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The Synthesis of Certain Novel [1,3]-Oxazine Derivatives and its Anti-malarial activity through Molecular Docking

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A B S T R A C T

Malaria is the most important parasitic disease in humans, with transmission occurring in over 100 countries with a population of three billion people. It is caused by protozoan parasites of the genus *Plasmodium*. These parasites are transmitted from one person to another by the female anopheles mosquito. Protein PfGST (*Plasmodium falciparum* Glutathione S-Transferase) present in the malarial parasite and as it is not the target of present antimalarial drugs but have highly lethal to human life, there comes the Molecular Docking to get better binding drugs to it. The synthetic (1,3)-oxazine derivatives has shown a better activity than the standard drug Artemisinin by Molecular Docking studies. These are one of the paramount heterocyclic classes which have drawn attention towards its synthesis because of their innumerable biological activities like antiviral, antitumour, antimalarial, antimicrobial, antitubercular and analgesics. These can be synthesized by the help of several mechanisms like Mannich reaction and Betti reaction, etc., Normally, oxazines are prepared by using expensive and toxic reagents. To reduce the cost and use of toxic reagents, novel [1,3]oxazine derivatives were synthesized by using primary amines, aldehydes, phenols and a catalyst with solvent. Currently, drug resistance is the major cause reducing the ability of the drug to treat the disease effectively. The objective of this article is to provide a precised view on the synthesis of [1,3]oxazine derivatives and their malarial activity by docking studies.

Keywords: Malaria, protozoa, PfGST, (1,3)-oxazine, Molecular Docking, Artemisinin, Mannich reaction, Betti reaction.

INTRODUCTION:

Malaria, caused by *Plasmodium* parasites. It is one of the world's deadliest infectious disease, killing over a million people each year, mainly women and young children in Africa and South East Asia.

- There are four species of plasmodium namely *P. falciparum, P. ovale, P. vivax* and *P. malariae*. In above mentioned, only *P. falciparum*accounts for over 95% of all malaria cases mainly in Africa and affects more children under five years of age.[1]
- The increasing of resistant strains to chloroquine has raised the search of new potential drugs and the artemisinin like substances are promising candidates in order to control this epidemic and intensive research is being made on cyclic-peroxy compounds.
- Combination therapy also decreases pressure on the rising demand of artemisinin, as it is a natural product in short supply, thus it is necessary to search an alternate drug.
- Docking procedures permits virtually screening a database of compounds and predict the strongest conformations. Docking allows screening a database of compounds and calculating the strongest binders based on various scoring functions. It explores ways in which two molecules, such as drugs and an enzyme, fit together and docks to each other well. The molecule may bind to receptor and modify their function. The interaction of drug and receptor complex was identified via docking and their relative stabilities were evaluated using molecular dynamics and also evaluated their binding affinities using free energy simulations.[2]

Almost all severe forms and deaths from malaria are caused by *P. falciparum. Rarely, P. vivax* or *P. ovale* produce serious complications, debilitating, relapses, and even death.

The major complications of severe malaria include cerebral malaria, pulmonary edema, acute renal failure, severe anemia, and/or bleeding. Acidosis and hypoglycemia are the most common metabolic complications. Any of these complications can develop rapidly and progress to death within hours or days. In many patients, several of these complications exist together or evolve in rapid succession within a few hours. In clinical practice, patients must be assessed for any of these signs or symptoms that suggest an increased risk for developing complications and must be treated immediately. In various studies risk factors for severe malaria and death include age greater than 65 years, female sex (especially when associated with pregnancy), nonimmune status, coexisting medical conditions, no antimalarial prophylaxis, delay in treatment, and severity of the illness at admission (coma, acute renal failure, shock, pulmonary edema, coagulation disorders). In tropical countries with a high transmission of malaria (hyperendemic areas), severe malaria is predominantly a disease of young children (1 month to 5 years of age).

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MATERIALS:

Docking steps

Protein selection:(NCBI) National Center for Biotechnology Information, Research Collaboratory for Structural Bioinformatics (RCSB) Protein Data Bank (PDB)[3]

Ligand optimization:to predict a position and orientation of a small molecule (ligand) to a larger receptor molecule (macromolecule) with minimum binding energy

Autodock: perform computational molecular docking of small molecules to proteins, DNA, RNA and other important macromolecules

Pymol:3D visualization of proteins

Molgrow: detects and visualizes the interactions between ligand & protein and helps to find number of hydrogen bonds

Websites used in docking

SwissADME:used to evaluate pharmacokinetics, drug-likeness and medicinal chemistry friendliness of ligands.[4]

Protein – ligand interaction profiler: detects and visualizes the interactions between ligand & protein and helps to find number of hydrogen bonds.[5]

Hyperchem: 3D visualization of ligand

RSCB PDB:protein downloading (homology modeling done)[6]

CASTp 3.0: (Computed Atlas of Surface Topography of Proteins) to study surface features and functional regions of proteins (active sites of protein)

Open Babel: change file format

SPDBV: (Swiss-PdbViewer) used to perform homology modelling of a protein

SAVES 6.0: Verification of protein structures: patterns of nonbonded automatic interactions

METHODOLOGY

4.1 METHOD

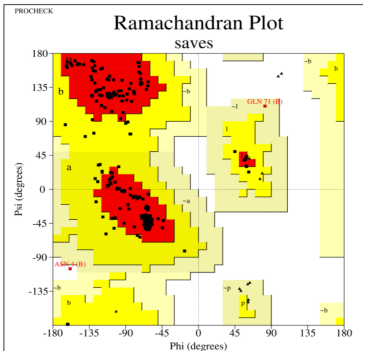
Homology modelling

The target sequence of PfGST was accrued from UniprotKB protein knowledge base and compelled by using NCBI PSI-BLAST to identify the template sequence. It was existing as a good sourcetopredictstructurebasedpharmacophoreanalysis.Further,thethree dimensionalproteinstructures were built by using Swiss PDB Viewer (SPDBV) and the protein structure by Modeller9.12

Ramachandran plot of model4ZXG

SAVES OF 4ZXG

VERIFY 3D- 94.95% of the residues have averaged 3D-1D score >=0.2; PASS **ERRAT**- Overall quality factor is 94.6092

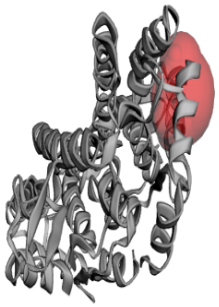


Plot statistics

Residues in most favoured regions [A,B,L]	325	92.1%
Residues in additional allowed regions [a,b,l,p]	26	7.4%
Residues in generously allowed regions [~a,~b,~l,~p]	1	0.3%
Residues in disallowed regions	1	0.3%
Number of non-glycine and non-proline residues	353	100.0%
Number of end-residues (excl. Gly and Pro)	13	
Number of glycine residues (shown as triangles)	17	
Number of proline residues	11	
Total number of residues	394	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

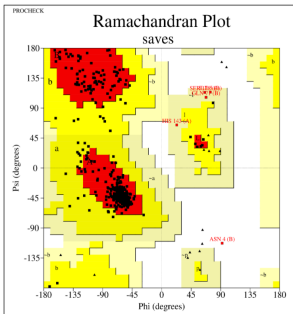
- Active site of 4ZXG protein predicted by CASTp server



ASP40, PHE42, VAL43, PHE45, LYS46 and LYS49

SAVES OF 3FR6

VERIFY 3D- 94.76% of the residues have averaged 3D-1D score >=0.2; PASS **ERRAT**- Overall quality factor is 94.6092.

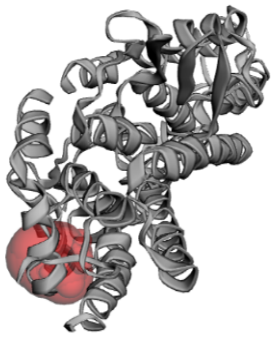


Plot statistics

Residues in most favoured regions [A,B,L]	320	88.2%
Residues in additional allowed regions [a,b,l,p]	38	10.5%
Residues in generously allowed regions [~a,~b,~l,~p]	4	1.1%
Residues in disallowed regions	1	0.3%
Number of non-glycine and non-proline residues	363	100.0%
Number of end-residues (excl. Gly and Pro)	6	
Number of glycine residues (shown as triangles)	20	
Number of proline residues	12	
Total number of residues	401	

Based on an analysis of 118 structures of resolution of at least 2.0 Angstroms and R-factor no greater than 20%, a good quality model would be expected to have over 90% in the most favoured regions.

- Active site of 4ZXG protein predicted by CASTp server



THR121, LEU124, ASN125, LEU128, PRO129, SER132, PRO177, SER178, SER179, LEU180, LYS181, ASN182 and PHE183

Ligand preparation:

The novel 1,3-oxazine analogues that is 4-methyl-3-(quinolin-2-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 3-(quinolin-2-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 4-ethyl-3-(quinolin-2-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 3-(quinolin-4-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 4-methyl-3-(quinolin-4-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 4-ethyl-3-(quinolin-4-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 4-propyl-3-(quinolin-2-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 4-propyl-3-(quinolin-4-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine, 2-(quinolin-4-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 1-methyl-2-(quinolin-4-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 1-ethyl-2-(quinolin-4-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 1-propyl-2-(quinolin-4-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 1-propyl-2-(quinolin-2-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 1-ethyl-2-(quinolin-2-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 1-methyl-2-(quinolin-2-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine, 2-(quinolin-2-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine were retrieved from PubChem database (http://pubchem.ncbi.nlm.nih.gov/search/search.cgi). The ligand optimization was performed by Hyperchem Professional7.0.

Molecular Docking

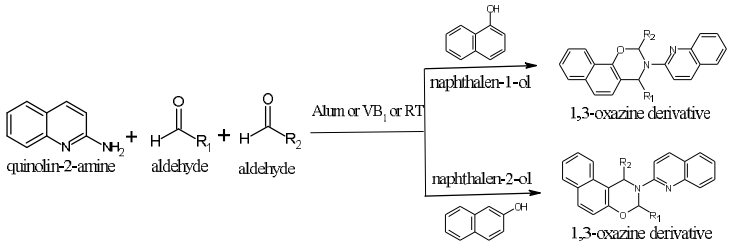
The docking evaluation of sixteen ligands (1,3-Oxazine derivatives) were docked with PfGST protein using Auto dock tools. Molecular docking studies were carried out using Auto dock 4.2 and Auto Dock Tools 1.5.4 from the Scripps Research Institute, http://www.scripps.edu/mb/olson/doc/autodock.[7]

The Lamarckian genetic algorithm was used for ligand conformational searching. The local search algorithm, which builds a population of individuals (genes), each being a different random conformation of the docked molecule. The grid was generated around the active site at 80 × 80 × 80 to calculate molecular simulation using AMBER tools, showed auto grid of active site residues around the complex structure. There were 150 populations with a mutation rate of 0.02, crossover rate of 0.8 and default grid spacing 0.375Å were used as parameters settings for docking. Consequently, these simulations were performed using up to 2.5 million energy evaluations with a maximum of 27,000 generations and each simulation was performed by 10 times that yielded 10 docked conformations. At last, the lowest energy conformations were regarded as the binding conformations between ligands and the protein.

General procedure of [1,3]-Oxazine derivative:

An efficient and convenient synthesis of 1,3-oxazine derivatives has been achieved by the one-pot, multicomponent condensation of α- or β-naphthol, an aniline and formaldehyde using catalyst in water as a universal solvent.

The product is extracted with ethyl acetate. The organic layer is washed with brine and dried over anhydrous Magnesium sulfate. The solvent is removed under reduced pressure to afford solid/ viscous 1,3- oxazine derivatives.[8]

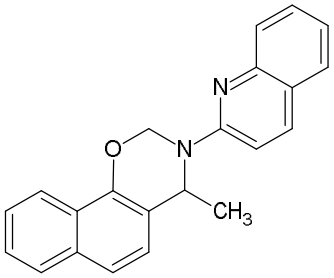


Method of collection of data:

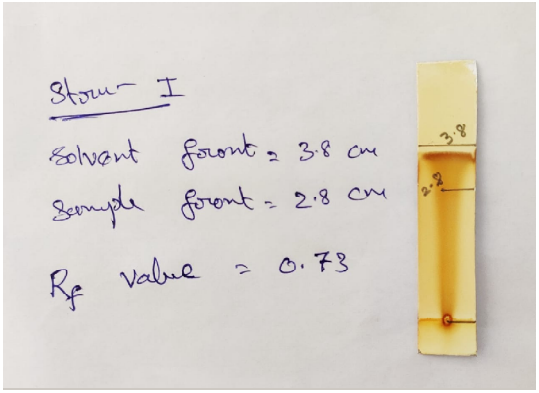
Practical data will be obtained from laboratory-based studies

- Synthesis
- Testing purity by TLC
- Characterization of compounds by IR, NMR and Mass spectroscopy.
- Biological activity confirmed by docking studies

Synthesis of 1,3-Oxazine derivatives from Aminoquinoline:
Structure I:4-methyl-3-(quinolin-2-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine

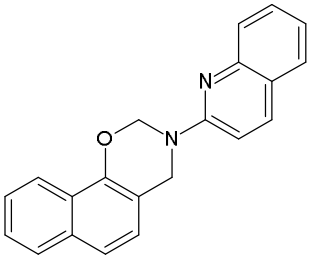


Mobile phase preparation: n-Hexane: Ethyl acetate (7:3)

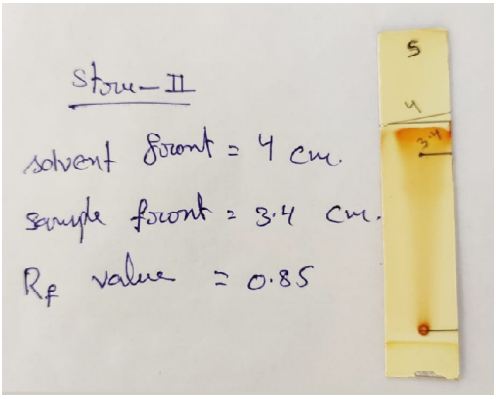


RF of Structure 1 in mobile phase = 2.8/3.8 = 0.73

Structure II:3-(quinolin-2-yl)-3,4-dihydro-2*H*-naphtho[2,1-*e*][1,3]oxazine

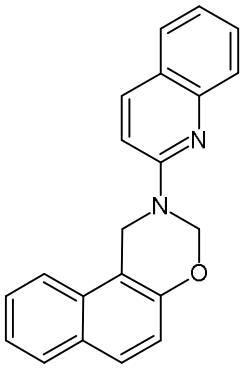


Mobile phase preparation: n-Hexane: Ethyl acetate (8:2)

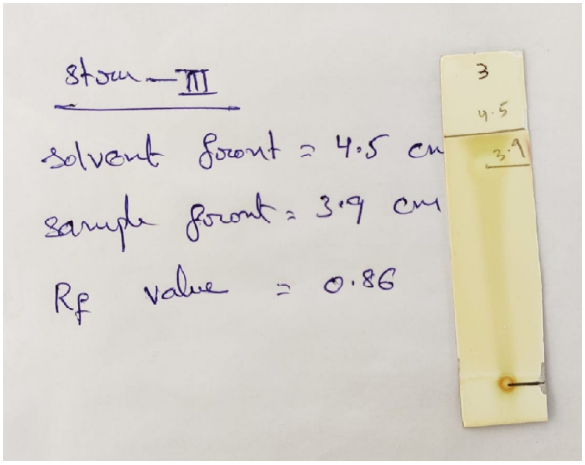


RF of 1B compound in mobile phase =3.4/4.0 = 0.85

Structure III:2-(quinolin-2-yl)-2,3-dihydro-1*H*-naphtho[1,2-*e*][1,3]oxazine



Mobile phase preparation: n-Hexane: Ethyl acetate (8:2)



RF of 1B compound in mobile phase =3.9/4.5 = 0.86

RESULTS AND DISCUSSION

Ligand	Binding free energy (Kcal/mol)	RMSD (A)	Inhibition constant KI (µm)	No. of Hydrogen Bonds
1A	-5.09	129.972	186.53	2
1B	-4.57	130.941	449.47	3
1C	-5.14	129.175	169.50	0
2A	-4.38	132.203	615.48	1
2B	-4.58	133.305	437.31	2
2C	-4.79	137.802	305.70	0
1D	-4.46	138.380	541.76	0
2D	-4.98	134.719	224.33	1
3A	-4.59	129.435	433.74	0
3B	-4.56	129.421	457.21	0
3C	-5.40	131.542	109.42	1
3D	-5.45	130.844	101.09	0
4A	-5.12	128.968	175.37	0
4B	-4.63	137.776	401.98	0
4C	-4.56	136.517	451.72	1
4D	-4.78	132.768	314.99	4
Standard	-4.69	128.865	367.52	4

Solubility data of the compounds:

Solvents	Compound 1A	Compound 1B	Compound 4D
Methanol	Insoluble	Insoluble	Insoluble
Ethanol	Insoluble	Insoluble	Insoluble
n-Hexane	Insoluble	Insoluble	Insoluble
Ethyl acetate	Completely soluble	Completely soluble	Completely soluble
CCl_4	Sparingly soluble	Completely soluble	Sparingly soluble
Toluene	Insoluble	Completely soluble	Completely soluble
Chloro-benzene	Completely soluble	Completely soluble	Completely soluble
Petroleum ether	Insoluble	Insoluble	Insoluble
Water	Insoluble	Insoluble	Insoluble

Physical data of the compound:

Physical properties	**Compound 1A**	**Compound 1B**	**Compound 4D**
Molecular Formula	$C_{22}H_{18}N_2O$	$C_{21}H_{16}N_2O$	$C_{21}H_{16}N_2O$
Molecular Weight	326.39112	312.36454	312.36454
Appearance	Brown color shiny crystal	Brown color shiny crystal	Green color shiny crystal
Melting Point	130-134℃	100-104℃	90-94 ℃
Rf Value	0.73	0.85	0.86
Yield	80%	80%	40%

ADMET prediction of Ligand molecules:

Ligand	**Carcinogenicity**	**Oral acute toxicity**	**LogS (water solubility)**
1A	0.5163	0.6142	-3.4641
1B	0.5734	0.6476	-3.2269
1C	0.5533	0.6503	-3.6295
1D	0.5986	0.6011	-3.8770
2A	0.6032	0.7048	-3.3377
2B	0.5528	0.6831	-3.6648
2C	0.5858	0.6965	-3.8107
2D	0.6207	0.6541	-4.0145
3A	0.6032	0.7048	-3.3377
3B	0.5528	0.6831	-3.6648
3C	0.5858	0.6965	-3.8107
3D	0.6207	0.6541	-4.0145
4A	0.5986	0.6011	-3.8770
4B	0.5533	0.6503	-3.6295
4C	0.5163	0.6142	-3.4641
4D	0.5734	0.6476	-3.2269

Conclusion:

The Protein-Ligand interaction plays a significant role in structure based drug designing. In the present work we have taken the receptor PfGST and identified the drugs that were used against Malaria.After analyzing the data and the result obtained1A Compound, 1B Compound and 4D Compound was showing the least binding energy and least inhibition constant which considered this compound as a best molecule binding to the PfGST protein which can be considered as antimalarial lead molecule. When the receptor (4ZXG) was docked with the drug Artemisinin the energy value obtained was (-4.69). When the designed drugs (compound 1A, 1B AND 4D) docked against the same receptor, the energy value obtained was (-5.09, -4.57 and -4.78) respectively. From this we can conclude that some of the designed drugs are better than the commercial drugs available in the market. Then, we have synthesized these drugs which shown better activity than the marketed drug and characterised them. We may proceed to work further in the future to test these synthesized drugs in wet lab for their pharmacokinetics properties and research can be proceed for clinical trials.
Remaining compounds which were docking has least binding activity compared to above three compounds by the data obtained by autodocking studies.

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