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DESIGN AND SYNTHESIS OF TRIAZINE DERIVATIVES AS ANTIMALARIAL AGENTS

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ABSTRACT

Malaria imposes great socio-economic burden on humanity, and with six other diseases (diarrhoea, HIV/AIDS, tuberculosis, measles, hepatitis B, and pneumonia), accounts for 85% of global infectious disease burden. Drug resistance in malarial parasite has become one of the most important problems in the disease control in recent years. Resistance has been reported in almost all the antimalarial drugs, including artemisinin. Considering this aspect, development of new drugs is of primary importance. The computer aided drug designing (CADD) is an advanced, novel and convenient technique. With the help of CADD, one can design drug molecules and also predict the drug-receptor binding. In the current study, a series of triazine derivatives were designed as inhibitors of wild *Pf*DHFR and quadruple mutant *Pf*DHFR enzyme. Compounds with good G-score were synthesized on the laboratory scale. The compounds would be evaluated for antimalarial activity, *in vivo* against *Plasmodium berghei* and *in vitro* against chloroquine resistant W2 strain of *Plasmodium falciparum*.

KEYWORDS: Malaria, drug resistance, computer aided drug designing, *Plasmodium falciparum*, triazines.

INTRODUCTION

Malaria imposes great socio-economic burden on humanity, and with six other diseases (diarrhea, HIV/AIDS, tuberculosis, measles, hepatitis B, and pneumonia), accounts for 85 % of global infectious disease burden.

According to the latest WHO estimates, at the beginning of 2016, malaria was considered to be endemic in 91 countries and territories, down from 108 in 2000. There were 212 million cases of malaria in 2015 and 429 000 deaths.[1] According to the World Malaria Report 2014, 22% (275.5 million) of India's population live in high transmission (> 1 case per 1000 population) areas, 67% (838.9 million) live in low transmission (0–1 cases per 1000 population) areas and 11% (137.7 million) live in malaria-free (0 cases) areas.[2] In 2013, 0.88 million cases have been recorded, with 128 million tests being conducted on the suspected cases, with *P. falciparum* causing 53% and *P. vivax* causing 47% of the infections. The incidence of malaria in India accounted for 58% of cases in the South East Asia Region of WHO.[2]

The principal causative organism of malaria is one-celled obligate intra-erythrocytic protozoan of the genus Plasmodium. Human malaria is caused by four different species of Plasmodium: *Plasmodium falciparum, Plasmodium malariae, Plasmodium ovale* and *Plasmodium vivax*. *P. falciparum* is more prevalent than other species. In recent years, sporadic cases of travellers' malaria due to *P. knowlesi* have been reported.[3] Young children, pregnant women, people who are immunosuppressed and elderly travellers are particularly at risk of severe disease. Malaria, particularly *P. falciparum*, in non-immune pregnant travellers increases the risk of maternal death, miscarriage, stillbirth and neonatal death.[3]

There are only a limited number of drugs which can be used to treat or prevent malaria. The most widely used are quinine and its derivatives and antifolate combination drugs.[4]

Major hurdle in the treatment of malaria is the drug resistance in malarial parasites. Resistance has been reported for almost all the antimalarial drugs. Resistance to artemisinin, the core compound in WHO recommended combination treatments (ACT) for uncomplicated resistant malaria, has been detected in five countries of south-east Asia: Cambodia, Laos, Myanmar, Thailand and Vietnam.[2] The emergence of resistance provides physicians with a real challenge to successfully treat the malarial infection. In view of the wide spread of resistant strains and absence of any effective vaccine, there is an urgent need for the development of newer antimalarial agents, which will be effective against resistant strains of malaria.

Dihydrofolate reductase (DHFR)

Dihydrofolate reductase is an enzyme that reduces dihydrofolic acid to tetrahydrofolic acid, using NADPH as an electron donor. It has a critical role in regulating the amount of tetrahydrofolate in the cell. Tetrahydrofolate and its derivatives are essential for purine and thymidylate synthesis, which are important for cell proliferation and cell growth. Thus, DHFR plays a central role in the *de novo* synthesis of purines, thymidylic acid and certain amino acids.

Malarial DHFR is significantly divergent from the human version of the enzyme, differing both in sequence as well as structure by having a second enzyme thymidylate synthase, conjoined to it. The sequences of the human and malarial DHFR enzymes align at 33% of the residues and are only 20% similar. However, both enzymes still perform the same reaction using identical substrates. The divergent sequence and regulation of the two enzymes create opportunities for drug selectivity.[5]

The inhibition of DHFR enzyme prevents dTMP production, thereby halting the process of DNA synthesis, cell division and reproduction, which ultimately leads to parasitic cellular death. The schematic representation of mechanism of action of DHFR inhibitors is shown in Figure 1.

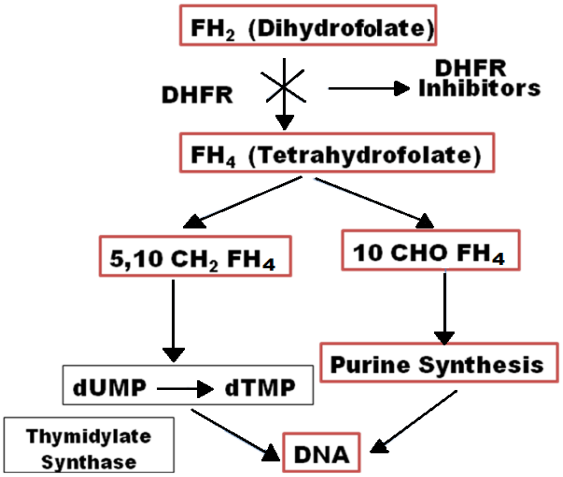


Figure 1: Mechanism of action of DHFR inhibitors.

It is the target for antifolate antimalarial drugs like pyrimethamine and proguanil. Efficiency of DHFR inhibitors as antimalarials has been decreased significantly due to the resistance caused by quadruple mutations (4 point mutation) in the enzyme. This quadruple mutation prevents drug binding to the enzyme and thus retains enzyme activity. The quadruple mutant of *Pf*DHFR has four point mutations within the active site: N51I, C59R, S108N, and I164L.[6] These mutations cause preferential decrease in binding affinities of inhibitors, mainly increasing the steric hindrance, while biological substrates are less affected.

A recent report[7] on the crystal structure of *Pf*DHFR-TS from wild type and resistant quadruple mutants in a complex with pyrimethamine and the antifolate WR99210, revealed features that could be exploited to overcome drug resistance. Hence, quadruple mutant DHFR enzyme could be targeted to invent new inhibitors.

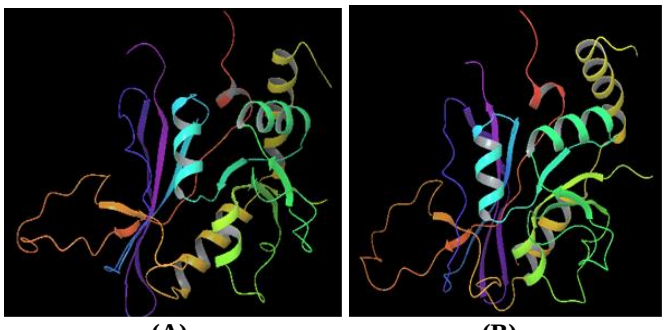
MATERIALS AND METHODS

***In silico* molecular modelling**

Software used for the docking study was *Maestro 11*, from Schrödinger, USA. Hundreds of triazine derivatives were built *in silico.* These built structures were further converted to their low energy 3D conformers. Two protein structures of *Pf*DHFR enzyme, wild type and mutant type, were downloaded from the Research Collaboratory for Structural Bioinformatics (RCSB), Protein Data Bank (PDB). They are:

- **1J3I:** Wild-type *Pf* DHFR-TS complexed with WR99210, NADPH, and dUMP
- **1J3K:** Quadruple mutant (N51I+C59R+S108N+I164L) *Pf* DHFR-TS complexed with WR99210, NADPH, and dUMP

These enzyme structures were pre-processed by using ***Protein Preparation Wizard.*** Various parameters like assign bond orders, add hydrogens, treat metal, find overlapping of amino acid chains, delete water molecules and orientation of amino acids were selected for pre-processing of protein. The energy minimization of the enzyme was done using OPLS-2005 force field to get the refined and optimized structure of the protein. **Figures 2 (A) and (B)** depict the final optimized ribbon structures of DHFR enzymes, 1J3I and 1J3K, respectively.



(A) **(B)**

Figure 2: Refined and optimized structures of DHFR enzymes: (A) 1J3I (B) 1J3K.

Program ***Receptor Grid Generation*** was used for generation of grid. The proteins with PDB ID 1J3I and 1J3K have co-crystallised reference inhibitor, WR99210, placed in the active pocket of the protein. Grid box was generated around this co-crystallised inhibitor as shown in Figure 3:

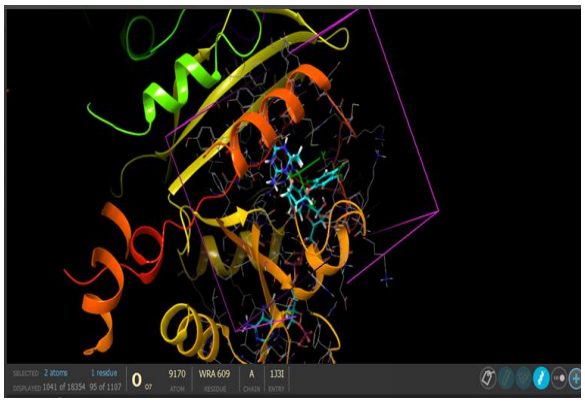


Figure 3: Generation of grid box around co-crystallised inhibitor WR99210.

The docking protocol was validated by super positioning of de-docked and re-docked enzymes.

A virtual library of more than hundred triazine derivatives was designed *in silico* using the program ***Lig Prep*** and docked into active site of both the enzymes, using the program ***Glide***. Based on the input from protein- ligand interaction, 10 compounds were selected for synthesis in the laboratory. Criteria for selection were G-score of the compounds and contacts formed by the compounds with the enzyme.

Synthesis

Compounds showing G-scores closer to the G score of inhibitor were successfully synthesized in the laboratory using inexpensive reagents and with good yields (Table 1) as per the scheme shown in Figure 4. All the chemicals and reagents used in current study were of Analytical (AR) and Laboratory (LR) grades. All synthesized compounds were characterized by melting point and R_f values from TLC. The structures were confirmed by recording their FTIR, NMR or mass spectra. All the reactions were monitored using aluminium-supported precoated thin layer chromatography (TLC), silica gel 60G F254 plates (Mercks), with a hexane: ethylacetate solvent system. The spots were visualized using an ultraviolet (UV) lamp. Melting points of the compounds were determined by using programmable melting point apparatus and are uncorrected. The NMR spectra were recorded from Punjab University, Chandigarh, India, on Bruker Avance II 400 NMR spectrometer using appropriate deuterated solvents. The chemical shifts are expressed in δ ppm and splitting patterns are designated as s: singlet; d: doublet; q: quartet and m: multiplet. The FTIR spectra were recorded on a JASCO FT/IR-4100 spectrometer, from H. K. College of Pharmacy, Mumbai, India. The mass spectra were recorded on Waters-Q-TOF Micromass (ESI-MS) spectrometer, from Punjab University, Chandigarh, India.

Synthetic procedures

General procedures for synthesis of compounds C1-C10

Synthesis of diamino derivatives of triazine[8]: A solution of a desired amine (0.002 mol) and sodium acetate (0.0025 mol) was prepared in a mixture of glacial acetic acid (AcOH) and water and then added slowly into the solution of cyanuric chloride (0.001 mol) in AcOH. Resulting solution was stirred for around 4 h. Precipitate obtained at the end of the reaction was filtered and sufficient washings with water were given to make it free of AcOH.

Synthesis of triamino derivatives of triazine (symmetrical and unsymmetrical)

Symmetrical triamino derivatives of triazine[8]: Cyanuric chloride (0.001 mol) was added in one portion to a solution of an amine (0.003 mol) in AcOH. The mixture was refluxed for around 4 h with stirring. Precipitate obtained was processed in the same way as that in diamino derivative of triazine.

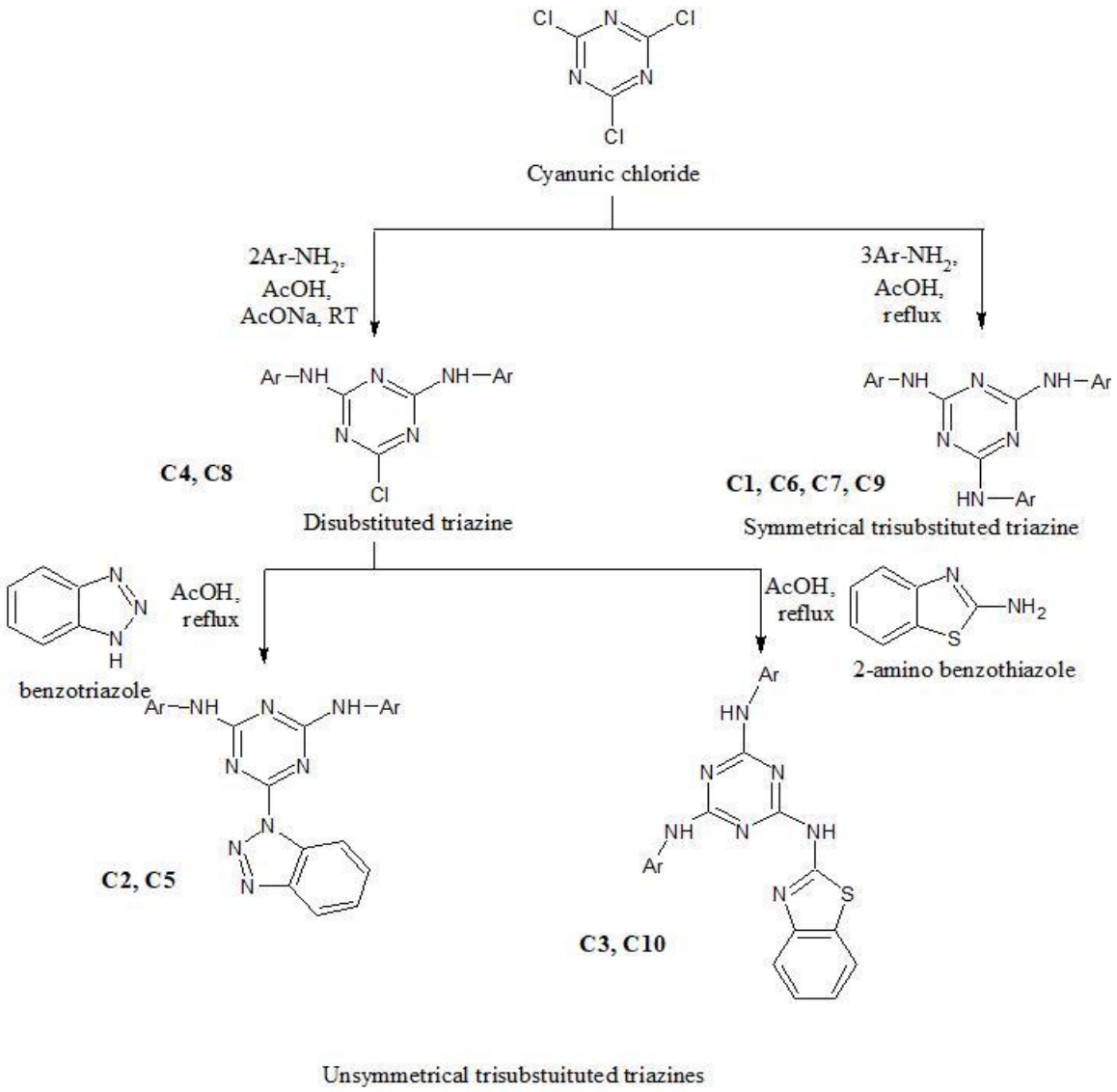


Figure 4: Scheme for synthesis.

Unsymmetrical triamino derivatives of triazine: Symmetrical diamino triazine (0.001mol) was added in one portion to a solution of an amine (0.001mol), other than the one used for synthesis of symmetrical diamino derivatve of triazine, in AcOH. Rest of the procedure was same as that given for the synthesis of symmetrical triamino derivative of triazine.

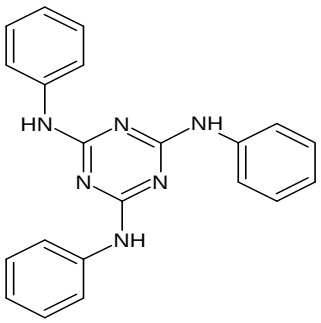
The solid products obtained in all the reactions were dried and purified by recrystallization using suitable solvents.

Table 1: G score and physical properties of the synthesised compounds.

Compound	G score 1J3I	G score 1J3K	Molecular weight	Melting point (^{O}C)	% Yield
C1	-7.215	-6.227	354.30	232-234	82
C2	-7.897	-6.936	380.13	>300	72
C3	-7.166	-7.824	411.37	>300	64
C4	-7.512	-6.456	366.61	162-166	85
C5	-7.451	-7.500	449.24	288-292	79
C6	-6.883	-7.349	457.71	>300	92
C7	-7.414	-6.360	444.39	125-127	68
C8	-7.129	-6.980	357.73	199-201	82
C9	-7.078	-6.824	411.24	269-271	93
C10	-7.117	-8.121	447.35	>300	68
Inhibitor WR99210	-8.075	-8.140			

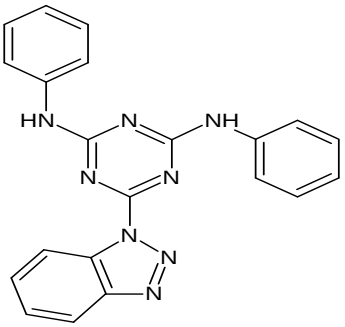
Structure and spectral details of the synthesised compounds

C1: *N*, *N*', *N*''-triphenyl-1, 3, 5-triazine-2, 4,6-triamine



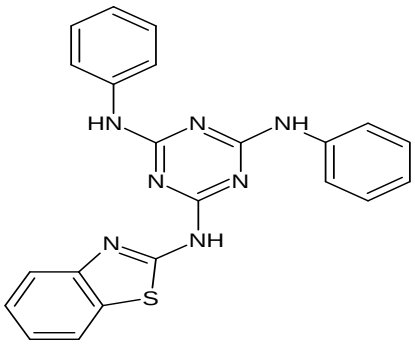
FTIR (KBr cm^{-1}); 3391 (N-H), 3029 (C-H), 1654 (C=C), 1599 (N-H bend), 1432 (C-C), 1254 (C-N), 790 (C-S). ESI-MS (M+1, *m/z*): 355.28.

C2: 6-(1*H*-benzotriazol-1-yl)-*N*,*N*'-diphenyl-1,3,5-triazine-2,4-diamine



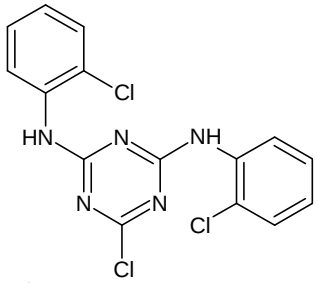
FTIR (KBr, cm^{-1}); 3459 (N-H), 3019 (C-H), 1668 (C=C), 1621 (N-H bend), 1443 (C-C), 1315 (C-N). ESI-MS (M+1, *m/z*): 381.45.

C3: N^2-(1, 3-benzothiazol-2-yl)-N^4, N^6-diphenyl-1,3,5-triazine-2,4,6-triamine



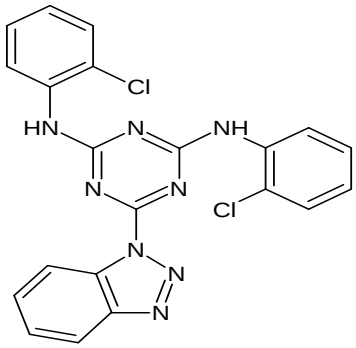
FTIR (KBr, cm^{-1}); 3410 (N-H), 3025 (C-H), 1689 (C=C), 1621 (N-H bend), 1486 (C-C), 1254 (C-N), 790 (C-S). ESI-MS (M+1, *m/z*): 412.40.

C4: 6-chloro-*N*,*N*'-bis(2-chlorophenyl)-1,3,5-triazine-2,4-diamine



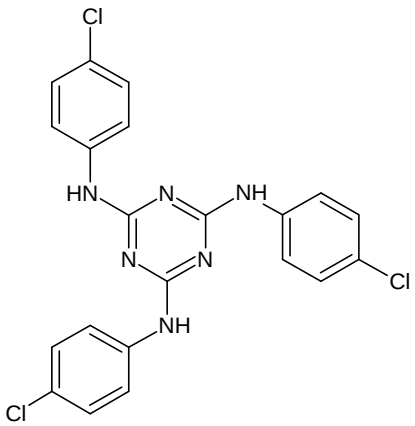
FTIR (KBr, cm^{-1}); 3328 (N-H), 3016 (C-H), 1635 (C=C), 1615 (N-H bend), 1429 (C-C), 1284 (C-N), 560 (C-Cl). ^{13}C NMR (DMSO): δ 126.97, 127.28, 128.62, 129.48, 134.50, 135.44 (C_{Ar}), 164.9 (C-Cl), 168.83 (C_{Ar}-Cl).

C5: 6-(1*H*-benzotriazol-1-yl)-*N*,*N*'-bis(2-chlorophenyl)-1,3,5-triazine-2,4-diamine



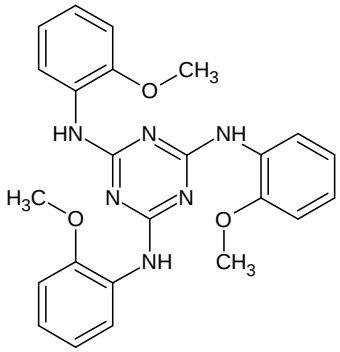
FTIR (KBr, cm^{-1}); 3385 (N-H), 3091 (C-H), 1689 (C=C), 1620 (N-H bend), 1441 (C-C), 1291 (C-N), 799 (C-Cl). ESI-MS (M^+, *m/z*): 449.34.

C6: *N*,*N*',*N*''-tris(4-chlorophenyl)-1,3,5-triazine-2,4,6-triamine



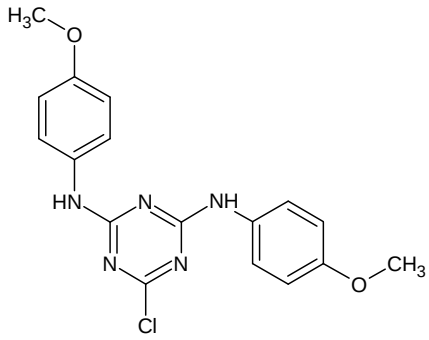
FTIR (KBr, cm^{-1}); 3391 (N-H), 3016 (C-H), 1661 (C=C), 1588 (N-H bend), 1497 (C-C), 1264 (C-N), 690 (C-Cl). ESI-MS (M^+, *m/z*): 457.22.

C7: *N*, *N*', *N*''-tris(2-methoxyphenyl)-1,3,5-triazine-2,4,6-triamine



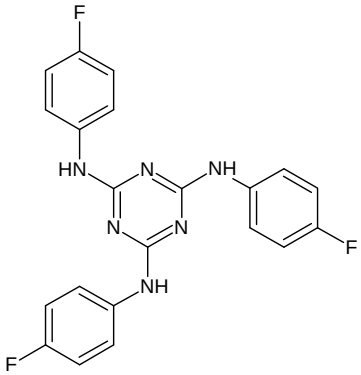
FTIR (KBr, cm^{-1}); 3384 (N-H), 3024 (C-H), 1677 (C=C), 1594 (N-H bend), 1424 (C-C), 1291 (C-N), 1029 (C-O), 1457 (C-H bend), 2935 (C-H), ESI-MS (M+1, *m/z*): 445.41.

C8: 6-chloro-*N*, *N*'-bis(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine



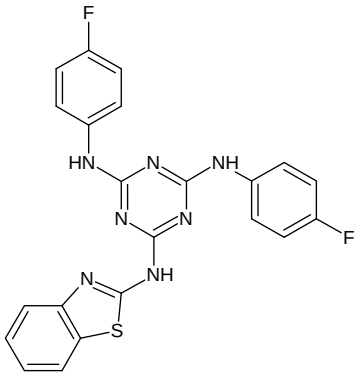
FTIR (KBr, cm^{-1}); 3280 (N-H), 3087 (C-H), 1675 (C=C), 1623 (N-H bend), 1469 (C-C), 1298 (C-N), 1191 (C-O), 1465 (C-H bend), 2914 (C-H). ^{13}C NMR (DMSO): δ 55.15 (OCH_3), 113.72, 122.65, 122.86, 131.18 (C_{Ar}), 155.58 (C_{Ar}-OCH_3), 163.70(C-Cl).

C9: *N*, *N*', *N*''-tris(4-fluorophenyl)-1,3,5-triazine-2,4,6-triamine



FTIR (KBr, cm^{-1}); 3446 (N-H), 3025 (C-H), 1652 (C=C), 1584 (N-H bend), 1459 (C-C), 1260 (C-N), 1070 (C-F). ESI-MS (M^+, *m/z*): 411.54.

C10: N^2-(1, 3-benzothiazol-2-yl)-N^4, N^6-bis(4-fluorophenyl)-1,3,5-triazine-2,4,6-triamine



FTIR (KBr, cm^{-1}); 3322 (N-H), 3191 (C-H), 1670 (C=C), 1644 (N-H bend), 1414 (C-C), 1294 (C-N), 1158 (C-F), 801 (C-S). ESI-MS (M^+, *m/z*): 447.55.

RESULTS AND DISCUSSION

The docking protocol was validated by super-imposing de-docked and re-docked inhibitor. The RMSD values for both wild and mutant variety was well within the limits (<2). The values of the G scores for the inhibitor bound to 1J3I and 1J3K were compared with those reported in the literature. The scores are shown in Table 2:

Table 2: Validation of docking protocol

	1J3I (Wild)	1J3K (Quadruple mutant)
G score (Literature)[9]	-8.707	-8.708
G score (Current study)	-8.075	-8.140
RMSD	1.2724	0.6589

Figure 5 shows the interaction between the compounds C6 and various amino acids present in the enzyme 1J3I.

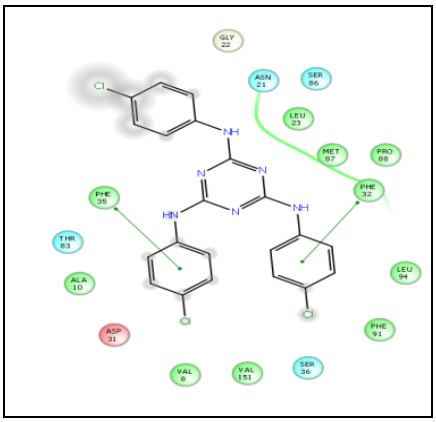


Figure 5: Binding mode of compound C6 with 1J3I and its interaction with amino acids.

Ten compounds showing docking scores closer to that of the inhibitor were shortlisted for synthesis. Various solvents were tried for carrying out the reaction and the

solvent that was finalised was acetic acid. Reactions using acetic acid as a solvent were fast and gave good yields. Acetic acid being cheaper and non-organic ('green solvent') gave added advantage of reaction being economical and eco-friendly.

CONCLUSION

Drug discovery is a challenging process due to complexity of biological systems. Synthesis of compounds with selective action on specific enzymes can be used to get good therapeutic activity with fewer side effects. Triazine derivatives with various amino substitutions were subjected to *in silico* molecular modelling, as falcipain DHFR inhibitors. The binding mode of these compounds with both wild and mutant *Pf*DHFR enzymes was studied using *Glide* module of the molecular modelling software *Maestro 11* from Schrödinger, USA. Based on the docking results, 10 compounds were selected for synthesis. They were synthesized by using inexpensive reagents and simple reaction conditions. Products were obtained in good yields. These products will be further taken up for *in vivo* antimalarial studies in animal models and *in vitro* enzyme inhibition studies. Correlation of results obtained in docking studies and antimalarial activity will be established in order to design and synthesize more potent antimalarials.

REFERENCES

1. World Malaria Report 2016. Available at http://apps.who.int/iris/bitstream/10665/252038/1/9789241511711-eng.pdf.
2. World Malaria Report 2014. Available at http://apps.who.int/iris/bitstream/10665/144852/2/9789241564830_eng.pdf.
3. Malaria - World Health Organization. Available at www.who.int/ith/diseases/malaria/en/.
4. Bloland PB, Drug resistance in malaria. Available at www.who.int/csr/resources/publications/drugresist/malaria.pdf.
5. Zhang K and Rathod PK, Divergent regulation of dihydrofolate reductase between malaria parasite and human host. Science, 2002; 296(5567): 545-547.
6. Sirawaraporn W, Sathitkul T, Sirawaraporn R, Yuthavong Y, Santi D, Antifolate-resistant mutants of *Plasmodium falciparum* dihydrofolate reductase. Proc. Natl. Acad. Sci. USA, 1997; 94(4): 1124-1129.
7. Huang F, Tang L, Yang H, Zhou S, Liu H, Li J, Guo S, Molecular epidemiology of drug resistance markers of *Plasmodium falciparum* in Yunnan Province China. Malaria Journal, 2012; 11: 243.
8. Kolmakov KA, An efficient, "green" approach to aryl amination of cyanuric chloride using acetic acid as solvent. J. Heterocyclic Chem., 2008; 45: 533-539.
9. Adane L, Bhagat S, Arfeen M, Bhatia S, Sirawaraporn R, Sirawaraporn W, Chakraborti AK , Bharatam PV, Design and synthesis of guanylthiourea derivatives as potential inhibitors of *Plasmodium falciparum* dihydrofolate reductase enzyme. BioOrg & Med. Chem. Letters, 2014; 24: 613-617.