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ARTICLE TYPE

Triazine-pyrimidine based molecular hybrids: Synthesis, docking studies and antimalarial activity evaluation

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Abstract: A series of novel triazine-pyrimidine hybrids have been synthesized and evaluated for their *in vitro* antimalarial activity. Some of the compounds showed promising antimalarial activity against both CQ-sensitive and CQ-resistant strains at micro molar level with high selectivity index. All the compounds displayed better activity (IC_{50} = 1.32-10.70 μM) than the standard drug pyrimethamine (>19 μM) against chloroquine-resistant (W2) strain. All the tested compounds were nontoxic against mammalian cell lines. Further, docking studies of the best active compounds were performed on both wild type and quadruple mutant (N51I, C59R, S108N, I164L) Pf-DHFR-TS using Glide to analyse the interaction of the compounds in the binding site of the protein. The binding poses of compounds **14** and **19** having high Glide XP score and lowest Glide energies shows comparable and efficient binding pattern similar to the DHFR substrate (dihydrofolate) in wild type and mutant DHFR active site. The pharmacokinetics property analysis of best active compounds using ADMET prediction attests to the possibility of developing compound **14** as a potent antimalarial lead.

Introduction

Malaria is the third most infectious disease after tuberculosis and HIV/AIDS, which affects over 100 countries in Africa, Asia and South America.[1] Despite intensive efforts of eradication in the early 1960s, malaria remains a major public health problem till date. According to World Health Organization (WHO) nearly 300-500 million people get infected with malaria every year throughout the world. The mortality rate is estimated to be around 1.1 million deaths per year, mostly children under the age of five. Eighty percent of malaria cases worldwide occur in Africa, remaining two third cases are found in six countries and India is one of them. There are four major species of the malaria parasite, of which *Plasmodium falciparum* causes the most virulent forms of malaria and is responsible for more than 95% malaria related deaths. Due to unavailability of effective vaccines, chemotherapy remains the only option for treatment of malaria. After the discovery of quinine in the late 1600s, a huge number of potent antimalarial agents such as chloroquine, amodiquine, primaquine, pamaquine, mefloquine and related compounds were developed. Chloroquine (CQ) has been the mainstay of malaria therapy for decades because of its efficacy, safety and low cost until the emergence and spread of CQ-resistance. Pyrimethamine-sulfadoxine (fansidar) was another best therapeutic option after CQ but rendered ineffective in most of malaria endemic regions due to spread of resistance. Currently, natural endoperoxide, artemisinin and its semi-synthetic derivatives (artemether, arteether and artesunate) are the most potent and fast acting antimalarials effective against resistant strains of *P. falciparum*. In order to combat with the resistance problems combination therapy has been introduced by WHO in which artemisinin and its analogue in combination with 4-aminoquinoline antimalarials are used to treat malaria. Although artemisinin combination therapy (ACT) is well tolerated and is nearly 95% effective in treating malaria, but its use is limited in some regions due to some serious issues like the higher cost of treatment, safety in pregnancy etc.[2-7] In addition, resistance to artemisinin derivatives has also been reported in Southeast Asian countries which may continue to increase, subsequently making malaria chemotherapy more complicated.[5-8]

The dihydrofolate reductase (DHFR) is one of the well-defined and explored target in malarial chemotherapy. Pyrimethamine and cycloguanil (Fig. 1) are potent DHFR inhibitors and are clinically used for the treatment of *P. falciparum* malaria.[9,10] Unfortunately, point mutations at certain amino acid residues in the surroundings of the active site of *P. falciparum* DHFR have resulted in resistance, compromising the clinical effectiveness of these drugs.[11-13] Despite this, the folate pathway remains a good target for malarial chemotherapy because the enzyme is limited in its mutational capability, owing to the loss in enzyme function. WR99210 (Fig. 1) having a flexible linker is found to be effective at nano molar concentration even against the strains which are highly resistant to other DHFR inhibitors. It is believed that the exceptional high activity of WR99210 is due to its highly flexible nature which helps to bind it into the active site of the target.[14] Unfortunately, WR99210 exhibits unacceptable gastro-intestinal (GI) intolerance. Recently a new DHFR inhibitor, P218 (Fig. 1) has been developed by BIOTEC pharmaceuticals.[15,16] It inhibits blood stage growth of drug-resistant malarial parasites with IC_{50} value of 6 nM. It has also been the most active antifolate agent against liver stage of *P. yoelii* (IC_{50} < 10 nM).[17]

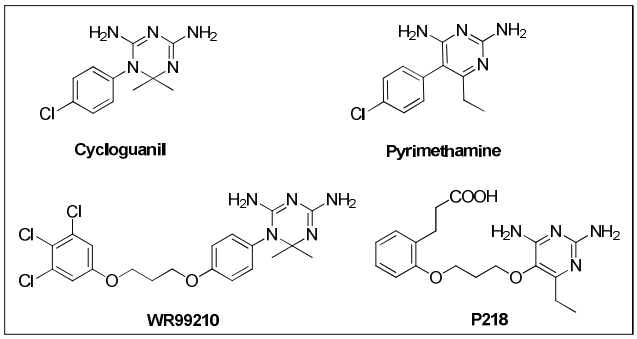


Figure 1: DHFR inhibitor based antimalarial drugs

Apart from this, a large number of structurally similar compounds such as triazine[18-20] and pyrimidine derivatives[21-23] have been synthesized, and some of these compounds have shown very potent antimalarial activity against both CQ-sensitive and CQ-resistant strains. Cyclogunil and pyrimethamine represent the triazine and pyrimidine chemical class of compounds, respectively. To combat with the increasing resistance problems, there is an urgent need to develop a potent safe and cost-effective antimalarial agent. As a part of our ongoing malaria research programme,[24-30] we became interested to join triazine and pyrimidine moieties together in a single molecule by a flexible linker to provide enough flexibility like WR99210 so that it can easily fit into the binding pocket of the target and as a result may provide a better antimalarial activity profile. To the best of our knowledge, this is the first report of covalent hybrids having triazine and pyrimidine pharmacophores together. All the synthesized compounds were characterized by various spectroscopic techniques.

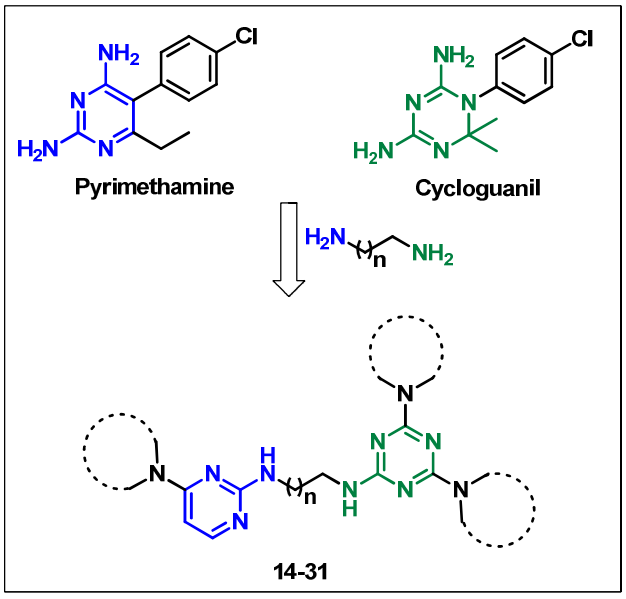


Figure 2: Design strategy for the synthesis of novel triazine-pyrimidine hybrids

Chemistry

The triazine-pyrimidine hybrids (**14-31**) were synthesized as shown in three different schemes 1, 2 and 3. Firstly, 2,4-dichloropyrimidine (**1**) was treated with different secondary amines in the presence of triethyl amine in THF at 0 °C to RT to give compounds **2-4** in high yield with small amount of its regioisomer (scheme 1).[31] The 2,4,6-trichloro-1,3,5-triazine shows temperature dependent nucleophilic substitution reactions being the first chlorine replaceable at 0 °C, second chlorine at room temperature and the third one at higher temperature. The disubstituted products **6** and **7** were obtained by the reaction of triazine (**5**) with two equivalents of morpholine and diethylamine, respectively at 0 °C to RT (< 30 °C) in the presence of K_2CO_3 using THF as solvent (scheme 2).[32,33] Thereafter, the third chlorine of resulting disubstituted-triazines was reacted with various alkyl diamines to give trisubstituted triazines (**8-13**) with a free terminal NH_2 group (scheme 2).[34] This reaction was carried out at reflux condition in THF using K_2CO_3 as a base. Finally, compounds **2-4** and **8-13** synthesized under schemes 1 and 2 were coupled together in the presence of K_2CO_3 using NMP as a solvent at reflux condition to give the desired triazine-pyrimidine hybrid molecules **14-31** (scheme 3). All the compounds were purified by column chromatography using $MeOH/CHCl_3$ as eluent and characterized by various spectroscopic techniques.

Biological Activity

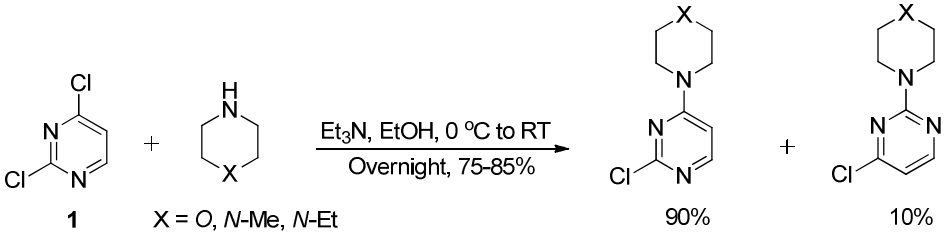
In Vitro Antimalarial Activity

The antimalarial activity was determined by measuring plasmodial LDH activity as described in literature.[35] A suspension of red blood cells infected with D6 or W2 strain of *P. falciparum* (200 µL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 µg/mL amikacin) was added to the wells of a 96-well plate containing 10 µL of serially diluted test samples. The plate was flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2 and incubated at 37 ºC, for 72 h in a modular incubation chamber (Billups-Rothenberg, CA). Parasitic LDH activity was determined according to the procedure of Makler and Hinrichs.[36] Briefly, 20 µL of the incubation mixture was mixed with 100 µL of the Malstat™ reagent (Flow Inc., Portland, OR) and incubated at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) was then added and the plate is further incubated in the dark for 1 h. The reaction was then stopped by the addition of 100 µL of a 5% acetic acid solution. The plate was read at 650 nm. Chloroquine and pyrimethamine were included in each assay as antimalarial drug controls. IC_{50} values were computed from the dose response curves. To determine the selectivity index of antimalarial activity of compounds, *in vitro* cytotoxicity of these compounds against mammalian cells was also determined. The assay was performed in 96-well tissue culture-treated plates as described earlier.[37] Vero cells (monkey kidney fibroblasts) were seeded to the wells of 96-well plate at a density of 25,000 cells/well and incubated for 24 h. Samples at different concentrations were added and plates were again incubated for 48 h. The number of viable cells was determined by Neutral Red assay. The IC_{50} values were obtained from dose response curves.

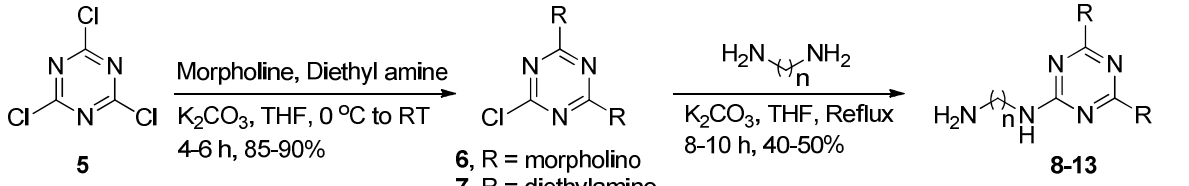
Docking studies

Antifolates act by inhibiting dihydrofolate reductase activity of *Plasmodium falciparum* bifunctional enzyme dihydrofolate reductase-thymidylate synthase (PfDHFR-TS). The four point mutations in codons 51, 59, 108, and 164 (N51I, C59R, S108N,

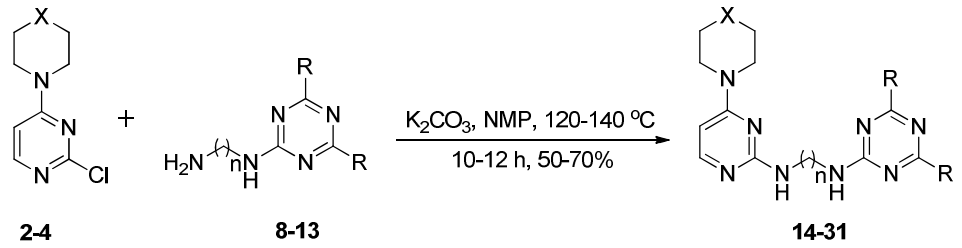
and I164L) has been found in the DHFR domain of PfDHFR-LS gene from the clinical isolates of dihydrofolate resistant parasite.[38] In the present work we have studied the binding pattern, ADMET properties of novel triazine-pyrimidine hybrids with PfDHFR-TS. The 2D structures of all the compounds were generated by drawing on ChemBioDraw Ultra 12.0 (www.cambridgesoft.com). Ligprep module implemented in Schrödinger was used to generate energy minimized 3D structures. Partial atomic charges were computed using the OPLS_2005 force field. The correct Lewis structure, tautomers and ionization states (PH 7.0±2.0) for each of these ligands were generated and optimized with default settings (Ligprep 2.5, Schrödinger, LLC, New York, NY, 2012).The 3D crystal structures of wild type PfDHFR-TS (PDB ID:3QGT; resolution 2.30 Å) and quadruple mutant (N51I+C59R+S108N+I164L) PfDHFR-TS (PDB ID:3QG2; resolution: 2.30 Å), was retrieved from protein data bank (www.rcsb.org). The proteins were prepared for docking using the Protein Preparation Wizard (Maestro 10.0 Schrödinger, LLC, New York, NY, 2012). Water molecules within 5 Å of the protein structures was considered. Bond order and formal charges were assigned and hydrogen atoms were added to the crystal structure. Further to refine the structure OPLS-2005 force field parameter was used to alleviate steric clashes and the minimization was terminated when RMSD reached a maximum cutoff value of 0.30 Å.



Scheme 1



Scheme 2



14, R = morpholino, X = O, n = 2	**23**, R = diethylamino, X = O, n = 2
15, R = morpholino, X = O, n = 3	**24**, R = diethylamino, X = O, n = 3
16, R = morpholino, X = O, n = 4	**25**, R = diethylamino, X = O, n = 4
17, R = morpholino, X = *N*-Me, n = 2	**26**, R = diethylamino, X = *N*-Me, n = 2
18, R = morpholino, X = *N*-Me, n = 3	**27**, R = diethylamino, X = *N*-Me, n = 3
19, R = morpholino, X = *N*-Me, n = 4	**28**, R = diethylamino, X = *N*-Me, n = 4
20, R = morpholino, X = *N*-Et, n = 2	**29**, R = diethylamino, X = *N*-Et, n = 2
21, R = morpholino, X = *N*-Et, n = 3	**30**, R = diethylamino, X = *N*-Et, n = 3
22, R = morpholino, X = *N*-Et, n = 4	**31**, R = diethylamino, X = *N*-Et, n = 4

Scheme 3

The location of co-crystallized ligand pyrimethamine in both wild and mutant protein structures were used to choose the center and size of the receptor grid, which was generated using Glide 5.8 (Schrödinger, LLC, New York, NY, 2012) with default settings for all parameters. The grid size was chosen sufficiently large to include all active site residues involved in substrate binding. The cofactor, NADH in the PfDHFR-TS wild and mutant structures were also considered as part of the receptor protein. All ligand conformers were docked to each of the receptor grid files (PfDHFR-TS wild and mutant structures) using Glide extra precision (XP) mode. Default settings were used for the refinement and scoring.

***In silico* ADMET prediction**

The pharmacokinetic profile of compounds showing good antimalarial activity was predicted by using programs Qikprop v3.5 (Schrödinger, Inc., New York, NY, 2012). All the compounds were prepared in neutralized form for the calculation of pharmacokinetic properties by QikProp using Schrodinger's Maestro Build module and LigPrep, saved in SD format. The programs QikProp utilizes the method of Jorgensen[39] to compute pharmacokinetic properties and descriptors such as octanol/water partitioning coefficient, aqueous solubility, brain/blood partition coefficient, intestinal wall permeability, plasma protein binding and others.

Results and discussion

The triazine-pyrimidine hybrids were evaluated for their *in vitro* antimalarial activity against both CQ-sensitive (D6 clone) and CQ-resistant (W2 clone) strains of *P. falciparum* using choroquine and pyrimethamine as standard drugs. Cytotoxicity was determined against Vero cells (Table 1). All the compounds exhibited promising antimalarial activity with IC_{50} values < 5 μM against chloroquine sensitive strain (D6) except compounds **16**, **17** and **23**. Four compounds **14**, **27**, **28** and **30** displayed potent antimalarial activity with IC_{50} values ranging from 1.18 μM to 1.57 μM towards CQ-sensitive strain with high selectivity index, while other compounds showed moderate to good antimalarial activity. None of these compounds showed any cytotoxicity to mammalian kidney fibroblast (Vero cells). In case of CQ-resistant strain, compounds have also shown significant activity (IC_{50} =<10 μM). It is interesting to note that all the compounds found to be more active than the standard drug pyrimethamine against chloroquine-resistant (W2) strain. Compound **14** has shown potent activity against both the strains.

The activity profile of these compounds against CQ-sensitive strain of *P. falciparum* clearly indicates that the compounds having *N*-methyl or *N*-ethyl groups at pyrimidine nucleus were found to be more active than the compounds having a morpholine ring (**15** *vs* **18** and **21**, **16** *vs* **19**, **22**). Similarly compounds having the diethyl amino group at triazine nucleus (**24-31**) were found to be more active than the respective compounds having a morpholine ring at the triazine nucleus (**15-22**) with the exception of compounds **14** and **23** where compound **14** was more active than the compound **23**. Compounds (**27**, **28**, **30** and **31**) having diethyl group at triazine nucleus and *N*-methyl or *N*-ethyl groups at pyrimidine nucleus were more potent than other compounds. Although, in the case of CQ-resistant strain, no uniform pattern was observed, yet compounds with long carbon chain between triazine and pyrimidine ring showed good activity than the compounds having short carbon chain. Amongst all, compound **14** was found to be the most potent compound with IC_{50} value of 1.18 μM against chloroquine-sensitive strain (D6) and 1.32 μM against chloroquine-resistant (W2) strain.

Table 1: *In-vitro* antimalarial activity and cytotoxicity of triazine-pyrimidine hybrids

Compd	*P. falciparum* (D6 Clone)		*P. falciparum* (W2 Clone)		Cytotoxicity (VERO cells)
	IC_{50} (μM)	SI	IC_{50} (μM)	SI	IC_{50} (μM)
14	1.18	>8.53	1.32	>7.62	>10.07
15	4.41	>2.21	7.48	>1.30	>9.78
16	6.21	>1.52	8.83	>1.07	>9.50
17	>9.80	1.00	>9.80	1.00	>9.80
18	3.88	>2.45	4.60	>2.06	>9.52
19	2.15	>4.30	2.62	>3.53	>9.26
20	4.54	>2.09	5.52	>1.72	>9.52
21	3.67	>2.52	5.86	>1.58	>9.26
22	4.32	>2.08	4.60	>1.96	>9.02
23	10.29	>1.03	>10.70	1.00	>10.70
24	3.05	>3.40	7.39	>1.40	>10.37
25	2.22	>4.53	9.98	>1.00	>10.07
26	3.46	>3.00	4.86	>2.13	>10.40
27	1.28	>7.88	3.07	>3.28	>10.09
28	1.54	>6.36	4.04	>2.42	>9.80
29	4.85	>2.08	7.96	>1.26	>10.09
30	1.57	>6.24	4.79	>2.04	>9.80
31	2.50	>3.80	4.70	>2.02	>9.52
CQ	0.04	>1500	0.39	>150	>60
Pyr	0.01	1820	*	-	18.2

IC_{50}: the concentration that causes 50% growth inhibition; SI: selectivity index (IC_{50} for cytotoxicity to Vero cells/IC_{50} for antimalarial activity, * not active upto 19 μM.

The molecular docking studies of best active compounds (**14**, **19**, **25**, **27**, **28**, **30** and **31**) were performed in the binding pocket of both the wild type Pf-DHFR-TS (PDB ID: 3QGT) and quadruple mutant Pf-DHFR-TS (N51I, C59R, S108 N, I164L, PDB ID: 3QG2) structures. The results of docking studies and the docked conformations of best scored ligands (**14** and **19**) in the active site of wild and mutant Pf-DHFR-TS are summarized in Table 2 and Figures 3 and 4. These docking results clearly indicate that the most active compounds in the study exhibited significant binding affinities towards the wild (Glide energy range -57.84 $kcalmol^{-1}$ to -14.52 $kcalmol^{-1}$) and quadruple mutant (Glide energy range -57.11 $kcalmol^{-1}$ to -23.65 $kcalmol^{-1}$) Pf-DHFR-TS structures and the energy ranges are comparable to the standard Pf-DHFR inhibitors (pyrimethamine, cycloguanil and WR99210) and the

native DHFR substrate, dihydrofolate (Table 2).

Table 2: Glide docking scores (kcal mol^{-1}) and docking energies of best active molecules along with the reference compounds (Pyrimethamine, Cycloguanil and WR99210) and dihydrofolate bound to wild and mutant PfDHFR-TS binding site

Compounds	Docking results with wild PfDHFR					Docking results with mutant PfDHFR				
	XP G Score	Van der Waals energy	Coulomb Energy	Glide Energy	XP H-bond	XP G Score	Van der Waals energy	Coulomb Energy	Glide Energy	
14	-6.25	-51.18	-6.65	-57.84	-1.99	-6.50	-41.17	-9.5	-57.11	
19	-4.71	-46.48	-6.08	-46.31	-1.04	-6.1	-29.81	-4.98	-39.09	
25	-2.13	-21.04	-1.98	-14.76	-0.12	-2.98	-23.51	-3.42	-28.03	
27	-3.04	-28.90	-3.26	-28.78	-0.23	-3.92	-28.08	-3.14	-37.65	
28	-3.22	-23.76	-2.32	-21.43	-0.26	-3.17	-23.74	-3.26	-29.73	
30	-2.75	-32.65	-2.09	-26.54	-0.17	-3.36	-29.54	-3.65	-32.54	
31	-1.98	-22.45	-0.97	-14.52	-0.18	-2.05	-21.62	-3.05	-23.65	
Dihydrofolate	-9.33	-52.14	-14.19	-64.84	-3.10	-11.00	-43.68	-17.61	-61.30	
Pyrimethamine	-9.04	-31.70	-15.51	-44.91	-2.64	-9.39	-33.65	-12.06	-43.55	
Cycloguanil	-8.94	-30.12	-10.74	-38.55	-2.86	-8.95	-34.30	-8.60	-46.6	
WR99210	-4.84	-51.18	-6.91	-37.03	-2.09	-5.48	-27.37	-8.07	-34.30	

Figures 3 and 4 illustrates the predicted binding poses for compounds **14** and **19** showing hydrogen bonding along with π-π interactions and the van der Waals interactions, with the expected binding pattern as observed for PfDHFR inhibitors and dihydrofolate in the wild type and mutant PfDHFR protein.[24] Compound **14**, showing lowest binding energy (-57.84 kcalmol^{-1}) and considerable high Glide XP score (-6.50 kcalmol^{-1}) for mutant PfDHFR, bind deep in the DHFR binding site forming hydrogen bond between linker NH group of **14** and carboxylate oxygen side chain of Asp54 in both wild type and mutant PfDHFR. The morpholine rings attached to triazine moiety of the compound **14** lie in the opposite end of the active site forming charge mediated hydrogen bond between one of the morpholine ring oxygen heteroatom and side chain nitrogen atom of Arg122 (Fig. 3B). Further, a π-π interaction between aromatic ring of Phe58 and triazine ring of compound was observed. Similar interaction pattern was observed for compound **14** (Glide energy: -57.84 kcalmol^{-1}) in the binding site of wild type PfDHFR (Figure 3A). Compound **19** predicted to have low binding energy and high glide score in wild type and mutant PfDHFR (Table 2), show H-bonding pattern between linker NH group of compound and carboxylate oxygen side chain of Asp54 and charge mediated H-bond between morpholine oxygen and Arg122 side chain (Fig. 4B).

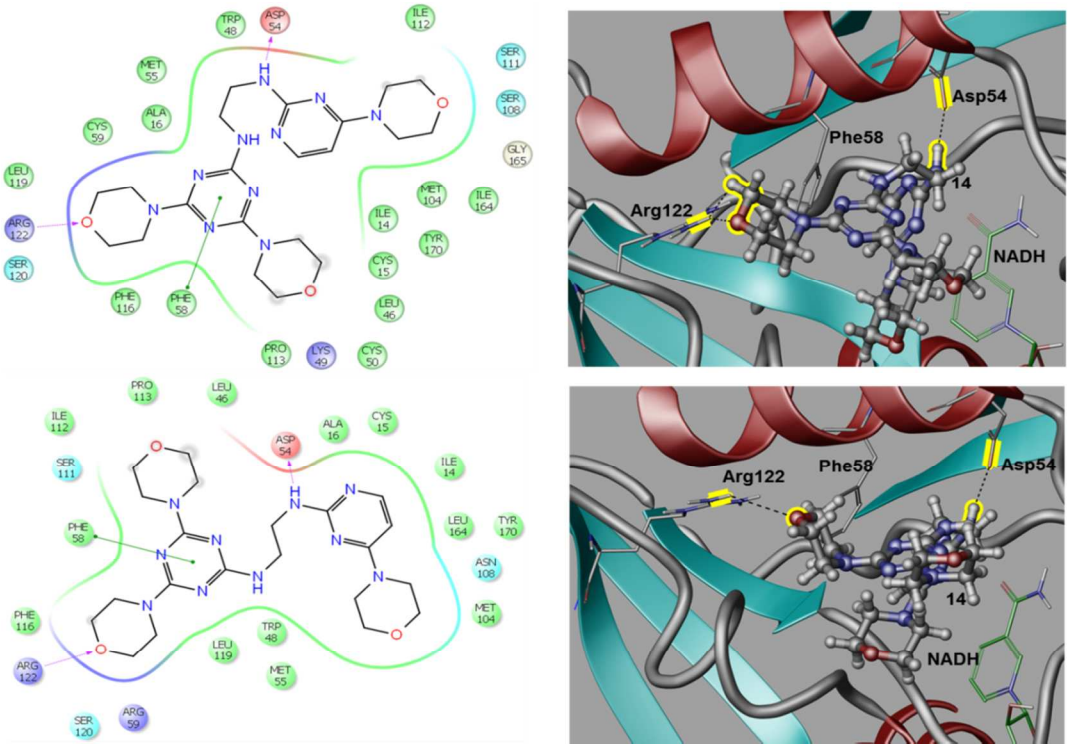


Figure 3: 2D and 3D docking pose showing interaction for compound 14 in the binding site of (A) wild (PDB ID:3QGT) and (B) mutant PfDHFR-TS (PDB ID:3QG2)

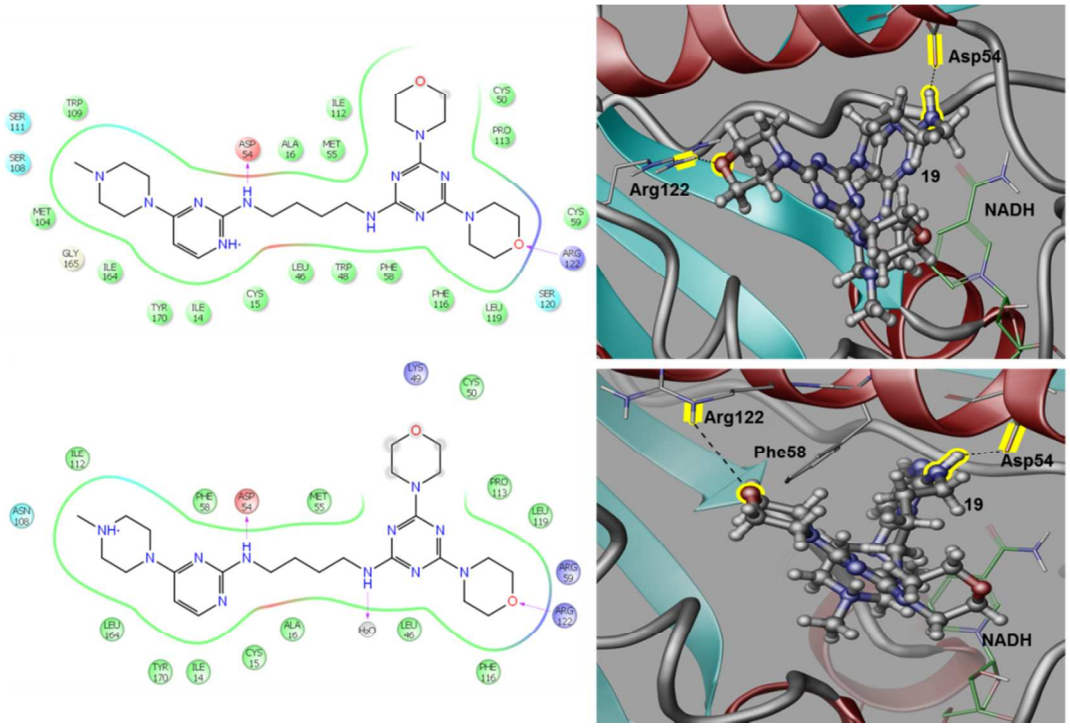


Figure 4: 2D and 3D docking pose showing interaction for compound 19 in the binding site of (A) wild (PDB ID:3QGT) and (B) mutant PfDHFR-TS (PDB ID:3QG2)

The influence of quadruple mutations (N51I, C59R, S108N, I164L) in DHFR is attributed to the movement in the active site residues and interferes in the inhibitor binding. The active site residue Asp54 forming H-bond with test compounds **14** and **19**, has been reported crucial for inhibitors and substrate (dihydrofolate) binding, lie in the proximity of residues 51 and 59. C59R mutation does not cause any significant changes in the protein structure and causes no close contacts with the inhibitors. N51I causes movement in main chain atoms of residues 48-51. Moreover, the function of the residue Asp54 is preserved in the mutant protein and not affected by the two proximal mutations N51I and C59R.[40] Further, I164L mutation causes shifts in the residues 164-167 and affects the active site gap, causing steric interactions of Phe58 with small inhibitors such as pyrimethamine and cycloguanil.[40] Also, the *p*-chloro-phenyl moiety in pyrimethamine and cycloguanil cause steric interference with the side chain of Asn108 in the active site modified by the first mutation S108N. In contrast, WR99210 endowed with long and flexible side chain could avoid such steric interactions forming effective binding with mutant protein. The test compounds **14** and **19** having flexible linker similar to WR99210, form π-π interaction Phe58, thus avoiding steric clash with the aromatic side chain of Phe58. Furthermore, the oxygen atom in the morpholine side chain linked to triazine nucleus of compounds **14** and **19** forms H-bond interaction with evolutionary conserved Arg122. Such charge mediated interaction of Arg122 is important and observed with α-carboxylate of DHFR substrate dihydrofolate. The binding of morpholine oxygen atom and side chain of Arg122 provides rigid docking site by restricting the mobility of flexible linker between the two rings in the designed inhibitors. Several observations have shown that drug molecules designed to occupy the surface volume of the native substrate of the protein will be less susceptible to resistance occurring due to steric clashes in the mutated protein binding site.[41,42]

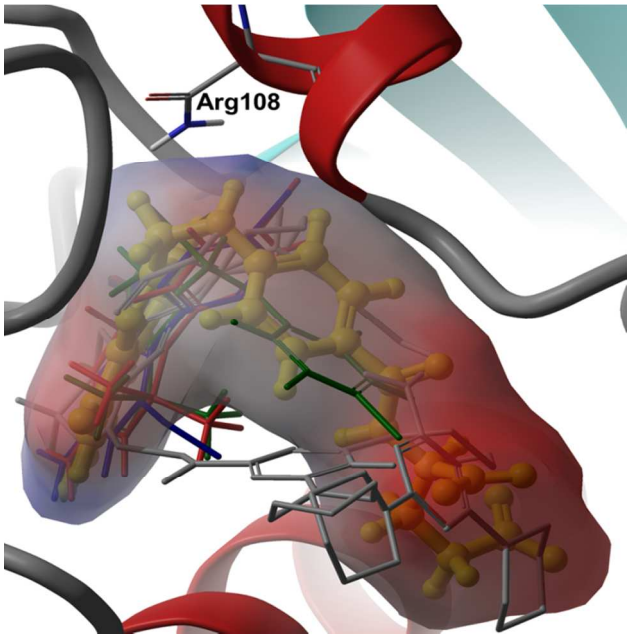


Figure 5: Superposition of most active docked test compounds (represented as grey sticks), Pyrimethamine (blue sticks), Cycloguanil (red sticks), WR99210 (green sticks) and the PfDHFR substrate dihydrofolate (yellow ball and stick) bound to the binding site of quadruple mutant (PDB ID: 3QG2) showing the fitting of the test compounds in the substrate surface.

Thus, it is desirable to explore the binding pattern of novel lead compound in the preliminary stages of drug design against mutant proteins. The molecular overlay of docking poses of active test compounds along with reference molecules on the dihydrofolate surface envelope clearly shows that the test compounds occupy the similar volume as that of the protein substrate unlike the dihydrofolates and avoid the steric clash with the side chain of Asn108 (Fig. 5). The results from the present study gives us important preliminary information to design novel compounds with similar scaffold that may lead to better active compounds which would be a scope for our future communication.

The result of ADMET predictions by Qikprop v3.5[43] is presented in Tables 3 and 4. Different pharmacokinetic parameters of the compounds which showed good inhibitory potential in malarial parasites were calculated. The most important of these parameters together with its permissible ranges are listed in the Tables 3 and 4.

Table 3: Prediction of Lipinski's 'Rule of 5' for the active test compounds[a]

Compd	mol_MW	Donor HB	Accpt HB	QPlogPo/w	Rule Of Five
14	472.55	2	12	2.127	1
19	513.64	2	12	2.56	2
25	472.63	2	9	4.532	1
27	471.65	2	9	4.358	1
28	485.68	2	9	4.361	1
30	485.68	2	9	4.728	1
31	499.70	2	9	4.667	1
Pyr	248.71	4	3	1.809	0
Cg	253.73	5	3	0.888	0

[a] All values calculated by QikPropv 3.5 and the explanations of the descriptors are given in the text, Pyr = Pyrimethamine, Cg = Cycloguanil

As a preliminary test of the drug-likeness of the compounds, we calculated Lipinski's rule of 5 using QikProp, requiring compounds to have no more than 5 and 10 hydrogen bond donors (donorHB) and acceptors (accptHB), respectively, molecular weights (mol_MW) less than 500 amu, and partition coefficients between octanol and water (QPlog P(oct/wat)) less than 5. Table 3 shows the Qikprop results for various parameters of Lipinski's rule of 5. An orally active compound should not have more than one violation of these rules. In the present study, all the active test compounds showed value for Lipinski's rule of 5 violations less than the maximum permissible value of 4, indicating that these active test compounds are endowed with drug likeness properties. All the compounds have shown Lipinski's rule of 5 of 1 except compound **19** showing 2 violations owing to mol_MW > 500 and accptHB > 10. Prediction of oral drug absorption (Percent Human Oral Absorption) was highly satisfactory for all the test compounds with the exception of compound **19** showing moderate value. Studies have suggested that oral bioavailability is influenced by compound's flexibility and can be measured by the number of rotable bonds (<15) and polar surface area (70 Å^2 –200 Å^2) and it has been emphasized that this approach should be considered with caution with respect to choice of descriptor algorithm used and also because other factors can have significant influence on bioavailability.[44] However, along with polar surface area criterion, a total sum of H-bond donors and acceptors criterion (≤12) can be used, which is algorithm independent.[50] In the present study, all the test compounds have a number of rotatable bonds <15 and polar surface area falls satisfactorily in the permissible range (Table 4). Similarly, molecules obeying Lipinski's rule of 5 could be viewed more likely to have good intestinal absorption or permeation which is confirmed by the predicted Caco-2 cells permeability (QPPCaco), used as a model for the gut–blood barrier.[45] QPPCaco predictions for all the test compounds showed very good values except for compound **19**, **27** and **31** having moderately good values for Caco-2 cells permeability which is comparable to the value predicted for the drug pyrimethamine. Further, QPlogKhsa, the prediction for human serum albumin binding and all inhibitors were predicted which lie within the expected range for 95% of known drugs (-1.5 to 1.5). Also, the QikProp descriptor for brain/blood partition coefficient (QPlogBB) and the blood-brain barrier mimic MDCK cell permeability (QPPMDCK) show satisfactory predictions for all the test compounds and the reference compounds. Further, aqueous solubility (QPlogS) parameter for the test compounds were assessed and all the compounds were predicted to have QPlogS values in permissible range. Furthermore, QPlogHERG descriptor for the prediction of IC_{50} value of HERG K^+ channel blockage was predicted for the test compounds. Compounds **14** and **25** have been predicted to possess values in permissible range comparable to reference compounds Pyrimethamine and Cycloguanil (Table 4).

Table 4: Calculated ADMET properties

Compound	[a]PercentHumanOralAbsorption (>80%-high,<25% poor)	[a]QPPCaco nms^{-1} (<25 poor, >500 great)	[a]QPlogBB (-3.0-1.2)	[a]QPPMDCK (<25 poor >500 great)	[a]QPlogKhsa (-1.5to1.5)	[a]QPlogHERG (concern below -5)	QPlogS (-6.5-0.5)	[a]PSA (7.0 – 200.0)	[a]#rotor (0 – 15)
14	85.123	1899.318	-0.416	989.649	-0.283	-4.251	-3.589	103.834	5
19	61.96	368.938	-0.32	186.276	0.007	-5.674	-3.557	102.564	7
25	100	2146.387	-0.909	1129.502	0.382	-4.903	-4.887	83.75	13
27	87.557	484.098	-0.598	249.85	0.554	-6.464	-5.163	80.387	12
28	88.046	514.064	-0.568	266.608	0.52	-5.977	-4.404	81.384	13
30	90.327	523.024	-0.635	271.635	0.653	-6.588	-5.496	79.854	13
31	89.246	476.685	-0.78	245.718	0.619	-5.988	-4.628	81.492	14
Pyrimethamine	84.346	412.287	-0.78	468.849	-0.243	-4.318	-2.978	73.731	4
Cycloguanil	68.814	111.854	-0.17	126.604	-0.306	-4.578	-1.596	76.262	2

[a] Calculated using QikProp v 3.5. Range/recommended values calculated for 95% known drugs.

Conclusions

In summary, we report synthesis, docking studies and antimalarial activity evaluation of triazine-pyrimidine molecular hybrids. The *in vitro* evaluation of these hybrids against D6 and

W2 strains of *P. falciparum* depicted activity in the micromolar range with no cytotoxicity against VERO mammalian cell lines. The active molecules were docked in the active site of wild type and quadruple mutant PfDHFR-TS protein to study the binding pattern of test molecules with DHFR. Compounds **14** and **19** were found to show good binding with wild type and mutant DHFR proteins with interaction pattern comparable to that of DHFR inhibitors and native DHFR substrate. Moreover, the test compounds exhibited efficient binding with the mutant protein avoiding steric clashes resulting from the amino acid mutations. The calculated ADMET parameters for the test compounds validated good pharmacokinetic properties for compound **14**, making it as an important candidate in the antimalarial drug discovery process.

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