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# 4-Aminoquinoline-1,3,5-triazine: Design, synthesis, *in vitro* antimalarial activity and docking studies

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A series of hybrid 4-aminoquinoline 1,3,5-triazine derivatives was synthesized and their chemical structure were confirmed by $^{1}$H-NMR, $^{13}$C-NMR, FT-IR and mass spectrometric analyses. *In vitro* antimalarial activity of these compounds was evaluated against chloroquine-sensitive (3D-7) and chloroquine resistant (RKL-2) strains of *P. falciparum*. Results showed that all compounds had considerable antimalarial activity against both the strains and further docking studies were performed on both wild type (1J3I.pdb) and quadruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb) *pf*-DHFR-TS to quantify the structural parameter necessary for the activity.



## Introduction

Growing levels of antibiotic resistance have resulted in significant morbidity and mortality worldwide and significant evidence has emerged of the resistance of *Plasmodium falciparum* (malignant tertian malaria) to almost all antimalarial agents.[1–3] Unfortunately, despite the indisputable need for newer therapeutic agents, drug discovery for malaria is very challenging for several reasons, such as (i) low levels of medical care as malaria is prevalent in poor resource countries, (ii) non-availability of oral administered drugs and (iii) unaffordable therapy *etc.*[4] One of the possible drug discovery approaches for such an infectious disease is by combining two pharmacophoric groups that may act as a dual drug targeting more than one site.[5] As a consequence, a few aminoquinoline- and trioxane-based hybrid molecules have already been developed[6,7] and some are in clinical trials as antimalarial agents.[8] To revalidate an earlier concept and in continuation of our research program to develop novel hybrid molecules, we have already reported 4-aminoquinoline-triazine,[9,10] thiazole-1,3,5-triazines,[11–13] 1,3-thiazine-1,3,5-triazine,[14] 1,3,4-thiadiazole-1,3,5-triazine,[15] phenyl thiazolyl-1,3,5-triazine derivatives.[16] We herein devised a new series of hybrid target molecules using 2-(piperazin-1-yl) ethylamine as a linker to connect covalently 1,3,5-triazine and 4-aminoquinoline. These identified hybrid molecules were screened for their antimalarial activity against laboratory adapted 3D-7 (chloroquine sensitive) and RKL-2 (chloroquine resistant) strains of *Plasmodium falciparum*. Additionally, molecular docking studies were also performed to get insight of the essential key structural requirement for antimalarial activity.

## Result and discussion

### Chemistry

Cynauric chloride (2,4,6-trichloro 1,3,5-triazine), due to presence of three chloro groups offers numerous possible modifications to be carried out *via* nucleophilic substitution reactions. This structural parameter is quite important in terms of medicinal chemistry to explore the SAR and thus, the chemistry of 1,3,5-triazine has been exploited experimentally and has been the subject of many research and review articles. The synthesis of compound **3** was achieved by the nucleophilic substitution of 2-(piperazin-1-yl)ethanamine (**2**) with 4,7-dichloroquinoline (**1**) at 120–130 °C for 6–8 h. Whereas, 2,4,6-trichloro 1,3,5-triazine (**4**) was allowed to react with different aromatic amines (**a–j**) in the presence of a saturated solution of $NaHCO_3$ to afford monochloro di-substituted 1,3,5-triazine **5(a–j)** derivatives. Later, the desired compounds **6(a–j)** were synthesized by refluxing the monochloro di-substituted 1,3,5-triazine **5(a–j)** with 7-chloro-*N*-(2-(piperazin-1-yl)ethyl)quinolin-4-amine (**3**) in 1,4-dioxane for 6–7 h as shown in Scheme 1. The completion of reaction was ascertained on the basis of TLC using appropriate solvent systems. FT-IR spectra of compounds

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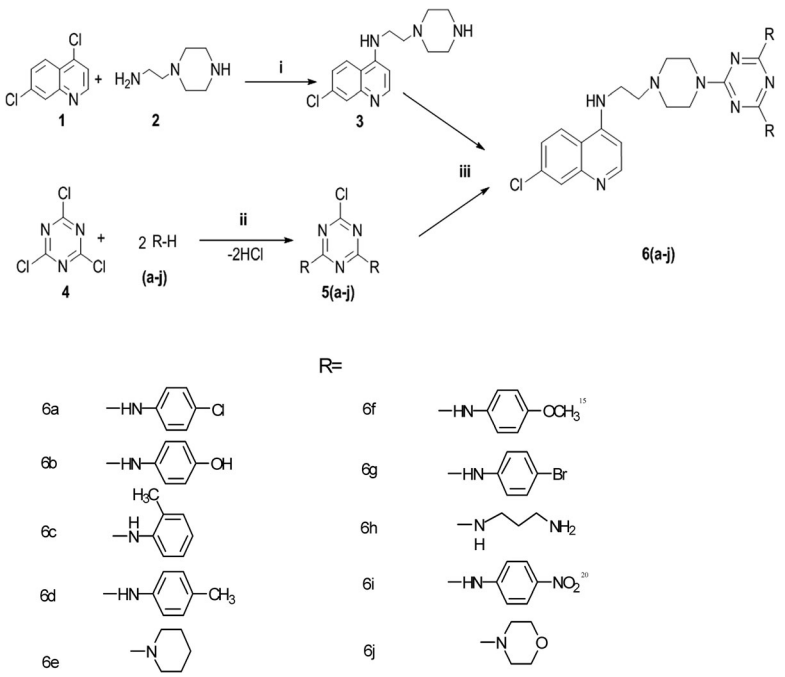


**Scheme 1** *Reagents and conditions*: R–H (a–j) various amines, (i) reflux 1 h at 80 °C followed by 6–8 h at 120–130 °C (ii) 1,4-dioxane 0–5 °C 1 h, 40–45 °C, 3 h, $NaHCO_3$ (iii) 1,4-dioxane 120–130 °C, 6–7 h, $K_2Co_3$.

**6(a–j)** are presented in the experimental section. The C═N group of 1,3,5-triazine was observed at 1675–1250 $cm^{-1}$. A strong band at 1475 $cm^{-1}$ is characteristic of the stretching frequencies of the Ar C═C group. The FT-IR spectra show a band at 850–670 $cm^{-1}$ corresponding to the aromatic ring. The Ar C–H group absorption is observed as a distinct band at 2965 $cm^{-1}$, whereas the NH linkage present on 1,3,5-triazine ring is observed at 3450–3100 $cm^{-1}$.

Hybrid derivatives show a characteristic strong band at 1650–1683 $cm^{-1}$, which is attributed to the C═N stretching vibrations. The $^1$H-NMR spectra shows a doublet at 8.65–7.57 ppm corresponding to the presence of the quinoline ring. A distinct resonance at 2.14 ppm is due to the presence of the NH group situated on the carbon atom of the s-triazine ring. The resonance due to Ar–H is observed at 6.83 to 8.30 ppm for the disubstituted 1,3,5-triazine derivatives **5(a–j)**. This is further supported by the resonance at 3.56 and 2.53 ppm for the piperazine protons and 3.45–2.78 ppm for methylene protons. Finally structures of all compounds were confirmed on the basis of mass spectra and elemental analysis.

**Table 1** *In vitro* antimalarial activity of hybrid derivatives **6(a–j)**

| | % Dead asexual parasites | | | |
|---|---|---|---|---|
| Compound | 3D-7[a] 5 μg $mL^{-1}$ (Dose) | 3D-7[a] 50 μg $mL^{-1}$ (Dose) | RKL-2[b] 5 μg $mL^{-1}$ (Dose) | RKL-2[b] 50 μg $mL^{-1}$ (Dose) |
| **6a** | 13.5 | 25.5 | 13.5 | 29 |
| **6b** | 11 | 23.5 | 7.5 | 15.5 |
| **6c** | 16.5 | 29.5 | 11 | 22 |
| **6d** | 15 | 24 | 9.5 | 17 |
| **6e** | 20.5 | 30.5 | 10 | 17 |
| **6f** | 15.5 | 36 | 16 | 26.5 |
| **6g** | 18 | 25 | 15.5 | 30.5 |
| **6h** | 26.5 | 36 | 11 | 24 |
| **6i** | 15.5 | 31 | 10 | 15.5 |
| **6j** | 6 | 18 | 8 | 16 |
| Chloroquine (CQ) (0.7 μg $mL^{-1}$) | 51 | — | | |
| Chloroquine (CQ) (1.2 μg $mL^{-1}$) | — | 46 | | |
| Proguanil (>200 μg $mL^{-1}$) | 50 | 50 | | |

[a] Wild malaria parasite (chloroquine sensitive). [b] Mutant malaria parasite (chloroquine resistant).

### Antimalarial activity and structure–activity relationship

The antimalarial screening results of compounds **6(a–j)**, against chloroquine-sensitive (3D-7) and chloroquine resistant (RKL-2) strains at 5 μg $mL^{-1}$ and 50 μg $mL^{-1}$ dosages are presented in Table 1 using chloroquine and proguanil as standard drugs. It was observed that the entire set of analogues **6a–j** displayed killing of parasitemia ranging from 18–36% at 50 μg $mL^{-1}$ and 6–26.5% at 5 μg $mL^{-1}$ against 3D-7, whereas, 16–30.5% at 50 μg $mL^{-1}$ and 7.5–15.5% at 5 μg $mL^{-1}$ were reported against RKL-2 strains. Results revealed that aliphatic analogous, such as 1,3-diamino propane containing 1,3,5-triazine (**6h**) showed the highest activity against chloroquine-sensitive (3D-7) but low activity against chloroquine resistant (RKL-2) strains. The replacement of aliphatic with aromatic groups **6(a–g)** and **6(i–j)**, displayed mid to moderate activity against both the strains of *P. falciparum*. Compound **6a** having a *p*-Cl substituent at the phenyl ring connected to 1,3,5-triazine showed 13.5% parasitemia kill against both the strains of *P. falciparum* at 5 μg $mL^{-1}$, while incorporation of *p*-Br at the phenyl ring (**6g**) displayed 18 and 15.5% parasitemia kill against chloroquine-sensitive and

 

chloroquine resistant strains at 5 μg $mL^{-1}$ and compound **6f** having a substituent (*p*-$OCH_3$) showed similar antimalarial activity with 15.5–16% parasitemia kill against both strains. The replacement of the *para* methoxy group with *p*-OH (**6b**) and *p*-$NO_2$ (**6i**) showed mild to moderate activity against both the strains. Compounds **6c** and **6d** containing electron donating substituents (*p*-$CH_3$ and *o*-$CH_3$) at the phenyl ring connected to 1,3,5-triazine showed similar activity against a chloroquine-sensitive strain with 15–16.5% of parasitemia kill but with the loss of the antimalarial activity against a chloroquine resistant strain at 5 μg $mL^{-1}$ with 9.5–11% of parasitemia kill only. The morpholine substituted derivative **6j** showed marginally less activity against both the strains with 6–8% of parasitemia kill. This was further subtantiated by the study carried out by Sunduru *et al.*[23] Introducing piperidine at 1,3,5-triazine **6e** showed improved activity with 20.5% parasitemia kill against a chloroquine sensitive strain but with loss of activity to 10% of parasitemia kill against a chloroquine resistant strain at 5 μg $mL^{-1}$. Among all derivatives, compounds **6h** and **6g** showed good activity against both strains at 5 μg $mL^{-1}$ concentration.

An SAR study indicated that aromatic derivatives, such as **6(a–j)** except **6h** showed mild to moderate activity but an aliphatic derivative **6h** showed good activity against a chloroquine sensitive strain. The compound with an electron withdrawing substituent at the phenyl ring, such as **6g** showed good activity against a chloroquine resistant strain at 5 μg $mL^{-1}$ concentration. However, introduction of basic moieties, like piperidine, morpholine, and 1,3-diaminopropane **6e**, **6h** & **6g** at the 1,3,5-triazine enhances the activity confirming that the presence of basic moieties at 1,3,5-triazine is imperative for antimalarial activity. Lastly, compounds **6c** and **6d** having electron donating groups at the *ortho* and *para* position on the phenyl ring displayed no antimalarial activity. Our results are in good agreement with the similar study carried out by Manoher *et al.*, which reported that the length of the covalent linker between piperazine and 4,7-dichloroquinoline moieties should be 2–3 carbon units for better activity.[22]

## Molecular docking study

The docking studies of target compounds were performed using the binding pocket of both the wild type (1J3I.pdb) and quadruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb) *pf*-DHFR. The docking results and docked conformations of ligands in the active site are illustrated in Table 2 and Fig. 1. These results disclosed that targeted molecules exhibited considerable and diverse binding affinities towards the wild (153.8 to −84.2) and quadruple mutant (−11.7 to −80.4) *pf*-DHFR-TS along with the formation of numerous hydrogen bonds and π–π, π–+, π–σ interactions. Further, compound **6f** exhibited higher antimalarial activity and considerably low binding energies (BE) for wild (−33.9) and mutant (−70.0) *pf*-DHFR-TS with the formation of one hydrogen bond with Ser111 through the involvement of the NH of the phenyl linkage to 1,3,5-triazine ring, while the σ–π bond with Ser167 through the involvement of the quinoline ring in wild type. The one hydrogen bond was observed between Leu164 and the phenyl ring of 1,3,5-triazine while, one σ–π interaction was revealed between the quinoline ring and Arg59 and Arg122 have been reported. Compound **6a** was reported to exhibit one hydrogen bond with Ser111 with the involvement of the amine on the phenyl ring of 1,3,5-triazine and π–π, +–π bonds through Phe58 and Lys49 with the phenyl ring of 1,3,5-triazine as well. In the quadruple mutant, it showed no hydrogen bond except the formation of +–π bonds through Arg59 with the phenyl ring of 1,3,5-triazine along with lower BE (−80.4). Introduction of a hydroxyl group (4-OH) on the phenyl ring of 1,3,5-triazine (**6b**) caused formation of new H-bonds between Asp54 and the hydroxyl group of the phenyl linked to 1,3,5-triazine and π–π, σ–π non-bonded interactions through the involvement of phenyl with Phe58 and 1,3,5-triazine with Leu46 in wild *pf*-DHFR-TS. The same ligand led to the creation of two hydrogen bonds between Asp54 and Ser111 and the hydroxyl group of the phenyl and the amine group of the 1,3,5-triazine as well as a π–π interaction with Phe58 in the mutant with lower binding energy (−17.2). Replacing *p*-hydroxy aniline with *o*-toluidine, **6c**, led to major variation in the binding energy and the absence of hydrogen bonds were reported by in both wild and mutant type, however, one π–π stacking interaction was observed between Phe58 and the phenyl of 1,3,5-triazine in wild type. Surprisingly, no H-bond was reported in compounds **6d** in the wild and mutant protein. Further, compound **6d** showed one π–π interaction between Phe58 and phenyl 1,3,5-triazine in wild type and π–σ, +–π of Phe116 andArg59 with quinoline in the mutant. Compound **6h** showed one hydrogen bond between Asp54 and Cys15 and the NH group of propane linked to 1,3,5-triazine in wild type; in mutant type formation of two hydrogen bonds between Asn108 and Lys49 with the nitrogen atom of the quinoline ring and the amine of the propane group linked to 1,3,5-triazine ring. In wild type, compound **6e** showed the formation of two hydrogen bonds with Ser167 and Ser108 and in addition to the π–σ interactions through the 1,3,5-triazine and piperidine linkage with Leu46 and Phe58. However, in the case of the mutant, no hydrogen bond was reported. The presence of a bromo group in compound **6g** exhibited H-bonds with Ser111 in wild and mutant type and additional π–π, π–+ interactions with Phe58, Lys49 and the phenyl of 1,3,5-triazine in wild type; it also showed π–+ interactions of Arg59 with the benzene ring in mutant type. Compound **6i** showed two hydrogen bond with Ser167 and Arg122 through involvement of the nitrogen atom of the quinoline ring and the nitro group of the phenyl linked to 1,3,5-triazine. It also exhibited two π–+ bonds with Arg122 and Phe116 utilising the phenyl ring and nitro group linked to the 1,3,5-triazine ring. In the case of the mutant, no hydrogen bond was reported. On the other hand, the introduction of morpholine on 1,3,5-triazine, **6j**, showed formation of the π–π interaction through involvement of the 1,3,5-triazine ring with the Phe116 in mutant *pf*-DHFR-TS.

In conclusion, we can say that most of the ligands exhibited a hydrophobic interaction between the phenyl ring on the ligand and the Phe58 residue as well as a σ–π interaction between the 1,3,5-triazine ring and the Leu46 residue in the wild type strain. Similarly in the case of the quadruple mutant

**Table 2** Docking interaction of hybrid derivatives **6(a–j)** in wild type and quadruple mutant *pf*-DHFR-TS

| | Wild type *pf*-DHFR-TS | | | Quadruple mutant *pf*-DHFR-TS | | |
|---|---|---|---|---|---|---|
| Compound | Donor/acceptor hydrogen bond | Non-bonded forces | Binding energy (kcal $mol^{-1}$) | Donor/acceptor hydrogen bond | Non-bonded forces | Binding energy (kcal $mol^{-1}$) |
| **6a** | 1,3,5-triazine-NH···SER 111 | π–π Benzene–PHE58<br>π–+ Benzene–LYS49 | | — | π–+, π–σ Benzene–ARG59 | −80.4 |
| **6b** | 1,3,5-triazine-phenyl OH···ASP54 | π–π Benzene–PHE58<br>π–σ Triazine-LEU46 | −31.7 | 1,3,5-triazine-phenyl-OH···OD-ASP54<br>1,3,5-triazine-NH···OG-SER111 | π–π Benzene–PHE58 | −17.2 |
| **6c** | — | π–π Benzene–PHE58 | −84.2 | — | — | −18.0 |
| **6d** | — | π–π Benzene–PHE58 | 153.8 | — | π–σ Quinoline–PHE116<br>π–+ Quinoline–ARG59 | −52.7 |
| **6e** | Quinoline-NH···SER167<br>Piperazine-NH···SER108 | π–σ Triazine–LEU46<br>π–σ Piperidine–PHE58 | −14.2 | — | — | −19.1 |
| **6f** | 1,3,5-triazine-phenyl NH···SER111 | π–σ Quinoline–SER167 | −33.9 | 1,3,5-triazine-phenyl NH···LEU164 | π–+ Quinoline–ARG122, ARg-159 | −70.0 |
| **6g** | 1,3,5-triazine-phenyl NH···SER111 | π–π Benzene–PHE58<br>π–+ Benzene–LYS49 | 52.2 | 1,3,5-triazine-phenyl NH···SER111 | π–+ Benzene–ARG59 | −42.4 |
| **6h** | 1,3,5-triazine-1,3-diamino propane NH···ASP54, CYS15 | — | 28.3 | Quinoline N···ASP108<br>1,3,5-triazine-1,3-diamino propane NH···LYS49 | — | −46.5 |
| **6i** | Quinoline N···SER167<br>1,3,5-triazine-phenyl NO···ARG122 | π–+ Benzene–ARG122<br>π–+ Benzene NO–PHE116 | −53.3 | — | — | −11.7 |
| **6j** | — | — | −24.8 | — | π–π Triazine–PHE116 | −59.7 |

of *pf*-DHFR-TS, a hydrophobic interaction was observed between the phenyl ring and Phe58 in addition to H-bond formation between the 1,3,5-triazine ring and Ser111. Additionally, the quinoline ring was engaged in the formation of a +–π interaction with Arg59.

## Experimental

All commercially available solvents and reagents of analytical grade were used without further purification. Melting points were determined on a Veego, MPI melting point apparatus and FT-IR (2.0 $cm^{-1}$, flat, smooth, abex) were recorded on a Perkin Elmer RX-I Spectrophotometer. $^1$H-NMR spectra were recorded on a Bruker Avance II 400 NMR and $^{13}$C-NMR spectra on Bruker Avance II 100 NMR spectrometer in DMSO-$d_6$ using TMS as internal standard. Mass spectra were obtained on VG-AUTOSPEC spectrometer equipped with electrospray ionization (ESI) sources. Elemental analysis was carried out on Vario EL-III CHNOS elemental analyzer.

The desired compounds **3**, **5(a–j)** and **6(a–j)** were obtained through the synthetic protocol as outlined in Scheme 1. Synthesis of compound **3** was achieved by the nucleophilic substitution of *N*-aminoethyl piperazine (**2**) at the fourth chloro of 4,7-dichloroquinoline (**1**). The syntheses of disubstituted *s*-triazines **5(a–j)** were accomplished by the nucleophilic substitution of the Cl atom of the 2,4,6-trichloro-1,3,5-triazine (cyanuric chloride) (**4**) with different primary and secondary amines (**a–j**). Finally, compounds **6(a–j)** were synthesized by incorporating a di-substituted 1,3,5-triazine moiety **5(a–j)** in the piperazine attached to 4-amino-quinoline pharmacophore (**3**).

**7-Chloro-*N*-(2-(piperazin-1-yl)ethyl)quinolin-4-amine (3).** A mixture of 4,7-dichloroquinoline (1 eq.) and *N*-aminoethyl piperazine (5 eq.) was heated slowly and raised to 80 °C over 1 h with stirring and subsequently at 120–130 °C for 6–8 h with continued stirring to drive the reaction to completion. The reaction mixture was cooled to room temperature and taken up in dichloromethane. The organic layer was successively washed with 5% aqueous $NaHCO_3$ followed by a water wash and finally with brine. The organic layer was dried over anhydrous $Na_2SO_4$ and solvent was removed under reduced pressure. The residue was precipitated by the addition of (80 : 20) hexane–chloroform to obtain compound **3**.

 

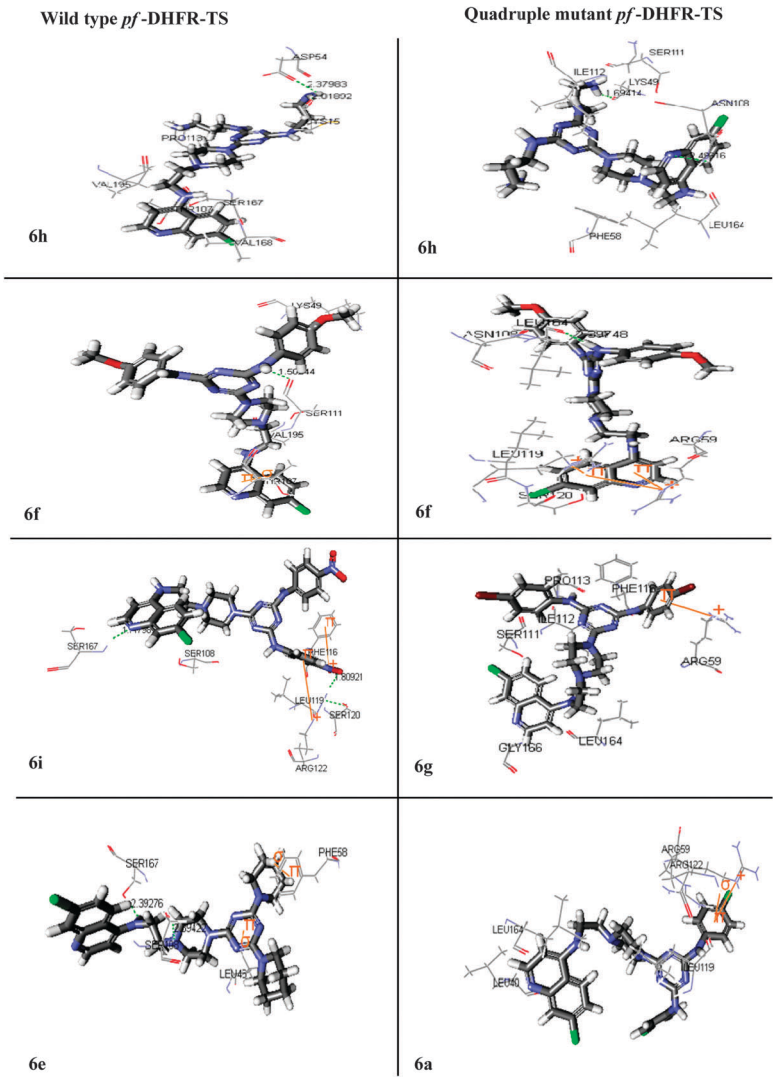


**Fig. 1** Docking image of hybrid 4-aminoquinoline 1,3,5-triazine derivatives (**6h**, **6f**, **6i** and **6e**) in wild and (**6h**, **6f**, **6g** and **6a**) in mutant *pf*-DHFR-TS.

Brown-crystals; yield: 78%; M.p: 139–140 °C; MW: 290.79; $R_f$: 0.73; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3450 (N–H stretch), 3022, 2950 (C–H stretch), 1652 (C═N stretch), 1217 (C–N stretch), 762 $cm^{-1}$; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.65 (d, 1H, $J$ = 5.02 Hz, quinoline), 8.12–7.91 (m, 3H, quinoline), 7.57 (dd, 1H, $J$ = 2.10, 8.62 Hz, quinoline), 3.48–3.32 (m, 4H, methylene), 2.73–2.47 (m, 4H, $2CH_2$, piperazine), 2.82–2.76 (m, 4H, $2CH_2$, piperazine), 2.14 (br–s, 1H, Quinoline, NH), 1.89 (s, 1H, piperazine NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 158.5, 152.1, 151.1, 136.2, 129.4, 129.2, 124.8, 121.6, 116.8, 61.2, 57.2, 55.5, 42.2; mass: 291 (M + 1); elemental analysis for $C_{15}H_{19}ClN_4$: calculated: C, 61.96; H, 6.59; N, 19.27; found: C, 60.84; H, 6.63; N, 19.26.

**General procedure for synthesis of disubstituted 1,3,5-triazine derivatives 5(a–j).** To a stirred solution of ice cold 2,4,6-trichloro-1,3,5-triazine (**4**) (0.1 mol) in acetone (25 mL), different anilines (**a–j**) (0.2 mol) were added drop wise at 0–5 °C. The resulting reaction mixture was stirred at 40–45 °C for 3 h followed by drop-wise addition of $NaHCO_3$ solution (0.1 mol) taking care that the reaction mixture did not become acidic. The completion of reaction was monitored by TLC using benzene : ethyl acetate (9 : 1) as mobile phase. The product was filtered and washed with cold water and recrystallized with ethanol to afford pure products **5(a–j)**.

**6-Chloro-$N^2$,$N^4$-bis(4-chlorophenyl)-1,3,5-triazine-2,4-diamine (5a).** White-crystals; yield: 75%; M.p: 135–137 °C; MW: 366.63; $R_f$: 0.48; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3243 (N–H secondary), 2965 (C–H broad), 1387 (aromatic –C═N); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.32 (d, 4H $J$ = 8.72 Hz, Ar–H), 7.08 (d, 4H $J$ = 8.52 Hz Ar–H), 4.82 (s, 2H, 2NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 168.4, 167.8, 137.1, 129.6, 127.7, 122.2; mass: 366 $(M + H)^+$; elemental analysis for $C_{15}H_{10}Cl_3N_5$: calculated: C, 49.14; H, 2.75; N, 19.10. Found: C, 49.17; H, 2.77; N, 19.15.

**4,4′-(6-Chloro-1,3,5-triazine-2,4-diyl)bis(azanediyl)diphenol (5b).** Black crystals; yield%: 67; M.p: 251–252 °C; MW: 329.74; $R_f$: 0.47; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3400 (OH aromatic), 3300 (N–H secondary), 3000 (C–H aromatic ring), 1675 (C═N), 1475 (C═C aromatic ring), 1250 (C–N aromatic ring); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.26–7.21 (m, 4H, 4CH, Ar–H), 6.93–6.84 (m, 4H, 4CH, Ar–H), 5.25 (s, 2H, Ar–OH), 4.13 (br, s, 2H, 2NH); $^{13}$C-NMR

(100 MHz, $CDCl_3$) $\delta$, ppm: 164.2, 160.5, 153.6, 131.5, 122.1, 116.7; mass 330.20 $(M + H)^+$; elemental analysis for $C_{15}H_{12}ClN_5O_2$: calculated: C, 54.64; H, 3.67; N, 21.24. Found: C, 54.63; H, 3.69; N, 21.23.

**6-Chloro-$N_2$,$N_4$-di-*o*-tolyl-1,3,5-triazine-2,4-diamine (5c).** Yellowish crystals; yield: 72%; M.p: 225–226 °C; MW: 332.80; $R_f$: 0.67; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3310 (N–H secondary), 3000 (C–H), 1620–1650 (C═C), 1605 (C═C aromatic ring), 1475 (C═C aromatic ring); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.10–7.07 (m, 4H, 4CH, Ar–H), 7.05–7.02 (m, 4H, 4CH, Ar–H), 5.24 (br, s, 2H, 2NH), 2.20 (s, 6H, $2CH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 168.3, 167.8, 141.5, 131.2, 129, 126.4, 123.8, 123.6, 17.2; mass 326.20 $(M + H)^+$; elemental analysis for $C_{17}H_{16}ClN_5$: calculated: C, 62.67; H, 4.95; N, 21.50. Found: C, 62.63; H, 4.98; N, 21.55.

**6-Chloro-$N^2$,$N^4$-di-*p*-tolyl-1,3,5-triazine-2,4-diamine (5d).** Pale-yellowish crystals; yield: 78%; M.p: 212–214 °C; MW: 332.80; $R_f$: 0.72; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3310 (N–H secondary), 3000 (C–H), 1650 (C═C), 1620–1475 (C═C aromatic ring); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.31–7.26 (m, 4H, 4CH, Ar–H), 7.06–7.02 (m, 4H, 4CH, Ar–H), 5.24 (br, s, 2H, 2NH), 2.53 (s, 6H, $2CH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 168.3, 167.8, 135.8, 131, 129.8, 21.5, 20.4; mass 326.20 $(M + H)^+$; elemental analysis for $C_{17}H_{16}ClN_5$: calculated: C, 62.67; H, 4.95; N, 21.50. Found: C, 62.63; H, 4.98; N, 21.55.

**2-Chloro-4,6-di(piperidin-1-yl)-1,3,5-triazine (5e).** Light brown crystals; yield: 64%; M.p: 256–258 °C; MW: 281.78; $R_f$: 0.73; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3000 (C–H), 1675 (C═N), 1475 (C═C aromatic ring), 1250 (C–N aromatic ring); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 3.71–3.67 (m, 8H, $4CH_2$, piperidin), 1.63–1.60 (m, 8H, $4CH_2$, piperidin), 1.57–1.54 (m, 4H, $2CH_2$, piperidin); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 164.2, 160.5, 52.1, 26.5, 24.5; mass 282.10 $(M + H)^+$; elemental analysis for $C_{13}H_{20}ClN_5$: calculated: C, 55.41; H, 7.15; N, 24.85. Found: C, 55.48; H, 7.13; N, 24.87.

**6-Chloro-$N^2$,$N^4$-bis(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (5f).** Yellow crystals; yield: 88%; M.p: 235 °C; MW: 357.79; $R_f$: 0.69; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3300 (N–H secondary), 3015 (C–H), 1670–1685 (C═N), 1630–1640 (C═C), 1585–1460 (C═C aromatic ring), 1100–1230 (C–N); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.52–7.49 (m, 4H, 4CH, Ar–H), 7.43–7.38 (m, 4H, 4CH, Ar–H), 5.49 (br, s, 2H, 2NH), 3.65–3.63 (s, 6H, $2OCH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 168.3, 167.8, 153.4, 131.1, 121.8, 115.1, 55.9; mass: 359 $(M + H)^+$; elemental analysis for $C_{17}H_{16}ClN_5O_2$: calculated: C, 57.07; H, 4.51; N, 19.57. Found: C, 57.02; H, 4.45, N, 19.58.

**$N^2$,$N^4$-Bis(4-bromophenyl)-6-chloro-1,3,5-triazine-2,4-diamine (5g).** Brownish-black crystals; yield: 81%; M.p: 169–170 °C; MW: 455.53; $R_f$: 0.35; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3350 (N–H secondary), 3015 (C–H broad), 1656 (C═C stretch); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.26–7.21 (m, 4H, 4CH, Ar–H), 7.06–7.02 (m, 4H, 4CH, Ar–H), 4.81 (br, s, 2H, 2NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 168.3, 167.8, 137.8, 132.4, 118.5, 116.8; mass: 455.85 $(M + H)^+$; elemental analysis for $C_{15}H_{10}Br_2ClN_5$: calculated: C, 39.55; H, 2.21; N, 15.37. Found: C, 39.53; H, 2.20; N, 15.34.

**$N^1$,$N^{1\prime}$-(6-Chloro-1,3,5-triazine-2,4-diyl)dipropane-1,3-diamine (5h).** Brown crystals; yield: 57%; M.p: 234–236 °C; MW: 259.74; $R_f$: 0.57; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3390 (N–H primary), 3300 (N–H secondary), 3000 (C–H), 1675 (C═N), 1475 (C═C aromatic ring), 1250 (C–N aromatic ring); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 5.10–5.08 (m, 4H, $2NH_2$), 4.13 (br, s, 2H, 2NH), 3.20–3.18 (m, 4H, $2CH_2$), 2.63–2.61 (m, 4H, $2CH_2$), 1.68–1.65 (m, 4H, $2CH_2$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 164.2, 160.5, 50.1, 29.6; mass 260.23 $(M + H)^+$; elemental analysis for $C_9H_{18}ClN_7$: calculated: C, 41.62; H, 6.99; N, 37.75. Found: C, 41.60; H, 7.03; N, 37.78.

**6-Chloro-$N^2$,$N^4$-bis(4-nitrophenyl)-1,3,5-triazine-2,4-diamine (5i).** Yellow-crystals; yield: 86%; M.p: 143–145 °C; MW: 387.74; $R_f$: 0.55; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3289 (N–H secondary), 3055 (C–H broad), 1548–1446 (aromatic C═N); $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 7.40–7.38 (m, 4H, 4CH, Ar–H), 7.32–7.30 (m, 4H, 4CH, Ar–H), 3.62 (br, s, 2H, 2NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 173.5, 168.8, 148.2, 143.1, 131.3, 126.2; mass 388.10 $(M + H)^+$; elemental analysis for $C_{15}H_{10}ClN_7O_4$: calculated: C, 46.46; H, 2.60; N, 25.29. Found: C, 46.48; H, 2.65; N, 25.26.

**6-Chloro-2,4-dimorpholino-1,3,5-triazine (5j).** White crystals; yield: 73.32%; M.p: 132–135 °C; FTIR (KBr) $cm^{-1}$ 2966 (C–H stretch), 1574 (C═N stretch), 1451 (C═C stretch), 1362 (C–N stretch), 1116; $^1$H-NMR (400 MHz, $CDCl_3$) $\delta$ ppm: 3.78–3.74 (m, 8H, $4CH_2$–O, morpholine), 3.70–3.67 (m, 8H, $4CH_2$–N, morpholine); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 169.6, 164.4, 66.5, 43.8; mass 286.10 $(M + H)^+$; elemental analysis for $C_{11}H_{16}ClN_5O_2$: C, 46.24; H, 5.64; N, 24.51. Found: C, 46.28; H, 5.58; N, 24.56.

**General procedure for synthesis of compounds 6(a–j).** A solution of disubstituted 1,3,5-triazines **5(a–j)** (0.01 eq.), piperazine attached to 4-aminoquinoline pharmacophore **3** (0.01 eq.) and $K_2CO_3$ (0.01 eq.) in 1,4-dioxane was refluxed for 6–7 h. The completion of reaction was monitored by TLC using ethanol : acetone (1 : 1) as mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was recrystallised from ethanol to afford the desired products **6(a–j)**.

**$N^2$,$N^4$-Bis(4-chlorophenyl)-6-(4-(2-((7-chloroquinolin-4-yl)amino)-ethyl)piperazin-1-yl)-1,3,5-triazine-2,4-diamine (6a).** White crystals; yield: 67%; M.P: 289–290 °C; MW: 620.96; $R_f$: 0.69; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3415 (N–H stretch secondary amine), 2937 (C–H stretch), 1583 (C═C stretch), 1368 (C–N stretch), 1012, 857, 637; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.72 (d, 1H $J$ = 6.63 Hz, quinoline), 8.03 (d, 1H $J$ = 8.58 Hz, quinoline), 7.92 (dd, 1H $J$ = 8.31, 1.52 Hz, Ar–H), 7.52 (d, 1H $J$ = 6.34 Hz, quinoline), 7.48–7.42 (m, 4H, 4CH, Ar–H), 7.46 (dd, 1H $J$ = 8.32, 1.90 Hz, Ar–H), 7.15–7.09 (m, 4H, 4CH, Ar–H), 3.82 (s, 2H, 2NH), 3.45 (d, 2H $J$ = 5.03 Hz, methylene), 3.25–3.19 (m, 4H, piperazine), 2.78 (d, 2H $J$ = 2.63 Hz, methylene), 2.53–2.47 (m, 4H, piperazine), 2.16 (br–s, 1H, quinoline, NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 178.3, 164.3, 158.5, 152.2, 151.1, 137.5, 136.3, 129.4, 128.2, 127.7, 124.7, 122.8, 121.6, 116.5, 94.4, 61.2, 56.2, 55.5, 54.2; mass: 622 $(M + 1)^+$; elemental analysis for $C_{30}H_{28}Cl_3N_9$: calculated: C, 58.03; H, 4.54; N, 20.30. Found: C, 58.01; H, 4.58; N, 20.42.

**4,4′-((6-(4-(2-((7-Chloroquinolin-4-yl)amino)ethyl)piperazin-1-yl)-1,3,5-triazine-2,4 diyl)bis(azanediyl))diphenol (6b).** Brown-crystals; yield: 72%; M.p: 292–294 °C; MW: 584.07; $R_f$: 0.62; FT-IR

($\nu_{max}$; $cm^{-1}$ KBr): 3487 (O–H stretch), 3389 (N–H stretch secondary amine), 1589 (C=C stretch), 1359 (C–N stretch), 1016, 853, 734; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.62 (d, 1H $J$ = 6.52 Hz, quinoline), 8.05 (dd, 1H $J$ = 8.53, 1.98 Hz, quinoline), 7.54 (dd, 1H $J$ = 8.67, 2.40 Hz, quinoline), 7.52–7.48 (m, 4H, 4CH, Ar–H), 7.45 (dd, 1H $J$ = 6.53, 1.90 Hz, quinoline), 6.84 (d, 1H $J$ = 4.84, Hz, quinoline), 6.43–6.41 (m, 4H, 4CH, Ar–H), 5.42 (s, 2H, 2OH, Ar–OH), 3.87 (s, 2H, 2NH), 3.60–3.56 (m, 4H, $2CH_2$, piperazine), 3.32 (d, 2H $J$ = 2.67 Hz, methylene), 3.08–3.02 (m, 4H, $2CH_2$, piperazine), 2.48 (d, 2H $J$ = 1.93 Hz, methylene), 2.15 (br–s, 1H, quinoline, NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 178.3, 164.3, 158.5, 152.2, 151.1, 148.5, 136.3, 131.3, 128.2, 125.3, 122.1, 121.6, 119.8, 116.7, 96.7, 60.2, 55.2, 51.3, 47.2; mass: 585 $(M + H)^+$; elemental analysis for $C_{30}H_{30}ClN_9O_2$: calculated: C, 61.69; H, 5.18; N, 21.58. Found: C, 60.98; H, 5.28; N, 21.59.

**6-(4-(2-((7-Chloroquinolin-4-yl)amino)ethyl)piperazin-1-yl)-$N^2$,$N^4$-di-*o*-tolyl-1,3,5-triazine-2,4-diamine (6c).** Light yellow crystals; yield: 68%; M.p: 287–288 °C; MW: 580.13; $R_f$: 0.47; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3426 (N–H stretch secondary amine), 2948 (C–H stretch), 1587 (C=C stretch), 1365 (C–N stretch), 1023, 862, 773; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.36 (d, 1H $J$ = 5.49 Hz, quinoline), 8.03 (dd, 1H $J$ = 8.51, 1.95 Hz, quinoline), 7.74–7.69 (m, 2H, 2CH, Ar–H), 7.48 (dd, 1H $J$ = 6.48, 1.88 Hz, quinoline), 7.36 (dd, 1H $J$ = 7.38, 2.46 Hz, quinoline), 7.01–6.95 (m, 2H, 2CH, Ar–H), 6.87 (d, 1H $J$ = 3.42 Hz, quinoline), 6.65–6.53 (m, 4H, 4CH, Ar–H), 3.82 (s, 2H, 2NH), 3.54–3.68 (m, 4H, piperazine), 3.32 (d, 2H $J$ = 2.63 Hz, methylene), 3,09–3.04 (m, 4H, piperazine), 2.51–2.45 (d, 2H $J$ = 1.97 Hz, methylene), 2.18 (br–s, 1H, quinoline NH), 2.15–2.12 (m, 6H, $2CH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 183.2, 178.6, 163.4, 156.7, 151.4, 143.3, 138.9, 134.6, 132.3, 129.4, 128.9, 127.8, 125.6, 124.5, 123.4, 121.6, 118.2, 96.7, 58.9, 53.4, 48.3, 21.2; mass: 581.18 $(M + H)^+$; elemental analysis for $C_{32}H_{34}ClN_9$: calculated: C, 66.25; H, 5.91; N, 21.73. Found: C, 66.27; H, 5.87; N, 21.76.

**6-(4-(2-((7-Chloroquinolin-4-yl)amino)ethyl)piperazin-1-yl)-$N^2$,$N^4$-di-*p*-tolyl-1,3,5-triazine-2,4-diamine (6d).** Yellow crystals; yield: 76%; M.p: 293–294 °C; MW: 580.13; $R_f$: 0.43; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3438 (N–H stretch secondary amine), 2942 (C–H stretch), 1585 (C=C stretch), 1369 (C–N stretch), 1027, 858, 782; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.42 (d, 1H $J$ = 5.38 Hz, quinoline), 7.49 (dd, 1H $J$ = 6.42, 1.85 Hz, quinoline), 7.32 (dd, 1H $J$ = 7.36, 2.43 Hz, quinoline), 8.08 (dd, 1H $J$ = 8.57, 1.92 Hz, quinoline), 6.87 (d, 1H $J$ = 3.53 Hz, quinoline), 7.76–7.69 (m, 4H, Ar–H), 6.57–6.52 (m, 4H, Ar–H), 3.38 (d, 2H $J$ = 2.68 Hz, methylene), 2.46 (d, 2H $J$ = 1.96 Hz, methylene), 3.57–3.62 (m, 4H, piperazine), 3.07–3.02 (m, 4H, piperazine), 2.18 (m, 3H, $CH_3$) 2.27 (br–s, 1H, quinoline NH); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 183.42, 172.64, 163.49, 156.72, 151.29, 138.92, 136.94, 132.31, 129.85, 127.32, 123.47, 121.42, 58.72, 51.38, 48.47, 23.51; mass: 581.10 $(M + H)^+$; elemental analysis for $C_{32}H_{34}ClN_9$: calculated: C, 66.25; H, 5.91; N, 21.73. Found: C, 66.24; H, 5.89; N, 21.74.

**7-Chloro-*N*-(2-(4-(4,6-di(piperidin-1-yl)-1,3,5-triazin-2-yl)piperazin-1-yl)ethyl)quinolin-4-amine (6e).** Light yellow crystals; yield: 63%; M.p: 278–279 °C; MW: 536.11; $R_f$: 0.41; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3417 (N–H stretch), 2942 (C–H stretch), 1583 (C=C stretch), 1363 (C–N stretch), 1021, 848, 764; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.54 (d, 1H, $J$ = 5.35 Hz, quinoline), 8.06 (d, 1H, $J$ = 2.03 Hz, quinoline), 7.47 (dd, 1H, $J$ = 2.05, 8.98 Hz, quinoline), 6.48 (d, 1H, $J$ = 5.34 Hz, quinoline), 4.75 (br s, 1H, NH), 3.85 (t, 4H, $J$ = 4.83 Hz, piperazine), 3.74 (t, 4H, $J$ = 5.52 Hz, piperazine), 2.02–1.98 (m, 2H, $CH_2$), 1.76–1.72 (m, 2H, $CH_2$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 186.84, 178.63, 161.21, 152.75, 151.36, 137.96, 131.27, 128.86, 124.62, 118.53, 101.28, 57.83, 55.29, 53.86, 52.19, 47.63, 26.75, 24.62; mass: 537.16 $(M + H)^+$; elemental analysis for $C_{28}H_{38}ClN_9$: calculated: C, 62.73; H, 7.14; N, 23.51. Found: C, 62.76; H, 7.13; N, 23.54.

**6-(4-(2-((7-Chloroquinolin-4-yl)amino)ethyl)piperazin-1-yl)-$N^2$,$N^4$-bis(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (6f).** Light brown crystals; yield: 76%; M.p: 302–303 °C; MW: 612.12; $R_f$: 0.58; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3408 (N–H stretch), 2958 (C–H stretch), 1650 (C=N stretch), 1589 (C=C stretch), 1358 (C–N stretch), 1009, 852, 768; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.65 (d, 1H, $J$ = 6.56 Hz, quinoline), 8.03 (d, 1H, $J$ = 2.01 Hz, quinoline), 7.49 (dd, 1H, $J$ = 2.03, 8.69 Hz, Ar–H), 7.02 (d, 1H, $J$ = 5.36 Hz, Ar–H), 4.38 (br s, 1H, NH), 3.83 (t, 4H, $J$ = 4.76 Hz, piperazine), 3.64 (t, 4H, $J$ = 5.47 Hz, piperazine), 3.45 (m, 2H, $CH_2$), 2.53 (m, 2H, $CH_2$), 3.87 (m, 3H, $OCH_3$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 184.24, 174.27, 157.83, 156.39, 154.58, 149.63, 132.43, 126.51, 123.58, 118.93, 115.75, 83.48, 55.86, 53.27, 52.43, 47.83; mass: 613.08 $(M + H)^+$; elemental analysis for $C_{32}H_{34}ClN_9O_2$: calculated: C, 62.79; H, 5.60; N, 20.59. Found: C, 62.81; H, 5.62; N, 20.57.

**$N^2$,$N^4$-Bis(4-bromophenyl)-6-(4-(2-((7-chloroquinolin-4-yl)amino)-ethyl)piperazin-1-yl)-1,3,5-triazine-2,4-diamine (6g).** Brown crystals; yield: 64%; M.p: 276–277 °C; MW: 709.86; $R_f$: 0.46; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3415 (N–H stretch), 2978 (C–H stretch), 1658 (C=N stretch), 1596 (C=C stretch), 1367 (C–N stretch), 1014, 858, 798; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.78 (d, 1H, $J$ = 6.63 Hz, quinoline), 7.53 (d, 1H, $J$ = 3.23 Hz, quinoline), 7.49 (dd, 1H, $J$ = 5.30, 8.79 Hz, quinoline), 7.32 (d, 1H, $J$ = 8.79 Hz, Ar–H), 7.15 (d, 1H, $J$ = 5.32 Hz, Ar–H), 4.32 (br s, 1H, NH), 3.63 (t, 4H, $J$ = 4.76 Hz, piperazine), 3.56 (t, 4H, $J$ = 5.47 Hz, piperazine), 3.38 (m, 2H, $CH_2$), 2.79 (m, 2H, $CH_2$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 182.35, 173.38, 156.73, 152.74, 149.38, 137.94, 134.82, 132.48, 128.41, 124.85, 121.62, 118.57, 117.52, 114.73, 55.52, 52.56, 51.13, 47.61; mass: 710.98 $(M + H)^+$; elemental analysis for $C_{30}H_{28}Br_2ClN_9$: calculated: C, 50.76; H, 3.98; N, 17.76. Found: C, 50.80; H, 3.96; N, 17.79.

**$N^1$,$N^{1\prime}$-(6-(4-(2-((7-Chloroquinolin-4-yl)amino)ethyl)piperazin-1-yl)-1,3,5-triazine-2,4-diyl)bis(propane-1,3-diamine) (6h).** Light brown crystals; yield: 67%; M.p: 246–248 °C; MW: 514.07; $R_f$: 0.42; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3458 (N–H stretch primary amine), 3312 (N–H stretch secondary amine) 2986 (C–H stretch), 1667 (C=N stretch), 1573 (C=C stretch), 1359 (C–N stretch), 1018, 863, 792; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.68 (d, 1H, $J$ = 5.45 Hz, quinoline), 7.81 (d, 1H, $J$ = 2.55 Hz, quinoline), 7.38 (dd, 1H, $J$ = 5.45, 8.51 Hz, quinoline), 7.10 (d, 1H, $J$ = 8.51 Hz, Ar–H), 6.83 (d, 1H, $J$ = 5.46 Hz, quinoline), 4.36 (br s, 1H, NH), 3.58 (t, 4H, $J$ = 3.11 Hz, piperazine), 3.28 (t, 4H, $J$ = 7.01 Hz, piperazine), 3.38 (t, 2H $J$ = 6.48 Hz, $CH_2$), 2.56 (d, 1H $J$ = 10.24 Hz, $CH_2$), 2.65 (d, 1H $J$ = 3.11 Hz, $CH_2$), 1.74 (t, 2H $J$ = 7.01 Hz, $CH_2$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 179.21, 164.25, 156.72,

152.13, 149.42, 134.94, 129.42, 124.53, 121.61, 117.58, 103.42, 55.27, 52.54, 51.10, 48.68, 39.42, 31.54; mass: 515.12 $(M + H)^+$; elemental analysis for $C_{24}H_{36}ClN_{11}$: calculated: C, 56.07; H, 7.06; N, 29.97. Found: C, 56.05; H, 7.04; N, 29.98.

**6-(4-(2-((7-Chloroquinolin-4-yl)amino)ethyl)piperazin-1-yl)-$N^2$,$N^4$-bis(4-nitrophenyl)-1,3,5-triazine-2,4-diamine (6i).** Yellow crystals; yield: 72%; M.p: 296–297 °C; MW: 709.86; $R_f$: 0.46; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3443 (N–H stretch secondary amine), 2963 (C–H stretch), 1683 (C=N stretch), 1528 (N–O stretch), 1475 (C=C stretch), 1245 (C–N stretch), 1009, 853, 784; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.65 (d, 1H, $J$ = 6.58 Hz, quinoline), 7.97 (d, 1H, $J$ = 2.12 Hz, quinoline), 7.61 (dd, 1H, $J$ = 1.93, 8.52 Hz, quinoline), 8.06 (d, 1H, $J$ = 8.45 Hz, Ar–H), 6.93 (d, 1H, $J$ = 1.76 Hz, Ar–H), 4.27 (br s, 1H, NH), 3.61 (t, 4H, $J$ = 3.69 Hz, piperazine), 3.36 (t, 4H, $J$ = 7.01 Hz, piperazine), 3.32 (t, 2H $J$ = 2.67 Hz, $CH_2$), 2.79 (t, 2H $J$ = 8.26 Hz, $CH_2$); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 182.36, 167.35, 154.53, 152.75, 151.78, 149.36, 146.24, 137.95, 134.84, 129.52, 123.49, 121.65, 119.23, 118.54, 99.45, 57.83, 55.52, 53.42, 51.63, 48.68; mass: 643.13 $(M + H)^+$; elemental analysis for $C_{30}H_{28}ClN_{11}O_4$: calculated: C, 56.12; H, 4.40; N, 24.00. Found: C, 56.14; H, 4.36; N, 23.97.

**7-Chloro-*N*-(2-(4-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperazin-1-yl)ethyl)quinolin-4-amine (6j).** White-crystals; yield: 65%, M.p: 306–307 °C; MW: 540.06; $R_f$: 0.41; FT-IR ($\nu_{max}$; $cm^{-1}$ KBr): 3352 (N–H stretch secondary amine) 2967 (C–H stretch), 1682 (C=N stretch), 1578 (C=C stretch), 1362 (C–N stretch), 1058, 876, 797; $^1$H-NMR (400 MHz, $CDCl_3$-$d_6$, TMS) $\delta$ ppm: 8.59 (d, 1H, $J$ = 5.42 Hz, quinoline), 8.10 (d, 1H, $J$ = 1.17 Hz, quinoline), 7.83 (dd, 1H, $J$ = 2.83, 1.91 Hz, quinoline), 7.06 (d, 1H, $J$ = 8.49 Hz, quinoline), 6.80 (d, 1H, $J$ = 2.82 Hz, quinoline), 4.39 (br s, 1H, NH), 3.69 (t, 4H, $J$ = 3.86 Hz, piperazine), 2.70 (t, 4H, $J$ = 6.12 Hz, piperazine), 3.38 (t, 2H $J$ = 2.67 Hz, $CH_2$), 2.79 (t, 2H $J$ = 2.67 Hz, $CH_2$), 3.18 (d, 1H $J$ = 10.24 Hz, Ar–H), 3.52 (d, 1H $J$ = 2.51 Hz, Ar–H); $^{13}$C-NMR (100 MHz, $CDCl_3$) $\delta$, ppm: 183.26, 177.63, 154.52, 152.75, 149.38, 134.92, 129.42, 124.83, 121,72, 117.58, 66.36, 55.21, 54.96, 51.26, 48.73, 46.53; mass: 541.12 $(M + H)^+$; elemental analysis for $C_{26}H_{34}ClN_9O_2$: calculated: C, 57.82; H, 6.35; N, 23.34. Found: C, 57.87; H, 6.34; N, 23.41.

## Antimalarial activity

### Preparation of parasites

The chloroquine sensitive 3D7 and chloroquine resistant RKL-2 strain (Raurkela, Orissa, India) of *P. falciparum* were routinely maintained in stock cultures in a RPMI-1640 medium supplemented with 25 mmol HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum. The asynchronous parasites of *P. falciparum* were synchronized after 5% D-sorbitol treatment to obtain only the ring stage parasitized cells. For carrying out the assay, the initial ring stage parasitaemia of 1.05% at 3% haematocrit in a total volume of 200 μL of medium RPMI-1640 was uniformly maintained.

### *In vitro* antimalarial efficacy test

The *in vitro* antimalarial assay was carried out according to microassay of Reickmann and co-workers in 96 well microtitre plates,[17] with minor modifications. A stock solution of 5 mg $mL^{-1}$ of each test compound was prepared in DMSO and subsequent dilutions were prepared with culture medium. The test compounds in 20 μL volume at 5 μg $mL^{-1}$ and 50 μg $mL^{-1}$ concentrations in triplicate wells were incubated with parasitized cell preparation at 37 °C in a $CO_2$ incubator set at 37 °C and 5% $CO_2$ level. After 40 h of incubation, the blood smears were prepared from each well and stained with Giemsa stain. The level of parasitemia in terms of % dead rings along and trophozoites was determined by counting a total of 100 asexual parasites (both live and dead) microscopically using chloroquine and proguanil as reference drugs.

## Molecular docking studies

The 3D X-ray crystal structure of wild type (1J3I.pdb) and quadruple mutant (N51I, C59R, S108 N, I164L, 3QG2.pdb) *pf*-DHFR-TS were used as starting models for this study. The protein was prepared, docked and the molecular dynamics simulation carried out. All computational analyses were carried out using Discovery Studio 2.5 (Accelrys Software Inc., San Diego; http://www.accelrys.com).

### Preparation of receptor

The target wild and quadruple mutant *pf*-DHFR proteins with removal of the co-crytallised ligands were taken and the bond order was corrected. The hydrogen atoms were added, and their positions were optimized using the all-atom CHARMm (version c32b1) forcefield with Adopted Basis set Newton Raphson (ABNR) minimization algorithm until the root mean square (r.m.s.) gradient for potential energy was less than 0.05 kcal $mol^{-1}$ $Å^{-1}$.[18,19] Using the 'Binding Site' tool panel available in DS 2.5, the minimized protein structures were defined as the receptor, the binding site was defined as the volume occupied by the ligand in the receptor, and an input site sphere was defined over the binding site with a radius of 5 Å. The center of the sphere was taken to be the center of the binding site, and side chains of the residues in the binding site within the radius of the sphere were assumed to be flexible during refinement of postdocking poses. The receptor having a defined binding site was used for the docking studies.

### Ligand setup

Using the built-and-edit module of DS 2.5, various ligands were built, all-atom CHARMm forcefield parameterization was assigned and then minimized using the ABNR method. A conformational search of the ligand was carried out using a stimulated annealing molecular dynamics (MD) approach. The ligand was heated to a temperature of 700 K and then annealed to 200 K. Thirty such cycles were carried out. The transformation obtained at the end of each cycle was further subjected to local energy minimization, using the ABNR method. The 30 energy-minimized structures were then superimposed and the lowest energy conformation occurring in the major cluster was taken to be the most probable conformation.

### Docking

Docking, a significant computational method was used to foretell the binding of the ligand to the receptor binding site

by varying the position and conformation of the ligand keeping the receptor rigid. The LigandFit[19] protocol of DS 2.5 was used for the docking of ligands with wild and quadruple mutant *pf*-DHFR proteins.[20] The LigandFit docking algorithm combines a shape comparison filter with a Monte Carlo conformational search to generate docked poses consistent with the binding site shape. These initial poses are further refined by rigid body minimization of the ligand with respect to the grid based calculated interaction energy using the Dreiding forcefield.[21] The receptor protein conformation was kept fixed during docking, and the docked poses were further minimized using an all-atom CHARMm (version c32b1) forcefield and smart minimization method (steepest descent followed by conjugate gradient) until the r.m.s. gradient for the potential energy was less than 0.05 kcal $mol^{-1}$ $Å^{-1}$. The atoms of the ligand and the side chains of the residues of the receptor within 5 Å of the center of the binding site were kept flexible during minimization.

## Conclusion

The present study describes the synthesis of hybrid compounds, 4-aminoquinoline and 1,3,5-triazine and their antimalarial evaluation against chloroquine sensitive (3D7) and chloroquine resistance (RKL-2) strains of *P. falciparum*. It was observed on the basis of results that compounds **6h** and **6e** exhibited considerable activity against wild type, while, the compounds **6g**, **6a** and **6f** exhibited enhanced activity in case of mutant strains. Thus, the present study provides a new approach for the ongoing malarial drug discovery initiatives.

## Conflict of interest

Authors declare no conflict of interest.

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## Notes and references

1 K. K. Modrzynska, A. Creasey, L. Loewe, T. Cezard, S. T. Borges, A. Martinelli, L. Rodrigues, P. Cravo, M. Blaxter, R. Carter and P. Hunt, *BMC Genomics*, 2012, **21**, 106.
2 World Malaria Report, World Health Organization, Geneva, Switzerland, 2010.
3 J. D. Maguire, I. W. Sumawinata, S. Masbar, B. Laksana, P. Prodjodipuro, I. Susanti, P. Sismadi, N. Mahmud, M. J. Bangs and J. K. Baird, *Lancet*, 2002, **360**, 58–60.
4 S. Nwaka, *Trans. R. Soc. Trop. Med. Hyg.*, 2005, **99**, S20–S29.
5 W. M. Francis and I. Akira, *Drug Dev. Res.*, 2010, **71**, 20–32.
6 P. M. O'Neill, A. Mukhtar, S. A. Ward, J. F. Bickley, J. Davies, M. D. Bachi and P. A. Stocks, *Org. Lett.*, 2004, **6**, 3035–3038.
7 S. Sabbani, P. A. Stocks, G. L. Ellis, J. Davies, E. Hedenstrom, S. A. Ward and P. M. O'Neill, *Bioorg. Med. Chem. Lett.*, 2008, **18**, 5804–5808.
8 L. von Seidlein, P. Milligan, M. Pinder, K. Bojang, C. Anyalebechi, R. Gosling, R. Coleman, J. I. Ude, A. Sadiq, M. Duraisingh, D. Warhurst, A. Alloueche, G. Targett, K. McAdam, B. Greenwood, G. Walraven, P. Olliaro and T. Doherty, *Lancet*, 2000, **355**, 352–357.
9 H. R. Bhat, S. K. Ghosh, A. Prakash, K. Gogoi and U. P. Singh, *Lett. Appl. Microbiol.*, 2012, **54**, 483–486.
10 H. R. Bhat, U. P. Singh, P. S. Yadav, V. Kumar, A. Das, D. Chetia, A. Prakash and J. Mahanta, *Arabian J. Chem.*, 2011, DOI: 10.1016/j.arabjc.2011.07.001.
11 U. P. Singh, H. R. Bhat, P. Gahtori and R. K. Singh, *In Silico Pharmacology*, 2013, **1**, 3.
12 S. Kumar, H. R. Bhat, M. K. Kumawat and U. P. Singh, *New J. Chem.*, 2013, **37**, 581–584.
13 U. P. Singh, H. R. Bhat and P. Gahtori, *J. Mycol. Med.*, 2012, **22**, 134–141.
14 U. P. Singh, M. Pathak, V. Dubey, H. R. Bhat, P. Gahtori and R. K. Singh, *Chem. Biol. Drug Des.*, 2012, **80**, 572–583.
15 V. Dubey, H. R. Bhat and U. P. Singh, *Chem. Biol. Drug Des.*, 2012, **80**, 598–604.
16 P. Gahtori, S. K. Ghosh, P. Pratap, A. Prakash, K. Gogoi and U. P. Singh, *Exp. Parasitol.*, 2012, **130**, 292–299.
17 K. H. Rieckmann, L. J. Sax, G. H. Campbell and J. E. Mrema, *Lancet*, 1978, **1**, 22–23.
18 B. R. Brooks, R. E. Bruccoleri, B. D. Olafson, D. J. States, S. Swaminathan and M. Karplus, *J. Comput. Chem.*, 1983, **4**, 187–217.
19 F. A. Momany and R. Rone, *J. Comput. Chem.*, 1992, **13**, 888–990.
20 C. M. Venkatachalam, X. Jiang, T. Oldfield and M. Waldman, *J. Mol. Graphics Modell.*, 2003, **21**, 289–307.
21 S. L. Mayo, B. D. Olafson and W. A. III Goddard, *J. Phys. Chem.*, 1990, **94**, 8897–8909.
22 S. Manohar, S. I. Khan and D. S. Rawat, *Bioorg. Med. Chem. Lett.*, 2010, **20**, 322–325.
23 N. Sunduru, M. Sharma, K. Srivastava, S. Rajakumar, S. K. Puri, J. K. Saxenac and P. M. S. Chauhan, *Bioorg. Med. Chem. Lett.*, 2009, **17**, 6451–6462.