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Antimalarial activity and docking studies of novel bi-functional hybrids derived from 4-aminoquinoline and 1,3,5-triazine against wild and mutant malaria parasites as *pf*-DHFR inhibitor

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Bi-functional conjugates comprised of 4-aminoquinoline and 1,3,5-triazine were synthesized through facile synthetic routes. These compounds were rigorously screened for determination of their antimalarial activity against wild and mutant cultured *Plasmodium falciparum*. The results disclosed that the conjugates have considerable antimalarial activity against both wild and mutant parasites with marked variation on changing the pattern of substitutions. The observed activity profiles were additionally substantiated by docking studies on both wild and quadruple mutant *P. falciparum* dihydrofolate reductase thymidylate synthase (*pf*-DHFR-TS).

Introduction

Malaria is a complex disease causing one of the major public health problems in developing countries. Recent statistics indicate that it accounted for about 219 million cases in 2010 (with an uncertainty range of 154 million to 289 million) and an estimated 660 000 deaths (with an uncertainty range of 610 000 to 971 000) and most of deaths prevail among children living in Africa, where a child dies every minute from malaria.[1] *Plasmodium falciparum* is the most virulent parasite of the malaria species, which is responsible for more than 95% of malaria related morbidity and mortality.[2] Modern medicinal chemistry explores the bi-functional enzyme dihydrofolate reductase-thymidylate synthase of the parasite *Plasmodium falciparum* (*Pf*-DHFR-TS) as an active binding site for 1,3,5-triazine derivatives.[3,4] The amino acids, Ala16, Asn51, Cys59, Ser108, and Ile164 are considered as active site residues for *Pf*-DHFR-TS.[5] Over the past few years, several computational techniques have been applied to understand better the *Pf*-DHFR-TS ligand interactions and intense research activity has been undertaken by both private companies and academic institutions, aimed at the discovery of new, effective DHFR inhibitors.[6]

The 4-amino-quinoline-type antimalarial drugs are supposed to exert their action through a π–π stacking interaction with the porphyrin ring system.[7–9] In this regard, researchers try to integrate this moiety with prevailing groups, for example 1,4-bis(3-aminopropyl)piperazine, istatin, β-carboline *etc.* to advantageously improve activity against the CQ-resistant strain.[10–14] 1,3,5-triazine, which is another core scaffold in clinically used antimalarial drugs, for instance in cycloguanil, chlorcycloguanil, clociguanil, and WR99210, has gained attraction due to its potential to deliver potent molecules.[14–16] Recently, conjugates of 4-aminoquinoline with 1,3,5-triazine have been employed widely as novel DHFR inhibitors.[16,18] Our research endeavour to discover newer hybrid molecules of 1,3,5-triazine as antibacterial,[19] antimalarial,[17] antifungal[20] agents continues. We herein disclose a new series of hybrid conjugates of N-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide and 1,3,5-triazine derivatives as core scaffolds. These molecules were tested for plausible antimalarial activity against 3D-7 (chloroquine sensitive) and RKL-2 (chloroquine resistant) strains of *P. falciparum*. Additionally, a molecular docking study was performed to elucidate the necessary key structural features of the potential antimalarial agents, see Fig. 1.

Results and discussion

Chemistry

The chemistry of this group of compounds has been studied intensively and has been the subject of many reviews. The various 2,4,6-mono, di- or tri-substituted, symmetrical and nonsymmetrical 1,3,5-triazines can be prepared with a high

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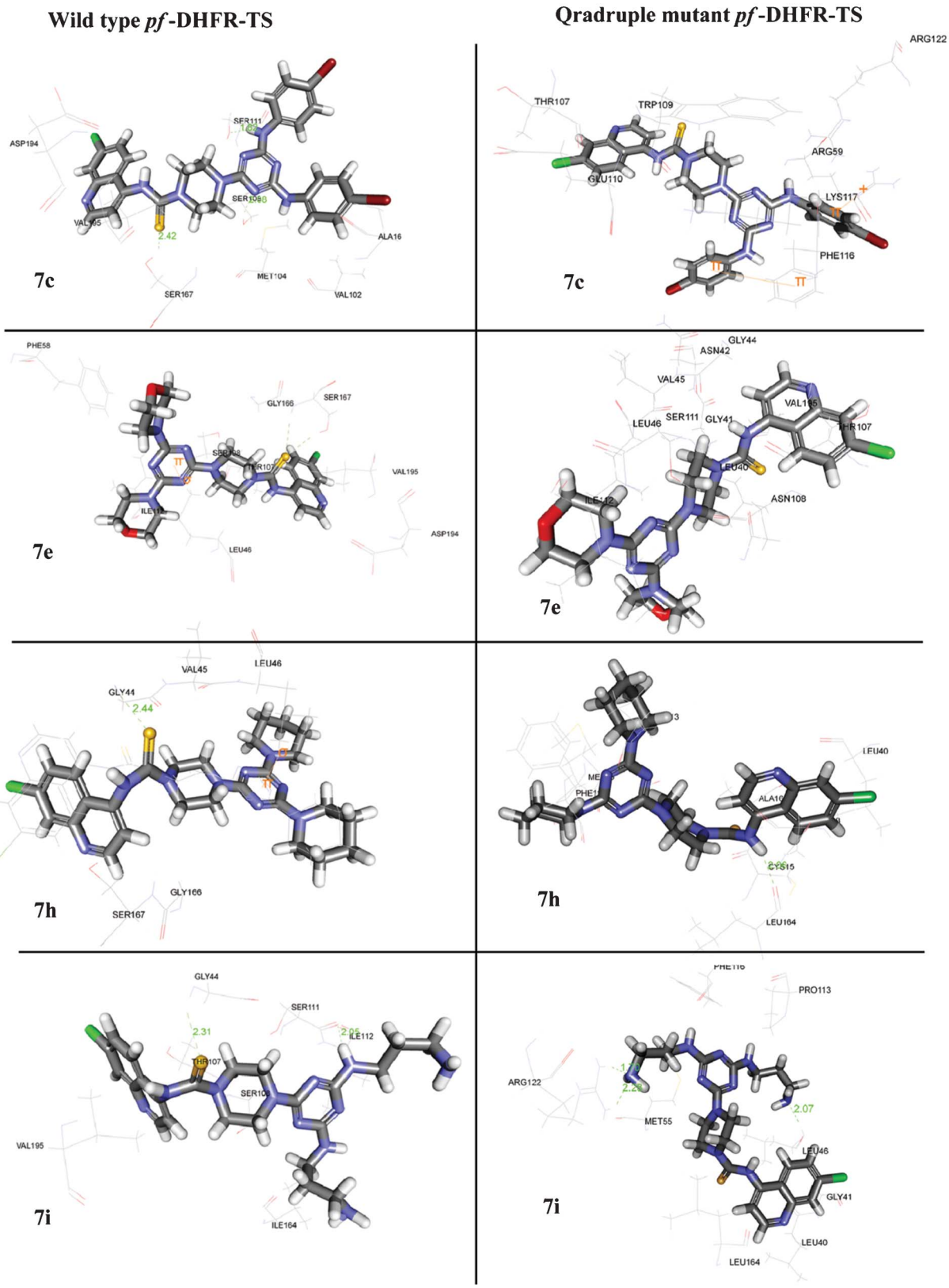
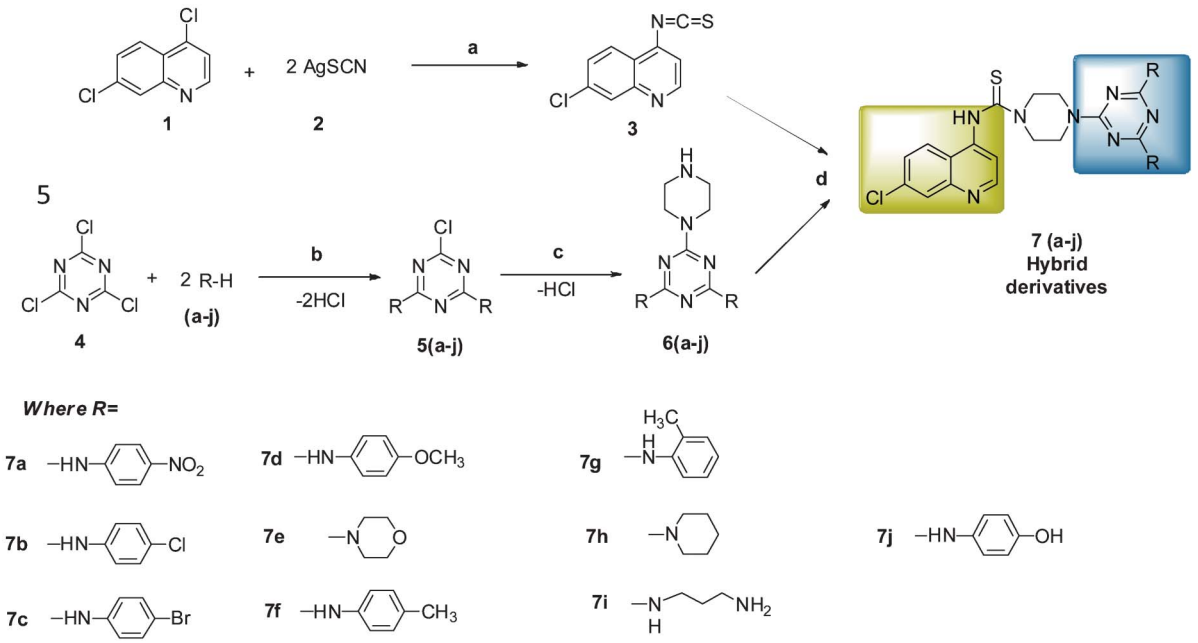


Fig. 1 Orientation of the most active hybrid compounds (**7c**, **7e**, **7h** and **7i**) in the active site of *pf*-DHFR-TS (wild and quadruple mutant).



Scheme 1 *Reagents and conditions*: (a) Reflux with stirring for 18 h at 90–120 °C. (b) Dry acetone, 0–5 °C, 1 h, then 40–45 °C for a further 3 h, $NaHCO_3$. (c) Piperazine, 1,4-dioxane, 120–130 °C, 5–6 h, K_2CO_3. (d) Dry acetone, 40–45 °C, 18 h.

level of sophistication by the nucleophilic substitution of chlorine atoms in 2,4,6-trichloro-1,3,5-triazine. Indeed, 2,4,6-trichloro-1,3,5-triazine is a cheap and commercially available reagent, which becomes the most intriguing starting approach for its wide application. This reaction therefore offers a convenient route for the preparation of substituted amino-1,3,5-triazines.

The synthesis of compound (**3**) was achieved by the nucleophilic substitution of silver thiocyanate (**2**) with the 4-chloro of 4,7-dichloroquinoline (**1**) in anhydrous toluene at 90–120 °C for 18 h. A controlled temperature of 40–45 °C, for 3–4 h in dry acetone drove the ease of replacement of the chlorine atoms in 2,4,6-trichloro-1,3,5-triazine to afford various di-substituted 1,3,5-triazine derivatives **5(a–j)**. The tri-substituted **6(a–j)** 1,3,5-triazine derivatives were obtained on refluxing **5(a–j)** in 1,4-dioxane for 6–7 h (Scheme 1). At the end, the desired compounds **7(a–j)** were furnished on stirring **6(a–j)** with 7-chloro-4-isothiocyanatoquinoline in dry acetone at 40–45 °C for 8–9 h. The structures of all the newly synthesized compounds were ascertained on the basis of FT-IR, ^{1}H-NMR, ^{13}C-NMR, mass and elemental analysis.

Bands in the FT-IR spectra of the products in the range 1548.28–1387.38 cm^{-1} are attributable to the aromatic C=N group of 1,3,5-triazine, whereas the C=C aromatic group appears nearer to 1475 cm^{-1}. Many strong absorption bands at 850–670 cm^{-1} confirm the existence of the aromatic ring. The ^{1}H-NMR spectra reveal a signal corresponding to quinoline at 7.27–8.85 ppm. For the tri-substituted 1,3,5-triazine, shielding for the bridged NH was usually observed, for instance the chemical shift of the –NH bridge was lowered approximately by 0.55 ppm in the case of **6(a–j)**. Moreover, all mass spectra and elemental analyses are found to be in agreement with the proposed structures.

Antimalarial activity and structure–activity relationship

The level of parasitemia in terms of % dead rings along with schizonts was determined for each compound **7(a–j)** along with the use of Chloroquine as the standard control against 3D-7 (Chloroquine sensitive) and RKL-2 (Chloroquine resistant) strains of *P. falciparum* (Table 1). Overall, the 3D-7 strain (avg% inhibition ± std dev = 13.44 ± 3.96) was found to be more sensitive as compared to the RKL-2 strain (avg% inhibition ± std dev = 7.50 ± 2.44) for the tested set of compounds. The % dead rings values for this series of compounds showed moderate activity with a wide degree of variation. It was found that the compounds **7c** and **7(h–j)** displayed substantial activity against both the strains and the remaining compounds demonstrated weak to moderate activities. Compound **7d** exhibited the weakest activity for the tested 3D-7 and RKL-2 strains.

It is imperative to determine the important structural features of these putative triazine hybrid compounds for antimalarial activity. The initial exploration performed was aimed at substituting the triazine core with the corresponding 4-aminoquinoline triazine template analogue. To this end, various 4-nitro, 4-chloro, 4-bromo, 4-methoxy, 4-methyl, 2-methyl, and 4-hydroxy anilines, morpholine, piperidine and aminopropyl amine substituted hybrid compounds were prepared as described above and characterized in terms of their *in vitro* antimalarial activity as well as their activity at the *pf*-DHFR receptor binding site through molecular docking. As shown in Table 1, these compounds exhibited good antimalarial activity, among them one compound, **7c**, has demonstrated the optimal level of paracitemia of 19.3 in terms of % dead rings along with schizonts, whereas the compound **7j** showed the level of paracetemia as 17.6 for the

Table 1 *In vitro* antimalarial activity of the hybrid derivatives **7(a–j)**

	Strains			
Compounds	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)
7a	11.6	22.6	4	11.5
7b	12.3	25	4.5	10
7c	19.3	34.6	11	16
7d	8.3	13.3	5.5	16.5
7e	12.3	28.6	7.5	18
7f	9	15.3	7.5	16
7g	10	18.6	6.5	16.5
7h	17	27.3	8.5	17.5
7i	17	29	10.5	17.5
7j	17.6	26.7	9.5	14
Chloroquine(CQ) (0.7 μg ml^{-1})	51		–	
Chloroquine(CQ) (1.2 μg ml^{-1})	–		50	
Proguanil (200 μg ml^{-1})	50		50	

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

same strain. Further to this encouraging result, a careful selection of the substituents of the pendent triazine ring was made to clarify the effects on biological activity.

Notably, the presence of an appropriate *ortho* substituent seems to escalate the *in vitro* potency; in fact, the corresponding methyl substituted compound **7f** was found to be less active than **7g**. Moreover, where the *para* substitution of the pendant phenyl ring is concerned, most of the substituents introduced were well tolerated, with the exception of the 4-methoxy, 4-methyl and 2-methyl groups, respectively, of compounds **7d**, **7f** and **7g**, whose lower activity is considered to be due to the high steric bulk hindrance of the methyl moiety.

The replacement of the phenyl ring by a 3-aminopropyl or morpholine was tolerated. In particular, as shown in Table 1, the 3-aminopropyl **7i** or morpholine **7e** derivative exhibited similar *in vitro* activity compared to the corresponding 4-bromo or 7-chloro derivatives. Whereas, the piperidino analogue was still active with a level of paracitemia (**7h**).

The SARs clearly postulate that 4-Br, 4-OH substitution play an essential role in escalating activity. Whereas, the similar effect was observed in the case of amino propyl and piperidino substitution. On the other hand, decline in antimalarial activity was observed for **7f**, **7g** and **7d** with 4-methyl, 2-methyl and 4-methoxy substitutions, respectively. It is imperative from this studies that electron withdrawing substituent such as bromo, hydroxy are endowed with high activity, whereas, electron donating substituent such as methyl and methoxy degraded antimalarial activity. Furthermore, amino propyl and piperidino substitution was well tolerated.

Molecular docking study

In order to gain insight into the key structural requirements and the basis of the distinct activity profile of the test compounds in both wild and mutant *P. falciparum* parasites, a molecular docking study was undertaken. The docking studies of the target compounds were performed into the binding pocket of both the wild type (1J3I.pdb) and quadruple mutant (N51I, C59R, S108N, I164L, 3QG2.pdb) *pf*-DHFR. The docking results and docked conformations of the ligands in the active site were illustrated in Table 2 and Fig. 1, respectively. These results showed that the targeted molecules were snugly fitted into the active site with considerable and diverse binding affinities towards the wild (−63.3 to −99.3) and quadruple mutant (43.6 to −80.0) *pf*-DHFR-TS along with the formation of numerous hydrogen bonds and π–π, π–+, and π–σ interactions. The results disclosed that compound **7c** has high antimalarial activity and very low binding energies for wild (−64.1) and mutant (−73.3) *pf*-DHFR-TS. It showed the generation of two hydrogen bonds with Ser108 and Ser167 through the involvement of 1,3,5-triazine and thioamide, respectively. The stability of these complexes (post docked ligand–receptor) and their low binding energies are attributed to the creation of greater intermolecular hydrogen bonding and non-bonding (π–π and covalent, +– π, and σ–π) forces amid the amino acid residues of both enzymes, as depicted in Fig. 1. The thioamide bridge used to join 4-aminoquinoline with 1,3,5-triazines was predicted to be predominantly engaged in hydrogen bonding with key amino acids (Ser167, Gly44) and minor bonding was disclosed through the participation of 1,3,5-triazine, the substituted phenyl and its amine linkage with Ser108, Arg106, Asp54 and Thr107, suggesting a role of biological activity for these compounds in wild malaria parasites. The formation of hydrophobic interactions was also shown by quinoline and the substituted phenyl ring with Phe58, and other non-bonded forces (σ–π) through the 1,3,5-triazine and quinoline with Leu46. On the contrary, for quadruple mutant *pf*-DHFR-TS, from the entire set of ligands, thioamide was again prevalent for the generation of H-bonds with Ser111, Asn108 and Leu164 and minor interactions through Arg59, Leu46, Lys49 and Arg122. Whereas, a hydrophobic interaction was reported for 1,3,5-triazine and the phenyl ring with Phe116 and Arg59, respectively. Additionally, the phenyl ring is also engaged in the formation of +–π interactions with Arg59.

Compound **7a** was revealed to make two hydrogen bonds with Arg106 and Thr107 with the involvement of the oxygen of the nitro present on the phenyl of 1,3,5-triazine. Additionally, it also created one σ–π bond through Leu46 with the quinoline

Table 2 Docking interaction of hybrid derivatives **7(a–j)** in wild 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})
7a	1,3,5-triazine-phenyl NO···ARG106:HH21 Triazine-phenyl NO···THR 107:HG1	π–σ Quinoline- LEU 46	−78.1	1,3,5-triazine-Phenyl-O···HE-ARG59	NO	−53.0
7b	Quinoline-S···HN-SER167	π–π Quinoline-PHE58	−63.3	Quinoline-S··· HD-ASN108	π–+ Benzene—ARG59	43.6
7c	Triazine-N···HG-SER-108 Quinoline-S···HG-SER167	NO	−64.1		π–π Benzene—ARG59	−73.3
7d	Triazine-N···HG-SER-108	π–σ Quinoline—THR107	−82.8		NO	−107.2
7e	Quinoline-S···HN-SER167	NO	−70.4		NO	−88.9
7f	NH···HO-SER111	π–π Quinoline—PHE58	−70.2	Quinoline-S··· HG-SER111	π–+ Benzene—ARG59	−162.5
7g	Quinoline-S···HN-GLY44 NH···HO-SER110	NO	−71.4		NO	
7h	Quinoline-S···HN-GLY44	π–σ Triazine—LEU46	−70.9	Quinoline-S··· HO-LEU164	NO	−61.1
7i	Quinoline-S···HN-GLY44 NH···HO-SER110	NO	−99.2	Amino-N··· HH-ARG122 Amino-N···HN-LEU46	NO	−80.0
7j	Quinoline-S···HG-SER167 1,3,5-triazine-phenyl-OH···HO-APS54	π–π Phenyl—PHE58	−93.3	Phenyl-O··· HE-ARG59 Phenyl-O··· HH-LYS49	π–π Triazine—PHE116	−48.36

ring. In the quadruple mutant, **7a** shows no hydrophobic interaction, and formed only one hydrogen bond, with Arg59, along with having lower binding energy (−53.0). On the contrary, the introduction of a halogen atom (4-Cl) on the phenyl ring of 1,3,5-triazine, **7b**, causes the formation of new H-bonds to Ser167 with the S of the thioamide linkage and a π–π non-bonded interaction through the involvement of quinoline with Phe58 in the wild *pf*-DHFR-TS. The same ligand led to the creation of a π–π interaction with Arg59 in the mutant, with lower binding energy (−43.6). On replacing *p*-Cl with another halogen (*p*-Br), **7c**, a minor variation in the binding energy and interactions was observed with an additional H-bond inbetween 1,3,5-triazine and Ser108, in the wild type, while no hydrogen bonds were reported with the same ligand in the mutant. It was surprising to see that no interactions were reported for **7d** and **7e** in the mutant, but again 1,3,5-triazine and the thioamide were involved in the generation of H-bonds with an additional σ–π interaction of Thr107 with quinoline in the wild type. In the case of compounds **7f** and **7g**, where a methyl group was present at the fourth and second positions of the phenyl, respectively, these compounds showed a diverse pattern of interaction with the binding site. In the wild type, compound **7f** formed a hydrogen bond with Ser111 with the additional creation of π–π interactions through quinoline with Phe58. Whereas, in the case of the mutant, **7f** caused the formation of a H-bond with Ser111 through involvement of the thioamide linkage with +–π interactions through Arg59. Thioamide was again disclosed as a common structural feature among the rest of the compounds (**7h**,**7i** and **7j**) for the creation of H-bonds through Gly44, Ser167 in the case of the wild type and Leu164 (for **7h**) for the quadruple mutant. The hydroxyl present in **7j** causes the formation of H-bonds with Arg59 and Lys49 in the mutant and Asp54 in the wild type with an additional H-bond with Ser167 through the thioamide.

These results corroborate the idea that the creation of H-bonds is the main predictor for the activity of the ligands. Nevertheless, the formation of other non-bonded forces (π–π, π–+, and π–σ) appeared to stabilize the post-docked conformation of the ligands in the inner groove of the binding site. Finally, the native ligands were allowed to dock into the active site of proteins (WR99210 and NDP 610, for wild type and quadruple *pf*-DHFR, respectively) for the validation of the docking protocol. The docked result was then compared with the crystal structure of the bound ligand–protein complex. It was revealed that the title ligands were bound in a similar fashion to the reference ligands with the root mean square deviation (RMSD) of >1.5 Å. It has been confirmed that the accuracy and performance of the LigandFit/Discovery Studio 2.5 was highly satisfactory.

Experimental

All commercially available solvents and reagents were of analytical grade and 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 Perkin Elmer RX-I Spectrophotometer. ^{1}H-NMR spectra were recorded on a Bruker Avance II 400 NMR and ^{13}C-

NMR spectra on a Bruker Avance II 100 NMR spectrometer in DMSO-d_6 using TMS as an internal standard. Mass spectra were obtained on a VG-AUTOSPEC spectrometer equipped with electrospray ionization (ESI) sources. Elemental analysis was carried out on a Vario EL-III CHNOS elemental analyzer.

The desired compounds **3**, **5(a–j)**, **6(a–j)** and **7(a–j)** were synthesised by the synthetic protocols as outlined in Scheme 1. The synthesis of compound (**3**) was achieved by the nucleophilic substitution of silver thiocynate (**2**) with the 4-chloro of 4,7-dichloroquinoline (**1**). The synthesis of di-substituted 1,3,5-triazines **5(a–j)** were accomplished by the nucleophilic substitution of the Cl atom of the 2,4,6-trichloro-1,3,5-triazine (**4**) with distinguished primary and secondary amines (**a–j**) as shown in Scheme 1. Whereas, the trisubstituted 1,3,5-triazines **6(a–j)** were acheived by the nucleophilic substitution (S_NAr) of the Cl atom of the disubstituted s-triazine **5(a–j)** with piperazine. Finally, new antimalarial compounds **7(a–j)** were synthesized by incorporating the tri-substituted 1,3,5-triazine moieties **6(a–j)** in the thiocynate attached to 4-aminoquinoline pharmacophore (**3**).

7-Chloro-4-isothiocyanatoquinoline (3)

A solution of 4,7-dichloroquinoline (**1**) (0.01 mol) and silver thiocynate (**2**) (0.02 mol) in anhydrous toluene was refluxed at 90–120 °C for 18 h. The completion of the reaction was monitored by TLC using ethanol : acetone (1 : 1) as the mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane and washed with brine and dried over Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the title compound (**3**).

Brown crystals, yield: 68%; M.p: 197–198 °C; MW: 220.68; R_f: 0.48; FT-IR (ν_{max}; cm^{-1} KBr): 3000 (C–H), 1690–1640 (C=N), 1630 (C=C), 1600 (C=C, aromatic ring), 1470 (C=C, aromatic ring), 1275 (C–N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.85 (d, 1H J = 4.97 Hz, quinoline ring), 8.40 (d, 1H J = 8.60 Hz, quinoline ring), 8.01 (d, 1H J = 1.96 Hz, quinoline ring), 7.76 (d, 1H J = 8.60 Hz, quinoline ring), 7.27 (d, 1H J = 4.97 Hz, quinoline ring); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 152.30, 138.40, 137.20, 135.20, 129.80, 129.10, 128.60, 124.10, 118.30; Mass: 221.60 $(M + H)^+$; Elemental analysis for $C_{10}H_5ClN_2S$: Calculated: C, 54.43; H, 2.28; N, 12.69. Found: C, 54.41; H, 2.23; N, 12.58.

General procedure for the synthesis of di-substituted 1,3,5-triazine derivatives 5(a–j)

Various distinguished anilines (**a–j**) (0.2 mol) were added into 100 mL of acetone, maintaining the temperature at 40–45 °C. A solution of 2,4,6-trichloro-1,3,5-triazine (**4**) (0.1 mol) in 25 mL of acetone was added, and stirred for 3 h followed by the dropwise addition of $NaHCO_3$ solution (0.1 mol) taking care that the reaction mixture does not become acidic. The completion of the reaction was monitored by TLC using benzene : ethyl acetate (9 : 1) as the 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-nitrophenyl)-1,3,5-triazine-2,4-diamine (5a). Yellow crystals, yield: 86%; M.p: 143–145 °C; MW: 387.74; R_f: 0.55; FT-IR (ν_{max}; cm^{-1} KBr): 3289.56 (N–H secondary), 3055.70 (C–H broad), 1548.28–1446.06 (aromatic C=N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.40–7.38 (m, 4H, 4xCH, Ar–H), 7.32–7.28(m, 4H, 4xCH, Ar–H), 3.62 (br, s, 2H,2x NH); ^{13}C-NMR (100MHz, $CDCl_3$) δ (ppm): 173.56, 168.85, 148.26, 143.16, 131.36, 126.23; 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-N^2,N^4-bis(4-chlorophenyl)-1,3,5-triazine-2,4-diamine (5b). White crystals, yield: 75%; M.p: 135–137 °C; MW: 366.63; R_f: 0.48; FT-IR (ν_{max}; cm^{-1} KBr): 3243.26 (N–H secondary), 2965.46 (C–H broad), 1387.38 (aromatic −C=N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.32–7.30 (m, 4H, 4xCH, Ar–H), 7.08–7.04 (m, 4H, 4xCH, Ar–H), 4.82 (br, s, 2H, 2xNH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.40, 167.85, 137.10, 129.60, 127.70, 122.25,; 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.

N^2,N^4-Bis(4-bromophenyl)-6-chloro-1,3,5-triazine-2,4-diamine (5c). Brownish black crystals, yield: 81%; M.p: 169–170 °C; MW: 455.53; R_f: 0.35; FT-IR (ν_{max}; cm^{-1} KBr): 3350.62 (N–H secondary), 3015.43 (C–H broad), 1656.15 (C=C stretch); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.26–7.21 (m, 4H, 4xCH, Ar–H), 7.06–7.02 (m, 4H, 4xCH, Ar–H), 4.81 (br, s, 2H, 2x NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.35, 167.85, 137.85, 132.45, 118.50, 116.80; 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.

6-Chloro-N^2,N^4-bis(4-methoxyphenyl)-1,3,5-triazine-2,4-diamine (5d). Yellow crystals, yield: 88%; M.p: 235 °C; MW: 357.79; R_f: 0.69; FT-IR (ν_{max}; cm^{-1} KBr): 3300 (N–H secondary), 3015 (C–H), 1670–1685 (C=N), 1630–1640 (C=C), 1585 (C=C aromatic ring), 1460 (C=C aromatic ring), 1100–1230 (C–N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.52–7.49 (m, 4H, 4xCH, Ar–H), 7.43–7.38 (m, 4H,4xCH, Ar–H), 5.49 (br, s, 2H, 2xNH), 3.65 (s, 6H, 2xOCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.35, 167.85, 153.40, 131.10, 121.80, 115.10, 55.90; 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.

6-Chloro-2,4-dimorpholino-1,3,5-triazine (5e). Buff white, yield: 73.32%, M.p: 132–135 °C; MW: 285.73; R_f: 0.62; FT-IR (ν_{max}; cm^{-1} KBr): 2966 (C–H stretch), 1574–1451, 1362 (C–N stretch), 1116(C–O stretch); ^{1}H-NMR (400 MHz, $CDCl_3$) δ (ppm): 3.78–3.74 (m, 8H, 4xCH_2-O, Morpholine), 3.70–3.67 (m, 8H, 4xCH_2-N, Morpholine); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 169.69, 164.48, 66.56, 43.86; 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.

6-Chloro-N^2,N^4-di-p-tolyl-1,3,5-triazine-2,4-diamine (5f). Pale yellowish crystals, yield: 78%; M.p: 212–214 °C; MW: 332.80; R_f: 0.72; FT-IR (ν_{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) δ (ppm): 7.31–7.26 (m,4H, 4xCH, Ar–H), 7.06–7.02 (m, 4H, 4xCH, Ar–H), 5.24 (br, s, 2H, 2xNH), 2.53 (s, 6H, 2xCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.35, 167.85, 135.85, 131, 129.85, 120.40, 21.5; 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-o-tolyl-1,3,5-triazine-2,4-diamine (5g). Yellowish crystals, yield: 72%; M.p: 225–226 °C; MW: 332.80; R_f: 0.67; FT-IR (ν_{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) δ (ppm): 7.10–7.07 (m, 4H, 4xCH, Ar–H), 7.05–7.02 (m, 4H, 4xCH, Ar–H), 5.24 (br, s, 2H, 2xNH), 2.20 (s, 6H, 2xCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.35, 167.85, 141.50, 131.20, 129, 126.40, 123.85, 123.60, 17.45; 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 (5h). Light brown, yield: 64%; M.p: 256–258 °C; MW: 281.78; R_f: 0.73; FT-IR (ν_{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) δ (ppm): 3.71–3.67 (m, 8H, 4xCH_2, Piperidine), 1.63–1.60 (m, 8H, 4xCH_2, Piperidine), 1.57–1.54 (m, 4H, 2xCH_2, Piperidine); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 164.20, 160.50, 52.10, 26.50, 24.50; 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.

N^1,$N^{1'}$-(6-Chloro-1,3,5-triazine-2,4-diyl)dipropane-1,3-diamine (5i). Brown crystals, yield: 57%; M.p: 234–236 °C; MW: 259.74; R_f: 0.57; FT-IR (ν_{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) δ (ppm): 5.10–5.08 (m, 4H, 2xNH_2), 4.13 (br, s, 2H, 2xNH), 3.20–3.18 (m, 4H, 2xCH_2), 2.63–2.61 (m, 4H, 2xCH_2), 1.68–1.65 (m, 4H, 2x CH_2); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 164.20, 160.50, 50.10, 29.60; 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.

4,4′-(6-Chloro-1,3,5-triazine-2,4-diyl)bis(azanediyl)diphenol (5j). Black crystals, yield: 67%; M.p: 251–252 °C; MW: 329.74; R_f: 0.47; FT-IR (ν_{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) δ (ppm): 7.26–7.21 (m, 4H, 4xCH, Ar–H), 6.93–6.84 (m,4H, 4xCH, Ar–H), 5.25 (s, 2H, Ar–OH), 4.13 (br, s, 2H, 2xNH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 164.20, 160.50, 153.60, 131.50, 122.10, 116.70; 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.

General procedure for the synthesis of tri-substituted 1,3,5-triazine derivatives. 6(a-j)

A solution of the di-substituted 1,3,5-triazine compound, **5(a–j)**, (0.01 mol), piperazine (0.01 mol) and K_2CO_3 (0.01 mol) in 1,4-dioxane was refluxed for 5–6 h. The completion of the reaction was monitored by TLC using benzene : ethyl acetate (9 : 1) as the mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was recrystallised by ethanol to afford the desired product **6(a–j)**

N^2,N^4-Bis(4-nitrophenyl)-6-(piperazin-1-yl)-1,3,5-triazine-2,4-diamine (6a). Yellow crystals, yield: 74%; M.p: 261–263 °C; MW: 437.20; R_f: 0.69; FT-IR (ν_{max}; cm^{-1} KBr): 3289.56 (N–H secondary), 3055.70 (C–H broad), 1675 (C=N), 1548.28–1446.06 (aromatic C=N), 1525 (NO2 aromatic), 1250 (C–N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.40–7.37 (m, 4H, 4xCH, Ar–H), 7.29–7.25 (m, 4H, 4xCH, Ar–H), 3.62 (br, s, 2H, 2xNH), 3.25–3.19 (m, 4H, 2xCH_2), 2.79–2.74(m, 4H, 2xCH_2), 1.95(s, 1H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 173.56, 168.85, 148.26, 143.16, 131.36, 126.23, 48.25, 45.80; Mass: 438.20 $(M + H)^+$; Elemental analysis for $C_{19}H_{19}N_9O_4$: calculated: C, 52.17; H, 4.38; N, 28.82. Found: C, 52.10; H, 4.39; N, 28.85.

N^2,N^4-Bis(4-chlorophenyl)-6-(piperazin-1-yl)-1,3,5-triazine-2,4-diamine (6b). White crystals, yield: 76%; M.p: 234–236 °C; MW: 416.31; R_f: 0.52; FT-IR (ν_{max}; cm^{-1} KBr): 3243.26 (N–H secondary), 3000 (C–H aromatic ring), 2965.46 (C–H broad), 1475 (C=C aromatic ring), 1387.38 (aromatic –C=N), 1250 (C–N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.32 (d, 2H J = 8.52 Hz, 2xCH, Ar–H), 7.29 (d, 2H J = 8.02 Hz, 2xCH, Ar–H), 7.08 (d, 2H J = 5.52 Hz, 2xCH, Ar–H), 7.02 (d, 2H J = 5.12 Hz, 2xCH, Ar–H), 4.82 (br, s, 2H, 2xNH), 3.25–3.15 (m, 4H, 2xCH_2), 2.79–7.72 (m, 4H, 2xCH_2), 1.95 (s, 1H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.40, 167.85, 137.10, 129.50, 127.70, 122.25, 48.30, 45.50; Mass: 438.20 $(M + H)^+$; Elemental analysis for $C_{19}H_{19}N_9O_4$: Calculated: C, 52.17; H, 4.38; N, 28.82. Found: C, 51.90; H, 4.36; N, 28.81.

N^2,N^4-Bis(4-bromophenyl)-6-(piperazin-1-yl)-1,3,5-triazine-2,4-diamine (6c). Brown crystals, yield: 63%; M.p: 258–259 °C; MW: 505; R_f: 0.48; FT-IR (ν_{max}; cm^{-1} KBr): 3350.62 (N–H secondary), 3015.43 (C–H broad), 1656.15 (C=C stretch), 1650 (C=N), 1475(C=C), 1250 (C–N); ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.26 (d, 2H J = 8.80 Hz, 2xCH, Ar–H), 7.21 (d, 2H J = 8.02 Hz, 2xCH, Ar–H), 7.06 (d, 2H J = 5.50 Hz, 2xCH, Ar–H), 6.95 (d, 2H J = 5.03 Hz, 2xCH, Ar–H), 4.81 (br, s, 2H, 2xNH), 3,28 (d, 4H J = 13.03 Hz, 2xCH_2), 2.91 (d,4H J = 13.29 Hz, 2xCH_2), 1.95 (s,1H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.35, 167.85, 137.85, 132.45, 118.50, 116.80, 48.20, 45.50; Mass: 506.20$(M + H)^+$; Elemental analysis for $C_{19}H_{19}Br_2N_7$: calculated: C, 45.17; H, 3.79; N, 19.41. Found: C, 45.10; H, 3.82; N, 19.40.

N^2,N^4-Bis(4-methoxyphenyl)-6-(piperazin-1-yl)-1,3,5-triazine-2,4-diamine (6d). Light yellow crystals, yield: 79%; M.p: 261–263 °C; MW: 407.47; R_f: 0.58; FT-IR (ν_{max}; cm^{-1} KBr): 3300 (N–H secondary), 3015 (C–H), 1670–1685 (C=N), 1630–1640 (C=C), 1585 (C=C aromatic ring), 1460 (C=C aromatic ring), 1100–1230 (C–N). ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 7.52 (d, 2H J = 5.49 Hz, 2xCH, Ar–H), 7.48 (d, 2H J = 5.01 Hz, 2xCH, Ar–H), 7.43 (d, 2H J = 8.68 Hz, 2xCH, Ar–H), 7.39 (d, 2H J = 8.76 Hz, 2xCH, Ar–H), 5.49 (br, s, 2H, 2xNH), 3.65 (s, 6H, 2x OCH3), 3.26 (d, 4H J = 13.03 Hz, 2xCH_2), 2.92 (d, 4H J = 13.29 Hz, 2xCH_2), 1.94 (s,1H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$) δ (ppm): 168.35, 167.85, 153.40, 131.10, 121.80, 115.10, 55.90, 47.70, 45.10; Mass: 408.50 $(M + H)^+$; Elemental analysis for $C_{21}H_{25}N_7O_2$: calculated: C, 61.90; H, 6.18; N, 24.06. Found: C, 61.94; H, 6.19, N, 24.05.

4,4′-(6-(Piperazin-1-yl)-1,3,5-triazine-2,4-diyl)dimorpholine (6e). White crystals, yield: 79%: M.p: 281–283 °C; MW: 335.40; R_f: 0.72; FT-IR (ν_{max}; cm^{-1} KBr): 2966 (C–H aromatic), 1574–1451 (C=C aromatic ring), 1362 (C–N), 1116; ^{1}H-NMR (400 MHz, $CDCl_3$) δ 3.78–3.73 (m, 8H, 4xCH_2-O), 3.70–3.64 (m, 8H,

4xCH$_2$-N), 3.26 (d, 4H, J = 13.03 Hz, 2xCH$_2$), 2.92 (d, 4H, J = 13.29 Hz, 2xCH$_2$), 1.94 (s,1H NH); ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 169.69, 164.48, 66.56, 47.60, 45.20, 43.86; Mass: 336.50 (M + H)$^+$; Elemental analysis for $C_{15}H_{25}N_7O_2$: calculated: C, 53.71; H, 7.51; N, 29.23. Found: C, 53.64; H, 7.57; N, 29.26.

6-(Piperazin-1-yl)-N^2,N^4-dip-tolyl-1,3,5-triazine-2,4-diamine (6f). Dark yellowish crystals, yield: 65%; M.p: 284–286 °C; MW: 375.47; R$_f$: 0.63; FT-IR (ν_{max}; cm^{-1} KBr): 3310 (N–H secondary), 3000 (C–H), 1605 (C=C aromatic ring), 1620–1650 (C=C), 1475 (C=C aromatic ring); ^{1}H-NMR (400 MHz, CDCl$_3$-d$_6$, TMS) δ (ppm): 7.31 (d, 2H J = 8.21 Hz, 2xCH, Ar–H), 7.28 (d, 2H J = 8.02 Hz, 2xCH, Ar–H), 7.23 (d, 2H J = 6.21 Hz, 2xCH, Ar–H), 7.06 (d, 2H J = 5.43 Hz, 2xCH, Ar–H), 5.24 (br, s, 2H, 2xNH), 3.26 (d, 4H J = 13.03 Hz, 2xCH$_2$), 2.92 (d, 4H J = 13.29 Hz, 2xCH$_2$), 2.53 (s, 6H, 2xCH$_3$), 1.94 (s,1H, NH); ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm):168.35, 167.85, 135.85, 131, 129.85, 120.40, 47.80, 45.30, 21.5; Mass: 376.45 (M + H)$^+$; Elemental analysis for $C_{21}H_{25}N_7$: calculated: C, 67.18; H, 6.71; N, 26.11. Found: C, 66.97; H, 6.73; N, 26.10.

6-(Piperazin-1-yl)-N^2,N^4-dio-tolyl-1,3,5-triazine-2,4-diamine (6g). Brown crystals, yield: 82%; M.p: 291–293 °C; MW:375.47; R$_f$: 0.46; FT-IR (ν_{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) δ (ppm): 7.12 (d, 2H J = 8.68 Hz, 2xCH, Ar–H), 7.10 (d, 2H J = 8.05 Hz, 2xCH, Ar–H), 7.08 (d, 2H J = 7.89 Hz, 2xCH, Ar–H), 7.05 (d, 2H J = 7.50 Hz, 2xCH, Ar–H), 5.24 (br, s, 2H, 2xNH), 3.26 (d, 4H J = 13.03 Hz, 2xCH$_2$), 2.92 (d, 4H J = 13.29 Hz, 2xCH$_2$), 2.20 (s, 6H, 2xCH$_3$), 1.94 (s,1H, NH); ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 168.35, 167.85, 141.50, 131.20, 129.20, 126.40, 123.85, 123.60, 47.5, 44.97, 17.30; Mass: 376.30 (M + H)$^+$; Elemental analysis for $C_{21}H_{25}N_7$: calculated: C, 62.67; H, 4.95; N, 21.50. Found: C, 62.69; H, 4.93; N, 21.51.

2-(Piperazin-1-yl)-4,6-di(piperidin-1-yl)-1,3,5-triazine (6h). Brown crystals, yield: 72%; M.p: 305–306 °C; MW: 323–325; R$_f$: 0.71; FT-IR (ν_{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) δ (ppm): 3.71–3.67 (m, 8H, 4xCH$_2$), 3.26 (d, 4H J = 13.03 Hz, 2xCH$_2$), 2.92 (d, 4H J = 13.29 Hz, 2xCH$_2$), 1.94 (s,1H, NH), 1.63–1.59 (m, 8H, 4xCH$_2$), 1.51–1.49 (m, 4H, 2xCH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm):164.20, 160.50, 52.10, 48.93, 45.65, 26.50, 24.50; Mass: 332.40 (M + H)$^+$; Elemental analysis for $C_{17}H_{29}N_7$: calculated: C, 61.60; H, 8.82; N, 29.58. Found: C, 61.56; H, 8.83; N, 29.56.

N^1,N$^{1'}$-(6-(Piperazin-1-yl)-1,3,5-triazine-2,4-diyl)dipropane-1,3-diamine (6i). Yellow brown crystals, yield: 69%; M.p: 324–325 °C;MW: 309.41; R$_f$: 0.52; FT-IR (ν_{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) δ (ppm): 5.10 (d, 4H J = 6.75 Hz, 2xNH$_2$), 4.13 (br, s, 2H, 2xNH)), 3.26 (d, 4H J = 13.03 Hz, 2xCH$_2$), 3.20 (d, 4H J = 6.74 Hz, 2xCH$_2$), 2.92 (d,4H J = 13.29 Hz, 2xCH$_2$), 2.63 (d, 4H J = 7.23 Hz, 2xCH$_2$), 1.94 (s,1H, NH), 1.68 (d, 4H J = 7.26 Hz, 2xCH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 164.20, 160.50, 50.10, 48.20, 45.60, 29.60; Mass: 310.40(M + H)$^+$; Elemental analysis for $C_{13}H_{27}N_9$: calculated: C, 50.46; H, 8.80; N, 40.74. Found: C, 50.43; H, 8.82; N, 40.76.

4,4′-(6-(Piperazin-1-yl)-1,3,5-triazine-2,4diyl)bis(azanediyl) diphenol (6j). Dark black crystals, yield: 82%; M.p: 287–289 °C; MW: 379.42; R$_f$: 0.56; FT-IR (ν_{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) δ (ppm): 7.32 (d, 2H J = 8.72 Hz, 2xCH, Ar–H), 7.26 (d, 2H J = 8.53 Hz, 2xCH, Ar–H), 6.93 (d, 2H J = 3.72 Hz, 2xCH, Ar–H), 6.84 (d, 2H J = 3.64 Hz, 2xCH, Ar–H), 5.25 (s, 2H, 2x Ar-OH), 4.13 (br, s, 2H, 2xNH), 3.26 (d, 4H J = 13.03 Hz, 2xCH$_2$), 2.92 (d, 4H, J = 13.29 Hz, 2xCH$_2$), 1.94 (s, 1H, NH); ^{13}C-NMR (100 MHz, CDCl$_3$) δ (ppm): 164.20, 160.50, 153.60, 131.50, 122.10, 116.70, 48.80, 45.30; Mass: 380.40 (M + H)$^+$; Elemental analysis for $C_{19}H_{21}N_7O_2$: calculated: C, 60.15; H, 5.58; N, 25.84. Found: C, 60.13; H, 5.59; N, 25.84.

General procedure for the synthesis of title compounds. 7(a–j)

A solution of compound (**3**) (0.01 mol) and the tri-substituted 1,3,5-triazine compounds **6(a–j)** (0.01 mol) in dry acetone was stirred at 40–45 °C for 18 h. The completion of the reaction was monitored by TLC using ethanol : acetone (1 : 1) as the mobile phase. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in dichloromethane, washed with brine and dried over anhydrous Na_2SO_4. The dried solution was concentrated under reduced pressure to obtain the titled compounds **7(a–j)**.

4-(4,6-Bis(4-nitrophenylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7a). Yellow crystals, yield: 58%; M.p: 230–232 °C; MW: 658.09; R$_f$: 0.81; FT-IR (ν_{max}; cm^{-1} KBr): 3300 (*N*-H secondary), 3000 (C–H aromatic ring), 1675 (C=N), 1640 (C=C), 1525 (NO$_2$ aromatic), 1475 (C=C aromatic ring), 1235 (C–N); ^{1}H-NMR (400 MHz, CDCl$_3$-d$_6$, TMS) δ (ppm): 8.82 (d, 1H J = 6.60 Hz, quinoline ring), 8.10 (d, 1H J = 8.43 Hz, quinoline ring), 8.03–7.98 (m, 4H, 4xC-H, Ar–H), 7.94 (d, 1H J = 8.35 Hz, quinoline ring), 7.35 (d, 1H J = 6.65 Hz, quinoline ring), 7.02 (d, 1H J = 6.25 Hz, quinoline ring), 6.90–6.87 (m, 4H 4xC-H, Ar–H), 4.13 (br, s, 1H, NH), 4.07–4.02 (m, 4H, 2xCH$_2$), 4.01 (s, 2H, 2xNH), 3.18–3.12 (m, 4H, 2xCH$_2$); ^{13}C-NMR (100 MHz, CDCl$_3$); δ (ppm): 181.40, 176.10, 167, 152.70, 150.70, 149.60, 145.35, 136.80, 134.90, 129.40, 126.70, 124.70, 121.60, 119.60, 118.20, 112.70, 56.70, 52.70; Mass: 659.09 (M + H)$^+$; Elemental analysis for $C_{29}H_{24}ClN_{11}S$: calculated: C, 51.90; H, 5.50; N, 21.68. Found: C, 51.85; H, 5.53; N, 21.70.

4-(4,6-Bis(4-chlorophenylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7b). Whitish yellow crystals, yield: 68%; M.p: 213–215 °C; MW: 636.99; R$_f$: 0.75; FT-IR (ν_{max}; cm^{-1} KBr): 3420 (N–H secondary), 3010 (C–H aromatic ring), 1675 (C=N), 1645 (C=C), 1470 (C=C aromatic ring), 1232 (C–N), 1007, 671 (Cl aromatic); ^{1}H-NMR (400 MHz, CDCl$_3$-d$_6$, TMS) δ (ppm): 8.76 (d, 1H J = 6.53 Hz, quinoline ring), 8.12 (d, 1H J = 8.43 Hz, quinoline ring),7.83 (d, 1H J = 6.58 Hz, quinoline ring), 7.68–7.63 (m, 4H, 4xC-H, Ar–H),7.13–7.09 (m, 4H, 4xC-H, Ar–H), 7.33 (d, 1H J = 6.42 Hz, quinoline ring), 6.87 (d, 1H J = 5.43 Hz, quinoline ring), 4.20–4.17 (m, 4H, 2xCH$_2$), 3.34–3.30 (m, 4H, 2xCH$_2$), 4.13 (br, s, 1H, NH), 3.98 (s, 2H, 2xNH); ^{13}C-NMR (100 MHz, CDCl$_3$); δ (ppm): 181.80, 176, 167.30, 152.70, 149.50, 149.30, 137.40, 134.9, 129.70, 129.20, 127.60, 124.80, 122, 121.60, 119.70, 113, 56.70, 52.20; Mass:

637.90 $(M + H)^+$; Elemental analysis for $C_{29}H_{24}Cl_3N_9S$: calculated: C, 55.35; H, 5.13; N, 17.23. Found: C, 54.68; H, 4.97; N, 19.79.

4-(4,6-Bis(4-bromophenylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7c). Yellowish brown crystals, yield: 71%; M.p: 315–317 °C; MW: 725.89; R_f: 0.76; FT-IR (ν_{max}; cm^{-1} KBr): 3435 (N–H secondary), 3025 (C–H aromatic ring), 1671 (C=N), 1647 (C=C), 1475 (C=C aromatic ring), 1236 (C–N), 1072, 769, 672; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.72 (d, 1H J = 6.57 Hz, quinoline ring), 8.23 (d, 1H J = 8.48 Hz, quinoline ring), 7.87 (d, 1H J = 6.65 Hz, quinoline ring), 7.53–7.46 (m, 4H, 4xC-H, Ar–H), 7.31 (d, 1H J = 6.52 Hz, quinoline ring), 7.01–6.95 (m, 4H, 4xC-H, Ar–H), 6.93 (d, 1H J = 5.37 Hz, quinoline ring), 3.87–3.83 (m, 4H, 2xCH_2), 3.33–3.29 (m, 4H, 2xCH_2), 4.10 (br, s, 1H, NH), 4.02 (s, 2H, 2xNH); ^{13}C-NMR (100 MHz, $CDCl_3$); δ,ppm: 180.40, 178.30, 167.20, 155.60, 149.20, 147.30, 138.40, 135.90, 132.80, 129.80, 125.30, 121.60, 119.50, 118.70, 115.70, 113.20, 56.40, 50.20; Mass: 726.90 $(M + H)^+$; Elemental analysis for $C_{29}H_{24}Br_2ClN_9S$: calculated: C, 46.67; H, 3.09; N, 19.40. Found: C, 46.60; H, 3.10; N, 19.56.

4-(4,6-Bis(4-methoxyphenylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7d). Yellowish crystals, yield: 66%; M.p: 320–322 °C; MW: 625.15; R_f: 0.73; FT-IR (ν_{max}; cm^{-1} KBr): 3410 (N–H secondary), 3018 (C–H aromatic ring), 1678 (C=N), 1650 (C=C), 1570, 1463 (C=C aromatic ring), 1236 (C–N), 1176, 1033, 671; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.79 (d, 1H J = 7.6 Hz, quinoline ring), 8.35 (d, 1H J = 8.49 Hz, quinoline ring), 7.61 (d, 1H J = 6.20 Hz, quinoline ring), 7.55–7.48 (m, 4H, 4xC-H, Ar–H), 7.28 (d, 1H J = 6.59 Hz, quinoline ring), 7.10–7.03 (m, 4H, 4xC-H, Ar–H), 6.85 (d, 1H J = 5.28 Hz, quinoline ring), 3.98–3.93 (m, 4H, 2xCH_2), 3.34–3.29 (m, 4H, 2xCH_2), 3.83 (t, 6H, 2xOCH_3), 4.20 (br, s, 1H, NH) 4.03 (s 2H, 2xNH); ^{13}C-NMR (100 MHz, $CDCl_3$); δ,ppm: 181.20, 178.30, 166.70, 155.20, 152.60, 149.20, 148.90, 139.40, 133.50, 131.20, 128.70, 127.60, 121.30, 119.60, 115.40, 113.70, 56.20, 54.90, 51.20; Mass: 626.10 $(M + H)^+$; Elemental analysis for $C_{31}H_{30}ClN_9O_2S$: calculated: C, 59.22; H, 6.57; N, 21.42. Found: C, 59.24; H, 6.56; N, 21.47.

***N*-(7-Chloroquinolin-4-yl)-4-(4,6-dimorpholino-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (7e).** Brown crystals, yield: 77%; M.p: 333–334 °C; MW: 557.08; R_f: 0.82; FT-IR (ν_{max}; cm^{-1} KBr): 3347 (N–H secondary), 3042 (C–H aromatic ring), 1689 (C=N), 1579 (C=C), 1444 (C=C aromatic ring), 1301, 1243 (C–N), 1218, 699; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.82 (d, 1H J = 7.40 Hz, quinoline ring), 8.28 (d, 1H J = 8.43 Hz, quinoline ring), 7.68 (d, 1H J = 6.40 Hz, quinoline ring), 7.23 (d, 1H J = 8.73 Hz, quinoline ring), 6.94 (d, 1H J = 5.38 Hz, quinoline ring), 4.25 (br, s, 1H, NH), 4.10–4.04 (m, 4H, 2xCH_2), 3.78–3.73 (m,8H, 4x CH_2), 3.58–3.51 (m, 8H, 4xCH_2), 3.08–3.02 (m, 4H, 2xCH_2); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 181.30, 179.40, 177.60, 160.50, 146.90, 142.40, 134.40, 130.50, 129.80, 125.30, 121.60, 113.60, 66.30, 56.20, 50.10, 48.70; Mass: 558.10 $(M + H)^+$; Elemental analysis for $C_{25}H_{30}ClN_9O_2S$: calculated: C, 46.12; H, 6.38; N, 24.33. Found: C, 46.10; H, 6.32; N, 24.30.

4-(4,6-Bis(p-tolylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7f). Yellowish crystals, yield: 64%; M.p: 342–343 °C; MW: 596.15; R_f: 0.83; FT-IR (ν_{max}; cm^{-1} KBr): 3405 (N–H secondary), 3020 (C–H aromatic ring), 1693 (C=N), 1556 (C=C), 1421 (C=C aromatic ring), 1261 (C–N), 1220, 1000.6, 671; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.86 (d, 1H J = 7.44 Hz, quinoline ring), 8.23 (d, 1H J = 8.54 Hz, quinoline ring), 7.75 (d, 1H J = 6.30 Hz, quinoline ring), 7.45–7.35 (m, 4H, 4xCH, Ar–H), 7.13 (d, 1H J = 8.14 Hz, quinoline ring), 7.06–7.04 (m, 4H, 4xCH, Ar–H), 6.93 (d, 1H J = 5.38 Hz, quinoline ring), 4.10 (br, s, 1H, NH), 4.05–4.01 (m, 4H, 2xCH_2), 3.92 (s, 2H, 2xNH), 3.17–3.07 (m, 4H, 2xCH_2), 2.43 (s, 6H, 2xCH_3); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 179.30, 176, 160.50, 153.30, 149.40, 146.90, 142.50, 140.40, 135.90, 131.70, 130.50, 129.80, 125.30, 121.20, 120.40, 113.70, 56.70, 52, 21.30; Mass: 597.20 $(M + H)^+$; Elemental analysis for $C_{31}H_{30}ClN_9S$: calculated: C, 61.96; H, 6.94; N, 20.65. Found: C, 61.98; H, 7.01; N, 20.64.

4-(4,6-Bis(o-tolylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7g). Yellowish crystals, yield: 71%; M.p: 396–398 °C; MW: 596.15; R_f: 0.78; FT-IR (ν_{max}; cm^{-1} KBr): 3404 (N–H secondary), 3020 (C–H aromatic ring), 1693 (C=N), 1556(C=C), 1421 (C=C aromatic ring), 1261 (C–N), 1225, 1020, 672; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.85 (d, 1H J = 7.44 Hz, quinoline ring), 8.35 (d, 1H J = 8.49 Hz, quinoline ring), 7.73 (d, 1H J = 6.32 Hz, quinoline ring), 7.28 (d, 1H J = 8.62 Hz, quinoline ring), 7.12–7.04 (m, 4H, 4xCH, Ar–H), 6.95 (d, 1H J = 5.37 Hz, quinoline ring), 6.69–6.56 (m, 4H, 4xCH, Ar–H), 4.06–3.98 (m, 4H, 2xCH_2), 3.17–3.12 (m, 4H, 2xCH_2), 2.15 (t, 6H, 2xCH_3), 4.05 (br, s, 1H, NH) 3.93 (s, 2H, 2xNH); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 179.30, 176, 167.50, 153.40, 149.20, 148.30, 142.50, 135.90, 130.50, 129.80, 129.40, 128.30, 126.50, 124.70, 123.70, 121.10, 119.70, 113.20, 56.80, 51.90, 17.70; Mass: 597.20 $(M + H)^+$; Elemental analysis for $C_{31}H_{30}ClN_9S$: calculated: C, 61.96; H, 6.94; N, 20.65. Found: C, 61.92; H, 7.05; N, 20.58.

***N*-(7-Chloroquinolin-4-yl)-4-(4,6-di(piperidin-1-yl)-1,3,5-triazin-2-yl)piperazine-1-carbothioamide (7h).** Brown crystals, yield: 66%; M.p: 270–272 °C; MW: 552.14; R_f: 0.73; FT-IR (ν_{max}; cm^{-1} KBr): 3415 (N–H secondary), 3032 (C–H aromatic ring), 1685 (C=N), 1565(C=C), 1475 (C=C aromatic ring), 1262 (C–N), 1228, 1027, 670; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.85 (d, 1H J = 7.44 Hz, quinoline ring), 8.12 (d, 1H J = 8.14 Hz, quinoline ring), 7.70 (d, 1H J = 6.32 Hz, quinoline ring), 7.43 (d, 1H J = 8.75 Hz, quinoline ring), 7.02 (d, 1H J = 5.63 Hz, quinoline ring), 4.03–4.01 (m, 4H, 2xCH_2), 3.75–3.71 (m, 8H, 4xCH_2), 3.18–3.15 (m, 4H, 2xCH_2), 1.63–1.61 (m, 4H 2xCH_2), 1.58–1.53 (m, 8H, 4xCH_2), 4.08 (br, s, 1H, NH); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 181.80, 180.50, 176.50, 152.30, 149.60, 148.30, 134.90, 129.40, 124.80, 121.60, 119.70, 114.10, 56.70, 54.80, 52.10, 25.50, 24.50; Mass: 553.10 $(M + H)^+$; Elemental analysis for $C_{27}H_{34}ClN_9S$: calculated: C, 56.86; H, 8.57; N, 22.63. Found: C, 57.01; H, 8.54; N, 22.64.

4-(4,6-Bis(3-aminopropylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7i). Yellow brown crystals, yield: 69%; M.p: 288–290 °C; MW: 530.09; R_f: 0.68; FT-IR (ν_{max}; cm^{-1} KBr): 3415 (N–H secondary), 3390 (N–H primary), 3030 (C–H aromatic ring), 1670 (C=N), 1555 (C=C), 1475 (C=C aromatic ring), 1269 (C–N), 1220, 1020, 670; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.78 (d, 1H J = 7.40 Hz, quinoline ring), 8.43 (d, 1H J = 8.31 Hz, quinoline ring),

7.78 (d, 1H J = 6.35 Hz, quinoline ring), 7.24 (d, 1H J = 8.74 Hz, quinoline ring), 6.94 (d, 1H J = 5.38 Hz, quinoline ring), 5.20 (d, 4H J = 2.34 Hz, 2xNH_2), 4.08 (br, s, 1H, NH), 4.03–3.98 (m, 4H, 2xCH_2), 3.95 (s, 2H, 2xNH), 3.18–3.12 (m, 4H, 2xCH_2), 3.35 (m, 4H, 2xCH_2), 2.68 (m, 4H, 2xCH_2), 1.83 (m, 4H, 2xCH_2); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 181.80, 176.50, 164.30, 152.70, 149.30, 148.70, 134.90, 129.60, 124.80, 121.60, 119.70, 112.90, 56.70, 52.50, 40.30, 31.50; Mass: 531.05 $(M + H)^+$; Elemental analysis for $C_{23}H_{32}ClN_{11}S$: calculated: C, 47.67; H, 6.64; N, 30.61. Found: C, 47.65; H, 6.67; N, 30.62.

4-(4,6-Bis(4-hydroxyphenylamino)-1,3,5-triazin-2-yl)-*N*-(7-chloroquinolin-4-yl)piperazine-1-carbothioamide (7j). Black crystals, yield: 74%; M.p: 278–280 °C MW: 600.09; R_f: 0.64; FT-IR (ν_{max}; cm^{-1} KBr): 3420 (N–H secondary), 3395 (OH aromatic ring), 3035 (C–H aromatic ring), 1676 (C=N), 1557 (C=C), 1477 (C=C aromatic ring), 1269 (C–N), 1210, 1003, 678; ^{1}H-NMR (400 MHz, $CDCl_3$-d_6, TMS) δ (ppm): 8.90 (d, 1H J = 7.50 Hz, quinoline ring), 8.28 (d, 1H J = 8.43 Hz, quinoline ring), 7.76 (d, 1H J = 6.33 Hz, quinoline ring), 7.53–7.48 (m, 4H, 2xCH_2, Ar–H), 7.23 (d, 1H J = 8.73 Hz, quinoline ring), 6.78 (d, 1H J = 5.32 Hz, quinoline ring), 6.75–6.68 (m, 4H, 2xCH_2, Ar–H), 5.40 (s, 2H, 2x Ar–OH), 4.05 (br, s, 1H, NH), 4.02–3.96 (m, 4H, 2xCH_2), 3.87 (s, 2H, 2xNH), 3.16–3.09 (m, 4H, 2xCH_2); ^{13}C-NMR (100 MHz, $CDCl_3$); δ (ppm): 181.80, 176.20, 167.30, 152.70, 149.50, 149.20, 148.50, 134.80, 131.50, 129.40, 124.80, 122.20, 121.60, 119.70, 115.90, 113.10, 56.70, 52.50; Mass: 601.01 $(M + H)^+$; Elemental analysis for $C_{29}H_{26}ClN_9O_2S$: calculated: C, 58.04; H, 4.37; N, 21.01. Found: C, 58.06; H, 4.39; N, 20.95.

Antimalarial activity

Preparation of parasites

The chloroquine sensitive 3D-7 and chloroquine resistant RKL-2 strain (Raurkela, Orissa, India) of *P. falciparum* were routinely maintained in stock cultures in the medium RPMI-1640 supplemented with 25 mmol of 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 0.8–1.5% 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 *et al.* (1978) in 96 well-microtitre plates,[21] with minor modifications. A stock solution of 5 mg mL^{-1} of each of the test samples was prepared in DMSO and subsequent dilutions were prepared with culture medium. The test compounds in 20 μL volume concentration at 5 μg mL^{-1} and 50 μg mL^{-1} in duplicate wells were incubated with the parasitized cell preparation at 37 °C in a candle jar. After 36–40 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 with Schizonts 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 were used as the starting model for this study. The protein was prepared, docked and the molecular dynamics simulation carried out. Computational analysis was 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 the 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} $Å^{-1}$.[22,23] Using the 'Binding Site' tool panel available in DS 2.5, the minimized protein structure was 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 the side chains of the residues in the binding site within the radius of the sphere were assumed to be flexible during the refinement of postdocking poses. The receptor with 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 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. LigandFit[23] protocol of DS 2.5 was used for the docking of ligands with wild and quadruple mutant *pf*-DHFR proteins.[24] 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.[25]

The receptor protein conformation was kept fixed during docking, and the docked poses were further minimized using the all-atom CHARMm (version c32b1) forcefield and smart minimization method (steepest descent followed by conjugate gradient) until the r.m.s gradient for 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 from the center of the binding site were kept flexible during minimization.

Conclusions

In conclusion, the present study describes the facile synthetic routes to give rise to bi-functional hybrid derivatives comprised of 4-aminoquinoline and 1,3,5-triazine and their evaluation as antimalarial agents against chloroquine sensitive (3D7) and chloroquine resistant (RKL-2) strain of *P. falciparum*. The observed activity profiles were further substantiated and illustrated using docking simulations performed using the Ligandfit module within Discovery Studio 2.5. Additional studies to develop newer analogues of this core scaffold are in progress and will be reported subsequently in the future.

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