Design, synthesis and biological evaluation of antimalarial activity of new derivatives of 2,4,6-s-triazine

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Abstract
Dihydrofolate reductase (DHFR) is an important enzyme for de novo synthesis of nucleotides in Plasmodium falciparum and it is essential for cell proliferation. DHFR is a well known antimalarial target for drugs like cycloguanil and pyrimethamine which target its inhibition for their pharmacological actions. However, the clinical efficacies of these antimalarial drugs have been compromising due to multiple mutations occurring in DHFR that lead to drug resistance. In this background, we have designed 22 s-triazine compounds using the best five parameters based 3D-QSAR model built by using genetic function approximation. In-silico designed compounds were further filtered to 6 compounds based upon their ADME properties, docking studies and predicted minimum inhibitory concentrations (MIC). Out of 6 compounds, 3 compounds were synthesized in good yield over 95% and characterized using IR, 1H NMR, 13C NMR and mass spectroscopic techniques. Parasitemia inhibition assay was used to evaluate the antimalarial activity of s-triazine compounds against 3D7 strain of P. falciparum. All the three compounds (7, 13 and 18) showed 30 times higher potency than cycloguanil (standard drug). It was observed that compound 18 was the most active while the compound 13 was the least active. On the closer inspection of physicochemical properties and SAR, it was observed that the presence of electron donating groups, number of hydrogen bond formation, lipophilicity of ligands and coulson charge of nitrogen atom present in the triazine ring enhances the DHFR inhibition significantly. This study will contribute to further endeavours of more potent DHFR inhibitors.

Keywords: Antimalarial, DHFR inhibitors, Molecular docking, s-Triazine, 3D7 strain

Introduction
Malaria is a protozoan disease caused by Plasmodium genus. According to WHO report entitled “World malaria report” (2015), 15 countries reported 80% of cases and 78% of deaths due to malaria in 2015 [1]. Malaria persists to be one of the critical public health problems in India. Around 1.13 million confirmed cases and 287 deaths were reported in 2015 by the National Vector Borne Disease Control Programme (NVBDCP), out of which 67.1% was due to Plasmodium falciparum [2]. Odisha, Jharkhand, Chhattisgarh, Madhya Pradesh, Karnataka and north-eastern states except Sikkim, Maharashtra and Rajasthan are high endemic areas in India.

Antifolate antimalarial drugs such as pyrimethamine and cycloguanil have been used in prevention and treatment of malaria. It is well known that folate metabolism is one of the most studied biochemical pathways of the parasite. Folate metabolism is a critical process being targeted to stop the proliferation of the parasite. The antimalarial activity of therapeutic agents that interfere with folate metabolism has been recognized since long. Two categories of antifolate antimalarial drugs were distinguished by their respective mechanisms of action. In the first category, the sulphamides and sulphones are chemical analogues of p-amino benzoic acid (PABA), an essential precursor for the de novo synthesis of folic acid. The second category includes a variety of drugs that inhibit dihydrofolate reductase (DHFR), the enzyme...
responsible for converting dihydrofolate to the biologically active tetrahydrofolate cofactor [3].

During the earlier trials of chloroguanide as an antimalarial drug on monkeys, rabbits and humans, triazine compounds were identified and isolated. Among these compounds, 2-amino-4-(p-chloro-anilino)-6,6-dimethyl-5,6-dihydro-1,3,5-triazine and p-chlorophenylbiguanide were isolated from the urine of monkeys treated with chloroguanide [4]. But both the compounds were found to be inactive against Plasmodium falciparum. However, an isomer of 2-amino-4-(p-chloro-anilino)-6,6-dimethyl-5,6-dihydro-1,3,5-triazine, 2,4-diamino-5-(p-chlorophenyl)-6,6-dimethyl-5,6-dihydro-1,3,5-triazine, isolated from the urine of rabbits [5] and humans [6] were found to be highly active. A large number of dihydrotriazines have been synthesized and many of them showed antimalarial activity [7, 8]. Further, several works have been reported to study the correlation between the structure and the antimalarial activity of triazine compounds. Based on these relationships, several triazine compounds have been synthesized and biologically evaluated for biochemical targets such as polyamine metabolism [9] and DHFR inhibition [10, 11, 12].

The synthesis of s-triazines and their pharmacological applications are well documented [13, 14, 15, 16]. Some s-triazine derivatives are reported to possess remarkable antitubercular [17], antimicrobial [18], antibacterial [19] and herbicidal activities [20]. Besides it, s-triazine compounds were found to be active as antitumorogenic agents, in chemotherapeutical preparations, active against viruses, protozoa, helminths, pharmacologically effective to treat cardiovascular, neuropsychotic disorders, or inflammatory processes, diuretics, antidepressive agents, etc. [21].

According to the literature [22, 23] s-triazine compounds fall into the second category that inhibits Plasmodium falciparum-DHFR. DHFR has received considerable attention as it is the target of cycloguanil (a triazine based antimalarial drug) and other antifolates. DHFR is used for prophylaxis and the treatment of Plasmodium falciparum infection [24]. The exponential increase in the emergence of antifolate resistance in Plasmodium falciparum has unfortunately compromised the clinical use of the currently used drugs and therefore highlights the urgent need for new effective antifolate antimalarials [25, 26].

During the last two decades, there has been tremendous progress in computational chemistry and Computer Aided Drug Design (CADD). CADD has played a major role in screening of new chemical entities. Under ligand based lead compounds optimization, QSAR study of the bioactive compounds plays a useful role for screening of new potential lead compounds. Therefore, the design of novel chemical entities which can affect selectively the parasite folate metabolism, may lead to discovery of better antimalarial drugs. In our previous reported study we had prepared and discussed 3D QSAR models using Genetic Function Approximation (GFA) method by employing data set of minimum inhibitory concentration (MIC) values of synthetic s-triazine compounds tested for DHFR inhibition against cycloguanil resistant strain of Plasmodium falciparum [27]. Using QSAR model no 1 (best model), a number of s-triazine compounds were designed by modifying the attached side chains to the carbon atoms of the triazine ring of the parent compound (Table 1).

Under the present study new s-triazine compounds were designed and their MIC values were predicted using same QSAR model. The designed compounds were also evaluated for ADME properties and docking score. Based upon these parameters 6 s-triazine compounds were selected for synthesis. Out of 6 compounds, 3 compounds were synthesized with yield percentage above 95%. The compounds were characterized using elemental analysis, IR, mass, 1HNMR and 13CNMR experimental techniques. The synthesized compounds were tested against the 3D7 strain of Plasmodium falciparum Rieckmann microassay [12, 16]. It was observed that all synthesized compounds possessed 30 times higher activity than the standard cycloguanil antimalarial drug.

**Materials and methods**

**Chemicals and techniques**

All chemicals used in the present study are of analytical grade purchased from Sigma Aldrich and Merck Chemical Company. All the solvents were used after distillation. All the synthesized compounds have been characterized from their analytical, physical and spectral (IR, 1HNMR, 13C-NMR) data. Infrared spectra (IR) spectra were recorded in KBr discs on an FT-IR Perkin-Elmer spectrum BX spectrophotometer. ESI–MS spectra were obtained using a VG Biotech Quattro mass spectrometer equipped with an electrospray ionization source in the mass range of m/z 100 to m/z 1000. 1H-NMR and 13C-NMR spectra were recorded on a Bruker NMR instrument 400 MHz and 100 MHz, respectively using CDCl3 and DMSO-d6 as solvents. Elemental analysis was performed on the elemental analyzer Gmbh variable system. All compounds gave satisfactory analytical results.

**ADME screening**

QikProp program from Schrödinger Mastero 9.7 [28] was employed to assess the absorption, distribution, metabolism, and excretion (ADME) properties of the compounds. QikProp predicts both pharmaceutically significant descriptors and physically relevant properties.
Table 1  Structural features of the designed inhibitors and predicted pMIC values

| Designed inhibitor | R group | Predicted log1/MIC | Designed inhibitor | R group | Predicted pMIC |
|-------------------|---------|--------------------|-------------------|---------|----------------|
| 1                 | NH.OH   | −4.47              | 12                | N.C.H.C.H.CH3 | −3.46          |
| 2                 | NH.NH2  | −3.84              | 13                | N.H.OH     | −0.90          |
| 3                 | NH.NH2  | −3.19              | 14                | N.H.NH2    | −2.93          |
| 4                 | NH.C.H3 | −3.41              | 15                | N.H.NH.C.H3 | −1.85          |
| 5                 | NH.NH.C.H3 | −2.62      | 16                | N.H.NH.C.H3 | −2.29          |
| 6                 | NH.NH.C.H3 | −2.78      | 17                | N.H.NH.C.H3 | −3.23          |
| 7                 | NH.NH2 | 0.65               | 18                | N.H.CH3    | 0.44           |
| 8                 | NH.NH2 | 0.19               | 19                | N.H.CH3    | −2.2           |
| 9                 | NH.NH2 | −0.13              | 20                | N.H.OTerm  | −3.88          |
| 10                | N.H.C.NH2 | 0.58       | 21                | N.H.SOH    | −4.05          |
| 11                | N.C.H3 | −2.76              | 22                | N          | −6.8           |
The program was processed in the normal mode, and 44 properties were predicted for the 22 s-triazine compounds. These predicted properties consist of principal descriptors and physicochemical properties with a detailed analysis of the octanol/water partition coefficient (QlogP), octanol/gas partition coefficient (QlogPc), water/gas partition coefficient (QlogPw), partition coefficient in cubic A0 (QPolr), % human absorption in the intestines (QP%), brain/blood partition coefficient (QlogBB), IC50 value of HERG K+ blockage channels (logHERG), skin permeability (QlogKp), binding to human serum albumin (QLogKhsa), apparent Caco-2 cell permeability in mm/s (QPPCaco), and apparent MDCK cell permeability in mm/s (QPPMDCK). Caco-2 cell line is model for the gut-blood barrier, while MDCK cell line is considered a good model for the blood–brain barrier. Besides, QikProp evaluates the acceptability of the compounds based on Lipinski’s rule of five [29], which is essential for rational drug design. Low permeability and/or poor absorption for compounds results when a compound violates one or more than one Lipinski’s rule of five (i.e. more than 5 hydrogen donors, the molecular weight is over 500, the logP is over 5 and the sum of N’s and O’s is over 10).

**Chemistry**

**General procedure for the synthesis of compounds (7, 13 and 18)**

The 2,4,6-trisubstituted-1,3,5-triazine compounds were synthesized by refluxing 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) with different nucleophiles (R). The mono-substituted triazine (4,6-dichloro-1,3,5-triazine (cyanuric chloride) with different nucleophiles were synthesized by refluxing cyanuric chloride with nitrophenyl)-1,3,5-triazin-2-amine) was synthesized by (solid). Yield 98%. Mp: 180–182 °C; IR (KBr, υmax in cm⁻¹): 3409 (OH, str.); 1631 (C=O, str.); 1591, 1326 (NO₂, str.); 1379 (s, 1H, NH); 13C NMR (100 MHz, CDCl₃) δ ppm: 172.1, 155.3, 141.8, 135.7, 130.4, 128.5, 115.9, 114.3, 82, 14.2, 56.3, 43.7, 37.6. Anal. Calcd. for C₁₉H₁₉N₇O₄: C: 58.47; H: 3.97; N: 25.83; found: C: 58.53; H: 4.01; N: 22.64. Mass spectrum (ESI) (M + H)+ = 442.1

**Pharmacology**

**Plasmodium parasite culture**

Stock culture of malaria parasite *Plasmodium falciparum* 3D7 strain was continuously maintained in vitro using the candle-jar method [30]. The *Plasmodium falciparum* 3D7 strain was maintained on human red blood cells. The aqueous culture media (960 mL) consisted of 10.4 g of RPMI-1640 with 40 mg of gentamicin and 5.94 g of HEPES buffer. The culture medium was reconstituted just before use by pouring sterile 5% sodium bicarbonate in ratio of 1:24 and the culture was further supplemented with 10% Bovine serum. The parasitemia culture was maintained in between 1 and 5% and routinely subculturing was performed on every fourth day. The hematocrit was maintained initially at 7%.

**Plasmodium dilutions preparation**

Each compound was dissolved separately in DMSO to obtain stock solutions of 1 mg/mL concentration. The graded concentration of each compound used was as follows: 10, 5, 2, 1, and 0.1 µg/mL. The working solutions of the desired concentration were prepared freshly by diluting the stock solutions of compounds. The final concentration of DMSO used in the culture media did not affect the parasite growth.

**Inhibitory concentration assay**

The minimum inhibitory concentrations of each compound were determined in vitro using a dose–response assay in 24-well tissue culture plates in triplicates. Synchronous parasites were prepared [31] to obtain parasitized cells harbouring only the ring stage and challenged with a graded concentration ranging from 0.1 to 10 µg/mL of the drug solution for 48 h at 37 °C by the candle-jar method [30]. The medium was changed...
routinely after 24 h in each of wells (with or without the drug). Thin smear with Giemsa-staining were prepared and analyzed to determine the percentage inhibition of parasitemia vis-a-vis the control.

**Plasmodium slide preparation**

The 96-well plates were taken out from the candle jar and the material from each well was transferred into the corresponding well labelled 1.5-mL microcentrifuge tube. After vortexing, the supernatant was pipetted out and the pellet was further spread thoroughly on a slide to prepare a thin blood smear slide for each well. Subsequently the smeared slides were air-dried, fixed with methanol and stained with Giemsa dye for 40 min. After staining, the excess dye was removed by washing the slides in running tap water and finally slides were again air-dried. The stained slides were examined in random adjacent microscopic fields to count the number of parasites equivalent to approximately 3000 erythrocytes at 100× magnification.

**Results and discussion**

**Design of new inhibitors**

In the QSAR model, the following properties appear in the top-most equations: χ(3) cluster, κ(1), Wiener index, Coulson charge on N3, Electrostatic charge on C2, Dipole moment (χ), Total dipole, Octupole moment and Total energy. This list indicates that structural (topological) as well as electronic factors contribute to the activity or inactivity of a given compound. However, we require a deeper introspection of the actual quantitative effect of these parameters on the activity value. Deciphering the information available from a QSAR model needs the study of coefficients of these properties as they appear in the top equations.

The most powerful factor here is the charge on the nitrogen atom of the triazine ring, sandwiched between the two side chains. This indicates that the electron density at the triazine group should not decrease. So it was rational to attach electron donating atoms in these side chains.

χ(3) cluster contributes negatively towards activity. It leads us to keep less clustering in the side chains. κ(1) has a positive coefficient, though of a comparatively lower value. It signifies that contacts of first degree between atoms are beneficial in improving the activity or we can say that branching is not a favourable trait. Clustering could result in bad grades. Very long chains are also not recommended as elongation of the side chain has no major effect on the electronic contribution towards activity. These points motivated us to choose simple 2–3 carbon atom chains to be introduced near the triazine moiety.

Considering the factors described above, a series of R groups were attached to the triazine ring. Structural features of the compounds so obtained are given in Table 1, along with the predicted pMIC values, based on the first QSAR equation, for the corresponding derivatives.

\[
Y = 0.2387(1) - 2.2704(3) - 0.3014\mu_T - 0.2207\mu_x - 0.07935q_c - 37.4695
\]

where, κ(1) is the shape descriptor, χ(3) is the molecular connectivity indices, μT is the total dipole moment, μx is the dipole moment in the X direction and q_c is the coulson charge on nitrogen atom.

It can be seen that substitution of electron donating groups at various positions lead to an increase in the activity of the derivatives. It is clear that attachment of an electron acceptor decreases the predicted value. An alcoholic group reduces pMIC to ~ 4.47 (compound 1). Replacement of –OH with –NH2 improves the activity to a small extent (compound 2). Therefore various kinds of groups, such as phenyl, heterocyclic aromatic and aliphatic 5–6 member rings, and small aliphatic chains, were taken and the –NH2 group was added at different positions on the chain and at rings so as to get higher pMIC values (example—compounds 3, 9). Addition of methylamine and ethylamine proved to be better than amine (e.g. compare the pairs compounds 5 and 6; 15 and 16). Elongation of chain length also results in slightly better activity. An additional methyl group in the chain causes a slight increment in the biological activity. This can be seen as we move from compound 2–3. However, clustering and branching of any kind is not at all beneficial. Whenever an isopropyl or isobutyl group is added instead of an ethyl or methyl group, the activity for the resulting compound decreases (as in case of 4, 11 and 12). In this course of action, we obtained new compounds which had better value than the existing compounds used in the QSAR study. Based on the overall analysis we can conclude that the compounds 7, 8, 9, 10, 13 and 18 (with pMIC: 0.65, 0.19, −0.13, 0.58, −0.90 and 0.44, respectively) are the most potent derivatives that could prove to be better drugs than the existing ones.

**ADME analysis and molecular docking**

In ADME screening, 44 parameters were calculated, which included molecular descriptors and pharmaceutically relevant properties like the partition coefficient (logPo/w) and water solubility (logS), critical for estimation of absorption and distribution of drugs within the body, the blood brain barrier permeability (logBB) which is prerequisite for the entry of drugs to the brain, (log Kp) predicted skin permeability, (logKhsa) prediction of binding to human serum albumin, (P_{caco}) model for the
gut-blood barrier, percentage of human oral absorption and Lipinski's rule of five was considered a parameter to
screen the best candidates out of 22 compounds.

It was observed that out of 22 compounds only 6 lead
compounds were found not violating any of ADME prop-
erty while the rest of 16 designed compounds have
violated few important ADME properties like $P_{\text{caco}}$ which is
predicted apparent Caco-2 cell permeability in nm/s and
are good model for gut-blood barrier, logKhsa that is the
prediction of binding of ligand to the human serum albu-
min which in turn influence the biodistribution of drug
in the blood and Lipinski's Rule. From Table 2, it was
observed that all 6 lead compounds also have percentage
human oral absorption more than 50% and allowed log-
Khsa values. Therefore, it is suggested that good binding
ability with human serum albumin and reasonably good
oral human absorption may result into better distribu-
tion and good absorption of these lead compounds. $P_{\text{caco}}$
values suggested that these lead compounds may result
into good absorption of these compounds through inte-
testine, which is must for good absorption of a drug through
oral route. However, additionally the docking study was
performed to predict how the designed potential antima-
larial compounds will bind to putative receptor (DHFR).
The binding ability of ligands to receptor protein was
determined on the basis of glide score found out by
molecular docking method performed by method given
in Additional file 1.

Table 2 displayed that all 6 hits have diverse glide scores
ranging from $-6.230$ to $-4.254$ which were higher vis-
a-vis that of cycloguanil standard antimalarial drug that
works through this pathway. The 2-dimensional interaction
maps suggested that in the docking site of DHFR,
both hydrophobic interactions and hydrogen bond-
ing were the dominant forces. It is well known through
various published works and our own experience that
hydrophobic and hydrogen binding interaction play piv-
otal role in complexation of ligands with proteins [32, 33].

Therefore, from the comparison of compounds selected
on the basis of predicted MIC values, docking score and
ADME analysis respectively, compound no 11, 15, 20
were ruled out of the 6 compounds selected on the basis
of predicted $pMIC$ values. However, we tried to synthe-
size all 6 lead compounds, but due to practical problems
it was not possible to synthesize all the selected comp-
ounds. Compounds 7, 13 and 18 are the only three
compounds which could be synthesized.

Figures 1, 2 and 3 showed the 3-dimensional docked
models for compounds 18, 7 and 13 in the binding site
of receptor protein DHFR. Compound 18 when docked
in the binding site (Fig. 1) formed the hydrogen bond
with LYS 359 involving oxygen atom of the nitro group,

| Table 2 The ADME properties and Glide score of the selected six lead candidates |
|---------------------------------|-----------------|----------|----------|----------|----------|----------|----------|
| Lead compounds | logPo/W | logS | PCaco | logKhsa | logBB | logKp | % human oral absorption | Glide score for inhibition of Pf-DHFR (Kcal/mol) |
| 13   | 2.421  | -5.30 | 27.88 | 0.09   | -3.01  | -3.93  | 54.03  | -6.23 |
| 20   | 3.841  | -6.17 | 55.04 | 0.58   | -0.72  | -3.15  | 100.00 | -5.41 |
| 7    | 4.614  | -6.39 | 184.56| 0.59   | -2.30  | -2.05  | 81.56  | -5.25 |
| 15   | 3.333  | -6.15 | 100.16| 0.31   | -2.42  | -3.06  | 69.31  | -4.74 |
| 11   | 2.778  | -4.19 | 619.07| 0.05   | -0.97  | -2.81  | 93.17  | -4.64 |
| 18   | 2.409  | -3.75 | 37.21 | 0.33   | -0.36  | -7.04  | 96.13  | -4.25 |
| Cycloguanil | -      | -     | -     | -      | -      | -      | -      | -4.17 |

a logPo/w ($-2.0$ to $6.5$)
b logS ($-6.5$ to $0.5$)
c $P_{\text{caco}}$ $< 25$ is very poor and $< 500$ is great
d logKhsa ($-1.5$ to $1.5$)
e % human oral absorption ($< 25$% is poor and $> 80$% is high)
sandwiched –NH– group with ILE357 and between one of ring nitrogen atoms of triazine ring and ASN330 of the receptor. While the two dimensional interaction map (Additional file 1: Figure S2a) indicated that compound 18 maintained hydrophobic interactions with seven interacting residues in the binding site. Besides, NH+ group of piperazine ring formed charged transition with ASP334. Similarly, one of the charged oxygen atom of nitro group formed salt bridge with LYS359.

During the docking of compound 13 with Plasmodium falciparum DHFR, it was observed that there was formation of four hydrogen bonds between the ligand and four interaction residues of the binding site. One of the phenol group formed hydrogen bond involving oxygen atom ILE357 (D chain) where oxygen atom act as donor and on the contrary, oxygen atom of other substituted phenol ring formed two hydrogen bonds separately with TYR365 and ASP212 respectively and act as hydrogen bond acceptor. The fourth hydrogen bond was formed between O atom of nitro group and residue of chain C ILE357 (Fig. 3 and Additional file 1: Figure S2c).

On comparing the two dimensional interaction maps of all three compounds two residues ILE357 and TYR356 were found common for hydrophobic interaction. While there was more than 80% commonness in the interacting residues on the binding sites which play an important role in hydrophobic interactions for compounds 7 and 13. Therefore, close examination of compounds 7 and 13 suggested that hydrophobic interaction play an equal role for both compounds but compound 13 has more hydrogen bond formation and highest potency.

Chemistry

The compounds 7, 13 and 18 were synthesized by refluxing 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) with different nucleophiles (R) (Scheme 1). The three compounds were synthesised by some modifications in the literature procedure, which exploits a nucleophilic substitution reaction under solid–liquid phase-transfer conditions. The reaction, which is carried out in the presence of K2CO3 and a catalytic amount of 18-crown-6, allows possibility to substitute, by employing the appropriate number of equivalents of the nucleophile and K2CO3, one, two, or all of the chlorine atoms of 2,4,6-trichloro-1,3,5-triazine, only by appropriate reaction temperature. Normally the first substitution in cyanuric chloride takes place at 0 °C, but the amino group of p-nitroaniline is highly deactivated due to the presence of a nitro group at the para position (Scheme 1 and synthetic scheme in Additional file 1). All the synthesized compounds were characterized by their spectroscopic data, such as IR, NMR, Mass Spectrometry and elemental analysis. The synthesized trisubstituted triazine compounds are shown in Table 3.

Spectroscopic characterization

The skeleton of all three final products (2,4,6-s-triazines) have been identified by mass spectrum. The mass spectrum of compound 7 showed a molecular ion peak at m/z = 486.6 amu corresponding to (M + H)+, which
confirms the proposed formula \((C_{25}H_{28}N_{9}O_{2})^+\) and its base peak was observed at \(m/z\) 363.2. Similarly, the molecular ion peak of compound 13 was found at 432.1 and for compound 18 at 442.9 (Additional file 1: Figure S3).

The IR spectra of the compound 7 (Additional file 1: Figure S4) shows a high intensity band at 3412 \(\text{cm}^{-1}\) due to the presence of intramolecular hydrogen bonding because of the presence of three NH groups. The absorption bands at 1537 and 1372 \(\text{cm}^{-1}\) are due to \(\text{NO}_2\) stretching vibrations. The IR bands at 1643, 1269, 2841 and 968 \(\text{cm}^{-1}\) are assigned to \(\text{C}=\text{N}, \text{C}–\text{N}, \text{CH}_2\) and \(\text{N}–\text{O}\) stretching vibrations, respectively. The IR peak at 730 \(\text{cm}^{-1}\) corresponds to a substituted pyridine ring, which further establishes the structure of compound 7. Similarly, Additional file 1: Figure S5 showed the IR spectrum of the compound 13 displayed the overlapped region of OH and NH stretching bands in the 3200–3400 \(\text{cm}^{-1}\) region due to the presence of two terminal phenolic groups and three NH groups which are symmetrically attached to the triazine ring. The IR peak at 3409 \(\text{cm}^{-1}\) corresponds to the O–H stretching vibration. The IR bands at 1631, 1261, 3081 and 1180 \(\text{cm}^{-1}\) are assigned to \(\text{C}=\text{N}, \text{C}–\text{N}, \text{Ar}–\text{H}\) and \(\text{C}–\text{O}\) stretching vibrations, respectively. Also, the presence of the \(\text{NO}_2\) group was confirmed by the presence of two IR stretching bands at 1591 and 1326 \(\text{cm}^{-1}\).
The IR spectrum of compound 18 (Additional file 1: Figure S6) indicated the absorption band at 3293 cm$^{-1}$ is related to the N–H stretching vibration. The absorption bands at 1559 and 1660 cm$^{-1}$ are due to C=$\text{N}$ stretching vibrations, confirming the presence of triazine rings in the compound; the IR bands at 1541 and 1317 cm$^{-1}$ are assigned to NO$_2$ stretching vibrations. The presence of the NO$_2$ group in the compound is further confirmed by the stretching vibration peak at 966 cm$^{-1}$ corresponding to the N–O stretching vibration. The structure of compound 18 was further confirmed by the presence of absorption bands at 1257, 2845 and 1365 cm$^{-1}$ due to C–N, CH$_2$ and tertiary N, respectively.

The $^1$H-NMR spectra have been recorded for compounds 7, 13 and 18. In compound 7, there were 6 aliphatic methylene groups were found in the region of δ 2.8–4.5 ppm. The 12 aromatic protons were confirmed by the integral of the $^1$H-NMR spectrum and were found to lie in the region of δ 6.3–8.1 ppm. The three NH groups were confirmed by a singlet at δ 5.6 ppm in the $^1$H-NMR spectrum of compound 7 (Additional file 1: Figure S7). The $^1$H-NMR spectra of compound 13 (Additional file 1: Figure S8) confirmed the presence of twelve aromatic protons in the region of δ 7.4–8.9 ppm. The spectrum showed a singlet at δ 9.16 ppm, which confirmed the presence of phenolic protons in the compound. Three different labile protons, belonging to three NH groups, were found as a singlet at δ 5.9 ppm, which further confirmed the structure of compound 13.

Similarly, the $^1$H-NMR spectra of compound 18 (Additional file 1: Figure S9), confirms the presence of three chemically non-equivalent methylene proton groups at δ3.9, 3.28 and 3.12 ppm.

The $^1$H-NMR signal at δ 3.5 ppm was caused due to D$_2$O exchange and confirms the presence of a labile proton attached to the nitrogen, as the NH group is sandwiched between the two aromatic rings in the compound. The multiplet in the region of δ 7.1–7.6 ppm confirms the presence of four aromatic protons. N terminal aliphatic methyl protons were also confirmed by its deshielded lower δ values.

The $^{13}$C-NMR spectra was recorded for three compounds; 7, 13 and 18. In compound 7, there were 6 aliphatic methylene groups were found in the region of δ 39.9–64.9 ppm and 19 aromatic carbons in the region of δ 79–172.8 ppm (Additional file 1: Figure S10). The $^{13}$C-NMR spectrum of compound 13 has showed 21 peaks of aromatic carbons falling in the region of δ 117.7–169 ppm (Additional file 1: Figure S11). In $^{13}$C-NMR spectrum of compound 18, 12 aliphatic methylene groups were found in the region of δ 29.5–82 ppm and 9 aromatic carbons in the region of δ 101.4–172.1 ppm (Additional file 1: Figure S12).

### Table 3 Synthesized trisubstituted triazine compounds

| Compound no. | HNu$_1$ | HNu$_2$ = HNu$_3$ |
|-------------|---------|------------------|
| 7           | ![structure](image1) |
| 13          | ![structure](image2) |
| 18          | ![structure](image3) |

### Table 4 MIC values of the synthesized 2,4,6-trisubstituted-1,3,5-triazine derivatives against 3D7 strain of *P. falciparum*

| Compound     | Minimum inhibitory concentration$^a$ |
|--------------|--------------------------------------|
| 7            | 4.466                                |
| 13           | 7.94                                 |
| 18           | 2.75                                 |
| Cycloguanil  | 255                                  |

$^a$ Minimum inhibitory concentration for the development of ring stage parasite into the schizont stage during 48 h incubation.

Antimalarial activity evaluation

All the prepared compounds 7, 13 and 18 were found to be active against the 3D7 strain (cycloguanil sensitive strain) of *Plasmodium falciparum* species and all the analogues show minimum inhibitory concentrations (MIC) in the low nano molar range from 2.75 to 7.94 μmol/L (Table 4).

The MIC values of the synthesized triazine derivatives were compared with the reported MIC value of cycloguanil, which is taken as a standard drug under the study. The MIC value of cycloguanil has been reported as 64 μg/mL (~255 nm) against the NF54 strain of *Plasmodium falciparum* species. Since 3D7 is a drug sensitive laboratory clone of the NF54 isolate and both of them are closely related to each other [34], the reported MIC value of 64 μg/mL was considered reference MIC for comparison with the MIC values of prepared s-triazine analogues against the 3D7 strain of the *Plasmodium falciparum* species. It was found, from our activity data of the three synthesized triazine derivatives that all three synthesized compounds
were more potent than cycloguanil and compound 18 is the most active out of the three compounds against the Plasmodium falciparum species, with an MIC value of 2.75 µmol/L. Compound 7, with a propyl group linked to 2-pyridine in the substituent part, was found to be less effective against the parasite, than the compound 18, as its MIC value is 4.466 µmol/L. Compound 13, was observed to be the least active against the parasite out of the three compounds, as it showed the highest MIC value of 7.94 µmol/L.

This data revealed that the compound 13 with an aromatic ring (with electron donating group) in the substituent part contributed positively towards the antimalarial action. While the ring consisting of electron withdrawing group may cause an increase in the MIC value as showed by compound 7. Another important feature to correlate biological activity is the number of hydrogen bond formations. In the docking data it was discussed previously that in compound 13 there were four hydrogen bond formations while numbers decreased for compound 18 and least for compound 7. In the same order, the biological activity varies for the prepared compounds highlighted and established the importance of hydrogen bonding for DHFR inhibition.

Another important dimension in the discussion, which is worth mentioning, is the correlation of two physicochemical properties calculated for these derivatives with MIC values. The first is the LogPo/w values, which describe the lipophilicity of a drug. The calculated LogPo/w values for the above-mentioned compounds 7, 13 and 18 are 4.61, 2.409 and 2.42, respectively. On matching these with the experimental MIC values of these compounds, it is found that the most potent compound is the least lipophilic one and the trend is that MIC follows those of the lipophilicity. This emphasizes the importance of lipophilicity in the antimalarial activity. The second useful physicochemical property is the coulson charge on the third nitrogen atom placed in the triazine ring of the basic structure of the compounds. On consideration of all five QSAR built models, it is found that out of the six important molecular descriptors, the descriptor which heavily contributes to the activity is the coulson charge of the nitrogen atom in triazine ring, which is sandwiched between two carbons atoms of same ring over which substitution were performed. The property, as obtained computationally, has values −0.3401, −0.3555 and −0.3479, respectively for the three synthesized compounds 7, 13 and 18. This property was found to be directly correlated to the MIC value of the compound and this is confirmed by our experimental results.

The smallest value of the coulson charge, −0.3555, was obtained for compound 13 and the least MIC value of 4.2 ± 0.02 nM is also found for the same compound. The other two compounds further confirm to the same trend. This validates the correlation between the structural features of the triazine analogues and their antiplasmodial activity against the Plasmodium falciparum species.

Conclusion
Therefore, in conclusion, the present methodology proved to be a facile and rapid procedure for the preparation of trisubstituted-1,3,5-triazine compounds. The compounds were designed based upon QSAR modelling and further screening was performed on designed inhibitors using ADME and docking studies. An attempt was made to synthesize six compounds, but practically three compounds were synthesized with significantly high yields in the range of 95–100% with high purity, as checked by thin layer chromatography (TLC). The triazine compounds were analysed satisfactorily, both by the spectral and analytical data. The IR, 1H-NMR and 13C-NMR data have been comprehensively discussed and complement each other for each compound. The triazine derivatives with desired structural features to promote requisite antimalarial property were shown to display desirable antimalarial activity and it was also confirmed using in silico investigations of synthesized inhibitors that hydrogen bonding, lipophilicity and coulson charge were found to correlate with the MIC values. The correlation was performed just to analyze the role of some important pharmacophoric features in DHFR inhibition.

Additional file

Additional file 1. Additional figures.

Authors' contributions
MP carried the synthesis and characterization of compounds and helped in writing the manuscript; HO has designed and conducted spectroscopic characterization, analyzed the results and finalized the manuscript; AKT helped in availability of characterization facilities and helped in in vitro study; DS has performed computational molecular docking studies; MS has performed QSAR and helped in molecular docking studies; RK has guided and did mentorship during the study and helped in finalizing the manuscript. All authors read and approved the final manuscript.

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Competing interests
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