International Journal of Entomology Research
www.entomologyjournals.com
ISSN: 2455-4758
Received: 30-09-2025, Accepted: 30-10-2025, Published: 14-11-2025
Volume 10, Issue 11, 2025, Page No. 57-63

In Silico screening and evaluation of antimalarial drugs and analogues against critical malarial targets

Priyanka Dash[1], Sunita Patra[2*], Subha Shree Satapathy[3], Subhasmita Pattnaik[1], Santhosh Goud[3], Subasini Patnaik[1]
[1] Department of Zoology, Berhampur University, Berhampur, Ganjam, Odisha, India
[2] Department of Zoology, University of Calcutta, Kolkata, West Bengal, India
[3] Department of Zoology, Khallikote Unitary University, Berhampur, Ganjam, Odisha, India

Abstract
Malaria remains a global health challenge, with rising drug resistance in *Plasmodium* parasites and insecticide resistance in vectors exacerbating the burden. *Plasmodium falciparum*, the deadliest species, is the primary cause of severe malaria and related deaths. Resistance to widely used antimalarials such as chloroquine, primaquine, mefloquine, and proguanil has rendered these treatments increasingly ineffective, highlighting the need for novel therapeutic strategies. This study utilizes an *in-silico* approach to identify analogues of these drugs, targeting key parasite proteins, including *Pf* CRT, Fe-II protoporphyrin IX, and *Pf* TS-DHFR. Molecular docking was employed to evaluate binding affinities and identify promising lead compounds with enhanced efficacy and reduced resistance potential. Integrating genomic and bioinformatics data facilitated the identification of parasite-specific targets essential for survival, minimizing off-target effects and resistance risks. The findings propose optimized formulations for targeted drug delivery, enhancing therapeutic efficacy while reducing side effects. This work underscores the role of bioinformatics and rapid drug design techniques in accelerating the discovery of safer, fast-acting, and cost-effective antimalarials to combat drug-resistant malaria effectively.

Keywords: Malaria drug resistance, *Plasmodium falciparum*, *in silico* drug design, molecular docking, rapid drug design (RDD)

Introduction

Malaria remains a significant global health threat, causing over 2 million deaths annually, with India bearing a high burden, especially among infants, children, and adults. The challenge of controlling malaria is exacerbated by the emergence of drug-resistant parasites, the lack of an effective vaccine, and the increasing resistance of vectors to insecticides. Malaria, caused by protozoan parasites of the *Plasmodium* genus, is predominantly transmitted by P. falciparum, P. vivax, P. ovale curtisi, P. ovale wallikeri, P. malariae, and P. knowlesi. Rare cases involving P. cynomolgi, P. brasilianum, and P. simium have also been reported in South America and Southeast Asia, but P. falciparum remains the leading cause of severe malaria and malaria-related deaths (Calderaro *et al*., 2024) [2].

Historically, malaria treatment has relied on antimalarial drugs, with early interventions dating back nearly 400 years when bark infusions from the Peruvian Amazon were used to combat the disease. This led to the isolation of quinine (QN) in 1820, which, despite its toxicity during prolonged use, remains essential for treating severe P. falciparum infections (WHO, 2010). In the mid-20th century, chloroquine (CQ), a 4-aminoquinoline, became a cornerstone of malaria treatment and prophylaxis, used alongside DDT as part of a global eradication campaign. Initially effective, resistance to CQ emerged within a decade, particularly in Southeast Asia and South America, eventually spreading globally and contributing to the failure of eradication efforts (Ige *et al*., 2024) [8]. This resistance significantly increased malaria cases and mortality rates. The subsequent introduction of drugs like sulphadoxine-pyrimethamine (SP) and mefloquine also faced resistance challenges shortly after their deployment, underscoring the need for combination therapies (Plowe, 2022) [9]. Artemisinin-based combination therapies (ACTs) are now the gold standard, pairing fast-acting artemisinin derivatives with longer-acting companion drugs to combat resistance (Erhunse and Sahal, 2021) [5].

Drug resistance remains a significant obstacle. Resistance to CQ and SP, two widely used antimalarials, is rising globally, leading to increased malaria-related morbidity and mortality (Fidock *et al*., 2004) [6]. For CQ, resistance is primarily driven by mutations in the *Plasmodium falciparum* chloroquine resistance transporter (Pf CRT), with the K76T mutation consistently observed. These mutations allow chloroquine efflux from the parasite's cytosol into the acidic digestive vacuole, rendering the drug ineffective (Cooper *et al*., 2005; Bray *et al*., 2005) [1, 3]. Similarly, antifolate drugs like pyrimethamine and cycloguanil face resistance due to mutations in dihydrofolate reductase (DHFR), a crucial enzyme in the folate pathway responsible for nucleotide synthesis. The folate pathway remains a critical target for antimalarial drugs, as its disruption halts parasite replication (Yuthavonga *et al*., 2012; Hyde, 2007) [7, 12].

Proguanil, an antifolate, blocks DHFR, inhibiting the biosynthesis of purines and pyrimidines essential for DNA synthesis and parasite reproduction in red blood cells (Senthilraja *et al*., 2012) [10]. Sulfa drugs like sulfadoxine target dihydropteroate synthase (DHPS), a parasite-specific enzyme involved in folate biosynthesis, further disrupting parasite metabolism (Hyde, 2007) [7]. Once widely used, chloroquine saw resistance develop rapidly, with initial cases reported in Southeast Asia, South America, and the

Western Pacific. Resistance eventually spread to Africa, where it contributed to higher malaria mortality.

Other antimalarials, including mefloquine and primaquine, target ferriprotoporphyrin IX. These drugs prevent the accumulation of hemoglobin breakdown products, such as undimerized Fe-II protoporphyrin IX, disrupting parasite metabolism (Dorn *et al*., 1998) [4].

The sequencing of the P. falciparum genome has provided valuable insights for developing new therapeutic approaches. Genomic data, coupled with bioinformatics tools, enable the identification of critical parasite-specific determinants that can serve as drug targets. Effective targets should play essential roles in the parasite life cycle, lack alternative bypass pathways, and be accessible within the parasite. They should also exhibit low potential for resistance development, be involved in rate-limiting biochemical processes, and have selective inhibitors for validation. High-throughput technologies, such as genome and cDNA sequencing, proteomics, structural genomics, and metabolic network analyses, support identifying and validating these targets.

This study employed an in-silico approach to identify chloroquine, primaquine, mefloquine, and proguanil analogs. These analogs aim to target Pf CRT, Fe-II protoporphyrin IX, and Pf TS-DHFR to discover potential lead molecules exhibiting high binding affinity. Designing optimized pharmaceutical formulations for targeted drug delivery can minimize side effects, improve therapeutic efficacy, and enhance drug effectiveness.

Rapid Drug Design (RDD) techniques like molecular docking are integral to drug discovery. Docking predicts the binding orientation of small molecules to their protein targets, providing insights into binding affinity and activity. This method facilitates the identification of fast-acting, cost-effective, and safer drug candidates that are suitable for single-dose administration and can be used in vulnerable populations, such as children and pregnant women. The development of such drugs is essential to combating the growing threat of drug-resistant malaria.

Materials and Methods

Molecular Docking Studies

Advances in bioinformatics and structural biology offer significant opportunities for identifying novel antimalarial drug targets. Antimalarial drugs target critical enzymes and pathways in *Plasmodium falciparum*, including dihydrofolate reductase (DHFR) and chloroquine resistance transporter (Pf CRT). This study employed molecular docking techniques to explore analogues of existing drugs, including proguanil, chloroquine, primaquine, and mefloquine, targeting proteins Pf TS-DHFR, Fe-II protoporphyrin IX, and Pf CRT. Tools such as AutoDock 4.2 and Discovery Studio Visualizer were used for docking studies. Active sites of target proteins were identified using CASTp, while the structural validation of modelled proteins was conducted using tools like PROCHECK, Verify 3D, and ProSA.

Target Proteins

- **Pf CRT:** Involved in chloroquine resistance.
- **Fe-II protoporphyrin IX:** Targeted by Primaquine and Mefloquine.
- **Pf TS-DHFR:** Targeted by Proguanil and its analogues

Data Collection

Docking scores, binding energies, ligand efficiencies, and inhibition constants were analyzed for each drug and its analogues. The analogues were selected from the ZINC database.

Results and Discussion

Structure Validation

The structure validation of Pf TS-DHFR, using various web servers, is represented in Table I. The PROCHECK results of Pf TS-DHFR reported 89.3% of residues in the core region with no residues in the disallowed region. Verify 3D represented 74.09 % of residues in the core region. ProSA represented the Z-Score as -5.74, which is acceptable and considered a structure. MolProbity reported all the residues in the favoured region with 0% Ramachandran outliers. Active/ Binding site of the reported Pf TS-DHFR target was predicted by CASTp represented in Table II. The analogues of proguanil with Zinc database IDs ZINC00001127 and ZINC16343331were considered for docking against Pf TS-DHFR. These analogues are said to have 99% identity with the drug proguanil. AutoDock 4.2 (autodock.scripps.edu/) that was used for docking studies revealed docking score with energy minimization values, Binding energy, Ligand Efficiency, Inhibition Constant and Electrostatic energy for proguanil and PfTS-DHFR interactions are represented at Table III.

Molecular Docking Result

Proguanil and Its Analogues

The docking score reported the binding energy of Pf TS-DHFR-Proguanil was -6.0 Kcal/mol, whereas docking with the analogues ZINC00001127, ZINC19144226, and ZINC16343331 was -6.6 and -5.54 Kcal/mol respectively.

TARGET NAME	Ligand (DRUGS) Analogues	Binding Energy (Kcal/mol)	Ligand Efficiency (Kcal/mol)	Inhibition constant
TsDHFR	Proguanil	-6.0	-0.23	40.11
	zinc_1127	**-6.6**	-0.39	14.59
	zinc_16343331	-5.54	-0.33	86.96

The results represent the analogues of Proguanil with the highest docking score and the best energy minimization values compared to the proguanil drug. This states that the analogues of proguanil drugs may be of therapeutic importance for malarial patients, as they have an effective role in clearing parasites from the liver. The compound (ligand)-target complex was performed and its interaction studies was visualized in Discovery Studio Visualizer, LigPlot and PyMol Visualizer were showed in Fig. 1.

Chloroquine and Its Analogues

The docking score reported the binding energy of Pf CRT-Chloroquine was -8.13 Kcal/ mol whereas docking with the analogues ZINC1530860, ZINC19144226, ZINC19144231

and ZINC1530861 was -8.02, -7.67, -8.09 and -7.41 Kcal/mol respectively.

TARGET NAME	Ligand (DRUGS)/ Analogues	Binding Energy (Kcal/mol)	Ligand Efficiency (Kcal/mol)	Inhibition constant
Pf CRT	Chloroquine	-8.13	-0.37	1.1
	zinc_1530860	-8.02	-0.36	1.32
	zinc_19144226	-7.67	-0.35	2.37
	zinc_19144231	-8.09	-0.37	1.18
	zinc_1530861	-7.41	-0.34	3.73

The results represent that the analogues of Chloroquine do not have highest docking score with best energy minimization values in comparison to the Chloroquine drug. This states that the analogues of chloroquine drug may be of less therapeutic importance as compared to the original drug. The compound (ligand)-target complex was performed and its interaction studies was visualized in Discovery Studio Visualizer, LigPlot and PyMol Visualizer were showed in Fig. 2.

Primaquine and Its Analogues

The Docking score reported the binding energy of Fe (II) protoporphyrin IX-Primaquine was -8.13 Kcal/mol, whereas docking with the analogues ZINC5319733, ZINC5320191 and ZINC1530862 was -6.69, -6.71 and -5.51 Kcal/mol respectively.

TARGET NAME	Ligand (DRUGS) Analogues	Binding Energy (Kcal/mol)	Ligand Efficiency (Kcal/mol)	Inhibition constant
Fe (II)-protoporphyrin IX	Primaquine	-5.53	-0.29	88.58
	zinc_5319733	-6.69	-0.26	12.56
	zinc_5320191	-6.71	-0.26	11.98
	zinc_1530862	-5.51	-0.29	92.18

The results represent that the analogues of Primaquine with highest docking score with best energy minimization values in comparison to the Primaquine drug. This states that the analogues of Primaquine drug may be of therapeutic importance for malarial patients. The compound (ligand)-target complex was performed and its interaction studies was visualized in Discovery Studio Visualizer, LigPlot and PyMol Visualizer were showed in Fig. 3.

Mefloquine and Its Analogues

The docking score reported the binding energy of Fe (II) protoporphyrin IX- Mefloquine was -5.81 Kcal/ mol whereas docking with the analogues ZINC32229196, ZINC32229200 and ZINC537964 was -5.28, -6.38 and -5.44 Kcal/mol respectively.

TARGET NAME	Ligand (DRUGS) Analogues	Binding Energy (Kcal/mol)	Ligand Efficiency (Kcal/mol)	Inhibition constant
Fe (II)- protoporphyrin IX	Mefloquine	-5.81	-0.31	55.49
	zinc_32229196	-5.28	-0.18	134.1
	zinc_32229200	-6.38	-0.22	21.2
	zinc_537964	-5.44	-0.21	103.04

The results represent that the analog of Mefloquine with highest docking score with best energy minimization values in comparison to the Mefloquine drug. This states that the analogues of Mefloquine drug may be of therapeutic importance for malarial patients. The compound (ligand)-target complex was performed and its interaction studies was visualized in Discovery Studio Visualizer, LigPlot and PyMol Visualizer were showed in Fig. 4.

This study demonstrates the utility of bioinformatics and molecular docking in identifying promising drug analogues for malaria treatment. Analogues of Proguanil, Primaquine, and Mefloquine showed potential as effective antimalarial agents. Future research should focus on ADME/T (Absorption, Distribution, Metabolism, Excretion/Toxicity) studies, *in vitro* antimalarial activity assays, and clinical trials to confirm their efficacy. Integrating computational and experimental approaches will expedite the development of safe, cost-effective, and highly efficient antimalarial therapies.

List of Tables and Figures

Table 1: The structure validation scores of Pf Ts-DHFR

Servers		Ts-DHFR		
PROCHECK	Most favored regions	10.3%		
	Core	89.3%		
	Generously allowed Regions	0.4%		
	Disallowed regions	0.0%		
	G-factor	Dihedrals: 0.00	Covalent: -0.19	Overall: -0.07
Verify 3D	Averaged 3D-1D Score > 0.2	74.09%		
ERRAT	Overall Quality	79.174%		
ProSA	Z-Score	-5.74		
MolProbity	Cβ deviations > 0.25 Å	0.00%		
	Residues with bad bonds	0.00%		
	Residues with bad angles	1.84		
	Ramachandran outliers	0.00%		

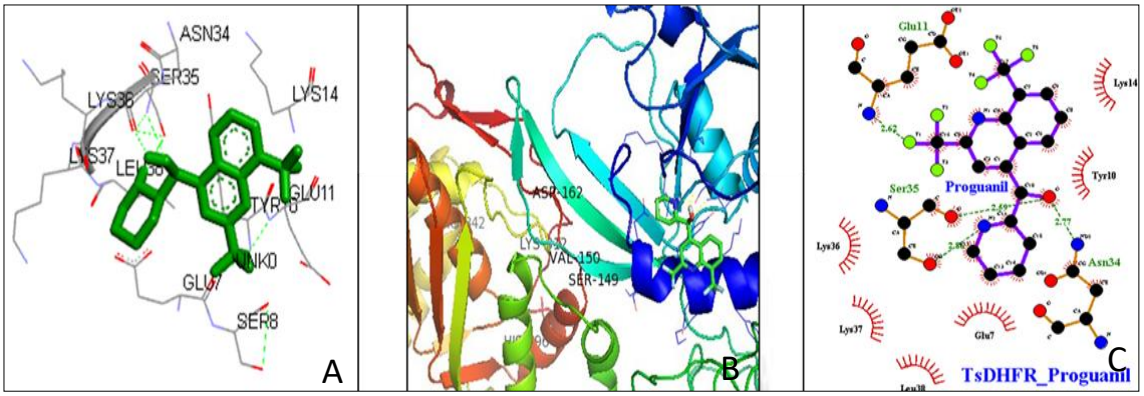
	Favored rotamers	92.07%		
ProQ	LGscore	4.192		
	MaxSub	0.315		
ProSA		-1.407		
Q mean score		0.64%		

Table 2: Active / binding site of the proteins predicted by using CASTp

Sl.no	Target name	Amino acid residues in binding pocket
1	PfTS- DHFR	THR2, THR3, LYS9, GLU7, ASN6, VAL5, TYR4, SER8, GLU11, LEU13, TYR10, ARG17, LYS12, LYS14, LYS16, TYR15
2	Fe (II)-protoporphyrin IX	LEU53, LYS57, TRP71, PHE75, ILE145, ILE151, PRO152, ASN190, LYS193, ALA194, LYS197
3	PfCRT	ILE66,LEU69,SER70,VAL73,MET74,ILE77,CYS101,MET104,P HE105, VAL108, PHE112, LEU 162, ILE166, ASN167, PHE169, PHE170, CYS171, ILE174, HIS180, ASN183, TYR184, GLY186, ALA 187, VAL 188, ILE190, VAL191, ILE 194, GLU198, PHE235, ALA247, PHE251, PHE255, CYS258, LEU259, LEU261, PRO262, THR265, LEU266, GLY286, CYS289, LEU290, ARG294, VAL298, TRP316, PHE319, PHE322, SER323, ASN326, ILE327, ASN330, LEU331, THR333, THR334, TYR335, ILE337, ASP338, SER341, THR342, TYR345, THR346, VAL348, SER349, ILE356, PRO354, GLY353, GLN352, CYS350

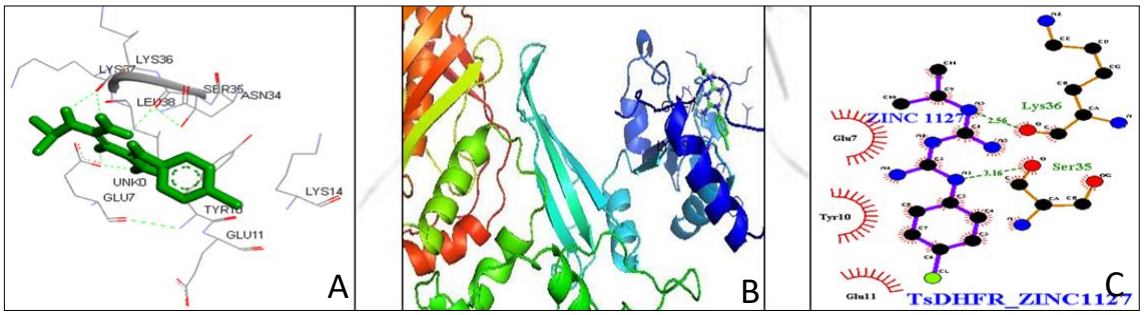
Table 3: Molecular docking analysis of 4 anti-malarial drugs and its analogues against 3 target proteins using AutoDock4.2 tool

Target name	Ligand (drugs) analogues	Binding energy (kcal/mol)	Ligand efficiency (kcal/mol)	Inhibition constant	Electrostatic energy	Hydrogen bond	Electrostatic interaction	Hydrophobic interaction
PfCRT	Chloroquine	8.13	0.37	1.1	0.03	CYS171, SER349		PHE170, PHE235, TYR345, THR346, CYC171, PHE112, PHE235,
	zinc_1530860	-8.02	-0.36	-1.32	-0.08	SER349, SER334	PHE251, PHE235	CYS171, PHE112, PHE235,
	zinc_19144226	-7.67	-0.35	2.37	0.39	SER349, THR342,	PHE235	PHE170, TYR345, ALA247, ILE337, VAL348, PHE251, VAL348
	zinc_19144231	-8.09	-0.37	1.18	-0.03	ASN167, THR333, SER349,	PHE251,	PHE251, PHE170, TYR345, THR346, CYS171, PHE235,
	zinc_1530861	-7.41	-0.34	3.73	-0.01	SER349, THR342, PHE235,	PHE251	PHE170, PHE251, PHE170, PHE235, TYR345, THR346, CYS171, PHE112, PHE235,
Fe (II)-protoporphyrin IX	Mefloquine	-5.81	-0.31	55.49	-1.39	ASN190, ASP78, PRO152, LYS193		TPR71, PRO152
	zinc_32229196	-5.28	-0.18	134.1	-0.09	LYS57, ASN129,		ILE145, ILE151, PRO152
	zinc_32229200	-6.38	-0.22	21.2	-0.16	LYS193, TRP71,		TRP71, LEU53, ILE145,



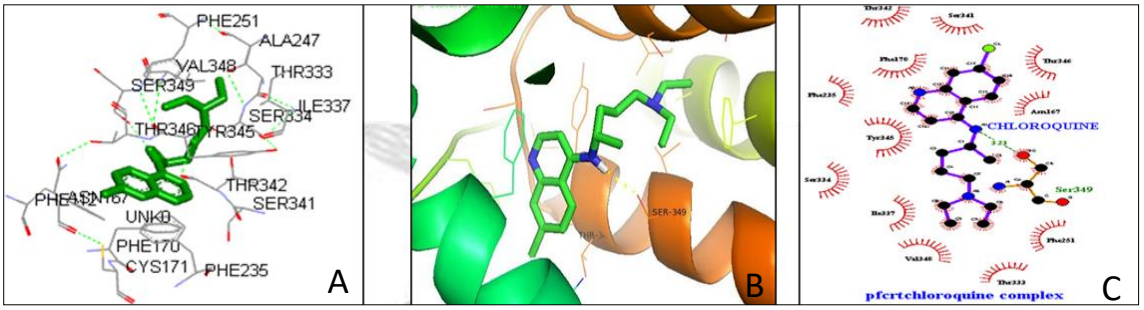
(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 1.1: Molecular interaction of Proguanil with Pf TS-DHFR



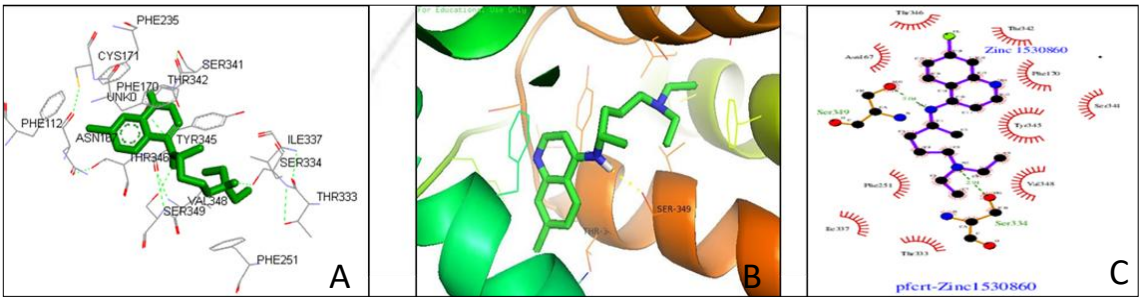
(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 1.2: Molecular interaction of Proguanil with zinc_1127 (analog of Proguanil)



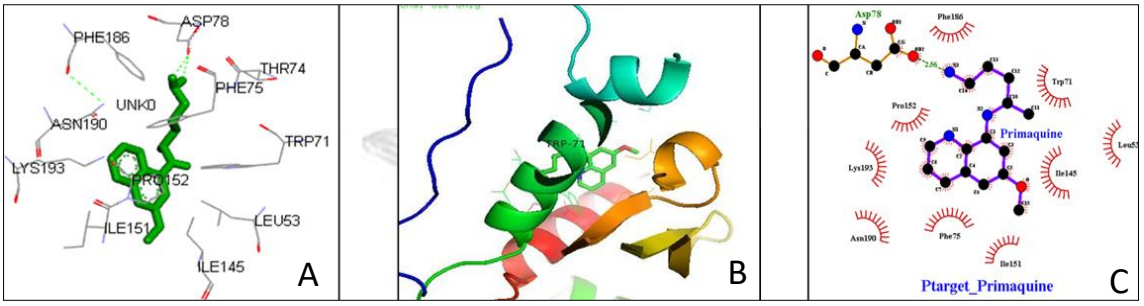
(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 2.1: Molecular interaction of Chloroquine with Pf CRT



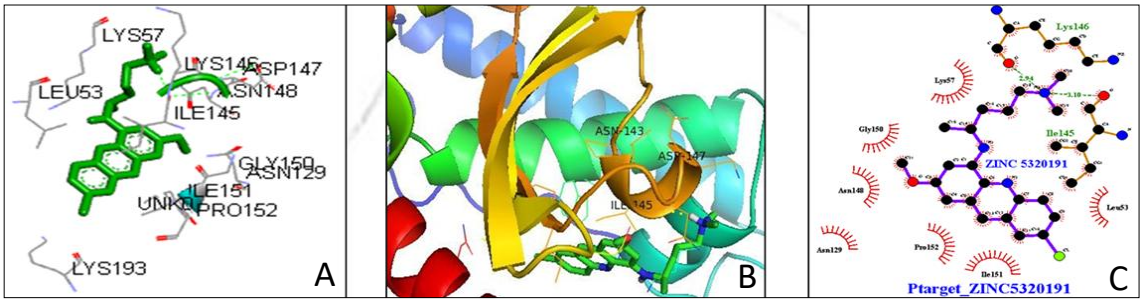
(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 2.2: Molecular interaction of Chloroquine with zinc_1530860 (analog of Chloroquine)



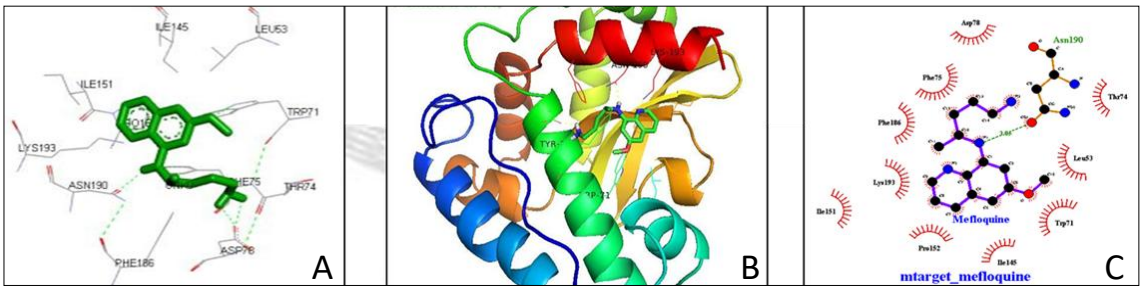
(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 3.1: Molecular interaction of Primaquine with Fe (II)-protoporphyrin IX



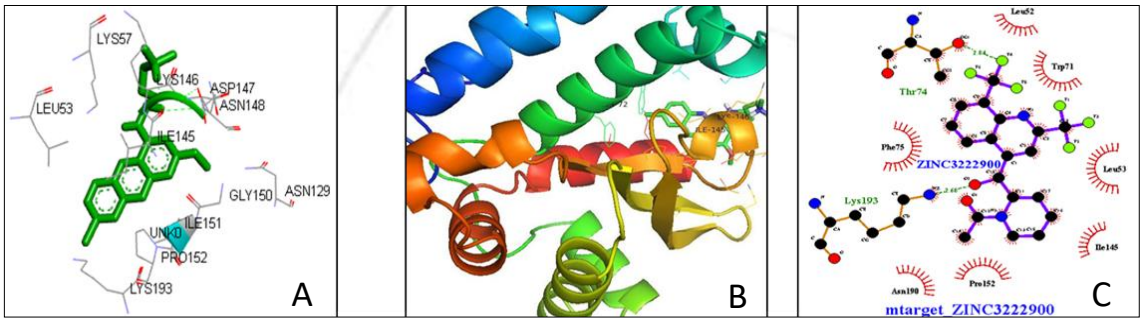
(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 3.2: Molecular interaction of Primaquine with zinc_5320191 (analog of Primaquine)



(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 4.1: Molecular interaction of Mefloquine with Fe (II)-protoporphyrin IX



(A. Discovery Studio Visualizer, B. PyMol, C. LigPlot)

Fig 4.2: Molecular interaction of Mefloquine with zinc_3222900 (analog of Mefloquine)

Conclusion

This study demonstrates that molecular docking can effectively identify promising analogues of existing antimalarial drugs. Among the compounds tested, analogues of Proguanil, Primaquine, and Mefloquine showed better or comparable binding affinity to key malaria targets than their parent drugs, indicating their potential to overcome current resistance issues. However, chloroquine analogues did not outperform the original drug, suggesting limited scope for improving its efficacy through structural modification. These findings provide a basis for selecting lead molecules for further *in vitro*, *in vivo*, and ADME/T studies to develop safer and more effective antimalarial therapies.

Acknowledgement

PD, SSP and SP express our heartfelt gratitude to Berhampur University for providing the essential resources, infrastructure, and academic support for this research. The guidance of the esteemed faculty and access to computational and laboratory facilities were instrumental in our study. SP acknowledge University of Calcutta, SSS and SG thankful to the Khallikote University for the support. We also thank the administrative and technical staff for their invaluable support in completing this work.

Table I: The structure validation scores of Pf Ts-DHFR.

Table II: Active / binding site of the proteins predicted by using CASTp.

Table III: Molecular docking analysis of 4 anti-malarial drugs and its analogues against 3 target proteins using AutoDock4.2 tool.

References

1. Bray PG, Martin RE, Tilley L, Ward SA, Kirk K, Fidock DA. Defining the role of PfCRT in *Plasmodium falciparum* chloroquine resistance. Molecular Microbiology,2005:56(2):323–333.
2. Calderaro A, Piccolo G, Chezzi C. The laboratory diagnosis of malaria: a focus on the diagnostic assays in non-endemic areas. International Journal of Molecular Sciences,2024:25(2):695.
3. Cooper RA, Hartwig CL, Ferdig MT. PfCRT is more than the *Plasmodium falciparum* chloroquine resistance gene: a functional and evolutionary perspective. Acta Tropica,2005:94(3):170–180.
4. Dorn A, Vippagunta SR, Matile H, Jaquet C, Vennerstrom JL, Ridley RG. An assessment of drug-haematin binding as a mechanism for inhibition of haematin polymerisation by quinoline antimalarials. Biochemical Pharmacology,1998:55(6):727–736.
5. Erhunse N, Sahal D. Protecting future antimalarials from the trap of resistance: Lessons from artemisinin-based combination therapy (ACT) failures. Journal of Pharmaceutical Analysis,2021:11(5):541–554.
6. Fidock DA, Rosenthal PJ, Croft SL, Brun R, Nwaka S. Antimalarial drug discovery: efficacy models for compound screening. Nature Reviews Drug Discovery,2004:3(6):509–520.
7. Hyde JE. Drug-resistant malaria – an insight. The FEBS Journal,2007:274(18):4688–4698.
8. Ige AA, Adedokun TT, Otutu O, Olorunfemi AB, Ayoola AO, Ojurongbe O. The role of the *Plasmodium falciparum* chloroquine resistance transporter gene in malaria drug resistance and its implication on malaria control in Africa. Pan African Journal of Life Sciences,2024:8(2):189–197.
9. Plowe CV. Malaria chemoprevention and drug resistance: a review of the literature and policy implications. Malaria Journal,2022:21(1):104.
10. Senthilraja P, Sunil KS, Kathiresan K. Potential of mangrove derived compounds against dihydrofolate reductase: An in-silico docking study. Journal of Computational Biology and Bioinformatics Research,2012:4(2):23–27.

11. World Health Organization. Lymphatic filariasis: progress report 2000–2009 and strategic plan 2010–2020. WHO, 2010.
12. Yuthavong Y, Tarnchompoo B, Vilaivan T, Chitnumsub P, Kamchonwongpaisan S, Charman SA, *et al*. Malarial dihydrofolate reductase as a paradigm for drug development against a resistance-compromised target. Proceedings of the National Academy of Sciences,2012:109(42):16823–16828.