Original Article

Novel Potent Inhibitors of *Plasmodium vivax* Dihydrofolate Reductase: An *in silico* Antimalarial Drug Discovery

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ABSTRACT

Objectives: In the present study, we targeted the dihydrofolate reductase enzyme that catalyzes the reduction of dihydrofolate to tetrahydrofolate which is required for the purines and pyrimidine synthesis. Malaria is one of the severe diseases throughout the world caused by blood-borne parasite *Plasmodium vivax*. **Materials and Methods:** Eighty-five parthenin analogs were docked against *P. vivax* and *Homo sapiens* dihydrofolate reductase proteins (PDB 2BL9 and 1KMS respectively) by using Maestro 9.6 program to evaluate the binding affinities of ligands with the protein. **Results and Discussion:** Docking analysis revealed some best hit ligands against *P. vivax* such as CID3467446 and CID56671343 but not inhibited the mammalian dihydrofolate reductase. The Dock score of parthenin analogs ranged from -7.31 to -9.3 while for standard dihydrofolate reductase inhibitors it was -4.78 to -8.04. Structural analysis of docked complexes of selected parthenin like compounds with *P. vivax* and mammalian dihydrofolate reductase revealed the involvement of Arg 115, Leu 136, Lys 138, Gly 175, Ser 117, Gln 177 and Ile 7, Ala 9, Thr 56, Ile 60, Pro 61 amino acid residues respectively in strong interactions. Absorption, distribution, metabolism, and excretion properties of best-docked compounds were predicted using QikProp application of Maestro 9.6. The results indicated that all the best-docked lead compounds followed Lipinski's rule of five. **Conclusion:** Based on the results of the present study it has been concluded that parthenin like compounds may serve as potent dihydrofolate reductase inhibition based anti-malarial drug lead.

Key word: Dihydrofolate reductase (DHFR), Malaria, Parthenin analogs (like compounds), Maestro 9.6, Antimalarial Drugs.

Submission Date: 01-06-2017;
Revision Date: 06-07-2017;
Accepted Date: 11-01-2018

DOI: 10.5530/ijper.52.1.14

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INTRODUCTION

Malaria is a life-threatening disease caused by *Plasmodium* parasites transmitted to people through malaria vectors. About three million peoples die and five million have been reported to be infected with malaria annually worldwide.[1] Absence of any effective malarial vaccine, chemotherapy plays crucial role in containment of the disease but unfortunately, drug-resistant strains of *Plasmodium* such as *P. vivax* have appeared against most of antimalarial introduced till date. Thus increased efforts in antimalarial drug discovery are urgently needed. The goal must be to develop safe and affordable new drugs to counter the spread of malaria parasites that are resistant to existing agents. The malaria parasite resides primarily with in the host erythrocyte, where it exploits host cell components to meet its needs for life-cycle development and degrade the haemoglobin content of infected erythrocyte cells leading to anaemia especially in children and pregnant women.[2,3] Tetrahydrofolate, is a coenzyme involved in amino acid and nucleotide metabolism. In *Plasmodium*, it can be synthesized either via a de novo or salvage pathway. Dihydrofolate reductase is one of the important folate pathway

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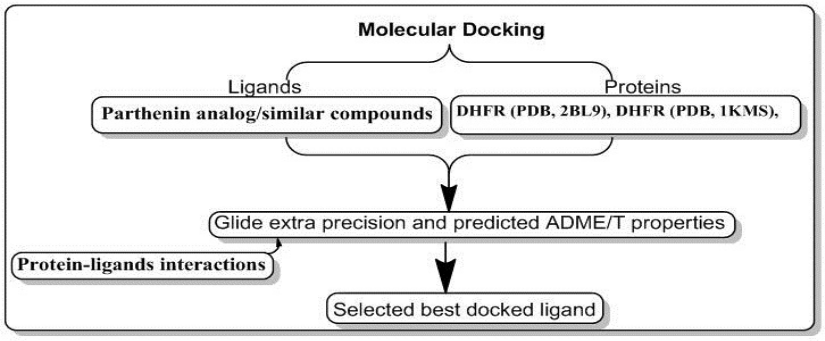


Figure 1: Workflow of study design.

enzyme used as traditional antimalarial targets. Unlike in mammalian cells these enzymes exist in malaria parasites as bi-functional enzymes. It catalyzes the NADPH-dependent reduction of H_2folate to H_4folate, a necessary co-factor for the biosynthesis of thymidylate, purine nucleotides, and certain amino acids.[4] 1,2,4-Triazole and 2,3-disubstituted quinazoline-4(3H)-one analogs are studies against DHFR and shown that it increases the potential activity against malarial.[5,6] Some of the most widely used antimalarial drugs inhibit folate metabolism such as sulfonamides and sulfones, usually combined with pyrimethamine or biguanides (DHFR inhibitors). Unfortunately, emergence of resistance against these drugs malaria chemotherapy and prevention has been hampered. It has been reported that resistance is determined by mutations, which do not have the same effect on all antifolates. This offers some hope for development of new DHFR inhibitors.[4] On the basis of above discussion DHFR may represent a potential target for malaria. *Parthenium hysterophorus* is a terrestrial weed found all around the world. *P. hysterophorus* confers many health benefits such as remedy for skin inflammation, rheumatic pain, diarrhoea, urinary tract infections, dysentery and neuralgia. Beside these several studies reported the anti-malarial potential of different parts of this plant. Parthenin is the major active phytoconstituents of *P. hysterophorus* leaf, stem flower and root.[7] Due to this we used different analogs of parthenin like compounds to target *P. vivax* DHFR enzyme in the present *in silico* study. *In silico* screening approach is the leading technique for preliminary identification of novel inhibitors for target proteins and predicting their biological binding mode. The majorities of anti-malarial drugs are small molecules designed to bind, interact and modulate the biological activity of the different pathogen proteins. Molecular docking inheres of three key consecutive goals; pose prediction, virtual screening and binding affinity evaluation. Computer aided drug designing is being exploited to identify hits, pick leads and optimize leads i.e. transform biologically active compounds into good drugs by enhancing their physicochemical, pharmaceutical and ADME\T (absorption, distribution, metabolism, excretion and toxicity) properties. Thus *in silico* modeling is used considerably to minimize risk, time and resource requirements of chemical synthesis and biological *in vitro* and *in vivo* testing.[8] In the present study we used Maestro 9.6 Schrodinger software to dock eighty five parthenin like compounds with malaria parasite and human DHFR protein. Furthermore QikProp application of Maestro 9.6 was used to predict ADME/T properties of best-docked analogs.

MATERIALS AND METHODS

Selection of Ligand and Protein molecules

GLIDE based molecular docking protocol adapted from our previous published literature with minor modifications.[9] (Figure 1). Selected ligand dataset for the study are given in Table 1.

PubChem molecules having molecular weight of <500 Da were screened in this study. Recommended values of estimated number of hydrogen bonds that would be accepted by the solute from water molecules in an aqueous solution was <10. For hydrogen bonds that would be donated by the solute to water molecules in an aqueous solution was <5. Averages values are taken over a number of configurations, so they can be non-integer.

All selected ligands were subjected to Ligprep wizard application of the Maestro 9.6 (Schrödinger Inc). Ligprep performs many corrections on the ligands, such as the addition of hydrogens, 2D to 3D conversion, corrected bond lengths and bond angles, low energy structure, stereochemistries and ring conformation. Ten tautomer's generated per ligands by using Maestro 9.6. The X-ray crystal structure of DHFR, PDB: 2BL9.[10] DHFR PDB: 1KMS.[11] retrieved from the protein data bank. Maestro

Table 1: List of PubChem molecules screened, with structural and physicochemical parameters.

S. No.	Molecule	Mol MW	Dipole	SASA	FOSA	FISA	PISA	D-HB	A-HB
1	23275702	290.358	6.92	501.933	321.143	111.692	69.099	0	5.7
2	23205	262.305	7.708	463.589	238.823	126.449	98.317	0	5.7
3	52167	262.305	4.478	462.106	232.043	128.879	101.184	0	5.7
4	88590	264.321	10.249	446.305	253.949	123.771	68.586	1	5.75
5	92119	246.305	9.786	458.22	250.566	102.738	104.916	0	5
6	93016	262.305	5.522	463.569	234.256	132.766	96.547	0	5.7
7	155585	278.304	3.912	460.576	211.41	178.439	70.727	0	6.4
8	158187	248.278	6.223	433.316	191.452	141.326	100.538	1	5.75
9	164614	262.305	4.161	459.175	249.736	137.775	71.663	0	5.7
10	174870	320.341	9.207	539.219	276.753	181.851	80.615	1	7.75
11	257272	246.305	9.989	465.024	256.95	106.975	101.099	0	5
12	257275	262.305	11.279	463.21	238.093	127.294	97.823	1	5.75
13	296217	344.407	4.834	585.451	372.161	114.347	98.943	0	7
14	442288	262.305	8.47	449.284	233.25	122.218	93.816	1	5.75
15	3467446	320.341	8.516	565.168	304.122	168.307	92.74	1	7.75
16	3482906	264.321	8.831	470.388	280.4	125.557	64.431	1	5.75
17	3482907	278.304	9.95	462.468	225.502	166.677	70.29	0	6.4
18	5317982	262.305	4.478	462.106	232.043	128.879	101.184	0	5.7
19	5318381	264.321	10.365	453.183	267.181	121.166	64.835	1	5.75
20	6325688	262.305	5.522	463.569	234.256	132.766	96.547	0	5.7
21	6442202	344.407	4.825	542.718	319.962	132.757	89.998	0	7
22	6610301	262.305	6.484	458.641	225.539	133.699	99.402	0	5.7
23	6713099	262.305	6.683	461.595	231.607	130.343	99.645	0	5.7
24	9993590	276.332	8.721	476.867	289.33	118.946	68.592	1	5.75
25	10265551	278.304	10.623	515.658	233.172	178.237	104.248	1	7.75
26	10333765	278.304	8.892	508.515	241.538	159.771	107.206	1	7.75
27	10356188	278.304	7.518	509.188	257.618	145.348	106.222	1	7.75
28	10400472	262.305	7.861	464.974	242.053	124.47	98.451	1	5.75
29	10400473	262.305	6.683	461.595	231.607	130.343	99.645	0	5.7
30	10989149	264.321	8.373	458.962	273.685	119.058	66.219	1	5.75
31	11302856	360.406	5.981	574.447	316.059	156.815	101.573	0	7.7
32	11552273	362.422	3.225	593.511	369.486	130.657	93.369	0	7.7
33	11660489	360.406	8.882	592.841	341.166	158.948	92.727	0	7.7
34	12310702	264.321	8.233	459.882	275.658	119.025	65.198	1	5.75
35	13918467	278.304	5.927	531.796	251.883	174.808	105.105	1	7.75
36	13918470	278.304	10.507	522.974	239.158	179.699	104.117	1	7.75
37	15139250	276.332	9.961	478.692	297.682	115.469	65.541	1	5.75
38	15605026	278.304	10.943	471.099	226.474	174.902	69.723	1	6.45
39	23246961	278.304	9.728	529.319	247.196	173.708	108.415	1	7.75
40	23246962	278.304	8.196	501.341	228.608	168.772	103.961	1	7.75
41	23275702	290.358	6.92	501.933	321.143	111.692	69.099	0	5.7
42	44176867	246.305	10.032	461.265	249.069	106.944	105.253	0	5
43	44383456	262.305	7.708	463.589	238.823	126.449	98.317	0	5.7
44	44444898	262.305	5.523	463.569	234.256	132.766	96.547	0	5.7
45	44468437	358.477	8.367	680.051	493.254	120.951	65.846	1	5.75

Continued...

Table 1: Cont'd.									
46	44470367	302.369	8.469	550.701	360.223	121.007	69.471	1	5.75
47	44470368	316.396	10.779	587.213	407.641	120.908	58.665	1	5.75
48	44470369	330.423	10.65	617.058	433.821	120.831	62.406	1	5.75
49	44567134	402.443	9.069	629.923	390.893	183.897	55.133	1	10.7
50	44567136	388.416	6.533	608.209	355.74	190	62.469	1	10.7
51	44583940	362.422	3.225	593.511	369.486	130.657	93.369	0	7.7
52	53298618	262.305	11.177	465.837	233.342	132.107	100.388	1	5.75
53	54671714	262.305	10.985	472.249	239.476	134.128	98.645	1	6.7
54	56664965	304.342	7.436	505.161	293.314	128.967	82.88	0	7
55	56668390	332.396	11.477	566.921	346.447	146.726	73.747	0	7
56	56671343	292.331	9.795	502.807	276.799	174.142	51.867	1	6.45
57	56678611	318.369	7.035	542.355	329.609	127.455	85.292	0	7
58	58399512	262.305	11.177	465.837	233.342	132.107	100.388	1	5.75
59	58399517	250.294	11.103	447.74	244.603	136.881	66.256	1	5.75
60	59513914	262.305	5.891	453.918	250.586	115.408	87.925	0	5.7
61	68034684	262.305	3.485	446.828	216.22	133.788	96.819	0	5.7
62	68152825	262.305	8.894	464.129	231.588	132.214	100.326	1	5.75
63	70498184	280.32	8.367	480.172	209.933	182.568	87.671	2	5.45
64	70498261	262.305	4.101	458.171	223.058	130.489	104.624	0	5.7
65	72786361	360.406	7.921	553.412	303.906	151.158	98.347	0	7.7
66	72791246	360.406	10.202	559.567	300.775	165.022	93.77	0	7.7
67	b*	362.422	6.128	583.404	343.848	151.244	88.312	0	7.7
68	a*	278.304	12.34	458.286	234.221	155.985	68.079	1	6.45
69	c*	402.443	10.701	653.753	406.752	195.922	51.079	1	10.7
70	75072308	388.416	8.961	629.686	368.517	190.013	71.156	1	10.7
71	76166589	262.305	11.317	460.569	234.575	128.355	97.639	1	6.7
72	d*	330.423	9.017	615.948	433.453	119.777	62.718	1	5.75
73	76390479	304.342	7.499	515.185	294.507	134.407	86.271	0	7
74	76391109	316.396	11.205	581.684	389.334	124.617	67.734	1	5.75
75	76391203	332.396	7.563	546.249	341.377	115.403	89.469	0	7
76	e*	358.477	9.053	682.214	495.198	124.834	62.182	1	5.75
77	76393399	318.369	11.364	538.702	336.122	109.771	92.809	0	7
78	76776246	250.294	11.369	446.519	249.288	131.625	65.607	1	5.75
79	77977597	280.32	3.808	458.115	201.696	168.282	88.136	2	5.45
80	78178185	248.278	4.2	436.341	193.611	146.186	96.543	1	5.75
81	78178433	262.305	6.603	452.273	257.132	123.522	71.619	0	5.7
82	78410193	344.407	5.913	575.505	362.161	118.382	94.962	0	7
83	85132821	262.305	7.341	463.328	237.639	128.251	97.437	1	5.75
84	90473934	262.305	10.718	460.849	231.846	128.38	100.623	1	5.75
85	442288	262.305	8.474	460.87	231.755	128.539	100.576	1	5.75

a* (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione
b* (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate
c* (3S, 4S, 9bR)-3-acetyl-6-(hydroxymethyl)-3, 9-dimethyl-2, 7-dioxo-2H, 3H, 3aH, 4H, 5H, 7H, 8H, 9bH- azuleno[4, 5-b]furan-4-yl (2E)-2-methylbut-2-enoate
d* (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione
e* (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione
mol_MW= Molecular Weight of the molecule; SASA= Solvent Accessible Surface Area; FOSA= Hydrophobic component of the SASA (saturated carbon and attached hydrogen); FISA= Hydrophilic component of the SASA (SASA on N, O, H on heteroatoms, and carbonyl C); PISA= π (carbon and attached hydrogen) component of the SASA; D-HB= Donar Hydrogen Bond; A-HB= Acceptor Hydrogen Bond

9.6 protein preparation wizard application performed for the correction of raw PDB structure; these are the addition of hydrogen atoms, assigning bond orders and bond length, creation of disulphide bonds, fixing of the charges and orientation of groups were included in to the protein molecules.

Molecular docking

Molecular docking studies using the chosen ligand molecules were conducted using Maestro 9.6.[12] Each of these compounds was docked into protein molecules, and the docking conformation possessing the lowest energy was fixed. After preparation of ligands and protein, optimized potential for liquid simulations (OPLS_2005) force field applied for local energy minimization (bond stretching energy) and geometry optimization.[13,14,15] After the execution OPLS_2005, a receptor-grid file was generated. Van der Waal radii of receptor atom by 1.00 Å and a partial atomic charge of 0.25 scaled for the generation of the receptor grid and consequently molecular docking was performed.

ADME/T properties studies

The majority of drug doesn't accomplish something in clinical trials due to deprived ADME/T properties. Therefore*, in silico* ADME/T (Absorption, Distribution, Metabolism, Excretion and Toxicity) predictive tools that could eliminate inappropriate compounds, before invested valuable time and money in primary testing of compounds. Computer based theoretical approaches transpire to be the best option for prediction of ADME/T, for new compounds. Thus, ADME/T properties of best-docked compounds were predicted using QikProp application of Maestro 9.6. ADME/T properties are prerequisite for the drug discovery and development process.[16,17]

RESULTS AND DISCUSSION

Analysis of docking results of promising compounds for *Plasmodium vivax* DHFR

Crystal structure of dihydrofolate reductase from *Plasmodium vivax*: pyrimethamine displacement linked with mutation-induced resistance in complex with inhibitors have been reported**,** which provide information about the exact location and composition of inhibitor binding pocket and opportunity to use the enzyme in a functional conformation.[10] We used X-ray structure of *Plasmodium vivax* DHFR in complex inhibitor (PDB id code 2BL9) for the docking study. Pyrimethamine (Pyr) targets dihydrofolate reductase of *Plasmodium vivax* (PvDHFR) as well as other malarial parasites, but its use as antimalarial is hampered by the widespread high resistance. Our selected dihydrofolate reductase of *Plasmodium vivax* structural insights suggest a general approach for developing new generations of antimalarial DHFR inhibitors that, by only occupying substrate space of the active site, would retain binding affinity with the mutant enzymes.[10] Molecular docking was performed using the extra precision (XP) mode of grid-based ligand docking with energetics (GLIDE). We also used known DHFR inhibitors for the comparison of results (Table 2, Figure 2). Our result highlighted that; CID3467446, (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione, CID3467446-2, CID56671343, (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2,5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6,5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate yielded a pre-eminent dock score for with proteins *Plasmodium vivax* DHFR -8.12, -8.77, -7.52, -7.42, -7.31Kcal/mol respectively (Table 2). Moreover, these compounds do not have better Gscore against human DHFR. Most of the interactions made by compounds with residues in active site of *Plasmodium vivax* DHFR seem to be hydrophobic in nature. Protein-ligand interactions of 2BL9 with compounds showed that amino acids Leu136, Ile155, and Val178 appeared in the hydrophobic interactions. Furthermore, amino acids Arg115, Leu136, Lus138, and Gly175 involved in back-bone hydrogen bonding of protein-ligand interactions (Figure 3)

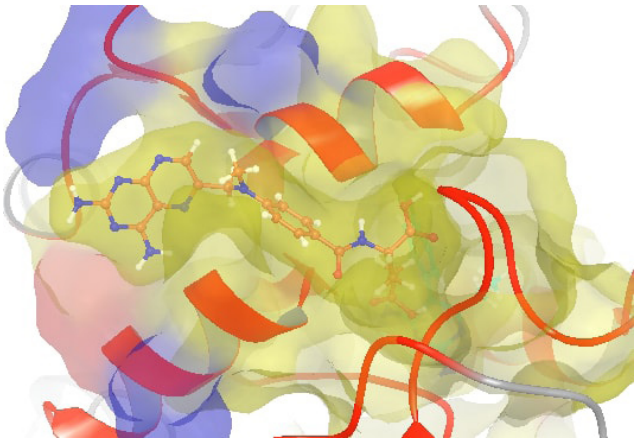
Interaction Modes between the parthenin like compounds and human DHFR

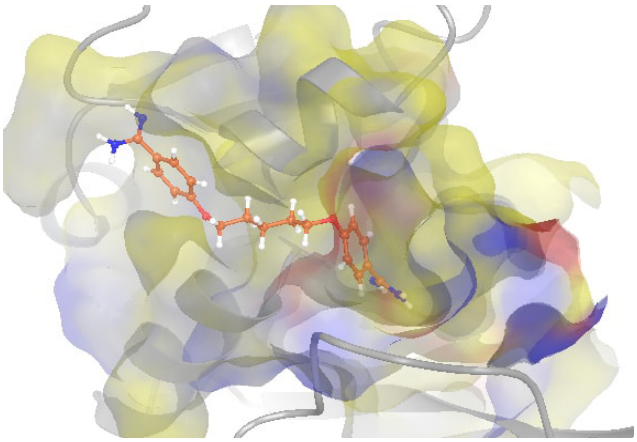
Molecular docking of *Plasmodium vivax* DHFR and human DHFR against natural compounds has been carried out. In the present investigation, our result highlighted that; (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione, (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione, (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione-2, (3S, 4S, 9bR)-3-acetyl-6-(hydroxymethyl)-3, 9- dimethyl-2, 7-dioxo-2H, 3H, 3aH, 4H, 5H, 7H, 8H, 9bH- azuleno[4, 5-b] furan-4-yl (2E)-2-methylbut-2-enoate, (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione -3 yielded a pre-eminent dock score for with proteins human DHFR -9.3, -9.04, -9.01, -9.03, -8.93Kcal/mol respectively (Table 3).

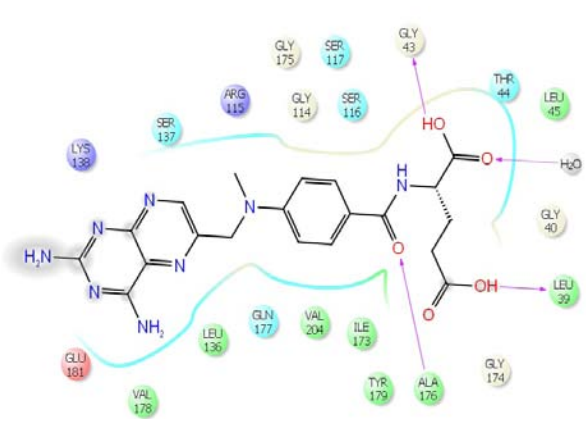
Table 2: Lowest binding energy for the ligand-DHFR (PDB, 2BL9) protein interaction as detected by GLIDE molecular docking.

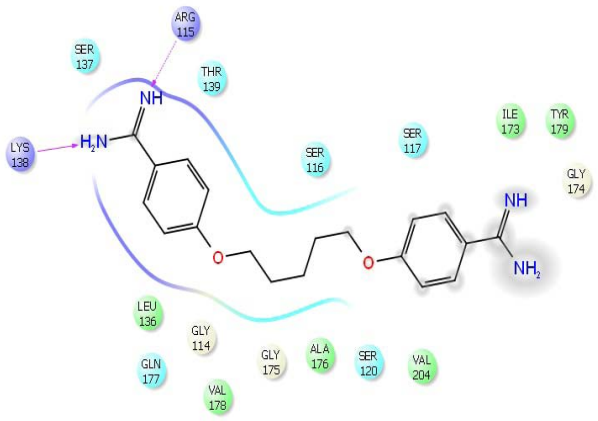
Ligand type	Compounds ID	GScore	Lipophilic Evdw	HBond	Electro	Protein-ligands interactions
DHFR Inhibitors (Control)	126941	-8.04	-4.22	-2.37	-1.06	Leu39, Gly43, and Ala176
	4735	-7.44	-5.12	-1.2	-0.88	Leu39, Arg115, and Lys138,
	5578	-5.08	-3.07	-1.46	-0.24	Asp153
	4993	-4.78	-3.08	-0.72	-0.61	Arg115, and Ser116
Compounds	3467446	-8.12	-3.33	-3.07	-1.34	Arg115, Leu136, Lys138, and Gly175
	a*	-8.77	-3.09	-2.12	-2.74	Arg115, Leu136, and Lys138
	3467446-2	-7.52	-3.25	-2.78	-1.07	Arg115, Ser117, Leu136, and Lys138
	56671343	-7.42	-2.99	-2.4	-1.51	Arg115, Leu136, and Lys138
	b*	-7.31	-3.09	-3.14	-1	Arg115, Leu136, and Gln177
	3467446-3	-7.28	-3.35	-2.63	-1.02	Arg115, Ser117, Leu136
	3482907	-7.25	-3.12	-2.73	-0.7	Arg115, Gln177
	3467446-4	-7.22	-3.13	-2.82	-0.99	Arg115, Ser116, Ser117, and Leu136
	3482907-2	-7.22	-3.46	-2.57	-0.5	Arg115 and Ser116
	77977597	-7.14	-2.45	-3.8	-0.85	Arg115, Leu136, and Gln177

a* (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione
b* (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate









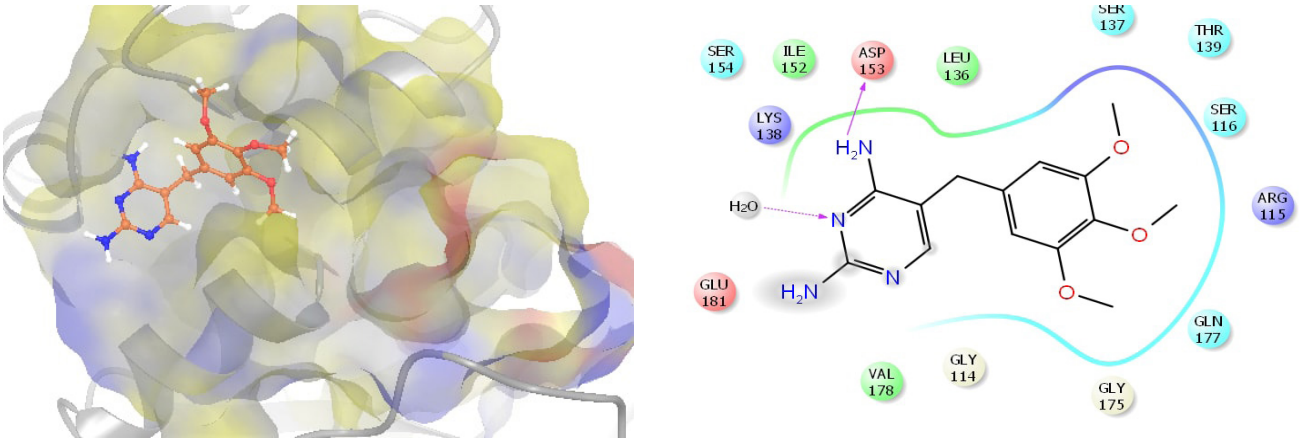


Figure 2: Interaction of *P. vivax* DHFR with standard antimalarial drugs.
(a) Ribbon presentation of DHFR (PDB, 2BL9) protein molecule with CID126941 (b) Protein-ligand interactions profile of 2BL9 with CID126941 (c) Ribbon presentation of DHFR (PDB, 2BL9) protein molecule with CID4735 (d) Protein-ligand interactions profile of 2BL9 with CID4735 (e) Ribbon presentation of DHFR (PDB, 2BL9) protein molecule with CID5578 (f) Protein-ligand interactions profile of 2BL9 with CID5578

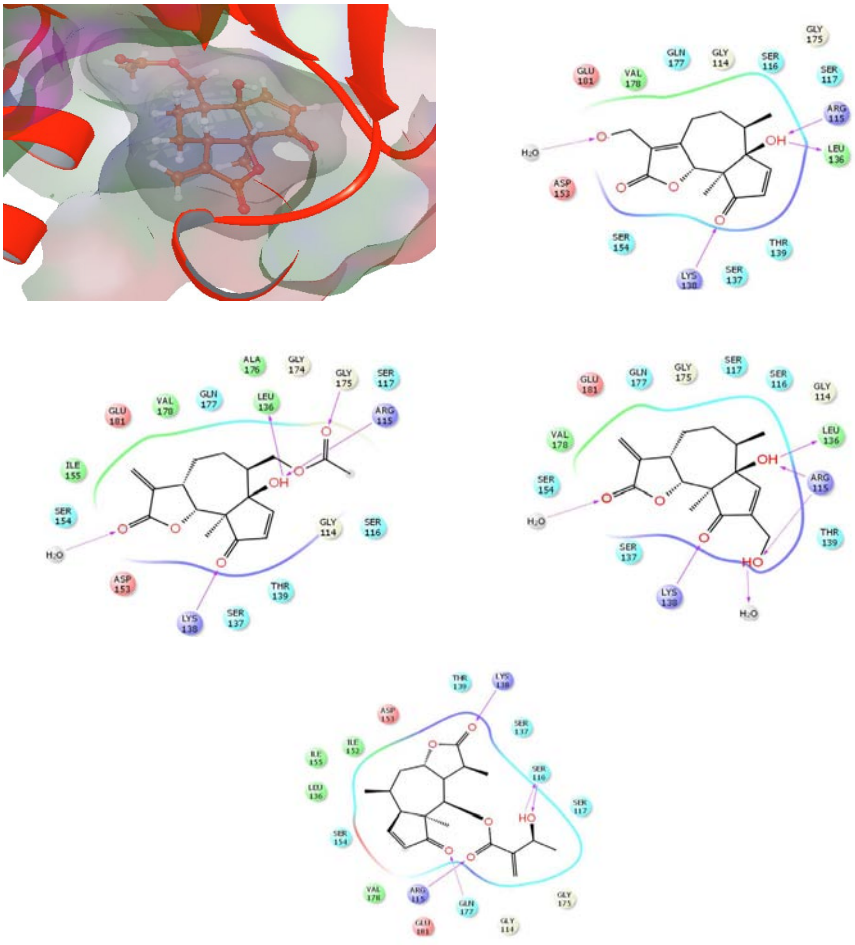


Figure 3: Interaction of *P. vivax* DHFR with parthenin like compounds.
(a) Ribbon presentation of DHFR (PDB, 2BL9) protein molecule with CID3467446 (b) Protein-ligand interactions profile of 2BL9 with CID3467446 (c) Protein-ligand interactions profile of 2BL9 with (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione (d) Protein-ligand interactions profile of 2BL9 with CID56671343 (e) Protein-ligand interactions profile of 2BL9 with (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate

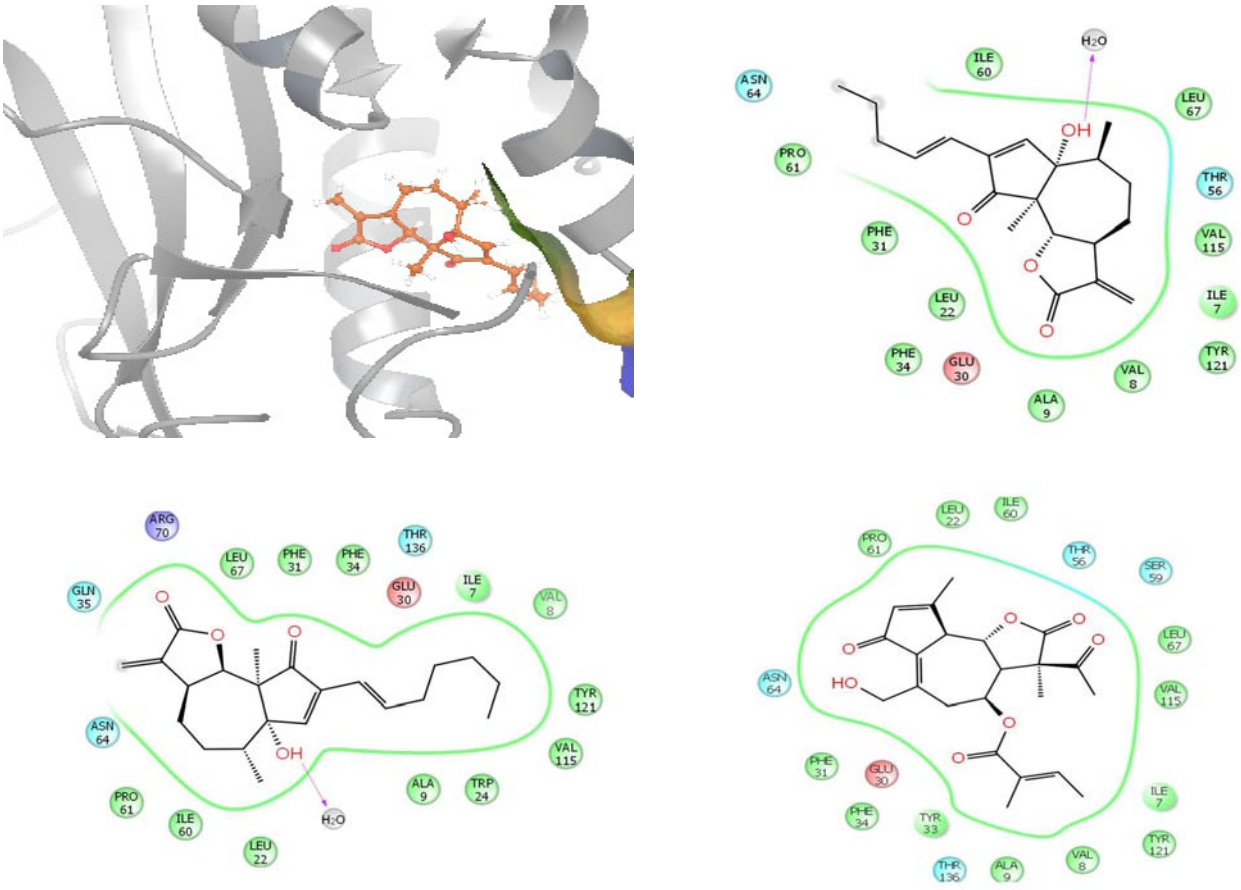


Figure 4: Interaction of *Homo sapiens* DHFR with parthenin like compounds(a) Ribbon presentation of DHFR (PDB, 1KMS) protein molecule with (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione (b) Protein-ligand interactions profile of 1KMS with (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione (c) Protein-ligand interactions profile of 1KMS with (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione (d) Protein-ligand interactions profile of 1KMS with (3S, 4S, 9bR)-3-acetyl-6-(hydroxymethyl)-3, 9- dimethyl-2, 7-dioxo-2H, 3H, 3aH, 4H, 5H, 7H, 8H, 9bH- azuleno[4, 5-b]furan-4-yl (2E)-2-methylbut-2-enoate.

Protein-ligand interactions delineate that the lipophilic, hydrogen bonding and π-π stacking interactions represent a central role in protein-ligand interactions at the active site. Molecular docking procedure identifies the docking free energy value (G-score) against these receptor protein molecules. Protein-ligands interactions highlighted the lipophilic, electrostatic and hydrogen bond interactions are a key player in protein-ligand interactions. All the compounds in the dataset were docked into the active site of human DHFR, using the same protocol. Figure 4 depicts the binding conformations of the parthenin like compounds in the binding pocket of the human DHFR. The active site of human DHFR comprises of mostly hydrophobic amino acids as Ile7, Ala9, Trp24, Phe31, Phe34, Thr56, Pro61, and Ile60, Val115 and these amino acid residues are involved in strong hydrophobic interactions with the parthenin like compounds. As expected, inhibitors used in this study bind to the same site like the docked ligand in the crystallographic complex. The inhibitors bind in a hydrophobic pocket adjacent to helix αB, with the 5-deazapteridine ring almost perpendicular to the 5-quinolylamino group. The 5-deazapteridine ring of the inhibitors forms hydrophobic contacts with Val8, Ile7 and Phe31. Notably, the human DHFR parthenin like compound inhibitors displayed same interaction Ile 7, Thr56 and Ile60 knowledgeable by some cycloguanil analogues which proved to be active against influenza virus and respiratory syncytial virus replication, via targeting the host (human) DHFR enzyme.[18] In another study 5-deazapteridine rings of DMDP (2, 4-diamino-5-methyl-5-deazapteridine) derivatives has been shown to bound human DHFR active site in an identical fashion, as reported in case of other inhibitors like methotrexate.[19] Recently, Viira *et al.* screened curcuminoids for their *in silico* antimalarial activity against *P. falciparum* and found 17 potential lead compounds, which they further tested

Table 3: Lowest binding energy for the ligand-DHFR (PDB, 1KMS) protein interaction as detected by GLIDE molecular docking.

Ligand type	Compounds ID	GScore	Lipophilic Evdw	HBond	Electro	Protein-ligands interactions
Compounds	d*	-9.3	-6.35	-0.7	-0.26	Ile7, Ala9, Thr56, and Ile60
	e*	-9.04	-6.13	-0.7	-0.4	Ile7, Ala9, Thr56, and Ile60
	e*-2	-9.01	-6.36	-0.54	-0.19	Ile7, Ala9, Thr56, and Ile60
	c*	-9.03	-6.04	-0.48	0.04	Ile7, Thr56, Pro61, and Ile60
	e*-3	-8.93	-6.23	-0.5	-0.24	Ile7, Thr56, Pro61, and Ile60
	d* -2	-8.82	-5.76	-0.7	-0.43	Ile7, Thr56, Pro61, and Ile60
	23275702	-8.76	-5.08	-0.95	-0.31	Ile7, Thr56, Pro61, and Ile60
	3482907	-8.7	-4.54	-1.44	-0.53	Ile7, Thr56, Pro61, and Ile60
	56671343	-8.6	-4.68	-1.44	-0.36	Ile7, Thr56, Pro61, and Ile60
	75072308	-8.6	-5.44	-0.53	-0.09	Ile7, Thr56, Pro61, and Ile60

c* (3S, 4S, 9bR)-3-acetyl-6-(hydroxymethyl)-3, 9-dimethyl-2, 7-dioxo-2H, 3H, 3aH, 4H, 5H, 7H, 8H, 9bH- azuleno[4, 5-b]furan-4-yl (2E)-2-methylbut-2-enoate
d* (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione
e* (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione
Molecule CID; Pubchem IDs, GScore; Glide extra precision scores (kcal/mol)., Lipophilic E Vdw; Chemscore lipophilic pair term and fraction of the total protein-ligand vdw energy., HBond; Hydrogen-bonding term., Electro; Electrostatic rewards., Protein ligands interaction; π-π stacking, π –cat interaction and hydrogen bond between the ligands and protein.

by using *in vitro* antimalarial assay and found that most of the lead compounds were potential antimalarial agents.[20]

ADME/T properties of leads molecules

ADME/T properties of lead compounds (Figure 6) were appraised by using the Qikprop application of Maestro 9.6[21]. Most attractive aspect of CID3467446, (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione, 3467446, 56671343, (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate, 3467446, 3482907, 3467446, 3482907, 77977597 are their admirable, Qplogpo/w, QplogHERG, QplogBB, QPP MDCK, Qplogkhsa, and Percentage of human oral value which satisfy the lipinski's rule of five (Table 4).

Moreover, polar surface area, high oral bioavailability, H-bond donors and acceptors are being imperative criteria for the development of therapeutic agents. Veber *et al.* reported that compounds having 10 or fewer rotatable bonds and polar surface area equal to or less than 140 Å (or 12 or fewer H-bond donors and acceptors) may have a high probability for best oral bioavailability in the rat.[22] Furthermore, it is also reported that polar surface area is inversely proportional to permeation rate.[23] These compounds have better SASA values that are claimed to be suitable for therapeutic agents. These results indicate that the compounds will have better penetration rate. Blood Brain Barrier (BBB) involves biochemical barriers such as metabolic enzyme systems and efflux transporters for the xenobiotics. hERG (human *Ether-a-go-go*-Related Gene) gene codes $K_v11.1$ protein (α subunit of K^+ channel). It conducts potassium ions out of the heart muscle cells and coordinates the heart's beating. *In silico* human colon adenocarcinoma (Caco-2) and Madin–Darby canine kidney (MDCK) epithelial cell models are used to evaluate drug's permeability and transporter interactions. Structure-based serum albumin binding model was used to determine the distribution and metabolism of lead compounds. This encompasses the *in silico* binding strength of lead compounds to human serum albumin. Human oral bioavailability deals with the information about fraction of an administered drug that reaches its site of action through systematic circulation, to exert its pharmacological and therapeutic effects. All these models unscrew the qualitative prediction and ranking of absorption, determining mechanism(s) of permeability, formulation effects on drug permeability, and the potential for transporter-mediated drug–drug interactions.

CONCLUSION

In this work, molecular docking studies were carried out to explore the binding mechanism of parthenin like compounds derivatives to the *P. vivax* and human DHFR enzyme to enable the design of new parthenin like compound-based human DHFR inhibitors. Both the binding conformation of parthenin like compounds and their binding free energies were predicted by molecular docking. Present study can be considered as an in silico

Table 4: Structural, physicochemical, biochemical, pharmacokinetics and toxicity properties of compound.

S.N.	Molecule	QP log $P_{o/w}$ (-2.0 to 6.5)	QPlog HERG (acceptable range: above -5.0)	QPP Caco (nm/sec) <25-poor >500- great	QP log BB (-3-1.2)	QPP MDCK (nm/sec) <25-poor >500- great	QPlog Khsa (Acceptable range: -1.5 to 1.5).	Percentage of human oral absorption; (<25% is poor and >80% is high)
1	3467446	1.023	-4.076	150.058	-1.362	63.677	-0.353	71.888
2	a*	0.865	-3.382	258.352	-0.974	114.557	-0.391	75.182
3	3467446	0.965	-4.163	121.167	-1.469	50.536	-0.351	69.886
4	56671343	1.251	-3.48	265.101	-1.062	117.795	-0.274	77.646
5	b*	1.56	-3.611	339.947	-0.953	154.121	-0.507	81.39
6	3467446	0.962	-4.026	150.658	-1.348	63.952	-0.377	71.558
7	3482907	0.574	-2.87	297.89	-0.833	133.619	-0.808	74.588
8	3467446	0.947	-3.855	170.727	-1.259	73.208	-0.395	72.447
9	3482907	0.54	-3.279	218.621	-1.014	95.639	-0.78	71.985
10	77977597	1.76	-1.282	53.449	-1.039	26.536	-0.339	68.179

a* (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione
b* (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate
QPlogPo/w (-2.0 to 6.5) Predicted octanol/water partition coefficient
QPlogHerg (acceptable range: above -5.0) Predicted IC50 value for blockage of HERG K+ channels;
QPPCaco (nm/sec) <25-poor >500- great Predicted apparent Caco-2 cell permeability in nm/sec. Caco-2 cells is a model for the gut blood barrier,
QPlogBB (-3-1.2) Predicted brain/blood partition coefficient;
QPPMDCK (nm/sec) <25-poor >500- great Predicted apparent MDCK cell permeability in nm/sec. MDCK cells are considered to be a good mimic for the blood-brain barrier.
Q P log Khsa-Prediction of binding to human serum albumin; (acceptable range: -1.5 to 1.5).
Percentage of human oral absorption; (<25% is poor and >80% is high)

approach to search for novel class of Dihydrofolate reductase (*Plasmodium vivax* and human) inhibitors using parthenin like compounds as a scaffold. Results obtained from molecular property analysis clearly indicate that some of the selected parthenin like compounds satisfies criteria's of Lipinski's rule of five, hence has potential to be utilized as effective anti-DHFR agents. The tetrahydrofolate synthesis and DHFR inhibition by parthenin like compounds represented in Figure 5.

Results from docking investigation of selected compounds on *Plasmodium vivax* DHFR point out that compound CID3467446, (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione, 3467446-2, 56671343, (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate can potentially be used as starting lead compound in developing anti-DHFR agents for *Plasmodium vivax*. Moreover, compound (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione, (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4,5-b]furan-2,9-dione, and (3S, 4S, 9bR)-3-acetyl-6-(hydroxymethyl)-3, 9- dimethyl-2, 7-dioxo-2H, 3H, 3aH, 4H, 5H, 7H, 8H, 9bH- azuleno[4, 5-b] furan-4-yl (2E)-2-methylbut-2-enoate can potentially be used as starting lead compound in developing anti-DHFR agents for mammals.

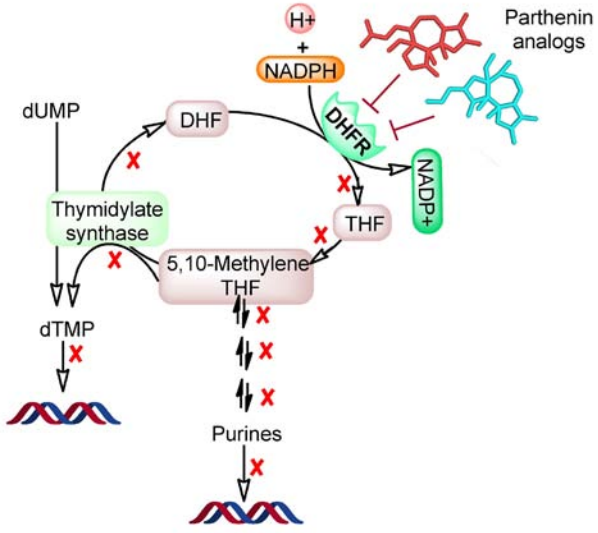


Figure 5: Parthenin like compounds inhibit dihydrofolate reductase (folate pathway) in *P. vivax*.

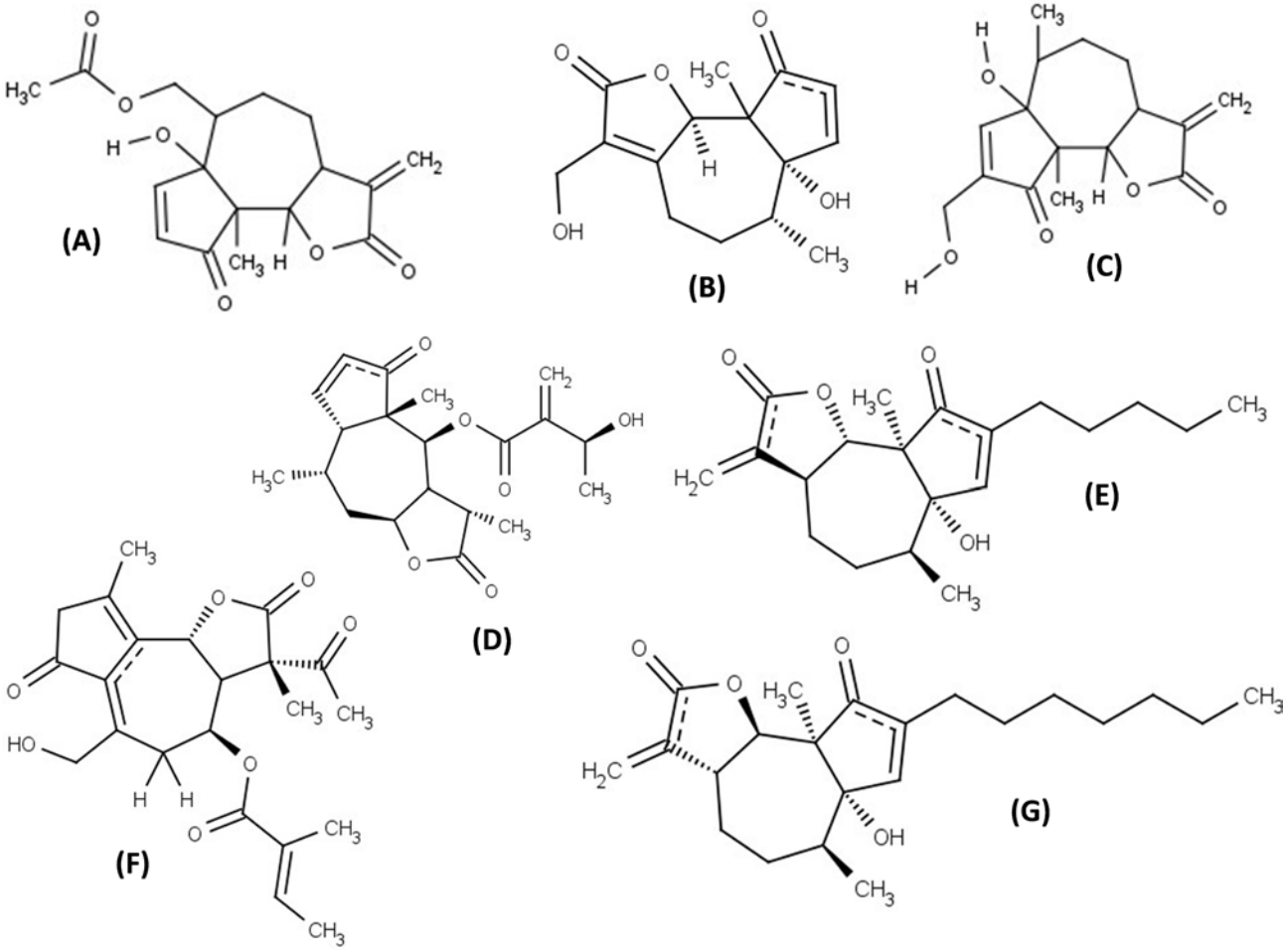


Figure 6: Chemical structure of the lead compounds. (A) AC1MRFOP (CID3467446) (B) (6R, 6aS, 9bR)-6a-hydroxy-3-(hydroxymethyl)-6, 9a-dimethyl-4H, 5H, 6H, 9bH-azuleno[4, 5-b]furan-2,9-dione (C) SCHEMBL4058769 (D) (3S, 3aS, 4S, 4aS, 7aR, 8S, 9aS)-3, 4a, 8-trimethyl-2, 5-dioxo-3H, 3aH, 4H, 7aH, 8H, 9H, 9aH-azuleno[6, 5-b]furan-4- yl (3S)-3-hydroxy-2-methylidenebutanoate (E) (3aS, 6S, 6aS, 9aR, 9bS)-6a-hydroxy-6, 9a-dimethyl-3-methylidene-8-pentyl-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione (F) (3S, 4S, 9bR)-3-acetyl-6-(hydroxymethyl)-3, 9- dimethyl-2, 7-dioxo-2H, 3H, 3aH, 4H, 5H, 7H, 8H, 9bH- azuleno[4, 5-b]furan-4-yl (2E)-2-methylbut-2-enoate (G) (3aR, 6S, 6aS, 9aR, 9bR)-8-heptyl-6a-hydroxy-6, 9a-dimethyl-3-methylidene-2H, 3H, 3aH, 4H, 5H, 6H, 6aH, 9H, 9aH, 9bH-azuleno[4, 5-b]furan-2,9-dione

Structural analysis of docked complexes of selected parthenin like compounds with *Plasmodium vivax* DHFR revealed strong chances of involvement of residues like Arg115, Leu136, Lys138, and Gly175 in inhibition. In addition to amino acids Leu136, Ile155, and Val178 appeared in the hydrophobic interactions. Moreover, the active site of human DHFR comprises of mostly hydrophobic amino acids as Ile7, Ala9, Trp24, Phe31, Phe34, Thr56, Pro61, and Ile60, Val115 and these amino acid residues are involved in strong hydrophobic interactions with the parthenin like compounds. The obtained results are expected to be useful for understanding not only the mode of inhibition but also in rapid and accurate prediction of the newly designed inhibitors. We also conclude that hydrophobic forces might play a highly influencing role in inhibition of *Plasmodium vivax* DHFR and successfully delineate specific functional groups that might be reSsponsible for hydrophobic effect of parthenin like compounds.

ACKNOWLEDGEMENT

PS and PPK acknowledge Indian Council of Medical Research (ICMR), India and UGC-CSIR, India respectively for providing the financial assistance in the form of Senior and Junior Research Fellowship. SK acknowledges Central University of Punjab for providing infrastructure facilities.

Conflicting Interest

The Authors declare no conflict of interest.

ABBREVIATIONS USED

DHFR: Dihydrofolate reductase; **DHF:** Dihydrofolate ; **dUMP:** Deoxyuridine monophosphate; **dTMP:** Deoxythymidine monophosphate; **THF:** Tetrahydro folate; **NADP+:** Nicotinamide adenine dinucleotide phosphate; **NADPH:** Nicotinamide adenine dinucleotide phosphate, reduced; **PDB:** The Protein Data Bank; **ADME/T:** Absorption, Distribution, Metabolism, Excretion and Toxicity.

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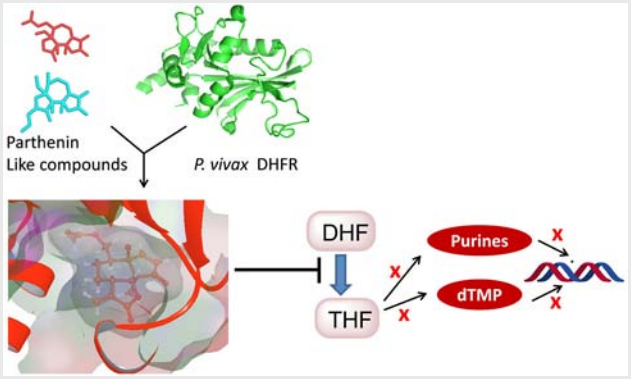
PICTORIAL ABSTRACT



SUMMARY

- Parthenin like compounds has the ability to bind selectively with *P. vivax* dihydrofolate reductase protein. In comparison to known inhibitors (Dock Score up to -8.04), parthenin like compounds showed better Dock Score (up to -9.3). Arg 115, Leu 136, Lys 138, Gly 175, Ser 117, Gln 177 amino acid residues of *P. vivax* dihydrofolate reductase protein are involved in strong interactions with lead compounds. All the best-docked lead compounds followed Lipinski's rule of five and showed drug-likeness, non-toxic, non-mutagenic and better biological properties. Thus parthenin like compounds showed possibilities to become potent anti-malarial agents of natural origin. Further *in vitro* and *in vivo* studies are required to study their potential.

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Cite this article: Pushpendra S, Prem KP, Kumar S. Novel Potent Inhibitors of *Plasmodium vivax* Dihydrofolate Reductase: An *in silico* Antimalarial Drug Discovery. Indian J of Pharmaceutical Education and Research. 2018;52(1):122-34.