IN SILICO EVALUATION OF MACROLIDE LIKE MOLECULES AS PROMISING ANTIMALARIAL AGENTS: A PHARMACOLOGICAL PERSPECTIVE

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Abstract

Malaria, caused by Plasmodium protozoa transmitted through mosquito bites, remains a significant global health concern despite extensive control efforts. The rise and dissemination of drug-resistant strains of the malaria parasite pose a substantial threat to human health. Therefore, there is an urgent need to explore alternative drug targets and develop effective antimalarial agents. This study aims to investigate the potential of macrolide molecules as promising candidates to combat drug resistance and reduce malaria-related mortality.

Using in silico approaches, macrolide molecules were selected based on their binding scores and protein-ligand interactions. The selected molecules underwent further analysis to assess their pharmacokinetic profiles and toxicity using computational methods. These investigations contribute valuable insights into important drug parameters.

By leveraging the recognized pharmacophore structure, the design of novel antimalarial drugs can be optimized. The findings of this study provide new avenues for research and development of macrolide-based antimalarial agents. These molecules hold the potential to overcome drug resistance and offer improved therapeutic outcomes. Through this in silico evaluation, a pharmacological perspective is presented to explore the antimalarial efficacy of macrolide molecules. By identifying new drug targets and developing lead compounds, this research offers a promising approach to addressing the global burden of malaria and reducing associated mortality rates. Further experimental validation is warranted to validate the potential of these macrolide molecules as effective antimalarial agents.

Keywords: Malaria, drug resistance, macrolide molecules, in silico, antimalarial agents, pharmacological perspective.

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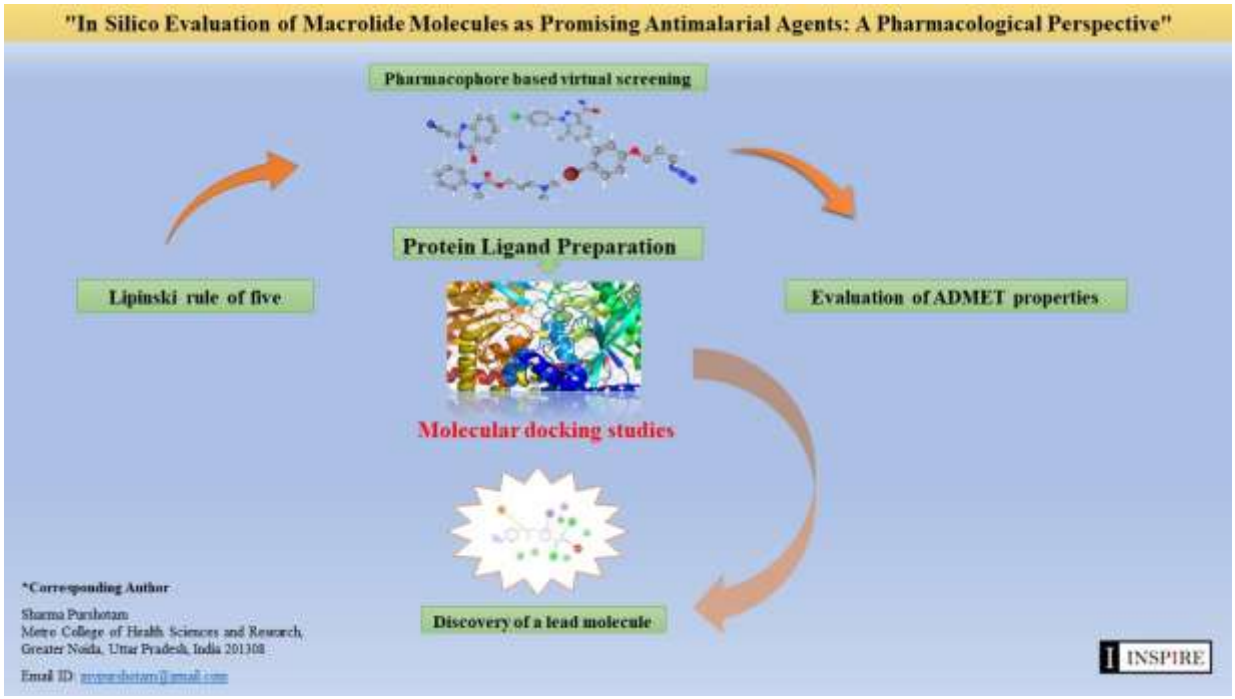
Highlights:

1. In-depth in-silico evaluation of macrolide molecules as potential antimalarial agents.
2. Comprehensive assessment of pharmacological properties for antimalarial drug development.
3. Exploration of novel drug targets to combat drug resistance in malaria.
4. Utilization of computational methods to identify lead macrolide candidates.
5. Insights into the pharmacokinetic profiles and toxicity of selected macrolide molecules.
6. Implications for the development of effective antimalarial therapies through in silico approaches.

Graphical Abstract



1. INTRODUCTION

Malaria is a mosquito-borne parasitic disease caused by intraerythrocytic protozoa of the *Plasmodium* genus. It remains to be a major cause of mortality worldwide despite intensive efforts to eliminate it. The emergence and spread of Malarial drug resistance is a major concern, threatening human health. This study raises new possibilities to investigate and identify new drug targets and the development of lead macrolide molecules to encounter drug resistance and reduce mortality by *Plasmodium* parasites. Molecules were selected according to score and protein-ligand interaction and selected molecules were analysed for pharmacokinetic profile and toxicity studies to investigate important drug parameters using in silico approaches. In designing novel antimalarial medicines, the recognized pharmacophore structure may be useful.

Drug-resistant strains can be managed by employing the approach of using antibiotics to enhance the efficacy of existing antimalarial medications. The three groups of antibiotics possess antimalarial activity, established in research using investigational malaria models: tetracyclines, macrolides and lincosamides [9,10]. To increase the effectiveness of current antimalarials, antibiotics have also been utilised in clinical practice. [11] The combination of tetracyclines with quinine and mefloquine has been effectively used in multidrug-resistant cases but it was discontinued as it led to the disposal of residue in cortical bones. [12] The antimalarial activity of azithromycin is a potential alternative treatment option, as it was recorded with a longer half-life and better bioavailability. [13,14] In the present study, we aimed to conduct comparative antimalarial in-silico studies of macrolide molecules' potential for development as effective antimalarials.

P. vivax epidemiology

Plasmodium vivax differs from falciparum malaria in terms of epidemiology and biology. Vivax malaria gives rise to a well-defined, recurring paroxysmal fever with a regular 48- hr periodicity. The fever is preceded by chills and is followed by excessive sweating. [15] Additionally, *P. vivax* sporozoites are more easily transmitted to Anopheles mosquito vectors than *P. falciparum* gametocytes, and they can do so at lower parasite levels. *P. vivax* sporozoites develop within the mosquito more quickly than *P. falciparum* sporozoites do at equal temperatures, which helps it exploit a larger geographic range. [16,17]. Relapse is an important aspect of the P. vivax life cycle following a primary infection by activation of dormant liver-stage parasites, known as hypnozoites. The hypnozoites begin multiple clinical attacks after a single bite of the *P. vivax*-infected mosquito. [18] The onset of clinical signs of malarial infection occurs quickly once the parasite's merozoite form, which invades red blood cells, moves from the first liver stage infection to the blood infection. *P. vivax* has a unique ability to infect reticulocytes [19,20].

Drug resistance

The treatment of malaria has been plagued by drug resistance to common antimalarial drugs.

Chloroquine (CQ) has been the frontline drug for treating asexual blood-stage P. vivax parasites. CQ was the preferred medication for treating P. vivax for a long time as it is efficient and inexpensive. [6] Maximum incidence of CQ-resistant to P. vivax was reported on the Northeastern coast of Indonesian Papua. [21] CQ treatment failure and resistance to primaquine failures have also been documented. Primaquine as an anti-relapse treatment has also failed to treat P. vivax malaria in various areas of Southwestern and North-eastern India. [22,23] Primaquine and artemisinin combination therapy is a common alternative treatment method in many areas across the world where P. vivax CQ resistance is observed. [24] In a study, it was found that an efficient antibacterial with a comparable treatment regimen to artesunate is azithromycin. The combination of azithromycin plus artesunate (AZ+AS) is potentially favourable. Adults in Asia have found AZ+AS to be an effective antimalarial treatment in clinical studies [25,26,27]; in pregnant women in Africa, AZ+AS appears promising when given with sulfadoxine-pyrimethamine [28]; and in Africa, azithromycin is an effective preventive against malaria when given as monotherapy.[29] The antibiotic azithromycin binds to the apicoplast ribosomal 50S subunit and prevents protein release from the ribosome, hence inhibiting apicoplast ribosomal protein synthesis of the asexual blood-stage parasite. [30,31,32] According to in vitro research, merozoites exposed to macrolide antibiotics exhibit a "delayed death" pharmacological effect. (Figure No.1) When exposed to macrolides, parasites go through their initial phase normally. However, due to the inheritance of a faulty apicoplast, parasite death is seen during the second post-treatment phase. [33,34,35]

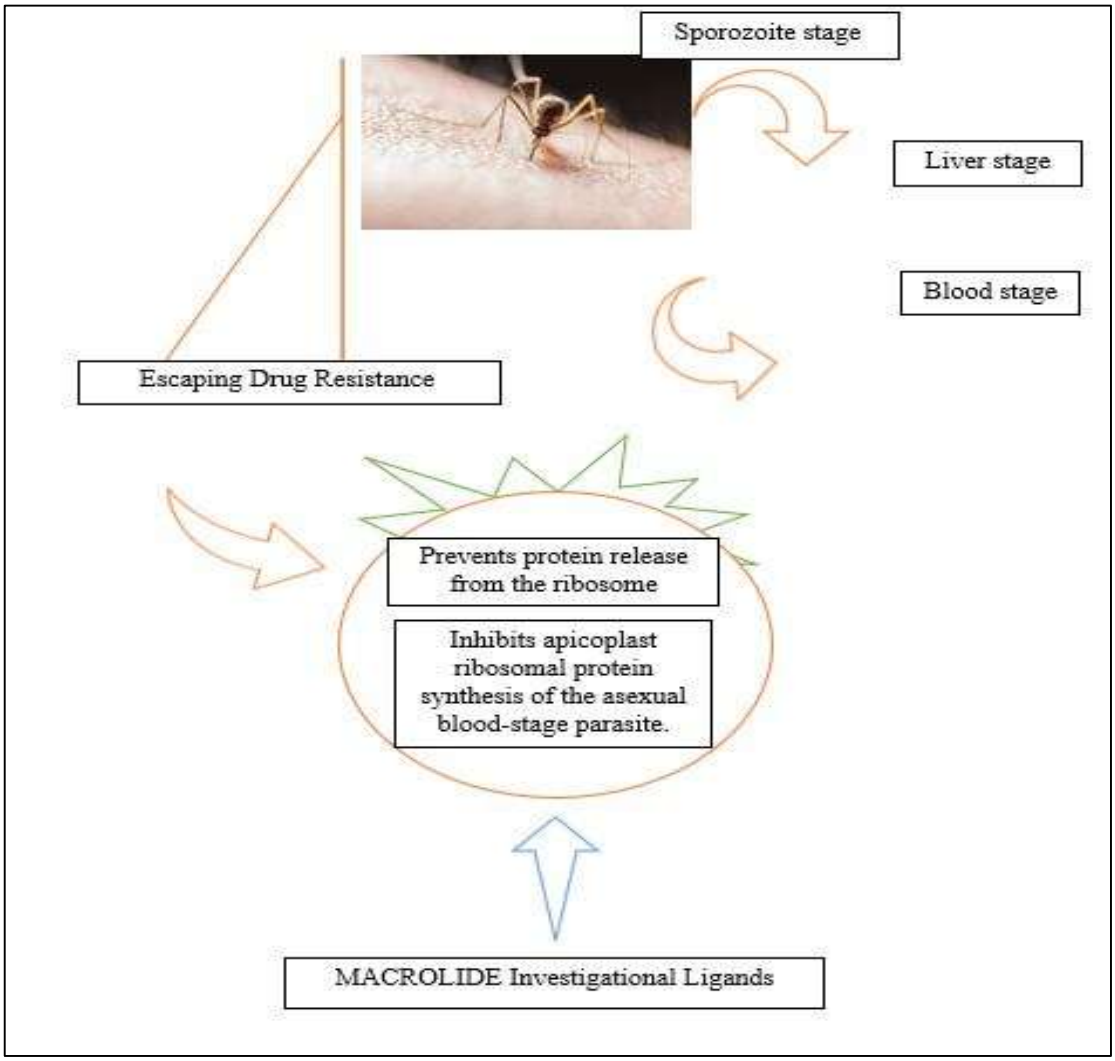


Figure 1: Merozoites exposed to macrolide antibiotic exhibit a "delayed death" pharmacological effect.

2. MATERIALS AND METHODS

The investigational ligand's (ILs) similarity was analysed using Lipinski rule-based filtering, synthetic accessibility, and in-silico analysis of adsorption, distribution, metabolism, and excretion to uncover viable therapeutic candidates.

2.1 Pharmacophore-based virtual screening

To conduct pharmacophore-based virtual screening of drug-like compounds, an online application called ZINC-pharmer was utilised from the ZINC database. To produce pharmacophore characteristics of the ligands, the mol 2 extension
file was fed into the ZINC-pharmer programme. Lipinski's rule was employed to determine the drug-like qualities. According to this criterion, the drug-like qualities of molecules should have the following parameters: log p-value less than five, molecular weight less than five hundred, H-bond acceptors fewer than ten, and H-bond donors fewer than five. The compounds with the highest number of hits were chosen to be input into a new database for additional interaction analysis.

2.2 Evaluation of Drug Likeness and ADMET

When searching for a potent lead molecule, it is essential to consider two major characteristics, namely drug-likeness and toxic impact. For this investigation, the drug-likeness of candidate compounds was determined by using the web application SwissADME. On the other hand, the pharmacokinetic profile prediction of prospective ILs was carried out with the assistance of the pkCSM online database. This method included the incorporation of predictions of toxicity, metabolism, distribution, absorption, and excretion.

2.3 Protein and Ligand Preparation

The three-dimensional crystal structure of the molecular target, merozoite surface protein MSPDBL2 from Duffy Binding Protein of P. vivax (PDB code: 6OAN) was retrieved from Protein Data Bank (PDB) for docking analysis. Before commencing the docking procedure, the expulsion of all water particles takes place and the missing charges, protonation states, and assigning of polar hydrogen to the receptor. Canonical smiles from PubChem were used to create investigational ligands, which were then transformed into pdbqt format by employing AutoDock tools. The best conformers were represented with the lowest binding energy.

2.4 Molecular docking

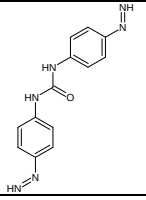
Docking calculations were performed with the Auto DOCK programme to locate potentially successful hit molecules for the subsequent stages of the drug discovery process. All of the chemicals that were retrieved were docked with the P. vivax Duffy Binding Protein, which has the PDB code 6OAN. In addition, a reference molecule known as azithromycin was made ready for the molecular docking process by removing all of the water molecules from it and then adding more polar hydrogens. After that, the programme is known as Discovery Studio was utilised so that the various binding modes of the interaction between the receptor ligands could be analysed and visualised.

3. RESULTS

3.1. Pharmacophore Modelling

The chemical structures were imported from PubMed in the SDF format. Then, the SDF format was converted into Mol2 format with Open Babel 2.3.1. The five drug molecules were employed as the input query on the PharmaGist webserver to build the best pharmacophore model. Further, the software ZINCPharmer was used to predict the physiochemical properties of investigational ligands (ILs). Table 1. The ILs were renamed as A1, A2, A3, A4 etc. (Table1. Presents the list ILs with their structure.) The hit compounds were screened through Lipinski's thumb rule of five. Given below is the table (Table 2.) of hit molecules selected for molecular docking studies as all ILs are satisfied by the Lipinski rule.

Table 1: ILs code with their IUPAC name and structure.

ILs code	IUPAC Name	Structure
A1	1,3-Bis[4-(methyldiazenyl)phenyl] urea	

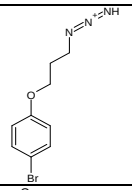
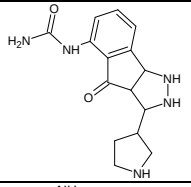
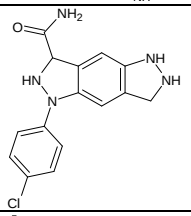
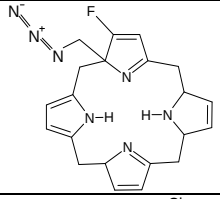
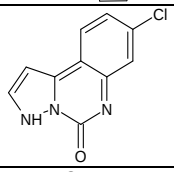
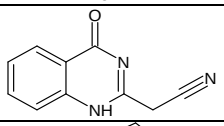
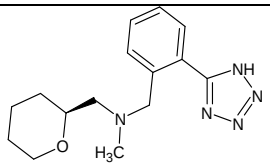
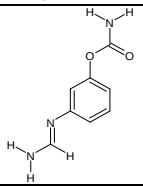
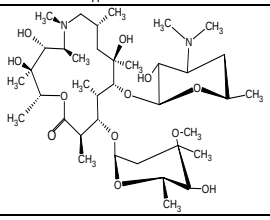
A2	1-(3-azidopropoxy)-4-bromobenzene	
A3	3-(1-methyl-1H-pyrrol-3-yl)-4-oxo-2H,4H-indeno[1,2-c] pyrazol-5-yl] urea	
A4	1-(4-chlorophenyl)-2H-pyrazolo[3,4-e] indazole-3-carboxamide	
A5	6-(azidomethyl)-7-fluoro-21,23-dihydro-5*H*-porphyrin	
A6	8-chloro-6H-pyrazolo [1,5-c] quinazolin-5-one	
A7	2-(4-oxo-3,4dihydro quinazolin-2-yl) acetonitrile	
A8	Methyl-[[(2*S*)-oxan-2-yl]methyl]-[[2-(1,2,3-triaza-4-azanidacyclopenta-2,5-dien-5-yl)phenyl]methyl]azanium	
A9	3-(dimethylamino)butyl methyl(phenyl) carbamate	
A10	Azithromycin (Reference)	

Table 2: Physiochemical properties of potential ILs.

Ligand name	**IUPAC name**	**Chemical formula**	**MW**	**NHD**	**NHA**	**Log P**
A1	1,3-bis [4 (methyl diazenyl) phenyl] urea	$C_{15}H_{16}N_6O$	296.33	2	5	3.01

Note: MW: molecular weight, NHD: No. of H-bond donors, NHA: No. of H-bond acceptors

A2	1-(3-azidopropoxy)-4-bromobenzene	$C_9H_{10}BrN_3O$	256.10	0	4	2.96
A3	3-(1-methyl-1H-pyrrole-3-yl)-4-oxo-2H,4H-indeno[1,2-c] pyrazol-5-yl] urea	$C_{16}H_{13}N_5O_2$	307.31	3	3	1.42
A4	1-(4-chlorophenyl)-2H-pyrazolo[3,4-e] indazole-3-carboxamide	$C_{15}H_{10}ClN_5O$	311.73	2	3	1.48
A5	6-(azidomethyl)-7-fluoro-21,23-dihydro-5*H*-porphyrin	$C_{21}H_{16}FN_7$	385.4	2	6	3.08
A6	8-chloro-6H-pyrazolo [1,5-c] quinazolin-5-one	$C_{10}H_6ClN_3O$	219.63	2	2	1.36
A7	2-(4-oxo-3,4dihydro quinazolin-2-yl) acetonitrile	$C_{10}H_7N_3O$	185.18	1	3	1.03
A8	Methyl-[[(2*S*)-oxan-2-yl]methyl]-[[2-(1,2,3-triaza-4-azanidacyclopenta-2,5-dien-5-yl)phenyl]methyl]azanium	C15H21N5O	287.36	5	1	2.11
A9	3-(dimethylamino)butyl methyl(phenyl) carbamate	$C_{14}H_{22}N_2O_2$	250.34	0	3	3.24
A10	Zithromax (Reference)	$C_{38}H_{72}N_2O_{12}$	748.98	5	14	4.76

3.2 Molecular docking

The electrostatic interactions, hydrogen bonding, hydrophobic interaction, and van der Waals forces are determined to predict the binding of the ligand. The binding energies of various ligands with the target proteins have been screened and enlisted in Table No. 3. The binding energy of the A1 ligand to the Duffy Binding Protein of P. vivax (PDB code: -6OAN) was -5.1 kcal/mol and that of Azithromycin was -10.5 kcal/mol. The main interacting residues in the binding site as per Azithromycin were **Trp358**, **Lys378**, **Cys377**, Leu379, Trp375, and other nearby residues. On the other hand, the A1 ligand forms van der Waals with Glu 481, Val 381, Arg 304, Ala 382, Cys 300, Ile 303, **Trp 358**, **Cys 377**, **Lys 378**, Lys 301, and Lys 297. The binding energy of the A1 ligand to the active site is even smaller than that of the reference drug molecule. From the binding energy point of view, investigational ligand A1 shows strong interactions with the surface targets of *P. vivax*

Table 3: Computation of binding energies with active site residue of ILs.

List of Investigational Ligands	Binding energies	Active sites residue atom range
A1	-5.1	Glu 481, Val 381, Arg 304, Ala 382, Cys 300, Ile 303, Trp 358, Cys 377, Lys 378, Lys 301, Lys 297
A2	-4.8	Ala 489, Asn 486, Leu 482, Phe 490, Tyr 400, Glu 493, Phe 485, Leu 404, Val 401, Asp 483, Glu 484
A3	-6.7	Ile385, Ala 463, Lys 347, Pro 387, Glu 386, Arg 391, Asp 312, Thr 317, Gln 388, Val 460, Met 319, Lys 456
A4	-7.5	Ile 322, Ser 325, Leu 245, Thr 251, Lys 248, Cys 246, Met 247, Cys 217, Asn 216, Tyr 324, Asn 218, Arg 221, Gln 244, Tyr 243, Arg 242, Glu 320
A5	-8.7	Arg 221, Tyr 219, Lys 220, Leu 286, Gln 244, Asn 218, Cys 217, Asn 216, Cys 246, Leu 245, Tyr 243

A6	-6.3	Arg 221, Asn 216, Gln 244, Asn 218, Lys 220, Tyr 219, Cys 217
A7	-5.9	Arg 391, Glu 386, Gln 388, Val 460, Met 319, Ile 389, Pro387, Tyr 390, Thr 317, Lys 347, Asp 312, Ile 385, Met 315
A8	-7.3	Glu 493, Phe 490, Tyr 400, Leu 404, Asp 483, Val 401, Leu 482, Ala 489, Asn 486, Glu 484, Phe 485
A9	-5.0	Ile385, Ala 463, Lys 347, Pro 387, Glu 386, Arg 391, Asp 312, Thr 317, Gln 388, Val 460, Met 319, Lys 456
A10	-10.5	Trp 358, Cys 377, Leu 379, Lys 378, Trp 375

Apart from binding energies, there is the significant importance of protein pocket matching in drug discovery. A binding pocket's functionality is determined by the collection of amino acid residues that surround it. It is evident from the Figure No. 2 that there are three major residues, Trp358, Cys377 and Lys378 involved in forming the van der Waals interaction with the reference molecule A10. This depicts that it is in a binding pocket and a similar pattern as that of the A10 molecule.

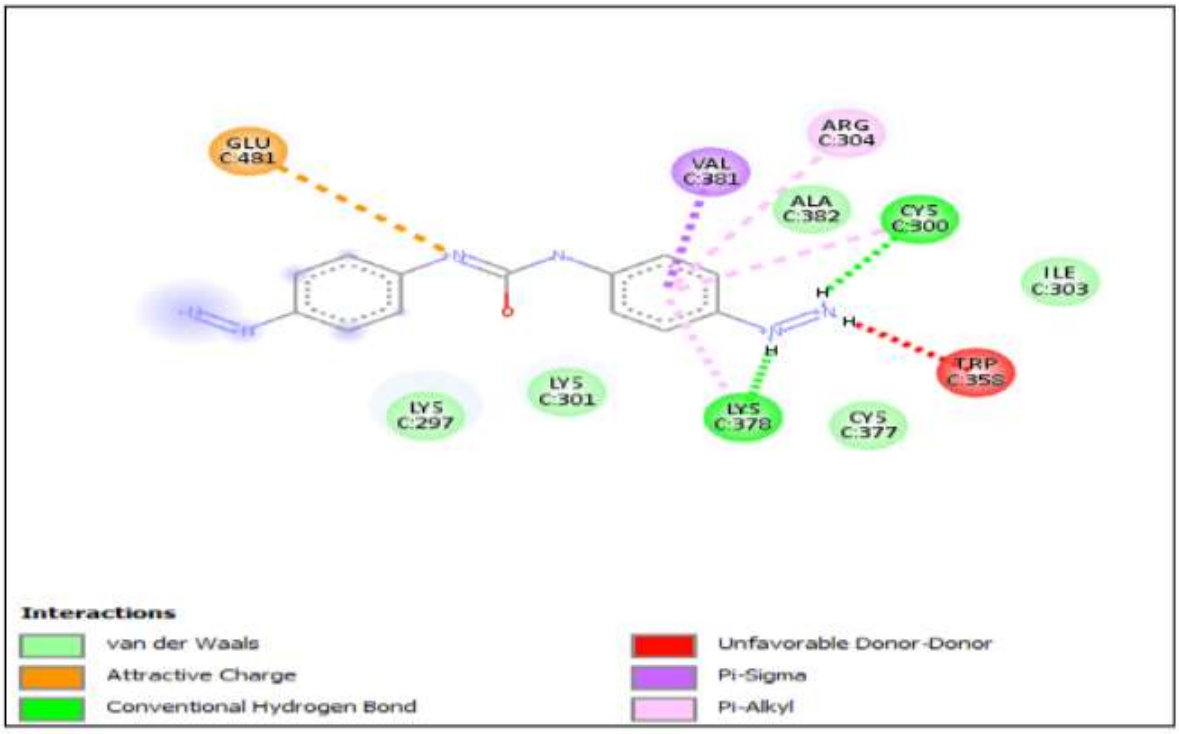


Figure 2: Visualization of interactions and binding region of A1 ligand with 6OAN.

3.3 ADMET Evaluation

Table 4 represents the data on ADMET properties including human intestinal absorption, human oral bio-availability, blood-brain barrier, P-glycoprotein substrate/inhibitor, sub-cellular localization, CYP substrate and inhibitor, carcinogenicity, and aquatic. ADME analysis is one of the important factors to analyse the pharmacokinetic properties of the molecules, which could be used as a drug. To calculate the indicators such as absorption, distribution, metabolism, excretion, and toxicity for ILs were calculated employing pkCSM, an online database.

Table 4: ADMET Evaluation

Name	Properties										
	Water solubility	Skin Permeability	BBB Permeability	CNS Permeability	CYP2D6 substrate	CYP3A4 substrate	Total Clearance	Max. Tolerated dose (human)	Oral Rat Acute Toxicity (LD50)	Hepatotoxicity	AMES toxicity
A1	-4.462	-2.977	-0.292	-1.778	No	Yes	0.229	0.12	2.108	No	No
A2	-2.871	-2.144	-0.078	-2.8	No	No	0.307	0.481	3.132	No	No
A3	-3.108	-2.735	-1.1	-2.444	Yes	Yes	0.329	0.633	2.72	Yes	No
A4	-3.366	-2.735	-1.23	-2.319	No	No	0.229	0.689	2.468	Yes	No
A5	-4.392	0.918	-0.612	-0.612	No	Yes	0.209	-0.829	2.917	Yes	No
A6	-3.593	-2.887	0.537	-2.829	No	No	0.236	0.191	2.572	Yes	No
A7	-2.744	-3.033	0.042	-2.345	No	No	0.7	0.661	1.73	No	No
A8	-1.962	-2.837	-0.002	-3.068	No	Yes	0.824	-0.274	2.732	Yes	No
A9	-2.522	-2.615	0.445	-2.544	No	No	0.727	0.073	3.209	No	No
A10	-4.133	-2.742	-1.857	-3.777	No	Yes	-0.424	1.027	2.769	Yes	No

4. CONCLUSION

This study used an in-silico approach to identify and develop lead macrolide molecules to encounter drug resistance to common antimalarial drugs. Based on the study's findings, it can be concluded that all the derivatives under investigation are projected to have a strong therapeutic profile. The pharmacokinetic profile and toxicity of derivatives were established. These hopeful findings can be seen in the larger context of combating the development of Plasmodium resistance against the majority of the medications now used to treat malaria. Overall, molecule A1 could be a possible antimalarial agent as it showed favourable properties. The findings are based on numerous factors we claim can lead to the disclosure of new drug applicants after in vivo investigations.

5. DISCUSSION

P. vivax is becoming a more well-known serious public health problem that affects numerous parts of the globe. Evidence indicates that it can contribute significantly to the severe malaria burden. Malaria intervention and control can no longer afford to overlook the impact of *P. vivax* if they want to advance the cause of local eradication and fulfil worldwide targets for reduced malaria disease and death. It is unusual biology and widespread distribution over the world pose hurdles to its eradication that well outweigh those of *P. falciparum*, its more well-known relative.

Abbreviations

CQ	Chloroquine
AS	Artesunate
AZ	Azithromycin
ILs	Investigational Ligands
PDB	Protein data bank
MW	Molecular Weight
NHD	No. of Hydrogen bond donors
NHA	No. of Hydrogen bond acceptors

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Conflict of Interest
The authors have no conflict of interest.

Author statement
All person who meets authorship criteria are listed as authors, and all authors certify that they have participated sufficiently in this work to take public responsibility for the content, including participation in the concept, design, analysis, writing, and critical revision of the manuscript.

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