory, antioxidant, anti-cardiovascular, anticancer, and other properties.[1,2]

Curry fish is a popular term for one of the sea cucumber species, *Sticophus hermanni*, which is common in shallow waters and coral reef regions.[3] Small blackish-brown papillae are on its lateral and dorsal portions, making this curry fish easily recognizable. It has a cylindrical shape with a pale yellow or yellowish-green color. Sea cucumbers, identified as potential sources of healthy nutrition with therapeutic benefits, contain various bioactive components such as vitamins, essential amino acids, fatty acids, glycosaminoglycans, carotenoids, keratin, glucosamine, peptides, chondroitin, glycosides, triterpene glycoside, minerals, cell growth factors, mucopolysaccharides, lectins, omega, collagen, quinoxaline, etc. Based on *in vitro* experiments, a prior study has demonstrated the substantial potential of curry fish for antimalarial action.[4-7] Quinoxaline has been identified as an active compound in sea cucumbers, including *S. hermanni*.[8] Benzene and pyrazine rings are both present in the quinoxaline. Synthesis of Quinoxaline 1,4-di-N-oxides (QdNOs) can occur by oxidizing both pyrazine ring nitrogens. Quinoxaline has exhibited antiparasitic properties, including effectiveness against malaria

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and schistosomes.[9-12] However, the mechanism as an antimalarial agent remains unexplored.

One of the impediments to malaria elimination is the issue of ACT or artemisinin combination treatment resistance.[3] ACT has played a significant role in managing various malaria infections for the last 20 years. Still, in the ensuing ten years, Southeast Asia's Mekong region noticed an increasing trend in artemisinin resistance.[13] The latest articles offer up-to-date details on the level of artemisinin resistance in India.[14] Other academic reports provide information on Africa and Rwanda areas.[15,16] Epidemiological data forecasts a "tsunami" of ACT resistance, also known as "super malaria," worldwide. According to a World Health Organization estimate, there were 247 million cases of malaria in 2021, up from 245 million cases in 2020. The rise from 568 thousand deaths in 2019 to 619 thousand deaths in 2021 was attributed to malaria. These facts indicate that the spread of malaria infection persists as an issue of concern for global health. Malaria infection is an international medical problem that leads to anemia and diminishes productive capacity and death rates, especially in susceptible communities like infants, youngsters, and gravid women.[16,17] The campaign against malaria will be jeopardized if ACT activity decreases. It is critical to develop alternate drug strategies to halt the ACT-resistant strain expansion.

Several medical researchers developed new antimalarial drugs through chemical changes to the existing medications.[18,19] Natural ingredients are also noteworthy as brand-new source materials of antimalarial agents.[20,21] Consequently, it is crucial to use novel, potent, inexpensive, safe, and accessible antimalarial agents.[22] Currently, the majority of antimalarial medications target the blood phase of the malaria development process, which results in symptoms. Otherwise, the non-blood (hepatic) stage persists unappealing due to the lack of clinical signs-the majority of antimalarial medicines act primarily by inhibiting parasites from performing many basic metabolic pathways. *P. falciparum* has several target proteins that play a role in metabolic processes; inhibiting these target proteins will suppress parasite replication and alleviate clinical symptoms. The key metabolic processes of the parasite, including oxidative stress, heme elimination process, the formation of fatty acid, and nucleic acid, provide a number of new drug design opportunities.[23] Several new drug design sites are the parasite's major metabolic pathways, including oxidative stress, heme detoxification, fatty acid synthesis, and nucleic acid synthesis.[24] Therefore, identifying drug targets has been possible by examining inhibitors specific to the malaria parasite's new target proteins.[25]

Discovering new antimalarials with knowledge of the *P. falciparum* genome or genetic information sequence will be easier. An *in silico* study is one way to use a computer program to predict how effectively an active ingredient can block the activity of a target protein in an organism.[26-28] Using an *in silico* study methodology, this study will investigate the mode of action of quinoxaline on proteins essential to *P. falciparum* metabolism.

MATERIALS AND METHODS

Quinoxaline Bioactive Compounds

A search for earlier studies exploring the active chemical components of *S. hermanni* led to the discovery of its bioactive compounds.

Structure of the *P. falciparum* Protein and Ligand

The method involved compiling a *P. falciparum* target protein database through a literature study approach and conducting a data search in the therapeutic target database. From the UniProt and PDB (Protein Data Bank) databases (accessed through http://www.rcsb.org), target protein structures and controls for each protein meeting the specified criteria were collected. The protein should comply with the following requirements: (1) it is essential for *Plasmodium* survival, and its suppression can lead to *P. falciparum* death; and (2) the protein target must have its native ligand, which can be utilized as a reference for the ligands analyzed in comparison to the 3D conformation.[29] Native ligands are employed in molecular docking research to confirm the binding between the active ingredient and the target protein. Molecules comprising the solvent and associated ligands were removed to enhance the protein structure. Using the binding cavities parameters of the Molegro virtual Docker five programs, protein structures that have been cleared of ligands, solvents, and other molecules are predicted to contain their active sites: five maximum cavities for van der Waals.[30] A molecule known as a ligand can interact with receptors and connect them to complex molecules to carry out biological functions. Drug substances engage in reversible complex formation with receptors to produce reaction-inducing effects.[31]

Ligand Preparation

The three-dimensional structures of quinoxaline (CID 7045) were obtained from the National Center for Biotechnology Information's (NCBI) PubChem database, serving as the native ligand for all protein targets of *P. falciparum*.

Molecules Interaction Prediction Analysis Using Molecular Docking

Molecular docking analysis is one of the most useful structure-based *in silico* tools for predicting interactions between drugs and biological receptor molecules. To perform molecular docking, it is frequently necessary to specify the receptor's orientation for the ligand molecules and apply a scoring system to ascertain the complementarity of the molecules. Five programs of the Molegro virtual docker were used to dock with different protein grids on each protein's active sites (binding cavities) (Table 1).[30] For Molegro's virtual docker, the docking requirements

include Score Function Moldock Score [Grid], MolDock SE, and a 0.30 grid resolution. The largest possible population is 50, with the highest number of runs set at 10, and the maximal number of iterations is 1500. Pose manufactured energy thresholds are set at 100 and 300; the neighbor distance factor is 1.00 with tries ranging from 10 to 30; the maximum number of steps for simplex evolution is 5; the energy threshold is 0.00; and the RMSD of the cluster equivalent pose is 1.

Pharmacokinetic and Toxicology Analysis

This study employed distance-based graph signatures and the pkCMS approach for predicting and optimizing the pharmacological and toxicological characteristics of small compounds.

Data Analysis

PyMol version 2.2 software was used to merge the docking results with protein (superimposed) using Molegro virtual docking version 5 results. Docking visualization was conducted in the Discovery Studio program version 21.1.1 to illustrate the interactions among 3D and 2D views.

RESULTS

The Active Component of *S. hermanni*

A prior assessment of the literature claims that *S. hermanni* contains a variety of therapeutic components, including quinoxaline derivatives with neuroprotective effects.[47,48] This study examines the quinoxaline bioactive component of *S.*

Table 1: Grid docking and native ligand of protein target.[30,31]

Protein	Native Ligand	PDB ID	References	X (Å)	Y (Å)	Z (Å)	Radius
P. falciparum protein kinase 5 (PfPK5).	2',3-dioxo-1,1',2',3-tetrahydro-2,3'-biindole-5'-sulfonic acid.	1V0O	32,33	22.62	5.53	34.72	7
P. falciparum casein kinase 2 alfa (PfCK2).	3FL5	3FL5	34	22.78	7.42	18.99	10
P. falciparum calcium-dependent protein kinase 1 (PfCDPK1).	P62344	P62344	35	-22.05	8.48	-8.6	15
Falcipain-3	3BPM	3BPM	36	6.07	17.54	-38.38	15
P. falciparum leucine aminopeptidase (PfLAP).	4X2T	4X2T	37	70.90	76.22	-17.21	12
P. falciparum M1 neutral aminopeptidase (PfA-*M1).*	3EBH	3EBH	38	17.89	5.34	9.48	15
P. falciparum erythrocyte membrane protein 1 (PfEMP1).	7FAP	7FAP	39	170.32	196.15	172.21	13
P. falciparum deoxyuridine 5′-triphosphate nucleotidohydrolase (PfdUTPase).	2,3-deoxy-3-fluoro-5-O-trityluridine	1VYQ	40	37.13	-9.8	11.66	11
P. falciparum dihydrofolate reductase (PfDHFR).	1J3K	1J3K	41,42	31.36	5.17	64.0	15
P. falciparum adenylosuccinate synthetase (PfADSS).	1P9B	1P9B	43	21.15	78.13	33.57	15
P. falciparum dihydroorotate dehydrogenase (PfDHODH).	6GJG	6GJG	37	13.12	-0.3	-0.97	10
P. falciparum β-hydroxy acyl-ACP dehydratase (PfFabZ).	1Z6B	1Z6B	44	12.59	38.78	69.31	10
P. falciparum phosphoethanolamine n-methyltransferase (PfPMT).	3UJ6	3UJ6	45	25.28	17.27	19.23	11
P. falciparum enoyl-acyl carrier protein reductase (PfENR).	2FOI	2FOI	46	51.4	87.94	37.12	11

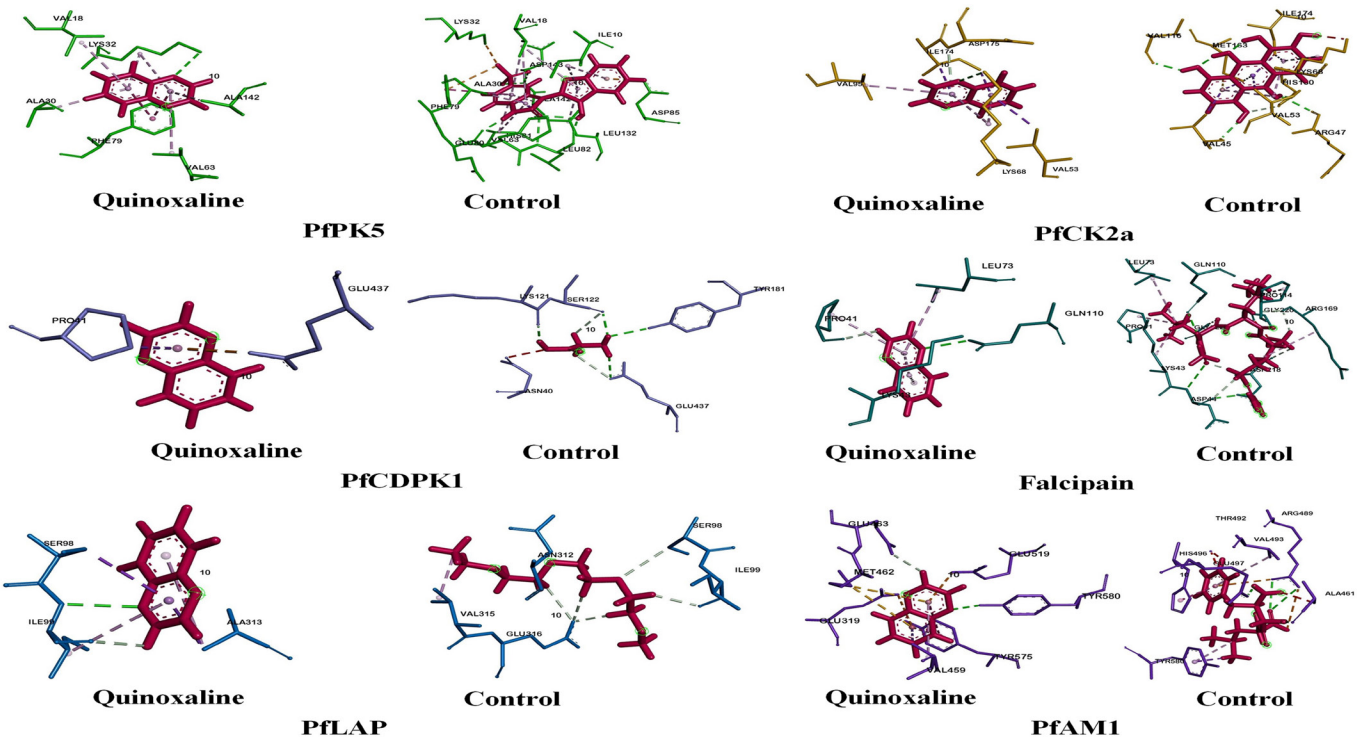

Figure 1: Interaction of quinoxaline with several *P. falciparum* proteins in 3D structure. Displayed proteins include PfPK5, PfCK2α, PfCDPK1, falcipain, PfLAP, and PfA-M1.

hermanni as ligands for fourteen protein targets of *P. falciparum*, building on prior research.

In this *in silico* investigation, the subsequent phase involved applying the molecular docking technique to examine the inhibitory activity of *S. hermanni's* quinoxaline bioactive compounds against the fifteen protein receptors of *P. falciparum*.

The results of the molecular docking of quinoxaline with fourteen protein receptors of *P. falciparum*, as presented in the preceding table and the three-dimensional images, demonstrate that quinoxaline can bind to all protein receptors at both distinct and identical locations as the controls (Table 3, Figures 1-3). Some interactions between quinoxaline bind PfENR, PfCK2α, and PfPK5 through the same locations as the controls, while the remaining protein receptors of *P. falciparum* bind with quinoxaline through different sites.

A precise and time-dependent orchestration of protein interactions supports cellular function. Binding affinity represents the capacity of the binding contact between a single biomolecule (such as a protein or DNA) and its ligand/binding partner (such as bioactive ingredients or inhibitors). To evaluate and report binding affinity, the equilibrium dissociation constant (Kd) is often used, classifying and quantifying the strengths of bimolecular interactions. The ligand's affinity for its target increases with decreasing Kd values. The weaker the target molecule and ligand's affinity and binding, the higher the Kd value.[58,59]

Displaying molecular structures with pkCSM expands the idea of cutoff scanning to characterize and determine the physicochemical and toxicological aspects of molecules. Nineteen predictors in pkCSM describe a compound's pharmacokinetic attributes, and ten predictors describe a molecule's toxicity. Pharmacological and toxicological analyses on quinoxaline used the pkCSM.

DISCUSSION

In general, the mechanism of action of currently used antimalarial drugs involves interference with major metabolic pathways in the parasite. Several new pathways are being explored for discovering and developing new antimalarial drugs by inducing cellular oxidative stress or interfering with heme metabolism, biosynthesis of fatty acids, nucleic acid, etc. Targeting appropriate proteins with drugs or active compounds can reduce disease progression and improve clinical manifestations. *Plasmodium falciparum* has multiple proteins involved in its metabolic process, including PfPK5, PfCK2, PfCDPK1, falcipain-3, PfLAP, PfA-M1, PfEMP1, PfdUTPase, PfDHFR, PfADSS, PfDHODH, PfFabZ, PfPMT, and PfENR (Table 1).

In organic chemistry, quinoxaline derivatives (C8H6N2) are known as benzopyrazine. Quinoxaline is a heterocyclic chemical with N replacements for carbon atoms in the naphthalene ring. Applications for quinoxaline analogues include the treatment of chronic metabolic diseases, cancer, and inflammation. They also have antibacterial, antifungal, and antiparasitic effects.[9] According to the findings of this research's prediction assay, quinoxaline

Table 2: Interaction of quinoxaline with protein receptors of *P. falciparum*.

Protein Receptors	Interaction	Distance (Å)	Category
PfPK5 Inhibiting DNA, RNA synthesis, and parasite proliferation.[49]	A:LYS32:NZ-:10:N1	30.882	Hydrogen bond
	A:PHE79-:10	377.115	Hydrophobic
	A:PHE79-:10	365.306	
	:10-A:VAL18	439.583	
	:10-A:ALA30	438.894	
	:10-A:LYS32	45.661	
	:10-A:LYS32	513.301	
	:10-A:VAL63	512.655	
	:10-A: ALA142	413.581	
PfCK2α Essential for the development of sexual parasites, both the regulatory and catalytic kinase subunits are necessary as substrates for phosphorylation.[50]	A:LYS68:NZ-:10:N1	30.459	Hydrogen bond
	A:ASP175:N-:10	366.348	
	A:VAL53:CG2-:10	386.299	Hydrophobic
	A:ILE174:CB-:10	375.379	
	A:ILE174:CD1-:10	346.433	
	:10-A:LYS68	470.366	
	:10-A:LYS68	495.478	
	:10-A:VAL95	502.685	
PfCDPK1 Regulating mRNA transcription and translation and macro- and microgametocyte formation.[51]	A:GLU437:OE2-:10	385.613	Electrostatic
	A:PRO41:CD-:10	396.412	Hydrophobic
Falcipain-3 Degradation of globin into amino acids.[29]	A:GLN110:NE2-:10:N1	270.337	Hydrogen bond
	:10:H6-A:PRO41:O	284.377	
	:10-A:PRO41	49.044	Hydrophobic
	:10-A:LYS43	444.168	
	:10-A:LYS43	440.499	
	:10-A:LEU73	509.507	
PfLAP Catalyzing the breakdown of amino acids into smaller peptides.[49]	C:ILE99:N-:10:N1	293.905	Hydrogen bond
	:10:H5-C:ILE99:O	259.087	
	C:SER98:CB-:10	39.282	Hydrophobic
	C:ALA313:CB-:10	363.973	
	:10-C:ILE99	519.693	
	:10-C:ALA313	526.749	
PfA-M1 Catalyzing the breakdown of amino acids into smaller peptides.[49]	A:TYR580:OH-:10:N1	260.712	Hydrogen bond
	:10:H6-A:GLU463:OE1	25.273	
	A:GLU319:OE2-:10	495.044	Electrostatic
	A:GLU519:OE2-:10	431.945	
	A:VAL459:CG2-:10	397.171	Hydrophobic
	A:MET462:SD-:10	461.094	Others
	A:MET462:SD-:10	483.678	
	A:TYR575-:10	567.337	Hydrophobic
	A:TYR575-:10	443.324	
	:10-A: VAL459	504.068	

Protein Receptors	Interaction	Distance (Å)	Category
PfEMP1 Infecting erythrocyte surface membrane protein that adheres to the blood vessel endothelium in infected individuals.[29]	A:GLN1036:NE2-:10	358.059	Hydrogen bond
	A:GLN1036:NE2-:10	397.611	
	A:ILE1849:CG2-:10	303.423	Hydrophobic
PfdUTPase Converting deoxyuridine triphosphate (dUTP) to deoxyuridine monophosphate (dUMP), a biomolecular component to create DNA nucleotides.[40]	B:THR123:OG1-:10:N1	28.873	Hydrogen bond
	:10:H5-B:THR97:O	253.797	
	A:ASP121:OD1-:10	392.378	Electrostatic
	A:ASP121:OD2-:10	425.551	
	A:THR44:CG2-:10	363.109	Hydrophobic
PfDHFR A molecule necessary for the de novo production of purines and amino acids, Catalyzing the NADPH-dependent conversion of dihydrofolate to tetrahydrofolate.[52]	A:ASN108:N-:10:N1	29.653	Hydrogen bond
	A:VAL168:N-:10:N2	310.752	
	A:GLY166:CA-:10	359.012	Hydrophobic
	:10-A: VAL168	50.514	
	:10-A: VAL169	548.714	
	:10-A: VAL195	533.175	
PfADSS An enzyme facilitating the conversion of the GTP-dependent inosine monophosphate (IMP) and aspartic acid to GDP during protein synthesis.[53]	A:LYS29:N-:10:N2	284.418	Hydrogen bond
	A:GLY30:N-:10:N2	264.193	
	A:ASP26:CA-:10:N1	292.302	
	A:THR55:N-:10	378.576	
	A:HIS54:CA-:10	399.887	Hydrophobic
	A:HIS54-:10	461.914	
	:10-A:LYS29	492.867	
PfDHODH An enzyme for pyrimidine base production.[54]	A:GLY478:N-:10:N1	285.984	Hydrogen bond
	A:TYR528:N-:10:N2	296.203	
	A:SER477:OG-:10	406.557	
	A:SER505:OG-:10	37.068	
	A:GLY507:N-:10	342.852	
	A:SER529:N-:10	365.242	
	A:SER529:OG-:10	384.206	
	A:GLY506:CA-:10	393.884	Hydrophobic
PfFabZ An enzyme involved in the fatty acid elongation cycle.[55]	A:ASN131:ND2-:10:N1	262.775	Hydrogen bond
	C:ASN131:ND2-:10	3.873	
	D:TYR100-:10	488.018	Hydrophobic
	D:TYR100-:10	494.093	
	:10-D: PRO101	504.675	
PfPMT An enzyme for lipid biosynthesis using a three-step, S-Adenosyl Methionine (SAM)-dependent methylation process to turn phosphoethanolamine into phosphocholine.[56]	A:ASP61:OD2-:10	380.473	Electrostatic
	:10-A:ILE36	516.974	Hydrophobic
	:10-A: ARG127	427.941	

Protein Receptors	Interaction	Distance (Å)	Category
PfENR An enzyme for reducing the trans-2-enoyl bond of the enoyl-acyl substrate carrier protein (ACP) to saturated acyl-ACP in completing the fatty acid elongation process.[57]	A:ALA169:N-:10:N2	294.809	Hydrogen bond
	:10:H5-A:ASN218:OD1	294.541	
	A:LEU216:CD2-:10	379.519	Hydrophobic
	A:TRP131-:10	4.183	
	A:TRP131-:10	454.676	
	:10-A: ALA169	439.442	
	:10-A: ALA169	479.361	
	:10-A: LEU216	455.191	
	:10-A: LYS240	516.959	

NB: Bolded amino acid residues represent the interaction between quinoxaline and the *P. falciparum* receptor through the same site as the controls.

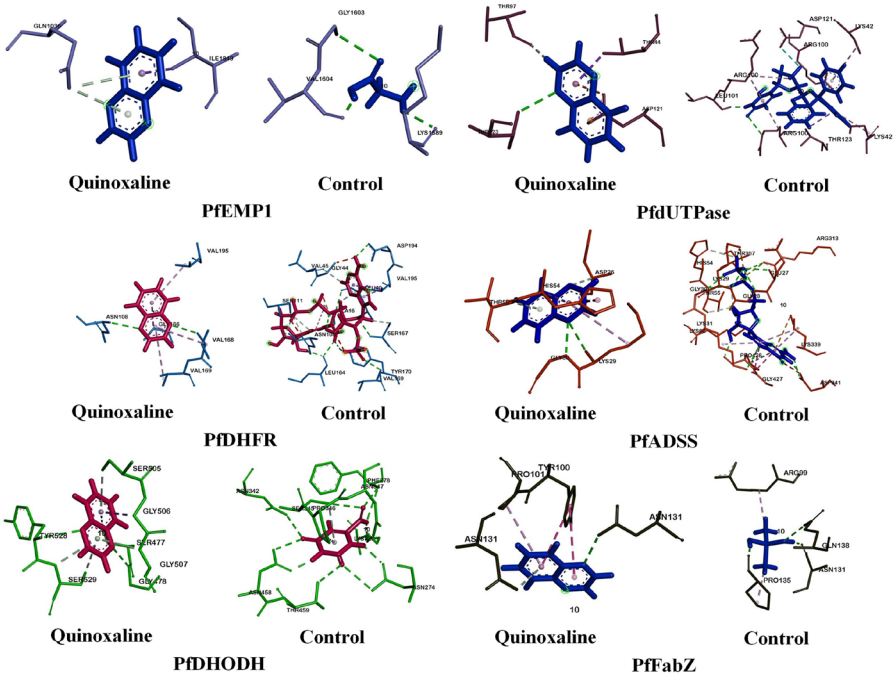

Figure 2: Interaction of quinoxaline with several *P. falciparum* proteins in 3D structure. Displayed proteins include PfEMP1, PfdUTPase, PfDHFR, PfADSS, PfDHODH, PfFabZ.

might obstruct all *P. falciparum* protein targets by binding to their inhibitor, active, substrate, and cofactor sites (see Figures 1-3, Table 2). This study found that quinoxaline derivatives and modifying quinoxaline structure have antimalarial action and significantly lower resistance indices than chloroquine.[9,10]

The molecular docking assay suggested that the number of bonds formed between quinoxaline and the target protein is smaller than that of the controls, except for the interaction with PfA-M1, where it produces more bonds than the controls. Additionally, PfFabZ has the same number of bonds as the controls (Figures 1 and 2, Table 3). Quinoxaline and *P. falciparum* proteins generally form fewer hydrogen bonds than the controls. Compared to the controls, only PfENR and PfDHODH have more hydrogen bonds. The types of bonds between proteins and ligands (bioactive substances) are elements that influence the binding affinity value. If bioactive chemicals can bind tightly via hydrogen bonds, they are predicted to bind strongly to the protein target receptor. Although the hydrogen bond is weaker than a covalent bond, its presence is nonetheless important, affecting the molecule's structure and properties. The design of therapeutic compounds with hydrogen bonds and how they interact with the human body's metabolism are current concerns in medicine and pharmacy.[60,61]

The quantity of the active compound's amino acid residues that bind to the target protein's active sites and how similar the active compound's amino acid residues are to those of the controls impact the molecular bond's strength.[60,61] This study demonstrates that quinoxaline interacts with three target proteins (PfPK5, PfCK2α, and PfENR) using the same amino acid residues as the controls (Figures 1 and 3, Table 2). Six amino

Table 3: Binding affinity of quinoxaline with protein targets of *P. falciparum*.

Sl. No.	Protein Receptors	Binding Affinity (K_d)	
		Control	Quinoxaline
1	PfPK5	-384.4	-129.2
2	PfCK2α	-291.6	-70.67
3	PfCDPK1	0	-127.4
4	Falcipain-3	-392.8	-166
5	PfLAP	-175	-134.6
6	PfA-M1	-266.6	-150
7	PfEMP1	0	-138.6
8	PfdUTPase	-516.8	-178.8
9	PfDHFR	99.45	-161.8
10	PfADSS	-529.8	-191.8
11	PfDHODH	0	-172.2
12	PfFabZ	-91.6	-105.4
13	PfPMT	-616.4	-174.8
14	PfENR	-358.6	-184

NB: Higher ligand affinities for their respective protein targets are indicated by Kd values in bold.

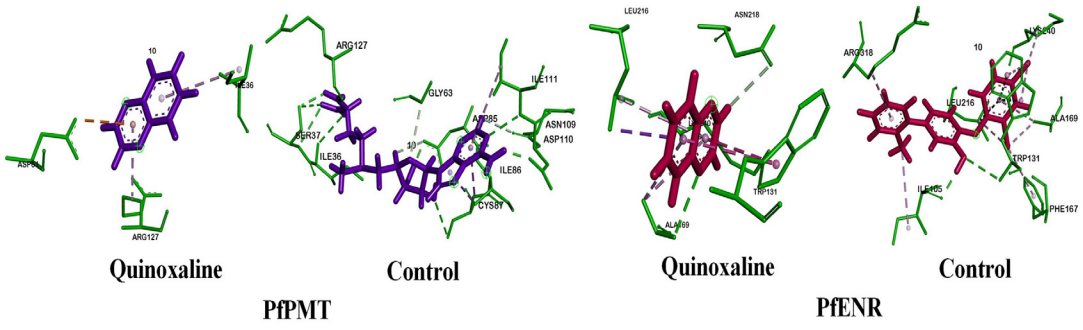

Figure 3: Interaction of quinoxaline with several *P. falciparum* proteins in 3D structure. Displayed proteins include PfPMT and PfENR.

acid residues between quinoxaline and PfPK5 are similar to the controls (A:PHE79-:10, 10-A:VAL18, 10A:ALA30, 10-A:VAL63: 10-A:ALA142) and are connected through hydrophobic bonds. Quinoxaline interacts with PfCK2α through two amino acid residues similar to the controls (10-A:LYS68 with a hydrophobic bond), and five amino acid residues are the same as the controls (A:TRP131 -:10; 10-A:ALA169: 10-A:LEU216 using hydrophobic bonds). In comparison, the remaining eleven target proteins interact via amino acid residues that differ from the controls (PfCDPK1, falcipain-3, PfLAP, PfA-M1, PfEMP1, PfdUTPase, PfDHFR, PfADSS, PfDHODH, PfFabZ, and PfPMT).

The magnitude of the connection between the target protein and other substances that act as ligands is defined as binding affinity. The binding affinity results between quinoxaline and protein targets and the control show that quinoxaline has a lower Kd value than the control, indicating a higher binding affinity for quinoxaline than the controls (five protein targets, including PfCDPK1, PfDHFR, PfDHODH, PfEMP1, and PfFabZ) (Table 3). Otherwise, the controls have a lower Kd value and higher affinity than quinoxaline in nine protein targets, such as PfPK5, PfCK2, falcipain-3, PfLAP, PfA-M1, PfdUTPase, PfADSS, PfPMT, and PfENR. A lower binding affinity value denotes a stronger bond between the target protein and its ligand (bioactive chemicals).[58,59]

Water solubility is a crucial element in the pharmacological reaction of medication following oral administration. Highly water-soluble drugs absorb well and are highly bioavailable. Increased plasma drug concentrations at the target site due to medication bioavailability and absorption allow for therapeutic effects.[62]

The Caco-2 model was used in preclinical investigations to forecast drug-induced gastrointestinal permeability.[63] Whenever the Papp of a compound exceeds 8×10^{-6} cm/s or >0.9, it is considered to have high Caco-2 permeability.[64] Quinoxaline exhibits high gastrointestinal permeability (value=1.835>0.9), classifying it

Table 4: Pharmacological and toxicological prediction on quinoxaline Using pkCSM approach

Parameter		Unit	Quinoxaline
Absorption capacity	Solubility in water	log mol/L	-0.986
	Permeability of Caco2	log Papp in 8×10^{-6} cm/s	1.835
	Gastrointestinal absorption	%	97.299
	Permeability of the skin	log Kp	-2.051
	Substrate of permeability-glycoprotein (P-gp).		No
	Inhibitor of Pgp I		No
	Inhibitor of Pgp II		No
Distribution capacity	Volume distribution steady state (VDss).	log L/kg	-0.283
	Fraction unbound	Fu	0.391
	Permeability of blood-brain barrier	log BB	0.024
	Permeability of the central nervous system.	log PS	-1.885
Metabolism capacity	Substrate of CYP2D6		No
	Substrate of CYP3A4		No
	Inhibitor of CYP1A2		Yes
	Inhibitor of CYP2C19		No
	Inhibitor of CYPC9		No
	Inhibitor of CYP3A4		No
Excretion capacity	Total renal and hepatic clearance	log mL/min/kg	0.187
	Substrate of renal organic cation transporter 2 (OCT2)		No
Toxicity properties	Ames mutagenic toxicity		No
	Maximum recommended tolerated dose (MRTD).	log mg/kg/day	0.597
	Inhibitor of hERG I channel		No
	Inhibitor of hERG II channel		No
	Oral acute toxicity (LD50)	mol/Kg	2.16
	Lowest observed adverse effect (LOAEL).	log (mg/Kg bw/day)	2.424
	Drug-induced liver injury.		No
	Sensitization of the skin.		Yes
	Toxicity effect of *T. pyriformis*	log ug/l	0.148
	Toxicity effect of minnow.	log mM	1.299

NB: Values or results in bold meet the pharmacokinetic and toxicity testing criteria.

as having good intestinal absorption in PkCSM if its percentage value is greater than 30% (a value of less than 30% indicates poor absorption). Based on PkCSM prediction analysis, quinoxaline demonstrates intestinal absorption of 97.299% (value>30%). Therefore, quinoxaline has strong intestine absorption qualities (Table 4).

If the logarithmic value of an active substance is < -2.5, it is declared to have good skin-permeable qualities.[64] Quinoxaline has a log value of -2.51, indicating poor skin permeability. Substrate P-glycoprotein is a member of protein transporters that regulates both the absorption and excretion of a range of medicines, so it impacts their plasma and tissue concentrations and overall effects.[65] P-glycoprotein I/II inhibitors can block or bypass P-gp outflow; when used with P-gp substrates, they can prevent substrate outflow and increase therapeutic benefits.[62,66] Quinoxaline does not affect intestinal absorption since it cannot be a P-glycoprotein substrate and inhibitor of P-glycoprotein I/II. In general, quinoxaline PkCMS results show good solubility and adequate intestinal absorption but poor skin permeability (Table 4).

The volume of distribution at steady state (VDSS) represents the total amount of medicine that must be circulated equally to achieve the identical level of concentration as blood plasma. The larger the VDSS amount, the greater the proportion of medication dispersed in tissues rather than plasma. Low VD compounds have logarithmic VD values less than -0.15, and high VD compounds have logarithmic VD values more than 0.45. The

fraction unbound represents the fraction able to cross/diffuse across the cell membrane; the higher the unbound fraction number, the more that diffuses into the cell.[64] Prediction analysis pkCSM of quinoxaline showed that this molecule has a 0.391 fraction unbound and a VDSS value of 0.283 (less than -0.15), indicating that the substance is dispersed in a small amount (Table 4).

The blood-brain barrier protects the brain's defense and homeostasis system by facilitating the movement of chemicals in and out of the brain. However, molecules with specific structures can penetrate the brain. Compounds capable of crossing the blood-brain barrier are predicted to exert pharmaceutical impacts on the nervous system. Chemical substances with a logarithm BB value <-1 are classified as having poor BBB distribution. In contrast, chemicals with a log BB value of more than 0.3 are classified as having an excellent BBB distribution.[64] Quinoxaline exhibits a moderate BBB distribution with a log BB value of 0.024. CNS permeability is a more accurate indicator of BBB distribution, describing the surface area of BBB permeability (LogPS). The interpretation of the results is that a compound can penetrate the CNS if LogPS >-2, but if LogPS is less than -3, the compound cannot penetrate the CNS. Quinoxaline can penetrate the CNS with a LogPS of -1.885 (LogPS >-2). According to the PkCMS distribution predictor, quinoxaline has limited tissue distribution ability but a decent ability to cross the BBB and CNS (Table 4).

The detoxifying enzyme cytochrome P450 is found in the liver. Cytochrome P450 is involved in the overall metabolism of medicines. On the other hand, P450 inhibitors have the potential to significantly alter the pharmacokinetics of medications. Therefore, it is crucial to ascertain whether the delivered molecule is a CYP2D6/CYP3A4 substrate expected to be metabolized by P450. Inhibitors of cytochrome P450 enzymes may interfere with drug metabolism and are not recommended. Therefore, assessment of the ability of the compound to inhibit cytochrome P450 (such as CYP1A2/CYP2C19/CYP2C9/CYP2D6/CYP3A4 isoforms) is critical.[62,64,66] According to the PkCMS metabolism predictor, quinoxaline can be an inhibitor of CYP1A2, affecting the metabolism of drugs by P450 (Table 4).

Drug clearance is the amount of drug eliminated from plasma in the vascular compartment per unit of time. The sum of all body clearances gives the total clearance, representing the removal of drugs from the core compartment regardless of the method of removal. A renal uptake transporter, Organic Cation Transport-2 (OCT2), is critical for drug distribution and renal clearance. OCT2 substrates can have significant side effects when combined with OCT2 inhibitors.[62,64] Based on the PkCMS excretion predictor, quinoxaline has a total clearance of 0.187 and is not predicted to be an OCT2 substrate (Table 4).

The Ames toxicology assay is a quick and accurate bacterial test that measures the bioactive substance's capacity to cause genetic transformation (mutation) at particular loci in numerous bacterial strains.[67] A chemical compound's estimated hazardous dose threshold for humans is known as the Maximum Tolerated Dose (MRTD). If the MRTD number is ≤0.447 logs, it is considered low, and if it is above 0.447 logs, it is considered high. HERG1/II inhibitors are potassium channel blockers that cause ventricular arrhythmias. [64] Predictions for quinoxaline using PkCSM indicate that the molecule has no Ames toxicity, a high MRTD, and is not an HERG1/II inhibitor (Table 4).

The LD_{50} assay aims to identify the dose of a substance that will result in 50% of rats dying.[68] Chronic oral toxicity (LOAEL) in rats is a test to determine the lowest dose of a compound given over the long term that causes adverse effects. Hepatotoxicity screening is important in the development of new drugs, as chemical substances must not cause damage to the liver. Skin sensitization is a test used to determine the ability of a compound to induce allergic contact dermatitis. *T. pyriformis* toxicity is an assessment of the toxicity of compounds to protozoan organisms; compounds are considered toxic if the measurement is larger than -0.5 log ug/l.[64] Minnow toxicology testing identifies the dose of a substance that can induce 50% death in laboratory animals (fathead minnows), and substances with a low LC_{50} (less than 0.5 mM or Log LC_{50} less than -0.3) have substantial acute toxicity.[64] Prediction of quinoxaline using PkCSM showed that the molecule has an LD_{50} of 2.16 mol/kg, LOAEL of 2.424 logs, promotes cutaneous sensitivity but has no hepatotoxicity or minnow toxicity (Table 4).

CONCLUSION

The molecular docking prediction analysis of quinoxaline indicates that this molecule has the potential to inhibit multiple protein receptors of *P. falciparum*, acting on inhibitor, active, substrate, and cofactor sites. However, quinoxaline's binding affinity to nine protein receptors of *P. falciparum* is weaker than the controls (PfPK5, PfCK2, falcipain-3, PfLAP, PfA-M1, PfdUTPase, PfADSS, PfPMT, and PfENR). The results of PkCSM pharmacokinetic analysis show that, in general, quinoxaline exhibits characteristics of water solubility, intestinal absorption, good excretion, and the ability to penetrate the BBB/CNS permeability. On the other hand, quinoxaline demonstrates poor tissue distribution ability. It also can interfere with P450 activity in the drug detoxification process. PkCSM toxicity prediction for quinoxaline indicates that this molecule has no toxic effects. However, it may induce skin sensitivity. Quinoxaline has great potential to be developed as an antimalarial drug. However, modifications to its chemical structure and combination with other substances are necessary to improve its binding affinity and distribution properties while considering its impact on P450 action.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PfPK5: *P. falciparum* protein kinase 5; **PfCK2α:** *P. falciparum* casein kinase 2 alfa; **PfCDPK1:** *P. falciparum* Ca-dependent protein kinase 1; **PfLAP:** *P. falciparum* leucine aminopeptidase; **PfA-M1:** *P. falciparum* neutral metallo aminopeptidase 1; **PfEMP1:** *P. falciparum* erythrocyte membrane protein 1; **PfdUTPase:** *P. falciparum* deoxyuridine 5′- triphosphate nucleotidohydrolase; **PfDHFR:** *P. falciparum* dihydrofolate reductase; PfADSS: *P. falciparum* adenylosuccinate synthetase; **PfDHODH:** *P. falciparum* dihydroorotate dehydrogenase; **PfFabZ:** *Plasmodium falciparum* β-hydroxy acyl-ACP dehydratase; **PfPMT:** *P. falciparum* phosphoethanolamine n-methyl transferase; **PfENR:** *P. falciparum* enoyl-acyl carrier protein reductase.

SUMMARY

Utilizing computational methods, an *in silico* investigation is a viable approach to prognosticate the efficacy of an active compound in inhibiting the activity of a certain protein within an organism. The active chemical quinoxaline has been found in various species of sea cucumbers, including *S. hermanni. In silico* analysis of this research indicates that quinoxaline has the potential to inhibit various protein receptors of *P. falciparum* by interacting with inhibitor, active, substrate, and cofactor binding sites. Nevertheless, the binding affinity of quinoxaline to nine protein receptors of *P. falciparum* appears relatively lower compared to the control group, which includes PfPK5, PfCK2, falcipain-3, PfLAP, PfA-M1, PfdUTPase, PfADSS, PfPMT, and PfENR. Curry fish quinoxaline exhibits modest pharmacokinetic qualities despite having no harmful effect and the ability to disrupt several *P. falciparum* metabolic pathways. However, it is necessary to modify the chemical structure of the molecule and combine it with other chemicals in order to enhance its binding affinity and distribution qualities.

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