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Original article

4-Anilinoquinoline triazines: A novel class of hybrid antimalarial agents

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ABSTRACT

A novel class of hybrid 4-anilinoquinoline triazines have been synthesized and evaluated in vitro for their antimalarial activity against CQ-sensitive 3D7 strain of *P. falciparum* as well as for their cytotoxicity toward VERO cell line. Five compounds (**19**, **20**, **23**, **41** and **45**) exhibited the antimalarial potency superior to CQ. Compounds **14** and **16** were found to be orally active at a dose of 100 mg/kg × 4 days against CQ-resistant strain of *P. yoelii*. Inhibition of β-hematin formation assay and molecular docking study has been conducted in order to gain insight into the mechanism of action of proposed targets for the 4-anilinoquinoline and triazine moiety of the hybrid compounds.

1. Introduction

Malaria, a deadly parasitic disease, is a major cause of concern to human health and economies of poor countries. The gravity of malaria reflects by the facts that 40% of the global population is at risk of infection, 1.5–2.5 million people die every year especially children under the age of five years [1]. There are four major species of the malaria parasite of which *Plasmodium falciparum* causes the most virulent form of malaria and is responsible for more than 95% of malaria-related morbidity and mortality [2]. Due to unavailability of effective vaccine, chemotherapy remains the only option for treatment of malaria [3]. Since the discovery of natural product quinine, structural modifications of its quinoline pharmacophore led to the development of most effective antimalarial agents namely chloroquine (CQ) mefloquine and amodiaquine (AQ) (Fig. 1) [4–6]. CQ has been the mainstay of malaria therapy for decades because of its efficacy, safety and low cost until the emergence and spread of CQ-resistance. Pyrimethamine-sulfadoxine (fansidar) was another best therapeutic option after CQ but rendered ineffective in most of malaria endemic regions due to spread of resistance [7,8]. Currently, natural endoperoxide artemisinin and its semi-synthetic derivatives (artemether, arteether and artesunate) are the most potent and fast acting antimalarials effective against resistant strains of *P. falciparum* [9]. In view of prevailing danger of resistance; combination therapy has been introduced as a measure to safeguard the few available antimalarial drugs. WHO recommended the use of artemisinin and its analogue in combination with 4-aminoquinoline antimalarials like lumefantrine and mefloquine [10,11]. However, the world-wide deployment of artemisinin based combination therapy is limited due to some serious issues like higher cost of treatment, safety in pregnancy and imbalance in demand than supply [12,13]. In addition, resistance to artemisinin derivatives was also emerged in Southeast Asian countries which will continue to evolve and spread, subsequently making malaria chemotherapy more complicated [14]. To keep pace with the continuously evolving resistant parasite, there is challenge and urgency to develop cost-effective and efficacious antimalarials with low potential of inducing resistance.

Despite the emergence of resistance to heme-targeted antimalarials mainly quinoline-based drugs, drug target heme is still being exploited extensively for designing new antimalarial agents because biochemical drug target heme cannot either be mutated or expressed by the parasite [15]. During the intraerythrocytic stage of life cycle, parasite invades the red blood cells of human host for feeding the hemoglobin which is a major source of nutrition for its growth and development. In the acidic food vacuole of the parasite, a toxic by-product free heme is generated as a result of hemoglobin

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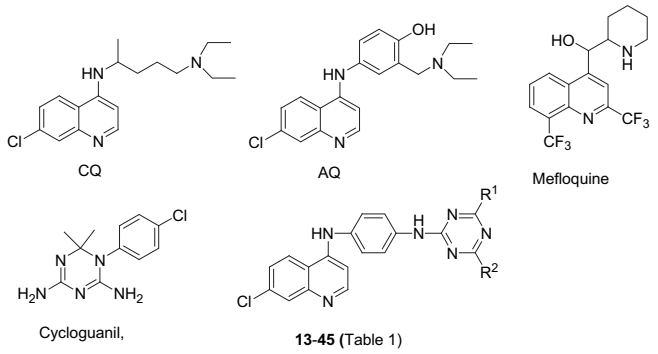


Fig. 1. Structures of CQ, AQ, mefloquine, cycloguanil and title compounds (**13**–**45**).

degradation into amino acids by enzymes aspartic, cysteine, and metallo proteases [16,17]. As a defense mechanism for its survival, parasite converts the free heme into non-toxic inert crystalline pigment called hemozoin [18]. CQ and other aminoquinoline drugs disrupt the conversion of toxic heme into hemozoin, consequently substantial accumulation of toxic heme lead to the death of the parasite [19–21]. Despite the persistent heavy drug pressure of CQ for several decades, the delayed emergence of resistance to CQ is considered due to the complexity of digestive vacuole environment and the immutable nature of heme target [22]. Multiple point mutations in *P. falciparum* chloroquine resistance transporter protein (*pfcrt*) conferred resistance to CQ characterized by the substantially reduced accumulation of CQ level in food vacuole. Interaction of CQ with *pfcrt* induces resistance very slowly to *P. falciparum* owing to the complexity in amino acid substitutions in pfcrt [23–25].

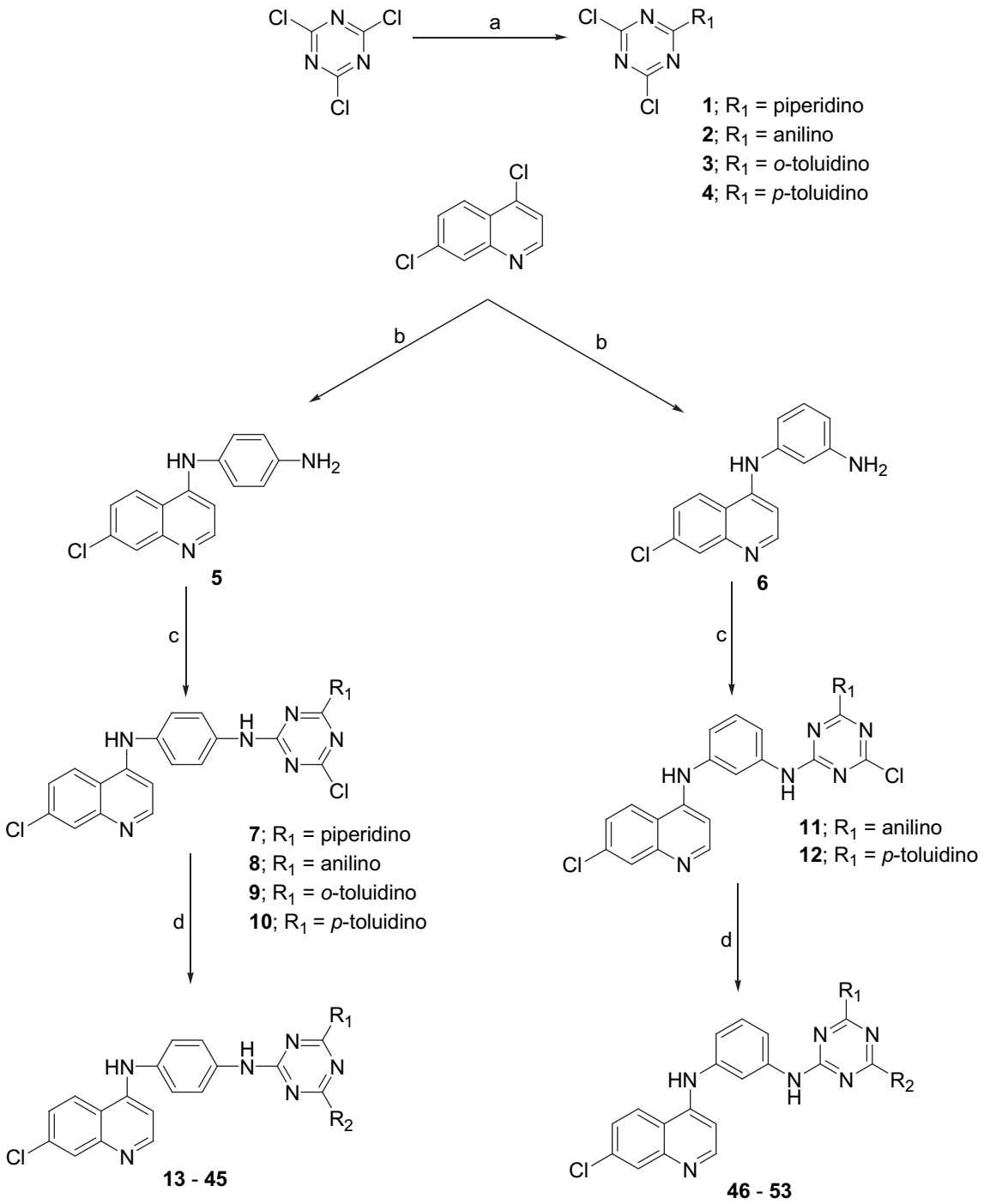
The comprehensive structure-activity relationship studies on CQ–Hematin binding have been explored to identify the optimal structural requirements for designing the new CQ-based antimalarial agents. It was established that the 7-chloro-4-aminoquinoline is critical for the antimalarial activity and basicity of the side chain nitrogen is also equally important for accumulation of drug within acidic food vacuole of the parasite [26,27]. Modification in the side chain of CQ led to the new CQ analogues with improved activity effective against CQ-resistant strain. Altering the chain length of CQ had the little effect on CQ-sensitive strain but produced the remarkable effect on the CQ-resistant strain suggesting that CQ-resistance is compound specific [28–30]. In addition to the side chain modified new CQ analogues; several functionalized 4-anilinoquinolines were identified as potential antimalarials. Amodiaquine is still clinically useful for low degree CQ-resistant parasites but it has some serious side effects like heptatotoxicity and agranulocytosis [31,32]. To prevent the formation of toxic metabolite, amodiaquine quinoneimine, a direct regioisomer of amodiaquine, isoquine was developed having potent in vitro activity against CQ-resistant parasites, however the unacceptable high first pass metabolism of isoquine to dealkylated metabolites did not allow further drug development process [33]. Modification of isoquine guided the discovery of drug candidate *N-tert*-butyl isoquine [34]. In addition, 4′-Fluoro-*N-tert*-butylamodiaquine was identified as a back-up compound for *N-tert*-butyl isoquine based on potent activity against CQ-sensitive and CQ-resistant parasites [35]. Thus, these findings manifest considerable scope for developing new antimalarial with quinoline nucleus.

Along with improvement of efficacy, preventing and delaying the emergence of drug resistance is an essential goal of antimalarial drug development. To combat the increasingly becoming resistant parasite, hybrid drug approach is gaining attention as a viable option for effective long term strategy of antimalarial chemotherapy [36]. Hybrid drug approach involves the incorporation of two drug pharmacophore in one single molecule with attention of dual drug action. Given the unique pharmacological effect of quinoline based antimalarials targeting the heme, quinoline moiety has been the integral component of designing hybrid antimalarials. It was exemplified by several potential antimalarials that include trioxaferroquines [37], trioxaquines [38], artemisinin-quinine hybrid [39], 4-aminoquinoline based tetraoxanes [40], clotrimazole-based 4-aminoquinolines [41], ferrocene-chloroquine analogues [42] inhibitors of glutathione reductase conjugated to a 4-aminoquinoline [43] and 4-aminoquinolines based on natural product scaffold isatin [44]. In addition, a chloroquine reversal agent hybrid of CQ-like moiety and imipramine was identified as potential antimalarial agent [45]. More recently, the dual function acridones as new antimalarial chemotype were discovered that combined the heme targeting character of acridones, together with a chemosensitizing component that counteracts resistance to quinoline antimalarial drugs [46].

The dihydrofolate reductase (DHFR) is one of the well-defined and successfully exploited targets in malarial chemotherapy. Pyrimethamine and cycloguanil (Fig. 1), the two important therapeutic drugs commonly employed for the prophylaxis and treatment of malaria target the DHFR. However, in the recent years, rapid spread of antifolate resistant *P. falciparum* seriously compromised the clinical utilities of these drugs and consequently necessitates the need to search for new potent antifolate antimalarials [8,47,48]. Toward this goal, additional pyrimethamine and cycloguanil analogues were identified as potential inhibitors of resistant DHFR [49–51]. Apart from this, structurally similar to cycloguanil, triazines have been reported to possess promising antimalarial activity [52]. As part of our research programme devoted to synthesis of various class of heterocycles as antimalarial agents, our group identified trisubstituted triazines [53], pyrazole based triazines [54], hybrid 4-aminoquinoline triazines [55], 9-anilinoacridinetriazines [56] and 4-aminoquinoline-based β-carbolines [57] as potential antimalarials. The 4-anilinoquinoline moiety has been shown to be responsible for CQ-sensitive and CQ-resistant activity in *P. falciparum*. We envisaged that combining two intrinsically active antimalarial moiety 4-anilinoquinoline and triazine would lead to develop more potent antimalarials. The present paper describes the synthesis and in vitro and in vivo antimalarial evaluation of 4-anilinoquinoline triazines. Inhibition of β-hematin formation assay and molecular docking study has been conducted in order to gain insight into the mechanism of action of proposed targets for the 4-anilinoquinoline and triazine moiety of the hybrid compounds.

2. Chemistry

Since malaria severely affects the health and economies of the poorest countries, low-cost of antimalarials are equally important alongside efficacy and safety. An easy access to the title compounds using readily available and inexpensive starting materials is a significant feature of our simple and efficient synthetic approach. The synthesis of title compounds **13**–**53** were achieved by a synthetic protocol as shown in Scheme 1.To begin with 4,6-dichloro-6-substituted-[1,3,5]triazine **1**–**4** were synthesized by nucleophilic substitution of cyanuric chloride with amines. Commercially available 4,7-dichloroquinoline was condensed with *p*-phenylenediamine/*m*-phenylenediamine in presence of catalyst *p*-TSA to afford the *N*-(7-chloro-quinolinyl-4-yl)-benzene-1,4-diamine **5** or *N*-(7-chloro-quinolinyl-4-yl)-benzene-1,3-diamine **6** [58]. The compound **5** or **6** was refluxed with 4,6-dichloro-6-substituted-[1,3,5]triazines in THF to



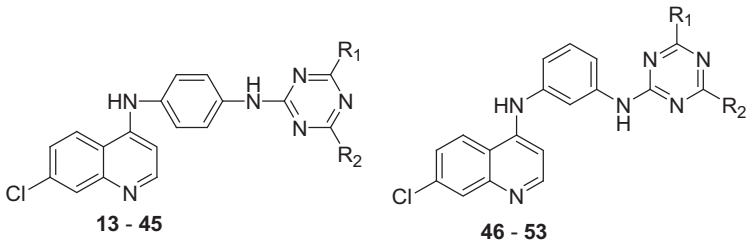
Scheme 1. Reagents and conditions: (a) anilines/piperidine, 0 °C, THF, 2 h; (b) *p*-phenylenediamine/*m*-phenylenediamine, *p*-TSA, EtOH, 3 h; (c) monosubstituted triazines, THF, reflux, 8 h; (d) various amines, THF, reflux, 5 h.

yield the corresponding 6-chloro-*N*-[4-(7-chloro-quinolinyl-4-yl-amino)-phenyl]- *N*′-substituted-[1,3,5]triazine-2,4-diamine **7**–**12**. The compounds (**7**–**12**) were subjected to nucleophilic substitution with various amines to furnish the title compounds **13**–**53** (Table 1).

3. Pharmacology

All the synthesized title compounds were evaluated in vitro for their antimalarial activity against CQ-sensitive strain 3D7 of *P. falciparum* using a standardized inexpensive assay based on SYBER Green I [59]. The IC_{50} values were calculated from experiments carried out in triplicate. The cytotoxicity of all the compounds was determined against VERO cell line using MTT assay [60]. The in vivo drug responses of selected compounds were evaluated in swiss mice infected with N-67 strain of *P. yoelii* which is innately resistant to CQ [62]. Effects of selected compounds upon the inhibition of β-hematin formation were investigated by using the β-hematin inhibitory (BHIA) assay [62].

Table 1
In vitro antimalarial activity of compounds against 3D7 strain of *P. falciparum* and their cytotoxicity on VERO cell lines.



Compound No.	R_1	R_2	IC_{50} (nM)[a]	SI[b]
13	piperidino	*N*-methyl piperazino	34.77	106.35
14	piperidino	*N*-ethyl piperazino	29.74	124.85
15	piperidino	*N*,*N*-diethylethylenediamino	38.77	59.71
16	piperidino	4-(3-aminopropyl)morpholino	23.13	170.18
17	piperidino	cyclohexylamino	313	189.52
18	piperidino	benzylamino	234.63	98.42
19	anilino	*N*-methyl piperazino	5.85	92.06
20	anilino	*N*-ethyl piperazino	5.92	192.66
21	anilino	n-butylamino	18.53	287.22
22	anilino	*N*,*N*-diethylethylenediamino	60.41	79.47
23	anilino	4-(3-aminopropyl)morpholino	3.01	154.29
24	anilino	cyclohexylamino	64.84	76.97
25	anilino	benzylamino	33.65	151.04
26	anilino	amino	41.5	132.63
27	anilino	methylamino	19.03	290.68
28	*o*-toluidino	*N*-methyl piperazino	18.85	16.33
29	*o*-toluidino	*N*-ethyl piperazino	102.43	2.58
30	*o*-toluidino	n-butylamino	31.52	821.15
31	*o*-toluidino	*N*,*N*-diethylethylenediamino	26.05	136.49
32	*o*-toluidino	4-(3-aminopropyl)morpholino	13.42	418.75
33	*o*-toluidino	cyclohexylamino	162.99	449.80
34	*o*-toluidino	benzylamino	27.78	2129.68
35	*o*-toluidino	amino	29.65	163.54
36	*o*-toluidino	methylamino	37.63	240.35
37	*p*-toluidino	*N*-methyl piperazino	12.44	36.39
38	*p*-toluidino	*N*-ethyl piperazino	11.90	35.61
39	*p*-toluidino	n-butylamino	44.49	101.03
40	*p*-toluidino	*N*,*N*-diethylethylenediamino	82.85	64.38
41	*p*-toluidino	4-(3-aminopropyl)morpholino	7.03	610.98
42	*p*-toluidino	cyclohexylamino	73.95	137.42
43	*p*-toluidino	benzylamino	63.50	79.44
44	*p*-toluidino	amino	11.88	134.89
45	*p*-toluidino	methylamino	6.41	491.91
46	anilino	*N*-methyl piperazino	47.17	223.40
47	anilino	*N*-ethyl piperazino	60.07	90.51
48	anilino	n-butylamino	26.30	156.25
49	anilino	*N*,*N*-diethylethylenediamino	291.73	135.37
50	anilino	4-(3-aminopropyl)morpholino	110.67	90.51
51	*p*-toluidino	*N*-methyl piperazino	43.17	226.18
52	*p*-toluidino	*N*-ethyl piperazino	40.95	229.50
53	*p*-toluidino	4-(3-aminopropyl)morpholino	34.56	321.48
CQ			8.15	8983

[a] IC_{50} (nM): concentration corresponding to 50% growth inhibition of the parasite.
[b] SI: Selectivity index (IC_{50} values of toxic activity/IC_{50} values of antimalarial activity).

4. Results and discussion

4.1. In vitro antimalarial activity

The antimalarial activity of all the synthesized hybrid 4-anilinoquinoline triazine derivatives was determined in vitro against the CQ-sensitive 3D7 strain of *P. falciparum*. In vitro activity results are summarized in Table 1. Considerable variations around the triazine nucleus of the hybrid compounds have been done in order to establish structure-activity relationship among the screened compounds. Among the 41 compounds evaluated, five compounds expressed the superior antimalarial potency to CQ, seven compounds exhibited the IC_{50} values ranging from 11.88 nM to 19.03 nM, 16 compounds displayed the IC_{50} values ranging from 23.13 nM to 44.49 nM, 7 compounds showed the IC_{50} values ranging from 47.17 nM to 82.85 nM and the rest six compounds showed the IC_{50} values ranging from 102.43 nM to 313 nM. As can be seen from Table 1, compounds having anilino unit as substituent at triazine nucleus have shown the superior potency among the screened compounds. While keeping *N*-methylpiperizino unit fixed, replacing the piperidino (**13**) with anilino (**19**) led to the six times increase in antimalarial potency (**13**, IC_{50} = 18.85 nM; **19**, IC_{50} = 3.15 nM). Further, introducing the methyl group at ortho (**28**) and para (**37**) position to the phenyl ring (**19**) resulted in three times and two times

decrease in antimalarial potency, respectively (**28**, IC_{50} = 10.41 nM; **37**, IC_{50} = 6.87 nM). In case of compounds having piperidino, anilino, and *p*-toluidino unit, replacement of *N*-mehtylpiperazino with *N*-ethyl piperizino had slight effect on the antimalarial activity pattern. Surprisingly, compounds having *o*-toluidino as substituent, replacing the *N*-methylpiperizino (**28**) with *N*-ethyl piperizino (**29**) produced dramatic decrease in the antimalarial activity (**28**, IC_{50} = 18.85 nM; **29**, IC_{50} = 102.43 nM). The IC_{50} values for compounds substituted with n-butylamino and *N,N*-diethylethylenediamino were relatively high. These results suggest that n-butylamino and *N,N*-diethylethylenediamino substituents were not favorable for the antimalarial activity. Compound **23** having anilino and aminopropyl morpholino substituents was the most potent among the evaluated compounds being the almost three times more active than CQ (**23**, IC_{50} = 3.01 nM). Aminopropyl morpholino in combination with piperidino (**16**), *o*-toluidino (**32**) and *p*-toluidino (**41**) produced good in vitro antimalarial activity with IC_{50} values of 23.13 nM, 13.42 nM and 7.03 nM, respectively. This observation indicates that aminopropyl morpholino substituent was highly favorable for antimalarial effect. Introducing the cyclohexylamino and benzylamino units to the triazine nucleus displayed the poor antimalarial activity. It might be attributed to the non-basic nature of these substituents. Compounds **26**, **35**, and **44** with amino group exhibited modest to good antimalarial activity (**26**, IC_{50} = 41.51 nM; **35**, IC_{50} = 29.65 nM; **44**, IC_{50} = 11.86 nM). Replacing the amino group with methylamino group, the corresponding compounds having anilino (**27**) and *p*-toluidino (**45**) led to the two times improvement of the antimalarial potency while the activity was considerably reduced for the compound substituted with *o*-toluidino group. A small set of molecules (**46**–**53**) with *m*-phenylenediamine linker were synthesized in order to investigate the effect of position of linkage on the antimalarial activity. Comparison of the antimalarial activity of compounds with the corresponding *p*-phenylenediamine derived compounds reflects that *m*-phenylenediamine linker significantly reduced the antimalarial potency. Compounds **46** and **47** were 8 and 10 times less active than compounds **19** and **20**, respectively. Similarly, Compounds **51** and **52** were almost 3 times less potent than compounds **37** and **38**. Compound **49** was virtually inactive with an IC_{50} value of 291.73 nM. Surprisingly, compound **50** with aminopropyl morpholine was considerably less active with an IC_{50} value of 110.67 nM given the highly favorable nature of aminopropyl morpholine on the antimalarial activity as exemplified by compounds with *p*-phenylenediamine linker. However, introducing the methyl group to the para position (**53**) of phenyl ring (**50**) resulted in 3 times improvement of the antimalarial activity.

The structure-activity relationship emerged from the activity results revealed that combination of aminopropyl morpholino with piperidino, anilino, *o*-toluidino and *p*-toluidino, combination of *N*-methylpiperizino with anilino, *o*-toluidino and *p*-toluidino, combination of amino with *p*-toluidino and combination of methylamino with anilino and *p*-toluidino at the 4- and 6-positions of the triazine nucleus were well tolerated for the in vitro antimalarial activity.

4.2. In vitro cytotoxicity

The cytotoxicity of all the synthesized hybrid 4-anilinoquinoline triazines was determined against VERO cells using MTT assay (Table 1) [60]. Compounds **19**, **20**, **23**, **41** and **45** which have shown in vitro antimalarial activity (IC_{50} range = 3.01–7.03 nM) superior to CQ possessed the selective index ranging from 92.06 to 610.98. Thus these compounds demonstrated the promising safe activity profile. Compounds **32**, **37**, **38**, and **44** which were slightly less active (IC_{50} range = 11.90–13.42 nM) than CQ displayed the fairly safe selectivity index range from 35.61 to 418.75. Compound **34** with an IC_{50} value of 27.78 nM exhibited the highest selectivity 2129.68 while compound **29** having an IC_{50} value of 102.43 nM was the most toxic with selectivity index 2.58. In general, most of the compounds having good in vitro activity endowed with fairly high selectivity index.

4.3. In vivo antimalarial activity

Compounds characterized by good in vitro antimalarial activity were selected for in vivo study. Compounds were tested in swiss mice infected with CQ-resistant N-67 strain of *P. yoelii* parasite (Table 2) [61]. Initially; in vivo activity of compounds was evaluated by intraperitoneal route at the dose 50 mg/kg × 4 days once daily. Compounds **14, 16**, and **23** provided the 100% protection to the treated mice at 50 mg/kg × 4 days while the rest of compounds only managed to exert suppressive effect. Compounds **14**, **16**, and **23** when further screened at 25 mg/kg by i.p. route exhibited the complete clearance of parasitemia on day 4 but none of the mice survived beyond day 28. It is interesting that compound **23** substituted with anilino unit was active by i.p route while introducing the methyl group (**41**) to the para position of phenyl ring (**23**) abolished the in vivo antimalarial activity. Compounds **14**, **16** and **23** active by i.p route were further assessed for oral activity. Compounds **14** and **16** having piperidino substituent provided the 100% protection to the treated mice at the dose 100 mg/kg for four days whereas compound **23** with anilino substituent was inactive. This observation suggests that oral activity of compounds **14** and **16** having piperidino substituent might be attributed to their more metabolic stability than compound **23** with anilino substituent. Though the efficacy of orally active compounds **14** and **16** was found to be modest, considering the hybrid nature of compounds able to overcome CQ-resistance, these molecules represent promising leads for the development of novel class of potent antimalarials.

4.4. β-Hematin inhibitory activity

The selected compounds were tested for their ability to inhibit the β-hematin formation by using the β-hematin inhibitory activity (BHIA) assay (Table 3) [62]. All the tested compounds showed a dose dependent inhibition in the BHIA assay and exhibited the stronger β-hematin inhibitory activity than CQ. Compounds **14**, **16**,

Table 2
In vivo antimalarial activity of selected compounds against CQ-resistant N-67 strain of *P. yoelii* in swiss mice.

Compound	Dose (mg/kg × 4 days)	% Suppression on day 4	Mice alive on day 28
14	50 (i.p.)	100	5/5
	25 (i.p.)	100	0/5
	100 (oral)	100	5/5
	50 (oral)	100	0/5
16	50 (i.p.)	100	5/5
	25 (i.p.)	100	0/5
	100 (oral)	100	5/5
	50 (oral)	100	0/5
20	50 (i.p.)	100	0/5
21	50 (i.p.)	100	0/5
23	50 (i.p.)	100	5/5
	25 (i.p.)	100	0/5
	100 (oral)	100	0/5
30	50 (i.p.)	100	0/5
41	50 (i.p.)	100	0/5
45	50 (i.p.)	100	0/5
48	50 (i.p.)	100	0/5
53	50 (i.p.)	100	0/5

Table 3
β-hematin inhibitory activity of selected compounds.

Compound	IC_{50}[a]
14	2.79
16	2.71
20	2.69
21	3.08
23	2.98
30	2.96
41	2.85
45	3.16
48	2.55
53	2.70
CQ	3.65

Data are the mean of three different experiments in triplicate.
[a] The IC_{50} represents the concentration of compound that inhibit β-hematin formation by 50%.

21 and **30** exerted the similar inhibitory effect on the β-hematin formation like compounds **20**, **23**, **41** and **45** but exhibited the decreased in vitro antimalarial activity (Table 1). These results suggested that apart from 4-anilinoquinoline triazine derivatives targeting heme, the possibility of other mechanism of action for the antimalarial activity cannot be ruled out.

4.5. Molecular docking studies

Molecular docking studies of 4-anilinoquinoline triazine derivatives were performed on the binding model based on the *P. falciparum* Dihydrofolate Reductase- Thymidylate Synthase (pfDHFR-TS) complexed With Wr99210 (Fig. 2) to identify a plausible binding mode (Fig. 3). LigandFit, a modern docking program within Cerius2 version 4.10 (Cerius2 Version 4.10 (2005), Accelrys Inc., San Diego, USA), was used for docking runs [63]. The representative compounds **14**–**16**, **18**, **20**, **21**, **23**, **30**, **33**, **40**, **41**, **45**, **48**, **53** were subjected to molecular docking studies. In this model, most of the derivatives bind in more or less similar fashion with its phenyl ring occupying the interior of the deep hydrophobic cleft, while the quinoline moiety containing lateral chain toward the solvent. The hydrophobic and π–π stacking interactions seem to be mainly responsible for orientation of these analogues in pfDHFR binding pocket. In order to compare the binding pattern of these molecules, the compound **45** that produced the best result in docking analysis was analyzed, along with the active site of the crystallographic structure of pfDHFR. All the amino acid residues which had interactions with *P. falciparum* DHFR were showed in Fig. 4. In the binding model of compound **45** and *P. falciparum* DHFR, carbonyl oxygen of Ser111 forms hydrogen bond with hydrogen of methylamino group substituent attached with triazine ring of compound **45** which is found to be conserved in other analogues also. Moreover, hydrophobic interactions probably existed between compound **45** and Ile14, Ala16, Leu56, Val45, Phe58 and Ile164 residues of active site while benzene ring act as possible basic and π-donor site and is involved in edge to face strong π–π stacking interactions with the residues of Phe58, and nicotinamide ring of

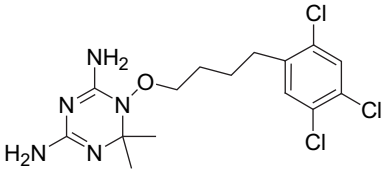


Fig. 2. Structure of WR99210.

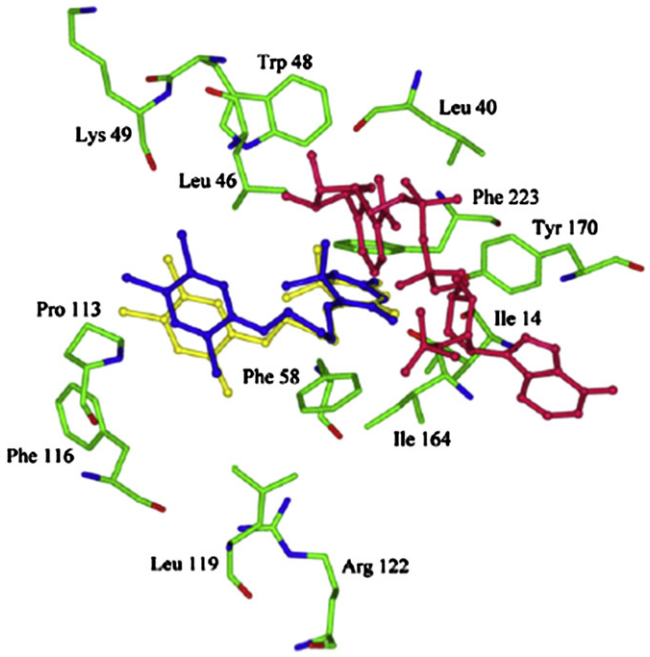


Fig. 3. Structure of WR99210 and its docked conformation in yellow. Crystal structure conformation of WR99210 (blue) in the pfDHFR. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

NADP. This edge to face or T shaped aromatic stacking interaction seems very important for pfDHFR inhibition and is responsible for the protein–ligand complex. Due to the non-aromatic and nonplanar nature of piperidine derivatives, compounds **14**, **15** and **16** were not able to accomodate inside the same binding pocket suggests that non-aromatic bulky substituents at this position may cause steric clashes with active site residues. As revealed from the docked conformation, the phenyl moiety attached with the aminoquinoline subunit make lipophilic interactions with Pro113 and Phe116 and it extends toward the solvent. It is hypothesized that these π–π stacking interactions are responsible for optimal orientation of 4-*p*-toluidino-6-methylamino triazine part of the hybrid compound into the binding pocket. These results, along with in vitro antimalarial activity of compounds against 3D7 strain of *P. falciparum* suggest that traizine moiety targeting the pfDHFR seems to account for in part the antimalarial activity of hybrid compounds.

5. Conclusions

Despite the extensive research efforts directed toward the development of new drugs, malaria continues to devastate the poorest countries due to the lack of affordable and efficacious drugs after the resistance to the most widely employed and successful drugs, CQ and fansidar. Therefore, there is challenge and urgency to develop the affordable, safe and effective drugs with low potential of inducing resistance. In search of new antimalarials, a new class of hybrid 4-anilinoquinoline triazine derivatives was synthesized using readily available and inexpensive chemicals. The assessment of compounds for in vitro antimalarial activity against 3D7 strain of *P. falciparum* led to the identification of five potent compounds superior to CQ. Most of the compounds have shown the fairly high selectivity index. Compounds **14** and **16** were found to be orally active at a dose of 100 mg/kg × 4 days in swiss mice infected with CQ-resistance N-67 strain of *P. yoelii*. Though the efficacy of orally active compounds **14** and **16** was modest, considering the hybrid nature of compounds able to overcome CQ-resistance, these

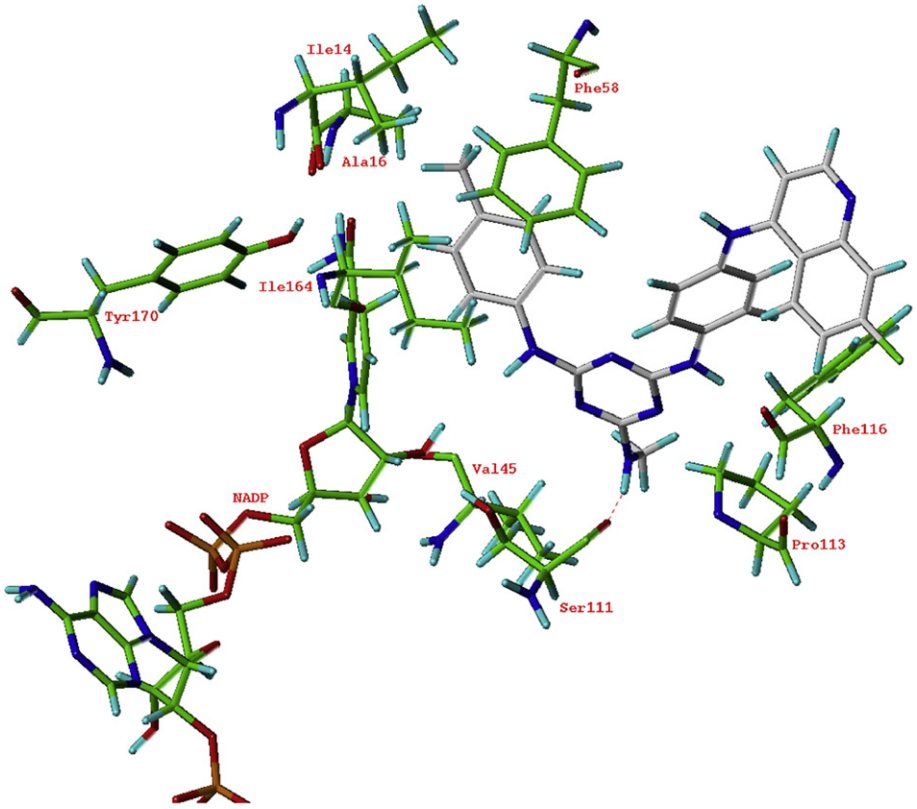


Fig. 4. Docked conformation of **45** in the pfDHFR.

molecules represent promising leads for the development of novel class of potent antimalarials.

6. Experimetal

6.1. General

IR spectra were recorded on Beckman Aculab-10, Perkin Elmer 881 and FTIR 8210 PC, Schimadzu spectrophotometers either on KBr discs or in neat. Nuclear magnetic resonance (NMR) spectra were recorded on either Bruker Avance DRX-300 MHz or Bruker DPX 200 FT spectrometers using TMS as an internal reference. The samples (dissolved in suitable solvents such as methanol/acetonitrile/water) were introduced into the ESI source through a syringe pump at the rate of 5 μl per min. The ESI capillary was set at 3.5 kV and the cone voltage was 40 V. The spectra were collected in 6 s scans and the print outs are averaged spectra of 6–8 scans. In case of multiplets the signals are reported as intervals. Signals were abbreviated as s, singlet; d, doublet; t, triplet; m, multiplet. The electron spray mass spectra were recorded on triple quadrupole mass spectrometer. EI mass spectra were recorded on JEOL JMS-D-300 spectrometer with the ionization potential of 70 eV and ES mass on Quantro-II, micro mass. Purity of all tested compounds was ascertained on the basis of their elemental analysis and was carried out on Carlo-Erba-1108 instrument. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

6.2. General procedure for the synthesis of monosubstituted triazine derivatives (*1*–*4*)

To an ice cold solution of cyanuric chloride (1.0 equiv.) and K_2CO_3 (2 equiv.) in dry THF was added dropwise the solution of desired amine in THF for 20 min and stirred for 2 h at room temperature. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was purified by column chromatography using ethylacetate–hexane (10:100) as eluents to afford the desired products **1**–**4**.

6.2.1. 2,4-Dichloro-6-piperidin-1-yl-[1,3,5]triazine (*1*)

Yield: 78%; mp 72–74 °C; MS: 233 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 3.83 (t, 4H, J = 5.7 Hz, N–CH_2). Anal. Calc. for $C_8H_{10}Cl_2N_4$: Calculated C: 41.22, H: 4.32, N: 24.04. Found C: 41.03, H: 4.21, N: 24.33.

6.2.2. (4,6-Dichloro-[1,3,5]triazin-2-yl)-phenyl-amine (*2*)

Yield: 92%; mp 136–138 °C; MS: 241 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.61 (bs, 1H, NH), 7.74 (d, 2H, J = 7.74 Hz, Ar–H), 7.41 (t, 2H, J = 7.59 Hz, Ar–H), 7.21 (t, 1H, J = 7.35 Hz, Ar–H). Anal. Calc. for $C_9H_6Cl_2N_4$: Calculated C: 44.84, H: 2.51, N: 23.24. Found C: 44.69, H: 2.37, N: 23.42.

6.2.3. (4,6-Dichloro-[1,3,5]triazin-2-yl)-o-tolyl-amine (*3*)

Yield: 90%; mp 132–134 °C; MS: 255 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.69 (d, 1H, J = 7.86 Hz, Ar–H), 7.3 (bs, 1H, NH), 7.29–7.21 (m. 3H, Ar–H). Anal. Calc. for $C_{10}H_8Cl_2N_4$: Calculated C: 47.08, H: 3.16, N: 21.96. Found C: 46.87, H: 3.26, N: 22.11.

6.2.4. (4,6-Dichloro-[1,3,5]triazin-2-yl)-p-tolyl-amine (*4*)

Yield: 94%; mp 142–144 °C; MS: 255 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 7.51 (bs, 1H, NH), 7.42 (d, 2H, J = 8.37 Hz, Ar–H), 7.22 (d, 2H, J = 8.37 Hz, Ar–H). Anal. Calc. for $C_{10}H_8Cl_2N_4$: Calculated C: 47.08, H: 3.16, N: 21.96. Found C: 47.29, H: 3.21, N: 21.78.

6.3. General procedure for the synthesis of 4-anilinoquinolines (*5* & *6*)

A solution of 4,7-dichloroquinoline (1.0 equiv.) and *p*-phenylenediamine or *m*-phenylenediamine (2.0 equiv.) in absolute

ethanol was refluxed in presence of p-TSA as a catalyst for 3 h. During the refluxing, precipitation of product occurred. The precipitate was collected through filtration, washed with ethanol and dried under vacuum to get the desired 4-anilinoquinoline **5** & **6** with excellent yields.

*6.3.1. N-(7-Chloro-quinolin-4-yl)-benzene-1,4-diamine (**5**)*

Yield: 95%; mp >210 °C; MS: 269 (M + 1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.54 (d, 1H, J = 5.31 Hz, Ar–H), 8.04 (d, 1H, J = 2.04 Hz, Ar–H), 8.85 (d, 1H, J = 8.97 Hz, Ar–H), 7.54 (d, 2H, J = 8.74 Hz, Ar–H), 7.49 (dd, 1H, J = 2.04, 8.97 Hz, Ar–H), 7.10 (d, 2H, J = 8.74 Hz, Ar–H), 6.83 (d, 1H, J = 5.31, Ar–H). Anal. Calc. for $C_{15}H_{12}ClN_3$: Calculated C: 66.79, H: 4.48, N: 15.58. Found C: 66.87, H: 4.29, N: 15.83.

*6.3.2. N-(7-Chloro-quinolin-4-yl)-benzene-1,3-diamine (**6**)*

Yield: 93%; mp 205–207 °C; MS: 270 (M + 1); ^{1}H NMR (300 MHz, DMSO-d_6): δ 8.56 (d, 1H, J = 5.32 Hz, Ar–H), 8.06 (d, 1H, J = 1.98 Hz, Ar–H), 7.94 (s, 1H, Ar–H), 7.87 (d, 1H, J = 8.94 Hz, Ar–H), 7.44 (dd, 1H, J = 1.98, 8.94 Hz, Ar–H), 7.34 (t, 1H, J = 7.98 Hz, Ar–H), 7.18 (d, 1H, J = 7.21 Hz, Ar–H), 6.96 (d, 1H, J = 7.22 Hz, Ar–H), 6.89 (d, 1H, J = 5.32 Hz, Ar–H). Anal. Calc. for $C_{15}H_{12}ClN_3$: Calculated C: 66.79, H: 4.48, N, 15.58. Found C: 67.06, H: 4.61, N, 15.49.

*6.4. General procedure for the synthesis of compounds (**7**–**12**)*

A reaction mixture of 4-anilinoquinoline (1.0 equiv.), mono-substituted triazine (1–4, 1.5 equiv.) and K_2CO_3 (2.0 equiv.) in dry THF was refluxed for 8 h. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in $CHCl_3$, washed with water and dried over Na_2SO_4. The solution was concentrated under reduced pressure and purified by column chromatography using MeOH-$CHCl_3$ (1:99) as eluents to afford the pure compounds (**7**–**12**).

*6.4.1. N-(4-Chloro-6-piperidin-1-yl-[1,3,5]triazin-2-yl)-N′-(7-chloro-quinolin-4-yl)-benzene-1,4-diamine (**7**)*

Yield: 72%; mp 135–138 °C; MS: 466 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.56 (bs, 1H, NH), 9.29 (bs, 1H, NH), 8.35 (d, 1H, J = 5.28 Hz, Ar–H), 8.25 (d, 1H, J = 9.03 Hz, Ar–H), 7.88 (d, 1H, J = 1.65 Hz, Ar–H), 7.68 (d, 2H, J = 7.65 Hz, Ar–H), 7.48 (d, 2H, J = 7.52 Hz, Ar–H), 7.35 (dd, 1H, J = 1.65 Hz, J = 9.03 Hz, Ar–H), 7.21 (d, 2H, J = 7.65 Hz, Ar–H), 7.04 (d, 2H, J = 7.52 Hz, Ar–H), 6.75 (d, 1H, J = 5.28 Hz, Ar–H), 2.35 (s, 3H, CH_3). Anal. Calc. for $C_{23}H_{21}Cl_2N_7$: Calculated C: 59.23, H: 4.54, N: 21.02. Found C: 59.41, H: 4.39, N: 21.18.

*6.4.2. 6-Chloro-N-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N′-phenyl-[1,3,5]triazine-2,4-diamine (**8**)*

Yield: 75%; mp 161–163 °C; MS: 474 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.91 (bs, 2H, NH), 8.40 (d, 1H, J = 5.49 Hz, Ar–H), 8.37 (d, 1H, J = 9.09 Hz, Ar–H), 7.87 (d, 1H, J = 2.07 Hz, Ar–H), 7.74–7.65 (m, 4H, Ar–H), 7.41 (dd, 1H, J = 2.07 Hz, J = 9.09 Hz, Ar–H), 7.30–7.25 (m, 4H, Ar–H), 7.03 (t, 1H, J = 7.32 Hz, Ar–H), 6.81 (d, 1H, J = 5.49 Hz, Ar–H). Anal. Calc. for $C_{24}H_{17}Cl_2N_7$: Calculated C: 60.77, H, 3.61, N: 20.67. Found C: 60.94, H, 3.52, N: 20.77.

*6.4.3. 6-Chloro-N-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N′-o-tolyl-[1,3,5]triazine-2,4-diamine (**9**)*

Yield: 71%; mp 167–169 °C; MS: 488 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.94 (bs, 1H, NH), 9.23 (bs, 1H, NH), 8.40–8.35 (m, 2H, Ar–H), 7.90 (d, 1H, J = 1.95 Hz, Ar–H), 7.76–7.63 (m, 3H, Ar–H), 7.43 (dd, 1H, J = 1.98 Hz, J = 8.92 Hz, Ar–H), 7.25–7.12 (m, 4H, Ar–H), 6.74 (d, 1H, J = 5.32 Hz, Ar–H), 2.31 (s, 3H, CH_3). Anal. Calc. for $C_{25}H_{19}Cl_2N_7$: Calculated: C: 61.48, H: 3.92; N: 20.08. Found C: 61.35, H: 4.05; N: 20.23.

*6.4.4. 6-Chloro-N-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N′-p-tolyl-[1,3,5]triazine-2,4-diamine (**10**)*

Yield: 72%; mp 172–174 °C; MS: 488 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$ + DMSO-d_6): δ 9.56 (bs, 1H, NH), 9.29 (bs, 1H, NH), 8.35 (d, 1H, J = 5.28 Hz, Ar–H), 8.25 (d, 1H, J = 9.03 Hz, Ar–H), 7.88 (d, 1H, J = 1.65 Hz, Ar–H), 7.68 (d, 2H, J = 7.65 Hz, Ar–H), 7.48 (d, 2H, J = 7.52 Hz, Ar–H), 7.35 (dd, 1H, J = 1.65 Hz, J = 9.03 Hz, Ar–H), 7.21 (d, 2H, J = 7.65 Hz, Ar–H), 7.04 (d, 2H, J = 7.52 Hz, Ar–H), 6.75 (d, 1H, J = 5.28 Hz, Ar–H), 2.35 (s, 3H, CH_3). Anal. Calc. for $C_{25}H_{19}Cl_2N_7$: Calculated C: 61.48, H: 3.92, N: 20.08. Found C: 61.67, H: 3.85, N: 20.11.

*6.4.5. 6-Chloro-N-[3-(7-chloro-quinolin-4-ylamino)-phenyl]-N′-phenyl-[1,3,5]triazine-2,4-diamine (**11**)*

Yield: 65%; mp 171–173 °C; MS: 474 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (d, 1H, J = 5.28 Hz, Ar–H), 8.07 (d, 1H, J = 2.04 Hz, Ar–H), 7.92 (s, 1H, Ar–H), 7.86 (d, 1H, J = 7.93 Hz, Ar–H), 7.56 (d, 2H, J = 7.86 Hz, Ar–H), 7.51 (dd, 1H, J = 2.04, 8.93 Hz, Ar–H), 7.34 (t, 1H, J = 7.86 Hz, Ar–H), 7.31–7.25 (m, 3H, Ar–H), 7.11 (bs, 1H, NH), 7.04–6.93 (m, 4H, Ar–H). Anal. Calc. for $C_{24}H_{17}Cl_2N_7$: Calculated C: 60.77, H: 3.61, N: 20.67. Found C: 60.45, H: 3.74, N: 20.89.

*6.4.6. 6-Chloro-N-[3-(7-chloro-quinolin-4-ylamino)-phenyl]-N′-p-tolyl-[1,3,5]triazine-2,4-diamine (**12**)*

Yield: 67%; mp 165–167 °C; MS: 488 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.59 (d, 1H, J = 5.32 Hz, Ar–H), 8.07 (d, 1H, J = 2.01 Hz, Ar–H), 7.93 (s, 1H, Ar–H).7.87 (d, 1H, J = 8.82 Hz, Ar–H), 7.49 (dd, 1H, J = 2.01, 8.82 Hz, Ar–H). 7.46 (d, 2H, J = 8.45 Hz, Ar–H), 7.35 (t, 1H, J = 8.1 Hz, Ar–H). 7.18 (d, 1H, J = 7.32 Hz, Ar–H), 7.11–7.07 (m, 3H, Ar–H), 6.97 (d, 1H, J = 5.32 Hz, Ar–H), 6.91 (bs, 1H, NH), 6.78 (bs, 1H, NH), 6.63 (bs, 1H, NH). Anal. Calc. for $C_{25}H_{19}Cl_2N_7$: Calculated C: 61.48, H: 3.92, N: 20.08. Found C: 61.27, H: 4.03, N: 20.21.

*6.5. General procedure for the synthesis of titled compounds (**13**–**53**)*

A solution of compound (**7**–**12**, 1.0 equiv.), desired amine (2.0 equiv.) and K_2CO_3 (2.0 equiv.) in dry THF was refluxed for 5 h. The reaction mixture was filtered and concentrated under reduced pressure. The resulting residue was dissolved in $CHCl_3$, washed with water and dried over Na_2SO_4. The dried solution was concentrated under reduced pressure. The resulting crude mass was purified by column chromatography using 2% MeOH/$CHCl_3$ as eluents to obtain the titled compounds (**13**–**53**).

*6.5.1. N-(7-Chloro-quinolin-4-yl)-N′-[4-(4-methyl-piperazin-1-yl)-6-piperidin-1-yl-[1,3,5]triazin-2-yl]-benzene-1,4-diamine (**13**)*

Yield: 92%; mp >230 °C; MS: 530 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.31 Hz, Ar–H)), 8.04 (d, 1H, J = 2.07 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.68 (d, 2H, J = 8.76 Hz, Ar–H), 7.48 (dd, 1H, J = 2.07 Hz, J = 9.00 Hz, Ar–H), 7.27 (d, 2H, J = 8.76 Hz, Ar–H), 6.84 (d, 1H, J = 5.31 Hz, Ar–H), 6.79 (bs, 1H, NH), 6.60 (bs, 1H, NH), 3.86 (t, 4H, J = 4.62 Hz, CH_2), 3.78 (t, 4H, J = 5.37 Hz, N–CH_2), 2.47 (t, 4H, J = 5.37 Hz, N–CH_2), 2.36 (s, 3H, CH_3), 1.68–1.61 (m, 6H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d6): δ 167.90, 167.56, 167.15, 154.13, 151.89, 140.61, 137.71, 135.92, 129.92, 127.95, 127.31, 126.65, 123.15, 120.86, 103.82, 57.54, 48.69, 46.94, 45.59, 28.54, 27.58. Anal. Calc. for $C_{28}H_{32}ClN_9$: Calculated C: 63.44, H: 6.08, N: 23.78. Found C: 63.26, H: 6.13, N: 23.95.

*6.5.2. N-(7-Chloro-quinolin-4-yl)-N′-[4-(4-ethyl-piperazin-1-yl)-6-piperidin-1-yl-[1,3,5]triazin-2-yl]-benzene-1,4-diamine (**14**)*

Yield: 92%; mp 215–218 °C; MS: 544 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.1 Hz, Ar–H), 7.88 (d, 1H, J = 9.03 Hz, Ar–H), 7.68 (d, 2H, J = 8.76 Hz, Ar–H), 7.49 (dd, 1H, J = 2.1 Hz, J = 9.03 Hz, Ar–H), 7.26 (d, 2H, J = 8.76 Hz, Ar–H), 6.84 (d, 1H, J = 5.34 Hz, Ar–H), 6.76 (bs, 1H, NH), 6.57 (bs, 1H, NH), 3.86 (t, 4H, J = 4.86 Hz, N–CH_2), 3.78 (t, 4H, J = 5.43 Hz, N–CH_2), 2.52–2.45 (m, 6H, N–CH_2), 1.68–1.61 (m, 6H, CH_2), 1.15 (t, 3H, J = 7.14 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.84, 163.62, 163.11, 150.59, 148.26, 147.42, 136.34, 133.89, 131.73, 127.51, 124.51, 123.34, 119.99, 119.22, 116.44, 100.53, 51.45, 51.16, 43.01, 41.87, 24.53, 23.61, 10.63. Anal. Calc. for $C_{29}H_{34}ClN_9$: Calculated C: 64.02, H: 6.30, N: 23.17. Found C: 64.27, H: 6.08, N: 23.42.

*6.5.3. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(2-diethylamino-ethyl)-6-piperidin-1-yl-[1,3,5]triazine-2,4-diamine (**15**)*

Yield: 85%; mp 150–151 °C; MS: 546 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.34 Hz, Ar–H), 8.04 (d, 1H, J = 2.04 Hz, Ar–H), 7.90 (d, 1H, J = 9.00 Hz, Ar–H), 7.69 (d, 2H, J = 7.92 Hz, Ar–H), 7.48 (dd, 1H, J = 2.04, J = 9.00 Hz, Ar–H), 7.26 (d, 2H, J = 7.92 Hz, Ar–H), 6.83 (d, 1H, J = 5.34 Hz, Ar–H), 6.8 (bs, 1H, NH), 6.67 (bs, 1H, NH), 5.42 (bs, 1H, NH), 3.78 (t, 4H, J = 5.21 Hz, N–CH_2), 3.48 (t, 2H, J = 5.52 Hz, CH_2), 2.70–2.55 (m, 6H, N–CH_2), 1.68–1.61 (m, 6H, CH_2), 1.07 (t, 6H, J = 4.65 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.51, 163.01, 150.48, 148.16, 147.57, 136.15, 133.92, 131.89, 127.31, 124.48, 123.30, 120.30, 119.42, 116.49, 100.44, 50.54, 45.49, 45.32, 43.01, 37.02, 24.55, 23.59, 10.16. Anal. Calc. for $C_{29}H_{36}ClN_9$: Calculated C: 63.78, H: 6.64, N: 23.08. Found C: 63.56, H: 6.92, N: 23.19.

*6.5.4. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(3-morpholin-4-yl-propyl)-6-piperidin-1-yl-[1,3,5]triazine-2,4-diamine (**16**)*

Yield: 87; mp 116–118 °C; MS: 574 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.31 Hz, Ar–H), 8.04 (d, 1H, J = 1.8 Hz, Ar–H), 7.89 (d, 1H, J = 9.0 Hz, Ar–H), 7.70 (d, 2H, J = 8.04 Hz, Ar–H), 7.47 (dd, 1H, J = 1.8 Hz, J = 9.0 Hz, Ar–H), 7.26 (d, 2H, J = 8.04 Hz, Ar–H), 6.84 (d, 1H, J = 5.31 Hz, Ar–H), 6.63 (bs, 1H, NH), 5.74 (bs, 1H, NH), 5.33 (bs, 1H, NH), 3.79–3.76 (m, 8H, N–CH_2 & O–CH_2), 3.53 (t, 2H, J = 6.03 Hz, N–CH_2), 2.52–2.48 (m, 6H, N–CH_2), 1.79 (quint, 2H, J = 6.03 Hz, CH_2), 1.68–1.61 (m, 6H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.50, 163.02, 150.57, 148.22, 147.48, 136.19, 133.90, 131.87, 127.43, 124.50, 123.27, 120.16, 119.42, 116.47, 100.46, 65.74, 55.59, 52.43, 42.99, 38.62, 24.70, 24.56, 23.60. Anal. Calc. for $C_{30}H_{36}ClN_9O$: Calculated C: 62.76, H: 6.32, N: 21.96. Found C: 62.95, H: 6.45, N: 22.15.

*6.5.5. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-cyclohexyl-6-piperidin-1-yl-[1,3,5]triazine-2,4-diamine (**17**)*

Yield: 85%, mp 156–158 °C; MS: 529 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51(d, 1H, J = 5.31 Hz, Ar–H), 8.02 (d, 1H, J = 1.68 Hz, Ar–H), 7.86 (d, 1H, J = 8.94 Hz, Ar–H), 7.65 (d, 2H, J = 8.64 Hz, Ar–H), 7.46 (dd, 1H, J = 1.68, J = 8.94 Hz, Ar–H), 7.23 (d, 2H, J = 8.64 Hz, Ar–H), 6.87 (bs, 1H, NH), 6.81 (d, 1H, J = 5.31 Hz, Ar–H), 6.59 (bs, 1H, NH), 4.83 (bs, 1H, NH), 3.81–3.74 (m, 5H, CH & CH_2), 2.05–2.02 (m, 2H, CH_2), 1.71–1.60 (m, 6H, CH_2), 1.45–1.33 (m, 2H, CH_2), 1.25–1.14 (m, 4H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 165.38, 164.48, 164.46, 152.22, 149.87, 149.28, 138.23, 134.13, 133.25, 127.95, 124.99, 124.68, 124.35, 120.54, 118.30, 101.22, 49.30, 43.87, 33.09, 25.82, 25.43, 24.83. Anal. Calc. for $C_{29}H_{33}ClN_8$: Calculated C: 65.83, H: 6.29, N: 21.18. Found C: 65.68, H: 6.47, N: 21.32.

*6.5.6. N-Benzyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-6-piperidin-1-yl-[1,3,5]triazine-2,4-diamine (**18**)*

Yield: 89%; mp 119–121 °C; MS: 537 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.31 Hz, Ar–H), 8.05 (d, 1H, J = 2.04 Hz, Ar–H), 7.88 (d, 1H, J = 8.97 Hz, Ar–H), 7.66 (d, 2H, J = 8.7 Hz, Ar–H), 7.48 (dd, 1H, J = 2.04 Hz, J = 8.97 Hz, Ar–H), 7.39–7.33 (m, 5H, Ar–H), 7.24 (d, 2H, J = 8.7 Hz, Ar–H), 6.83 (d, 1H, J = 5.31 Hz, Ar–H), 6.72 (bs, 1H, NH), 6.58 (bs, 1H, NH), 5.25 (bs, 1H, NH), 4.66 (s, 2H. CH_2), 3.78 (t, 4H, J = 4.92 Hz, N–CH_2), 1.65–1.61 (m, 6H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.97, 163.52, 163.12, 150.55, 148.23, 147.42, 138.14, 136.07, 133.91, 131.93, 127.47, 127.23, 126.24, 125.87, 124.51, 123.22, 120.08, 119.53, 116.47, 100.52, 43.55, 43.05, 24.53, 23.58. Anal. Calc. for $C_{30}H_{29}ClN_8$: Calculated C: 67.09, H: 5.44, N: 20.86. Found C: 67.28, H: 5.29, N: 20.73.

*6.5.7. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-methyl-piperazin-1-yl)-N′-phenyl-[1,3,5]triazine-2,4-diamine (**19**)*

Yield: 82%; mp >220 °C; MS: 538 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47 (d, 1H, J = 5.4 Hz, Ar–H), 8.00 (d, 1H, J = 1.98 Hz, Ar–H), 7.92 (d, 1H, J = 8.94 Hz, Ar–H), 7.65 (d, 2H, J = 8.73 Hz, Ar–H), 7.58 (d, 2H, J = 7.77 Hz, Ar–H), 7.46 (dd, 1H, J = 1.98 Hz, J = 8.94 Hz, Ar–H), 7.32 (t, 2H, J = 7.65 Hz, Ar–H), 7.26 (d, 2H, J = 8.73 Hz, Ar–H), 7.15 (bs, 1H, NH), 7.06 (t, 1H, J = 7.65 Hz, Ar–H), 6.83 (d, 1H, J = 5.4 Hz, Ar–H), 6.63 (bs, 1H, NH), 3.87 (t, 4H, J = 4.77 Hz, N–CH_2), 2.48 (t, 4H, J = 4.77 Hz, N–CH_2), 2.35 (s, 3H, N–CH_3). ^{13}C NMR (50 MHz, DMSO-d6): δ 165.44, 165.01, 164.96, 152.78, 150.42, 149.69, 141.04, 137.91, 134.70, 134.53, 129.24, 128.49, 125.58, 125.24, 124.78, 122.63, 121.83, 120.90, 118.89, 101.90, 55.31, 46.68, 43.67. Anal. Calc. for $C_{29}H_{28}ClN_9$: Calculated C: 64.74, H: 5.25, N: 23.43. Found C: 64.92, H: 5.13, N: 23.67.

*6.5.8. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-ethyl-piperazin-1-yl)-N′-phenyl-[1,3,5]triazine-2,4-diamine (**20**)*

Yield: 90%; mp 190–192 °C; MS: 552 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.4 (d, 1H, J = 5.4 Hz, Ar–H), 8.00 (d, 1H, J = 2.04 Hz, Ar–H), 7.93 (d, 1H, J = 8.97 Hz, Ar–H), 7.66 (d, 2H, J = 8.76 Hz, Ar–H), 7.58 (d, 2H, J = 7.83 Hz, Ar–H), 7.47 (dd, 1H, J = 2.04 Hz, J = 8.97 Hz, Ar–H), 7.32 (t, 2H, J = 7.75 Hz, Ar–H), 7.27 (d, 2H, J = 8.97 Hz, Ar–H), 7.16 (bs, 1H, NH), 7.06 (t, 1H, J = 7.41 Hz, Ar–H), 6.84 (d, 1H, J = 5.4 Hz, Ar–H), 6.64 (bs, 1H, NH), 3.88 (t, 4H, J = 4.29 Hz, N–CH_2), 2.54–2.42 (m, 6H, N–CH_2), 1.14 (t, 3H, J = 7.17 Hz, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6): δ 165.37, 165.01, 164.98, 152.75, 150.41, 149.71, 141.05, 134.72, 129.23, 128.48, 125.57, 125.24, 124.77, 121.82, 120.89, 118.75, 102.32, 53.11, 52.55, 43.76, 12.80. Anal. Calc. for $C_{30}H_{30}ClN_9$: Calculated C: 65.27, H: 5.48, N: 22.83. Found: C: 65.05, H: 5.32, N: 22.98.

*6.5.9. N-Butyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**21**)*

Yield: 87%; mp 125–127 °C; MS: 511 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.51 (d, 1H, J = 5.34 Hz, Ar–H), 8.01 (d, 1H, J = 2.01 Hz, Ar–H), 7.85 (d, 1H, J = 8.97 Hz, Ar–H), 7.63–7.58 (m, 4H, J = Ar–H), 7.45 (dd, 1H, J = 2.01 Hz, J = 8.97 Hz, Ar–H), 7.32 (t, 2H, J = 7.62 Hz, Ar–H), 7.23 (d, 2H, J = 8.85 Hz, Ar–H), 7.03 (t, 1H, J = 7.62 Hz, Ar–H), 6.92 (bs, 2H, NH), 6.81 (d, 1H, J = 5.34 Hz, Ar–H), 6.56 (bs, 1H, NH), 5.06 (bs, 1H, NH), 3.46 (t, 2H, J = 6.36 Hz, CH_2), 1.59 (quint, 2H, J = 7.62 Hz, CH_2), 1.42 (sext, 2H, J = 7.5 Hz, CH_2), 0.94 (t, 3H, J = 7.29 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d6): δ 171.35, 169.55, 156.92, 154.85, 154.58, 144.77, 142.07, 139.94, 139.27, 133.29, 130.31, 129.59, 128.57, 127.59, 126.42, 125.55, 123.37, 106.53, 45.79, 36.97, 25.30, 19.08. Anal. Calc. for $C_{28}H_{27}ClN_8$: Calculated C: 65.81, H: 5.33, N: 21.93. Found C: 65.59, H: 5.54, N: 22.15.

*6.5.10. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(2-diethylamino-ethyl)-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**22**)*

Yield: 78%; mp 130–132 °C; MS: 554 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.04 Hz, Ar–H), 7.90 (d, 1H, J = 9.00 Hz, Ar–H), 7.68–7.61 (m, 4H, Ar–H), 7.49 (dd, 1H, J = 2.04 Hz, J = 9.00 Hz, Ar–H), 7.34 (t, 2H, J = 8.22 Hz, Ar–H), 7.24 (d, 2H, J = 8.82 Hz, Ar–H), 7.07 (t, 1H, J = 8.22 Hz, Ar–H), 6.99 (bs, 2H, NH), 6.86 (d, 1H, J = 5.34 Hz, Ar–H), 6.60 (bs, 1H, NH), 3.54 (t, 2H, J = 6.09 Hz, NH–CH_2), 2.70 (t, 2H, J = 6.09 Hz, N–CH_2), 2.66 (quart, 4H, J = 6.99 Hz, N–CH_2), 1.07 (t, 6H, J = 6.99 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d6): δ 166.39, 164.75, 151.99, 149.87, 149.65, 139.69, 137.01, 135.29, 134.55, 128.96, 128.47, 125.66, 124.73, 123.37, 123.00, 121.74, 120.79, 118.47, 101.82, 52.13, 47.01, 38.76, 11.89. Anal. Calc. for $C_{30}H_{32}ClN_9$: Calculated C: 65.03, H: 5.82, N: 22.75. Found C: 64.78, H: 5.65, N: 22.97.

*6.5.11. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(3-morpholin-4-yl-propyl)-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**23**)*

Yield: 89%; mp 151–153 °C; MS: 582 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.31 Hz, Ar–H), 8.05 (d, 1H, J = 2.04 Hz, Ar–H), 7.90 (d, 1H, J = 8.9 Hz, Ar–H), 7.70 (d, 2H, J = 8.22 Hz, Ar–H), 7.63 (d, 2H, J = 7.89 Hz, Ar–H), 7.49 (dd, 1H, J = 2.04 Hz, J = 8.94 Hz, Ar–H), 7.33 (d, 2H, J = 7.62 Hz, Ar–H), 7.24 (d, 2H, J = 8.22 Hz, Ar–H), 7.07 (t, 1H, J = 7.38 Hz, Ar–H), 6.93 (bs, 2H, NH), 6.85 (d, 1H, J = 5.31 Hz, Ar–H), 6.64 (bs, 1H, NH), 6.62 (bs, 1H, NH), 3.78 (t, 4H, J = 4.47 Hz, O–CH_2), 3.59 (t, 2H, J = 5.88 Hz, NH–CH_2), 2.55–2.51 (m, 6H, N–CH_2), 1.82 (quint, 2H, J = 6.42 Hz, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.42, 164.79, 151.98, 149.68, 149.29, 139.48, 136.99, 135.68, 134.41, 129.13, 128.75, 126.18, 124.71, 123.39, 122.27, 122.06, 120.78, 118.24, 102.13, 67.38, 57.76, 54.09, 40.75, 25.82. Anal. Calc. for $C_{31}H_{32}ClN_9O$: Calculated C: 63.89, H: 5.48, N: 21.78.

*6.5.12. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-cyclohexyl-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**24**)*

Yield: 83%; mp 165–167 °C; MS: 537 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.31 Hz, Ar–H), 8.05 (d, 1H, J = 2.01 Hz, Ar–H), 7.89 (d, 1H, J = 8.97 Hz, Ar–H), 7.64–7.58 (m, 4H, Ar–H), 7.49 (dd, 1H, J = 2.01 Hz, J = 8.97 Hz, Ar–H), 7.33 (t, 2H, J = 7.65 Hz, Ar–H), 7.27 (d, 2H, J = 8.85 Hz, Ar–H), 7.07 (t, 1H, J = 7.32 Hz, Ar–H), 6.93 (bs, 2H, NH), 6.85 (d, 1H, J = 5.31 Hz, Ar–H), 6.60 (bs, 1H, NH), 5.04 (bs, 1H, NH), 3.90–3.87 (m, 1H, NH–CH), 2.10–2.07 (m, 2H, CH_2), 1.82–1.78 (m, 2H, CH_2), 1.44–1.37 (m, 2H, CH_2), 1.30–1.23 (m, 4H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d6): δ 170.52, 169.64, 156.92, 154.91, 154.71, 145.04, 142.33, 139.84, 139.16, 133.68, 133.19, 130.19, 129.59, 128.91, 127.39, 126.40, 125.67, 123.44, 106.44, 54.76, 38.35, 30.82, 30.37. Anal. Calc. for $C_{30}H_{29}ClN_8$: Calculated C: 67.09, H: 5.44, N: 20.86. Found C: 67.29, H: 5.63, N: 21.07.

*6.5.13. N-Benzyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**25**)*

Yield: 86%; mp 155–157 °C; MS: 545 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.07 Hz, Ar–H), 7.88 (d, 1H, J = 8.94 Hz, Ar–H), 7.64 (d, 2H, J = 8.41 Hz, Ar–H), 7.58 (d, 2H, J = 7.71 Hz, Ar–H), 7.48 (dd, 1J, J = 2.07 Hz, J = 8.94 Hz, Ar–H), 7.38–7.34 (m, 5H, Ar–H), 7.31 (t, 2H, J = 7.70 Hz, Ar–H), 7.24 (d, 2H, J = 8.41 Hz, Ar–H), 7.07 (t, 1H, J = 7.38 Hz, Ar–H), 7.01 (bs, 2H, NH), 6.84 (d, 1H, J = 5.34 Hz, Ar–H), 6.61 (bs, 1H, NH), 5.49 (bs, 1H, NH), 4.68 (s, 2H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.61, 164.76, 152.18, 149.85, 149.00, 139.22, 136.77, 135.67, 134.49, 129.16, 129.05, 127.76, 126.25, 124.63, 123.59, 121.94, 121.10, 118.22, 109.98, 102.26, 45.28. Anal. Calc. for $C_{31}H_{25}ClN_8$: Calculated C: 68.31, H: 4.62, N: 20.56. Found C: 68.38, H: 4.49, N: 20.81.

*6.5.14. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-phenyl-[1,3,5]triazine-2,4,6-triamine (**26**)*

Yield: 76%; mp 178–180 °C; MS: 455 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 8.57 (d, 1H, J = 9.09 Hz, Ar–H), 8.22 (d, 1H, J = 7.08 Hz, Ar–H), 8.00 (d, 1H, J = 1.89 Hz, Ar–H), 7.83 (d, 2H, J = 8.73 Hz, Ar–H), 7.71 (dd, 1H, J = 1.89, 9.09 Hz, Ar–H), 7.58 (d, 2H, J = 7.71 Hz, Ar–H), 7.42 (d, 2H, J = 8.73 Hz, Ar–H), 7.36–7.30 (m, 3H, Ar–H), 7.12 (t, 1H, J = 7.35 Hz, Ar–H), 6.83 (d, 1H, J = 7.08 Hz, Ar–H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 167.13, 165.45, 155.99, 143.74, 139.88, 139.46, 132.02, 129.23, 128.05, 127.00, 126.42, 123.91, 122.50, 121.97, 120.01, 116.71, 100.91. Anal. Calc. for $C_{24}H_{19}ClN_8$: Calculated C: 63.37, H: 4.21, N: 24.63. Found C: 63.29, H: 4.16, N: 24.48.

*6.5.15. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-methyl-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**27**)*

Yield: 78%; mp 158–161 °C; MS: 469 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (d, 1H, J = 5.31 Hz, Ar–H), 8.22 (d, 1H, J = 7.08 Hz, Ar–H), 8.01 (d, 1H, J = 1.98 Hz, Ar–H), 7.84 (d, 2H, J = 8.73 Hz, Ar–H), 7.70 (dd, 1H, J = 1.98, 9.08 Hz, Ar–H), 7.58 (d, 2H, J = 7.71 Hz, Ar–H), 7.43 (d, 2H, J = 8.73 Hz, Ar–H), 7.36–7.29 (m, 3H, Ar–H), 7.11 (t, 1H, J = 7.43 Hz, Ar–H), 6.84 (d, 1H, J = 5.31 Hz, Ar–H). ^{13}C NMR (50 MHz, DMSO-d_6): δ 167.08, 165.31, 156.01, 143.65, 139.73, 139.48, 132.13, 129.13, 127.92, 127.05, 126.39, 123.96, 122.48, 121.98, 119.96, 116.69, 101.13, 19.03. Anal. Calc. for $C_{25}H_{21}ClN_8$: Calculated C: 64.03, H: 4.51, N: 23.90. Found C: 64.25, H: 4.36, N: 24.12.

*6.5.16. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-methyl-piperazin-1-yl)-N′-o-tolyl-[1,3,5]triazine-2,4-diamine (**28**)*

Yield: 81%; mp 212–215 °C; MS: 552 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.07 Hz, Ar–H), 7.95 (d, 1H, J = 8.04 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.66 (d, 2H, J = 8.73 Hz, Ar–H), 7.50 (dd, 1H, J = 2.07 Hz, J = 9.00 Hz, Ar–H), 7.26–7.22 (m, 4H, Ar–H), 7.08 (t, 1H, J = 7.29 Hz, Ar–H), 6.87 (d, 1H, J = 5.34 Hz, Ar–H), 6.83 (bs, 1H, NH), 6.56 (bs, 2H, NH), 3.88 (t, 4H, J = 4.68 Hz, N–CH_2), 2.49 (t, 4H, J = 4.68 Hz, N–CH_2), 2.37 (s, 3H, CH_3), 2.35 (s, 3H, N–CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.68, 163.41, 162.93, 150.03, 147.94, 147.71 135.35, 134.15, 132.50, 129.14, 128.92, 126.51, 124.91, 124.98, 123.10, 122.55, 120.64, 119.82, 116.49, 100.21, 53.44, 47.35, 41.70, 16.69. Anal. Calc. for $C_{30}H_{30}ClN_9$; Calculated C: 65.27, H: 5.48, N: 2.83. Found C: 65.42, H: 5.62, N: 3.05.

*6.5.17. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-ethyl-piperazin-1-yl)-N′-o-tolyl-[1,3,5]triazine-2,4-diamine (**29**)*

Yield: 83%; mp 200–202 °C; MS: 566 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.07 Hz, Ar–H), 7.94 (d, 1H, J = 7.98 Hz, Ar–H), 7.89 (d, 1H, J = 8.97 Hz, Ar–H), 7.66 (d, 2H, J = 8.76 Hz, Ar–H), 7.49 (dd, 1H, J = 2.07 Hz, J = 8.97 Hz, Ar–H), 7.27–7.22 (m, 4H, Ar–H), 7.08 (t, 1H, J = 7.38 Hz, Ar–H), 6.97 (bs, 1H, NH), 6.85 (d, 1H, J = 5.34 Hz, Ar–H), 6.62 (bs, 2H, NH), 3.89 (t, 4H, J = 4.74 Hz, N–CH_2), 2.54–2.46 (m, 6H, N–CH_2), 2.35 (s, 3H, CH_3), 1.16 (t, 3H, J = 7.11 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 163.73, 163.56, 163.09, 150.62, 148.28, 147.25, 135.63, 133.98, 132.31, 129.18, 128.43, 127.59, 125.00, 124.63, 123.17, 122.90, 122.29, 119.99, 119.73, 116.49, 100.61, 51.41, 51.16, 42.01, 16.89, 10.69. Anal. Calc. for $C_{31}H_{32}ClN_9$: Calculated C: 65.77, H: 5.70, N: 22.27. Found C: 65.58, H: 5.93, N: 22.43.

*6.5.18. N-Butyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**30**)*

Yield: 87%; mp 110–112 °C; MS: 525 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.31 Hz, Ar–H), 8.05 (d, 1H, J = 2.01 Hz, Ar–H), 7.98–7.86 (m, 2H, Ar–H), 7.67 (d, 2H, J = 8.78 Hz, Ar–H),

7.49 (dd, 1H, J = 2.01 Hz, J = 8.91 Hz, Ar–H), 7.27–7.22 (m, 4H, Ar–H), 7.09 (t, 1H, J = 7.17 Hz, Ar–H), 6.98 (bs, 1H, NH), 6.82 (d, 1H, J = 5.31 Hz, Ar–H), 6.60 (bs, 1H, NH), 5.07 (bs, 1H, NH), 3.48 (t, 2H, J = 6.42 Hz, NH–CH_2), 2.34 (s, 3H, CH_3), 1.62 (quint, 2H, J = 6.81 Hz, CH_2), 1.42 (sext, 2H, J = 7.21 Hz, CH_2), 0.98 (t, 3H, J = 7.21 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$ + DMSO-d_6): δ 164.61, 163.49, 162.86, 150.11, 147.76, 135.30, 134.13, 132.52, 129.21, 126.63, 124.97, 124.51, 123.71, 123.07, 120.55, 116.47, 100.22, 39.36, 30.38, 18.77, 16.70, 12.48. Anal. Calc. for $C_{29}H_{29}ClN_8$: Calculated C: 66.34, H: 5.57, N: 21.34. found C: 66.25, H: 5.43, N: 21.49.

*6.5.19. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(2-diethylamino-ethyl)-N″-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**31**)*

Yield: 72%; mp 150–152 °C; MS: 68 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.1 Hz, Ar–H), 7.90–7.87 (m, 2H, Ar–H), 7.66 (d, 2H, J = 8.88 Hz, Ar–H), 7.49 (dd, 1H, J = 2.1 Hz, J = 8.88 Hz, Ar–H), 7.24–7.22 (m, 4H, J = Ar–H), 7.11 (t, 1H, J = 6.57 Hz, Ar–H), 6.92 (bs, IH, NH), 6.84 (d, 1H, J = 5.34 Hz, Ar–H). 6.62 (bs, 2H, NH), 5.72 (bs, 1H, NH), 3.54 (t, 2H, J = 5.46 Hz, NH–CH_2), 2.71–2.57 (m, 6H, N–CH_2), 2.34 (s, 3H, CH_3), 1.03 (t, 6H, J = 3.57 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.84, 163.67, 163.17, 159.98, 150.50, 148.22, 147.38, 135.44, 133.94, 132.48, 129.15, 127.40, 124.98, 123.08, 120.24, 119.91, 116.53, 100.52, 50.44, 45.44, 37.11, 34.14, 16.80, 10.28. Anal. Calc. for $C_{31}H_{34}ClN_9$: Calculated C: 65.54, H: 6.03, N: 22.19. Found C: 65.48, H: 6.17, N: 22.34.

*6.5.20. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(3-morpholin-4-yl-propyl)-N″-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**32**)*

Yield: 87%; mp 140–142 °C; MS: 596 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.06 (d, 1H, J = 2.07 Hz, Ar–H), 7.96–7.87 (m, 2H, Ar–H), 7.69 (d, 2H, J = 8.4 Hz, Ar–H), 7.50 (dd, 1H, J = 2.07 Hz, J = 8.94 Hz, Ar–H), 7.25–7.22 (m, 4H, Ar–H), 7.08 (t, 1H, J = 7.41 Hz, Ar–H), 6.93 (bs, 1H, NH), 6.83 (d, 1H, J = 5.34 Hz, Ar–H), 6.61 (bs, 2H, NH), 6.23 (bs, 1H, NH), 3.78 (t, 4H, J = 5.49 Hz, O–CH_2), 3.55 (t, 2H, J = 6.06 Hz, NH–CH_2), 2.55–2.50 (m, 6H, N–CH_2), 2.35 (s, 3H, CH_3), 1.81 (quint, 2H, J = 6.42 Hz, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.84, 163.32, 163.00, 150.50, 148.18, 147.34, 135.50, 133.97, 132.42, 129.17, 127.43, 124.97, 124.58, 123.07, 122.43, 120.14, 119.79, 116.49, 100.51, 65.72, 56.06, 52.43, 38.97, 24.32, 16.83. Anal. Calc. for $C_{32}H_{34}ClN_9O$: Calculated C: 64.47, H: 5.75, N: 21.15. Found C: 64.56, H: 5.89, N: 21.23.

*6.5.21. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-cyclohexyl-N″-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**33**)*

Yield: 81%; mp 160–162 °C; MS: 551(M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.07 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.68 (d, 2H, J = 7.98 Hz, Ar–H), 7.49 (dd, 1H, J = 2.07 Hz, J = 9.00 Hz, Ar–H), 7.27 (bs, 1H, NH), 7.25–7.22 (m, 4H, Ar–H), 7.08 (t, 1H, J = 7.2 Hz, Ar–H), 6.97–6.93 (m, 1H, Ar–H), 6.84 (d, 1H, J = 5.34 Hz, Ar–H), 6.60 (bs, 2H, NH), 5.04 (bs, 1H, NH), 3.89–3.86 (m, 1H, NH–CH), 2.34 (s, 3H, CH_3), 2.09–2.06 (m, 2H, CH_2), 1.82–1.76 (m, 2H, CH_2), 1.45–1.36 (m, 2H, CH_2), 1.26–1.18 (m, 4H, CH_2). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.02, 163.52, 163.13, 150.64, 148.27, 147.24, 135.31, 132.34, 129.28, 127.59, 125.03, 124.67, 123.15, 120.09, 119.62, 116.58, 100.53, 98.72, 31.95, 24.32, 23.73, 16.84. Anal. Calc. for $C_{31}H_{31}ClN_8$: Calculated C: 67.56, H: 5.67, N: 20.33. Found C: 67.67, H: 5.53, N: 20.43.

*6.5.22. N-Benzyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**34**)*

Yield: 86%; mp 145–147 °C; MS: 559(M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.31 Hz, Ar–H), 8.06 (d, 1H, J = 2.04 Hz, Ar–H), 7.89–7.86 (m, 2H, Ar–H), 7.64 (d, 2H, J = 8.28 Hz, Ar–H), 7.50 (dd, 1H, J = 2.04 Hz, J = 8.94 Hz, Ar–H), 7.38–7.32 (m, 4H, Ar–H), 7.25–7.20 (m, 5H, Ar–H), 7.09 (t, 1H, J = 7.53 Hz, Ar–H), 6.93 (bs, 1H, NH), 6.84 (d, 1H, J = 5.31 Hz, Ar–H), 6.58 (bs, 2H, NH), 5.41 (bs, 1H, NH), 4.68 (s, 2H, CH_2), 2.34 (s, 3H, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.97, 163.66, 163.10, 150.51, 148.19, 147.31, 137.57, 135.26, 133.97, 132.52, 129.20, 127.33, 126.03, 125.01, 124.57, 122.99, 120.18, 116.51, 100.54, 43.52, 16.80. Anal. Calc. for $C_{32}H_{27}ClN_8$: Calculated C: 68.75, H; 4.87, N: 20.04. Found C: 68.69, H; 5.04, N: 19.83.

*6.5.23. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**35**)*

Yield: 76%; mp 183–185 °C; MS: 469(M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.41 (d, 1H, J = 5.13 Hz, Ar–H), 8.27 (d, 1H, J = 9.00 Hz, Ar–H), 7.99 (d, 1H, J = 2.04 Hz, Ar–H), 7.90 (bs, 1H, NH), 7.78 (d, 1H, J = 7.71 Hz, Ar–H), 7.70 (d, 2H, J = 8.55 Hz, Ar–H), 7.44 (dd, 1H, J = 2.04 Hz, J = 9.00 Hz, Ar–H), 7.41 (bs, 1H, NH), 7.23–7.20 (m, 4H, Ar–H), 7.06 (t, 1H, J = 7.71 Hz, Ar–H), 7.00 (bs, 1H, NH), 6.77 (d, 1H, J = 5.13 Hz, Ar–H), 6.64 (bs, 1H, NH), 5.42 (bs, 2H, NH), 2.31 (s, 3H, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6): δ 167.93, 166.29, 165.37, 151.17, 150.57, 149.14, 138.46, 135.22, 134.00, 133.53, 130.98, 127.49, 126.88, 125.82, 125.63, 125.43, 124.98, 121.25, 118.57, 101.66, 18.99. Anal. Calc. for $C_{25}H_{21}ClN_8$: Calculated C: 64.03, H: 4.51, N: 23.90. Found C: 63.86, H: 4.72, N: 23.79.

*6.5.24. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-methyl-N″-o-tolyl-[1,3,5]triazine-2,4,6-triamine (**36**)*

Yield: 79%; mp 147–149 °C; MS: 483(M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.01 Hz, Ar–H), 7.88–7.85 (m, 2H, Ar–H), 7.64–7.67 (m, 2H, Ar–H), 7.49 (dd, 1H, J = 2.01 Hz, J = 8.97 Hz, Ar–H), 7.24–7.21 (m, 4H, Ar–H), 7.08 (t, 1H, J = 7.86 Hz, Ar–H), 6.95 (bs, 1H, NH), 6.83 (d, 1H, J = 5.34 Hz, Ar–H), 6.60 (bs, 2H, NH), 5.06 (bs, 1H, NH), 3.03 (s, 3H, NH–CH_3), 2.34 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 167.14, 165.24, 164.73, 152.03, 149.71, 149.23, 137.11, 137.02, 135.70, 134.21, 130.88, 128.83, 126.67, 126.22, 124.74, 122.07, 121.67, 118.20, 102.16, 28.14, 18.48. Anal. Calc. for $C_{26}H_{23}ClN_8$: Calculated C: 64.66, H: 4.80, N: 23.20. Found C: 64.73, H: 4.63, N: 23.41.

*6.5.25. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-methyl-piperazin-1-yl)-N′-p-tolyl-[1,3,5]triazine-2,4-diamine (**37**)*

Yield: 88%; mp 201–203 °C; MS: 552 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (d, 1H, J = 5.28 Hz, Ar–H), 8.05 (d, 1H, J = 2.01 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.68 (d, 2H, J = 8.7 Hz, Ar–H), 7.49–7.45 (m, 3H, Ar–H), 7.25 (d, 2H, J = 8.7 Hz, Ar–H), 7.16 (d, 2H, J 8.19 Hz, Ar–H), 6.86 (d, 1H, J = 5.28 Hz, Ar–H), 6.84 (bs, 1H, NH), 6.77 (bs, 1H, NH), 6.57 (bs, 1H, NH), 3.88 (t, 4H, J = 5.56 Hz, N–CH_2), 2.48 (t, 4H, J = 4.56 Hz, N–CH_2), 2.36 (s, 3H, N–CH_3), 2.34 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$ + DMSO-d_6): δ 170.24, 169.63, 156.96, 154.97, 154.70, 142.64, 142.54, 139.74, 136.47, 134.19, 133.14, 130.10, 129.54, 129.24, 126.25, 125.65, 123.51, 106.44, 60.08, 51.43, 48.38, 25.99. Anal. Calc. for $C_{30}H_{30}ClN_9$: Calculated C: 65.27, H: 5.48, N: 22.83. Found C: 65.05, H: 5.37, N: 22.63.

*6.5.26. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-ethyl-piperazin-1-yl)-N′-p-tolyl-[1,3,5]triazine-2,4-diamine (**38**)*

Yield: 90%; mp 192–194 °C; MS: 566 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.04 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.67 (d, 2H, J = 8.73 Hz, Ar–H), 7.49–7.44 (m, 3H, Ar–H), 7.27 (d, 2H, J = 8.73 Hz, Ar–H), 7.16 (d, 2H, J = 8.22 Hz, Ar–H), 6.90 (bs, 1H, NH), 6.85 (d, 1H, J = 5.34 Hz, Ar–H), 6.81 (bs, 1H, NH), 6.59 (bs, 1H, NH), 3.89 (t, 4H, J = 4.53 Hz, N–CH_2), 2.54–2.45 (m, 6H, N–CH_2), 2.34 (s, 3H, CH_3), 1.15 (t, 3H, J = 7.14 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$):

δ 165.36, 164.78, 164.72, 152.22, 149.89, 149.08, 137.18, 136.81, 135.63, 134.18, 132.93, 129.69, 129.05, 126.21, 124.81, 121.92, 121.72, 120.96, 118.21, 102.23, 53.05, 52.82, 43.75, 21.23, 12.35. Anal. Calc. for $C_{31}H_{32}ClN_9$: Calculated C: 65.77, H: 5.70, N: 22.27. Found C: 65.98, H: 5.56, N: 22.13.

*6.5.27. N-Butyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**39**)*

Yield: 84%; mp 141–143 °C; MS: 525 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 1.98 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.68 (d, 2H, J = 8.94 Hz, Ar–H), 7.49–7.46 (m, 3H, Ar–H), 7.27 (d, 2H, J = 8.94 Hz, Ar–H), 7.15 (d, 2H, J = 8.25 Hz, Ar–H), 7.02 (bs, 1H, NH), 6.85 (d, 1H, J = 5.34 Hz, Ar–H), 6.73 (bs, 1H, NH), 6.58 (bs, 1H, NH), 5.06 (bs, 1H, NH), 3.46 (t, 2H, J = 6.33 Hz, NH–CH_2), 2.34 (s, 3H, CH_3), 1.60 (quint, 2H, J = 7.59 Hz, CH_2), 1.45 (sext, 2H, J = 7.59 Hz, CH_2), 0.98 (t, 3H, J = 7.29 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.55, 164.78, 152.24, 149.91, 149.02, 136.67, 135.64, 134.30, 129.69, 129.11, 126.24, 124.73, 121.87, 120.90, 118.21, 102.23, 41.13, 32.19, 21.24, 20.54, 14.26. Anal. Calc. for $C_{29}H_{29}ClN_8$: Calculated C: 66.34, H: 5.57, N: 21.34. Found C: 66.52, H: 5.45, N: 21.38.

*6.5.28. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(2-diethylamino-ethyl)-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**40**)*

Yield: 71%; mp 128–130 °C; MS: 568 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.04 Hz, Ar–H), 7.90 (d, 1H, J = 9.00 Hz, Ar–H), 7.49 (d, 2H, J = 8.7 Hz, Ar–H), 7.49–7.45 (m, 3H, Ar–H), 7.26 (d, 2H, J = 8.7 Hz, Ar–H), 7.15 (d, 2H, J = 8.25 Hz, Ar–H), 7.05 (bs, 1H, NH), 6.85 (d, 1H, J = 5.34 Hz, Ar–H), 6.64 (bs, 1H, NH), 5.67 (bs, 1H, NH), 3.53 (t, 2H, J = 5.58 Hz, NH–CH_2), 2.70–2.57 (m, 6H, N–CH_2), 2.34 (s, 3H, CH_3), 1.05 (t, 6H, J = 4.11 Hz, CH_3). ^{13}C NMR (75 MHz, $CDCl_3$): δ 164.76, 163.19, 150.55, 148.26, 147.30, 135.37, 134.99, 133.94, 132.59, 131.44, 127.97, 127.47, 124.55, 123.06, 120.14, 119.69, 116.52, 100.56, 50.46, 45.42, 37.22, 19.52, 10.26. Anal. Calc. for $C_{31}H_{34}ClN_9$: Calculated C: 65.54, H; 6.03, N: 22.19. Found C: 65.36, H; 6.21, N: 22.04.

*6.5.29. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(3-morpholin-4-yl-propyl)-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**41**)*

Yield: 87%; mp 158–160 °C; MS: 596 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.31 Hz, Ar–H), 8.05 (d, 1H, J = 2.07 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.70 (d, 2H, J = 8.34 Hz, Ar–H), 7.50–7.45 (m, 3H, Ar–H), 7.26 (d, 2H, J = 8.34 Hz, Ar–H), 7.15 (d, 2H, J = 8.28 Hz, Ar–H), 6.93 (bs, 2H, NH), 6.84 (d, 1H, J = 5.31 Hz, Ar–H), 6.61 (bs, 1H, NH), 6.21 (bs, 1H, NH), 5.12 (bs, 1H, NH), 3.78 (t, 4H, J = 4.5 Hz, O–CH_2), 3.57 (t, 2H, J = 5.97 Hz, NH–CH_2), 2.54–2.50 (m, 6H, N–CH_2), 2.34 (s, 3H, CH_3), 1.81 (quint, 2H, J = 5.49 Hz, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.42, 164.68, 152.21, 149.88, 149.06, 137.06, 136.76, 135.64, 134.30, 133.07, 129.06, 126.22, 124.72, 121.95, 121.08, 118.21, 102.19, 67.42, 57.79, 54.11, 40.73, 25.95, 21.24. Anal. Calc. for $C_{32}H_{34}ClN_9O$: Calculated C: 64.47, H: 5.75, N: 21.15. Found C: 64.67, H: 5.83, N: 21.27.

*6.5.30. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-cyclohexyl-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**42**)*

Yield: 81%; mp 171–173 °C; MS: 551 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.34 Hz, Ar–H), 8.05 (d, 1H, J = 2.04 Hz, Ar–H), 7.89 (d, 1H, J = 8.97 Hz, Ar–H), 7.68 (d, 2H, J = 8.82 Hz, Ar–H), 7.49–7.45 (m, 3H, Ar–H), 7.27 (d, 2H, J = 8.82 Hz, Ar–H), 7.15 (d, 2H, J = 8.22 Hz, Ar–H), 6.93 (bs, 1H, NH), 6.85 (d, 1H, J = 5.34 Hz, Ar–H), 6.75 (bs, 1H, NH), 6.59 (bs, 1H, NH), 5.00 (bs, 1H, NH), 3.89–3.87 (m, 1H, NH), 2.34 (s, 3H, CH_3), 2.10–2.06 (m, 2H, CH_2), 1.82–1.77 (m, 2H, CH_2), 1.44–1.30 (m, 2H, CH_2), 1.27–1.19 (m, 4H, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.68, 164.83, 152.22, 149.90, 149.05, 136.88, 135.65, 134.29, 132.92, 129.69, 129.09, 126.24, 124.75, 122.16, 121.89, 121.55, 120.75, 118.20, 102.22, 50.16, 33.59, 26.06, 25.43, 21.23. Anal. Calc. for $C_{31}H_{31}ClN_8$: Calculated C: 67.56, H: 5.67, N: 20.33. Found C: 67.79, H: 5.59, N: 20.18.

*6.5.31. N-Benzyl-N′-[4-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**43**)*

Yield: 86%; mp 138–140 °C; MS: 559 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 5.28 Hz, Ar–H), 8.05 (d, 1H, J = 2.01 Hz, Ar–H), 7.88 (d, 1H, J = 9.00 Hz, Ar–H), 7.65 (d, 2H, J = 8.4 Hz, Ar–H), 7.49 (dd. 1H, J = 2.01 Hz, J = 9.00 Hz, Ar–H), 7.46 (d, 2H, J = 8.16 Hz, Ar–H), 7.38–7.30 (m, 5H, Ar–H), 7.24 (d, 2H, J = 8.4 Hz, Ar–H), 7.14 (d, 2H, J = 8.16 Hz, Ar–H), 6.93 (bs, 2H, NH), 6.84 (d, 1H, J = 5.28 Hz, Ar–H), 6.57 (bs, 1H, NH), 5.43 (bs, 1H, NH), 4.68 (s, 2H, CH_2), 2.33 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.48, 164.68, 151.97, 149.93, 149.65, 139.92, 137.19, 134.88, 134.20, 131.94, 129.23, 128.63, 128.27, 127.45, 125.24, 124.58, 123.82, 121.43, 120.82, 118.44, 101.48, 44.62, 21.00. Anal. Calc. for $C_{32}H_{27}ClN_8$: Calculated C: 68.75, H: 4.87, N: 20.04. Found C: 68.98, H: 5.09, N: 20.13.

*6.5.32. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**44**)*

Yield: 76%; mp 188–190 °C; MS: 469 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$ + CD_3OD): δ 8.37 (d, 1H, J = 5.58 Hz, Ar–H), 8.01 (d, 1H, J = 1.74 Hz, Ar–H), 7.98 (d, 1H, J = 8.88 Hz, Ar–H), 7.61 (d, 2H, J = 8.64 Hz, Ar–H), 7.44 (dd, 1H, J = 1.74 Hz, 8.88 Hz, Ar–H), 7.39 (d, 2H, J = 8.91 Hz, Ar–H), 7.23 (d, 2H, J = 8.64 Hz, Ar–H), 7.11 (d, 2H, J = 8.19 Hz, Ar–H), 6.79 (d, 1H, J = 5.58 Hz, Ar–H), 2.29 (s, 3H, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6): δ 167.71, 165.34, 165.28, 152.33, 149.16, 146.14, 139.29, 138.54, 136.52, 132.89, 131.38, 129.60, 126.54, 125.50, 125.03, 121.61, 121.08, 117.98, 101.48, 21.28. Anal. Calc. for $C_{25}H_{21}ClN_8$: Calculated C: 64.03, H: 4.51, N: 23.90. Found C: 64.21, H: 4.34, N: 24.16.

*6.5.33. N-[4-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-methyl-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**45**)*

Yield: 78%; mp 165–167 °C; MS: 483 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.55 (d, 1H, J = 6.30 Hz, Ar–H), 8.05 (d, 1H, J = 2.07 Hz, Ar–H), 7.89 (d, 1H, J = 9.00 Hz, Ar–H), 7.68 (d, 2H, J = 8.83 Hz, Ar–H), 7.49–7.46 (m, 3H, Ar–H), 7.24 (d, 2H, J = 8.83 Hz, Ar–H), 7.16 (d, 2H, J = 8.25 Hz, Ar–H), 6.94 (bs, 2H, NH), 6.85 (d, 1H, J = 6.30 Hz, Ar–H), 6.60 (bs, 1H, NH), 5.05 (bs, 1H, NH), 3.04 (s, 3H, CH_3), 2.34 (s, 3H, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6): δ 167.75, 165.98, 165.35, 152.31, 149.21, 146.11, 139.25, 138.51, 136.45, 132.95, 131.29, 129.63, 126.43, 125.62, 124.98, 121.63, 121.15, 118.05, 101.42, 21.23, 18.73. Anal. Calc. for $C_{26}H_{23}ClN_8$: Calculated C: 64.66, H: 4.80, N: 23.20. Found C: 64.89, H: 4.73, N: 23.47.

*6.5.34. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-methyl-piperazin-1-yl)-N′-phenyl-[1,3,5]triazine-2,4-diamine (**46**)*

Yield: 82%; mp 201–203 °C; MS: 538 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 5.28 Hz, Ar–H), 8.07 (d, 1H, J = 2.04 Hz, Ar–H), 7.92 (s, 1H, Ar–H), 7.87 (d, 1H, J = 8.97 Hz, Ar–H), 7.55 (d, 2H, J = 7.86 Hz, Ar–H), 7.50 (dd, 1H, J = 2.04 Hz, 8.97 Hz, Ar–H), 7.34 (t, 1H, J = 8.07 Hz, Ar–H), 7.30–7.24 (m, 3H, Ar–H), 7.18 (bs, 1H, NH), 7.09 (d, 1H, J = 5.28 Hz, Ar–H), 6.97–6.93 (m, 3H, Ar–H), 6.87 (bs, 1H, NH), 6.58 (bs, 1H, NH), 3.82 (t, 4H, J = 4.75 Hz, N–CH_2), 2.40 (t, 4H, J = 4.75 Hz, N–CH_2), 2.33 (s, 3H, N–CH_3). ^{13}C NMR (75 MHz, CD_3OD): δ 165.34, 164.85, 151.39, 150.21, 149.20, 141.50, 140.38, 139.91, 135.76, 129.69, 128.85, 126.89, 125.74, 123.69, 122.54, 120.57, 118.62, 117.54, 116.78, 115.25, 102.09, 54.64, 45.28, 42.96. Anal. Calc. for $C_{29}H_{28}ClN_9$:

Calculated C: 64.74, H: 5.25, N: 23.43. Found C: 64.91, H: 5.39, N: 23.26.

*6.5.35. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-ethyl-piperazin-1-yl)-N′-phenyl-[1,3,5]triazine-2,4-diamine (**47**)*

Yield: 85%; mp 191–193 °C; MS: 552 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.52 (d, 1H, J = 5.25 Hz, Ar–H), 8.04 (d, 1H, J = 1.89 Hz, Ar–H), 8.00 (s, 1H, Ar–H), 7.91 (d, 1H, J = 8.94 Hz, Ar–H), 7.53 (d, 2H, J = 7.83 Hz, Ar–H), 7.46 (dd, 1H, J = 1.89, 8.94 Hz, Ar–H), 7.33–7.21 (m, 5H, Ar–H & NH), 7.11 (s, 1H, NH), 7.04–7.02 (m, 2H, Ar–H & NH), 6.95–6.93 (m, 2H, Ar–H & NH), 3.81 (t, 4H, J = 4.73 Hz, N–CH_2), 2.48–2.41(m, 6H, N–CH_2), 1.12 (t, 3H, J = 7.17 Hz, CH_3). ^{13}C NMR (50 MHz, CD_3OD): δ 169.16, 168.57, 155.44, 153.51, 153.25, 144.99, 144.41, 143.30, 139.92, 133.96, 132.92, 131.59, 130.14, 127.18, 127.02, 124.80, 122.59, 121.40, 120.68, 118.92, 106.51, 56.67, 47.21, 15.59. Anal. Calc. for $C_{30}H_{30}ClN_9$: Calculated C: 65.27, H: 5.48, N: 22.83. Found C: 65.49, H: 5.31, N: 22.69.

*6.5.36. N-Butyl-N′-[3-(7-chloro-quinolin-4-ylamino)-phenyl]-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**48**)*

Yield: 87%; mp 128–131 °C; MS: 511 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.58 (d, 1H, J = 5.31 Hz, Ar–H), 8.06 (d, 1H, J = 2.07 Hz, Ar–H), 7.99 (s, 1H, Ar–H), 7.85 (d, 1H, J = 8.97 Hz, Ar–H), 7.55 (d, 2H, J = 7.78 Hz, Ar–H), 7.49 (dd, 1H, J = 2.07, 8.97 Hz, Ar–H), 7.32 (t, 1H, J = 7.98 Hz, Ar–H), 7.26–7.23 (m, 3H, Ar–H), 7.11 (bs, 1H, NH), 7.07 (d, 1H, J = 5.31 Hz, Ar–H), 6.97–6.93 (m, 3H, Ar–H & NH), 6.60 (bs, 1H, NH), 5.10 (bs, 1H, NH), 3.40 (q, 2H, J = 5.92 Hz, NH–CH_2), 1.58 (quint, 2H, J = 7.18 Hz, CH_2), 1.44 (sext, 2H, J = 7.45 Hz, CH_2), 0.92 (t, 3H, J = 7.35 Hz, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.17, 164.42, 151.71, 149.44, 148.86, 140.84, 140.39, 139.08, 135.87, 130.01, 129.04, 128.16, 126.27, 123.57, 122.43, 121.34, 118.51, 117.30, 116.74, 114.98, 102.59, 41.08, 31.96, 20.41, 14.06. Anal. Calc. for $C_{28}H_{27}ClN_8$: Calculated C: 65.81, H: 5.33, N: 21.93. Found C: 65.67, H: 5.51, N: 22.14.

*6.5.37. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(2-diethylamino-ethyl)-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**49**)*

Yield: 72%; mp 156–158 °C; MS: 554 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): 8.58 (d, 1H, J = 5.28 Hz, Ar–H), 8.06 (d, 1H, J = 2.04 Hz, Ar–H), 7.98 (s, 1H, NH), 7.90 (d, 1H, J = 8.7 Hz, Ar–H), 7.62 (d, 2H, J = 7.86 Hz, Ar–H), 7.50 (dd, 1H, J = 2.04, 8.7 Hz, Ar–H), 7.33 (t, 1H, J = 7.92 Hz, Ar–H), 7.29–7.23 (m, 3H, Ar–H), 7.13 (bs, 1H, NH), 7.11 (d, 1H, J = 5.28 Hz, Ar–H), 6.99–6.96 (m, 3H, Ar–H), 6.93 (bs, 1H, NH), 6.67 (bs, 1H, NH), 5.68 (bs, 1H, NH), 3.48 (q, 2H, J = 6.52 Hz, NH–CH_2), 2.59–2.55 (m, 6H, N–CH_2), 1.04 (t, 6H, J = 6.85 Hz, CH_3). ^{13}C NMR (50 MHz, DMSO-d_6): δ 166.56, 164.95, 161.99, 152.79, 150.47, 149.09, 142.23, 141.09, 134.72, 130.03, 129.11, 128.49, 125.65, 125.42, 122.40, 120.78, 119.20, 117.23, 116.80, 115.25, 102.77, 52.51, 52.36, 47.38, 36.37, 12.54. Anal. Calc. for $C_{30}H_{32}ClN_9$: Calculated C: 65.03, H: 5.82, N: 22.75. Found C: 64.86, H: 5.88, N: 22.91.

*6.5.38. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(3-morpholin-4-yl-propyl)-N″-phenyl-[1,3,5]triazine-2,4,6-triamine (**50**)*

Yield: 89%; mp 167–169 °C; MS: 582 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.56 (d, 1H, J = 5.04 Hz, Ar–H), 8.05 (d, 1H, J = 1.89 Hz, Ar–H), 7.98 (s, 1H, Ar–H), 7.91 (d, 1H, J = 9.42 Hz, Ar–H), 7.56 (d, 2H, J = 7.88 Hz, Ar–H), 7.49 (dd, 1H, J = 1.89, 9.42 Hz, Ar–H), 7.33 (t, 1H, J = 8.07 Hz, Ar–H), 7.30–7.27 (m, 3H, Ar–H), 7.13 (bs, 1H, NH), 6.98–6.96 (m, 4H, J = Ar–H & NH), 6.72 (bs, 1H, NH), 3.74 (t, 4H, J = 4.41 Hz, O–CH_2), 3.51 (q, 2H, J = 6.24 Hz, NH–CH_2), 2.63–2.46 (m, 6H, N–CH_2), 1.71 (quint, 2H, J = 6.52 Hz, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): δ 166.25, 164.67, 151.88, 149.61, 148.42, 141.14, 140.35, 139.43, 135.75, 130.02, 129.08, 128.68, 126.33, 123.37, 122.18, 120.76, 118.51, 117.01, 116.39, 114.77, 109.99, 102.93, 67.34, 57.63, 54.01, 40.66, 25.88. Anal. Calc. for $C_{31}H_{32}ClN_9O$: Calculated C: 63.96, H: 5.54, N: 21.66. Found C: 64.17, H: 5.37, N: 21.89.

*6.5.39. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-methyl-piperazin-1-yl)-N′-p-tolyl-[1,3,5]triazine-2,4-diamine (**51**)*

Yield: 82%; mp 187–189 °C; MS: 552 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): 8.58 (d, 1H, J = 5.28 Hz, Ar–H), 8.07 (d, 1H, J = 2.04 Hz, Ar–H), 7.93 (s, 1H, Ar–H), 7.87 (d, 1H, J = 8.82 Hz, Ar–H), 7.49 (dd, 1H, J = 2.04, 8.82 Hz, Ar–H), 7.43 (d, 2H, J = 8.34 Hz, Ar–H), 7.34 (t, 1H, J = 8.1 Hz, Ar–H), 7.18 (d, 1H, J = 7.32 Hz, Ar–H), 7.09–7.03 (m, 3H, Ar–H), 6.97 (d, 1H, J = 5.28 Hz, Ar–H), 6.90 (bs, 1H, NH), 6.79 (bs, 1H, NH), 6.60 (bs, 1H, NH), 3.80 (t, 4H, J = 4.72 Hz, N–CH_2), 2.39 (t, 4H, J = 4.72 Hz, N–CH_2), 2.32 (s, 3H, CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.27, 164.45, 152.18, 149.88, 148.13, 141.41, 136.73, 135.70, 132.92, 130.18, 129.62, 129.08, 126.43, 121.87, 120.86, 118.55, 116.86, 114.39, 103.14, 55.16, 46.54, 43.65, 21.08. Anal. Calc. for $C_{30}H_{30}ClN_9$: Calculated C: 65.27, H: 5.48, N: 22.83. Found C: 65.46, H: 5.69, N: 23.04.

*6.5.40. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-6-(4-ethyl-piperazin-1-yl)-N′-p-tolyl-[1,3,5]triazine-2,4-diamine (**52**)*

Yield: 86%; mp 163–165 °C; MS: 566 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): 8.59 (d, 1H, J = 5.28 Hz, Ar–H), 8.07 (d, 1H, J = 2.04 Hz, Ar–H), 7.95 (s, 1H, Ar–H), 7.87 (d, 1H, J = 8.94 Hz, AR-h), 7.49 (dd, 1H, J = 2.04 8.94 Hz, Ar–H), 7.43 (d, 2H, J = 8.31 Hz, Ar–H), 7.34 (t, 1H, J = 7.98 Hz, AR-h), 7.18 (d, 1H, J = 8.24 Hz, Ar–H), 7.09–7.03 (m, 3H, Ar–H), 6.97 (d, 1H, J = 5.28 Hz, Ar–H), 6.89 (bs, 1H, NH), 6.78 (bs, 1H, NH), 6.59 (bs, 1H, NH), 3.81 (t, 4H, J = 4.83 Hz, N–CH_2), 2.48–2.41 (m, 6H, N–CH_2), 2.20 (s, 3H, CH_3), 1.12 (t, 3H, J = 7.2 Hz, N–CH_3). ^{13}C NMR (50 MHz, $CDCl_3$): δ 165.19, 164.65, 152.23, 149.93, 148.11, 140.43, 136.75, 135.67, 132.89, 130.17, 129.62, 126.41, 121.86, 120.85, 118.58, 116.83, 114.35, 103.59, 52.93, 52.75, 43.70, 21.08, 12.29. Anal. Calc. for $C_{31}H_{32}ClN_9$: Calculated C: 65.77, H: 5.70, N: 22.27. Found C: 65.46, H: 5.91, N: 22.45.

*6.5.41. N-[3-(7-Chloro-quinolin-4-ylamino)-phenyl]-N′-(3-morpholin-4-yl-propyl)-N″-p-tolyl-[1,3,5]triazine-2,4,6-triamine (**53**)*

Yield: 89%; mp 145–147 °C; MS: 596 (M + 1); ^{1}H NMR (300 MHz, $CDCl_3$): 8.58 (d, 1H, J = 5.28 Hz, Ar–H), 8.06 (d, 1H, J = 2.04 Hz, Ar–H), 7.90 (d, 1H, J = 8.73 Hz, Ar–H), 7.49–7.43 (m, 3H, Ar–H), 7.33 (t, 1H, J = 7.95 Hz, Ar–H), 7.11–7.05 (m, 5H, Ar–H), 6.98 (d, 1H, J 5.28 Hz, Ar–H), 6.81 (bs, 1H, NH), 6.68 (bs, 1H, NH), 6.58 (bs, 1H, NH), 3.73 (t, 4H, J = 4.47 Hz, O–CH_2), 3.51 (q, 2H, J = 6.33 Hz, NH–CH_2), 2.44–2.34 (m, 6H, N–CH_2), 2.22 (s, 3H, CH_3), 1.77 (quint, 2H, J = 7.60 Hz, CH_2). ^{13}C NMR (50 MHz, $CDCl_3$): 166.29, 164.64, 152.25, 149.96, 148.16, 141.16, 140.42, 136.71, 135.64, 133.00, 130.09, 129.61, 126.36, 121.90, 120.90, 118.57, 116.32, 103.06, 67.38, 57.67, 54.03, 40.68, 25.84, 21.09. Anal. Calc. for $C_{32}H_{34}ClN_9O$: Calculated C: 64.47, H: 5.75, N: 21.15. Found C: 64.72, H: 5.61, N: 21.38.

7. Materials and methods

7.1. In vitro antimalarial activity assay

The in vitro antimalarial activity of compounds was determined by using a standardized inexpensive assay based on Syber Green I [59]. The compounds were dissolved in DMSO at 5 mg/ml. For the assays, fresh dilutions of all compounds in screening medium were prepared and 50 μl of highest starting concentration (500 ng/ml) was dispensed in duplicate wells in row B of 96 well tissue culture plate. The highest concentration for chloroquine was 25 ng/ml. Subsequently two fold serial dilutions were prepared up to row H (seven concentrations). Finally 50 μl of 2.5% parasitized cell

suspension containing 0.5% parasitemia was added to each well except 4 wells in row A which received non infected cell suspension. These wells containing non infected erythrocytes in the absence of drugs served as negative controls, while parasitized erythrocytes in the presence of CQ served as positive control. After 72 h of incubation, 100 μl of lysis buffer [20 mM tris (Ph 7.5), 5 mM EDTA, 0.008% (wt/vol) saponin, and 0.08% (vol/vol) Triton X − 100] containing 1 × concentration of SYBER Green I (Invitrogen) was added to each cell. The plates were re-incubated for 1 h at room temperature and examined for the relative fluoroscence units (RFUs) per well using the FLUOstar, BMG lab technologies. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis dose-response curves.

7.2. In vitro cytotoxicity assay

Cytotoxicity of the compounds was determined against VERO cell lines (C-1008; Monkey kidney fibroblast cells) using MTT assay [60]. A total of 1×10^4 cells/well were incubated with varying concentrations of compound for 72 h. The highest concentration of compound was 100 μg/ml. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis dose-response curves and represented the concentration of compound required to kill 50% of the fibroblast cells.

7.3. In vivo activity assay

The in vivo drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain) which is innately resistant to CQ [61]. The mice (22±2g) were inoculated with 1 × 106 parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0–3, once daily. The aqueous suspensions of compounds were prepared with a few drops of Tween 80. Initially, the efficacy of test compounds was evaluated at 50.0 mg/kg/day and required daily dose was administered in 0.2 mL volume via intraperitoneal route. The efficacy of test compounds was evaluated at 100 mg/kg/day and required daily dose was administered in 0.1 mL volume via oral route. Parasitemia levels were recorded from thin blood smears between days 4 and 6. The mean value determined for a group of 5 mice was used to calculate the percent suppression of parasitemia with respect to the untreated control group. Mice treated with CQ served as reference controls.

7.4. β-Hematin inhibitory activity assay

Male Swiss mice, weighing 15–20 g, were infected with 1×10^5 infected RBCs. Blood of infected animals at 50% parasitemia was collected by cardiac puncture in 2.0% citrate buffer and centrifuged at 5000 rpm for 10 min at 4 °C. The plasma was used in assay of β-hematin formation. The inhibition of β-hematin formation was measured as reported by pandey et al. (1999) [62]. The assay mixture contained 100 mM acetate buffer pH = 5.1, 50 μl plasma, 100 μM hemin as the substrate and 1–20 μg compound/drug in a total volume of 1.0 ml. The reaction mixture in triplicate was incubated at 37 °C for 16 h in a rotary shaker. The reaction was stopped by centrifugation at 10, 000 rpm for 10 min at 30 °C. The pellet was suspended in Tris–HCl (100 mM, Ph 7.4) containing 2.5% SDS and centrifuged as above. The pellet washed thrice and re-suspended in sodium bicarbonate buffer (100 mM, pH 9.1) containing 2.5% SDS. The pellet was washed thrice with distilled water (TDW) to remove free hemin attached to β-hematin. The pellet was solubilized in 50 μl of 2N NaOH and the volume was made to 1.0 ml with TDW and absorbance was measured at 400 nm.

7.5. Molecular docking study

LigandFit, a modern docking program within Cerius2 version 4.10 (Cerius2 Version 4.10 (2005), Accelrys Inc., San Diego, USA), was used for docking runs [63]. Reference protein coordinates for docking were taken from the X-ray structure of Plasmodium falciparum TS-DHFR in complex with the pfDHFR inhibitor WR99210. The site search was performed in the shape-based mode based on the crystal structure of the compound WR99210. All calculations were performed with the CFF 1.01 force field. Conformations were generated with Monte Carlo simulations (10,000 trials) and Flexible fit was selected. A grid resolution was set to 0.5 Å. Electrostatic energy was included in the calculation of the ligand internal energy. In order to avoid identical conformations, root mean square deviation threshold of 1.5 Å and a score threshold of 20 kcal/mol were used while saving the final conformations. The top 10 conformations were saved after rigid body minimizations of 1000 steps. Scoring was performed for each of the 10 saved ligand conformations using a set of scoring functions as implemented in Cerius2, including LigScore1, LigScore2,13 PLP1, PLP2,14 JAIN,15 PMF16 and LUDI.17 Consensus scoring function was also used to evaluate and rank the ligand binding affinities.

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References

[1] (a) P.M.S. Chauhan, S.K. Srivastava, Curr. Med. Chem. 8 (2001) 1535–1542;
(b) A. Kumar, S.B. Katiyar, A. Agarwal, P.M.S. Chauhan, Drugs Fut. 28 (2003) 243–255;
(c) A. Kumar, S.B. Katiyar, A. Agarwal, P.M.S. Chauhan, Curr. Med. Chem. 10 (2003) 1241–1253.
[2] U. Frevert, Trend Parasitol. 20 (2004) 417–424.
[3] V. Narasimhan, A. Attaran, Malar. J. 2 (2003) 8.
[4] M.H. Gelb, Curr. Opin. Chem. Biol. 11 (2007) 440–445.
[5] J. Wiesner, R. Ortmann, H. Jomaa, M. Schlitzer, Angew. Chem. Int. Ed. 42 (2003) 5274–5293.
[6] G.A. Biagini, P.M. O'Neill, P.G. Bray, S.A. Ward, Curr. Opin. Pharmacol. 5 (2005) 473–478.
[7] R.G. Ridley, Nature 415 (2002) 686–693.
[8] J.E. Hyde, Parasitol. Today 5 (1989) 252–255.
[9] P.M. O'Neill, G.H.A. Posner, J. Med. Chem. 47 (2004) 2945–2964.
[10] S. Yeung, W. Pongtavornpinyo, I.M. Hastings, A.J. Mills, N.J. White, Am. J. Trop. Med. Hyg. 71 (2004) 179–186.
[11] N.J. White, P.L. Olliaro, Parasitol. Today 12 (1996) 399–401.
[12] P.B. Bloland, M. Ettling, S. Meek, Bull. World Health Org. 78 (2000) 1378–1388.
[13] T.K. Mutabingwa, Acta Trop. 95 (2005) 305–315.
[14] R. Jambou, E. Legrand, M. Niang, N. Khim, P. Lim, B. Volney, M.T. Ekala, C. Bouchier, P. Esterre, T. Fandeur, O. Mercereau-Puijalon, Lancet 366 (2005) 1960–1963.
[15] (a) D. Payne, Parasitol. Today 4 (1988) 112–115;
(b) L.M.B. Ursos, P.D. Roepe, Med. Res. Rev. 22 (2002) 465–491.
[16] M. Krugliak, J. Zhang, H. Ginsburg, Mol. Biochem. Parasitol. 119 (2002) 249–256.
[17] A. Yayon, Z.I. Cabantchik, H. Ginsburg, EMBO J. 3 (1984) 2695–2700.
[18] S. Pagola, P.W. Stephens, D.S. Bohle, A.D. Kosar, S.K. Madsen, Nature 404 (2000) 307–310.
[19] A. Leed, K. DuBay, L.M. Ursos, D. Sears, A.C. de Dios, P.D. Roepe, Biochemistry 41 (2002) 10245–10255.
[20] A.C. de Dios, R. Tycko, L.M.B. Ursos, P.D. Roepe, J. Phys. Chem. A 107 (2003) 5821–5825.
[21] A. Dorn, S.R. Vippagunta, H. Matile, C. Jaquet, J.L. Vennerstrom, R.G. Ridley, Biochem. Pharmacol. 55 (1998) 727–736.
[22] M. Foley, L. Tilley, Int. J. Parasitol. 27 (1997) 231–240.
[23] A.B. Sidhu, D. Verdier-Pinard, D.A. Fidock, Science 298 (2002) 210–213.
[24] H. Zhang, M. Paguio, P.D. Roepe, Biochemistry (Mosc) 43 (2004) 8290–8296.
[25] T.N. Bennett, A.D. Kosar, L.M. Ursos, S. Dzekunov, S.A.B. Singh, D.A. Fidock, P.D. Roepe, Mol. Biochem. Parasitol. 133 (2004) 99–114.

[26] S.R. Cheruku, S. Maiti, A. Dorn, B. Scorneaux, A.K. Bhattacharjee, W.Y. Ellis, J.L. Vennerstrom, J. Med. Chem. 46 (2003) 3166–3169.
[27] T.J. Egan, R. Hunter, C.H. Kaschula, H.M. Marques, A. Misplon, J. Walden, J. Med. Chem. 43 (2000) 283–291.
[28] R.G. Ridley, H. Hofheinz, H. Matile, C. Jacquet, A. Dorn, R. Masciadri, S. Jolidon, W.F. Richter, A. Guenzi, M.A. Girometta, H. Urwyler, W. Huber, S. Thiathong, W. Peters, Antimicrob. Agents Chemother. 40 (1996) 1846–1854.
[29] P.B. Madrid, A.P. Liou, J.L. DeRisi, K. Guy, J. Med. Chem. 49 (2006) 4535–4543.
[30] K. Yearick, K. Ekove-Kovi, D.P. Iwaniuk, J.K. Natarajan, J. Alumasa, A.C. de Dios, P.D. Roepe, C.W. Wolf, J. Med. Chem. 51 (2005) 1995–1998.
[31] K.A. Neftel, W. Woodtly, M. Schmid, Br. Med. J. 292 (1986) 721–723.
[32] D.E. Lind, J.A. Levi, P.C. Vincent, Br. Med. J. 1 (1973) 458–460.
[33] P.M. O'Neill, A. Mukhtar, P.A. Stocks, L.E. Randle, S. Hindley, S.A. Ward, R.C. Storr, J.F. Bickley, I.A. O'Neill, J.L. Maggs, R.H. Hughes, P.A. Winstanely, P.G. Bray, B.K. Park, J. Med. Chem. 46 (2003) 4933–4945.
[34] P.M. O'Neill, B.K. Park, A.E. Shone, J.L. Maggs, P. Roberts, P.A. Stocks, G.A. Biagini, P.G. Bray, P. Gibbons, N. Berry, P.A. Winstanley, A. Mukhtar, R. Bonar-law, S. Hindley, R.B. Bambal, C.B. Davis, M. Bates, T.K. Hart, S.L. Gresham, R.M. Lawrence, R.A. Brigandi, F.M. Gomez-delas-Heras, D.V. Gargallo, S.A. Ward, J. Med. Chem. 52 (2009) 1408–1415.
[35] P.M. O'Neill, A.E. Shone, D. Tanford, G. Nixon, E. Adollahy, B.K. Park, J.L. Maggs, P. Roberts, P.A. Stocks, G. Biagini, P.G. Bray, J. Davies, N. Berry, C. Hall, K. Rimmer, P.A. Winstanley, S. Hindley, R.B. Bambal, C.B. Davis, M. Bates, S.L. Gresham, R.A. Brigandi, F.M. Gomez-delas-Heras, D.V. Gargallo, S. Parapini, L. Vivas, H. Lander, D. Taramelli, S.A. Ward, J. Med. Chem. 52 (2009) 1828–1844.
[36] J.J. Walsh, A. Bell, Curr. Pharm. Design 15 (2009) 2970–2985.
[37] F. Bellot, F. Cosledan, L. Vendier, J. Brocard, B. Meunier, A. Robert, J. Med. Chem. 53 (2010) 4103–4109.
[38] (a) O. Dechy-Cabaret, F. Benoit-Vical, A. Robert, B. Meunier, ChemBioChem 1 (2000) 281–283;
(b) A. Robert, O. Dechy-Cabaret, J. Cazelles, B. Meunier, Acc. Chem. Res. 35 (2002) 167–174;
(c) O. Dechy-Cabaret, F. Benoit-Vical, C. Loup, A. Robert, H. Gornitzka, A. Bonhoure, H. Vial, J.F. Magnaval, J.P. Seguela, B. Meunier, Chemistry 10 (2004) 1625–1636;
(d) F. Benoit-Vical, J. Lelievre, A. Berry, C. Deymier, O. Dechy-Cabaret, J. Cazelles, C. Loup, A. Robert, J.F. Magnaval, B. Meunier, Antimicrob. Agents Chemother. 51 (2007) 1463–1472;
(e) C. Singh, H. Malik, S.K. Puri, Bioorg. Med. Chem. 12 (2004) 1177–1182.
[39] J.J. Walsh, D. Coughlan, N. Heneghan, C. Gaynor, A. Bell, Bioorg. Med. Chem. Lett. 17 (2007) 3599–3602.
[40] I. Opsenica, D. Opsenica, C.A. Lanteri, L. Anova, W.K. Milhous, K.S. Smith, B.A. Salaja, J. Med. Chem. 51 (2008) 6216–6219.
[41] S. Gemma, G. Campiani, S. Butini, B.P. Joshi, G. Kukreja, S.S. Coccone, M. Burrutti, M. Persico, V. Nacci, I. Fiorini, E. Novellino, D. Taramerlli, N. Banilico, S. Parapini, V. Yardley, S. Croft, S.K. Maerk, M. Rottman, R. Brun, M. Coletta, S. Marini, G. Guiso, S. Caccia, C. Fattorusso, J. Med. Chem. 52 (2009) 502–513.
[42] (a) D. Dive, C. Biot, Chem. Med. Chem. 3 (2008) 383–391;
(b) M.A. Blackie, P. Beagley, S. L Croft, H. Kendrick, J.R. Moss, K. Chibale, Bioorg. Med. Chem. 15 (2007) 6510–6516.
[43] (a) E. Davioud-Charvet, S. Delarue, C. Biot, B. Schwobel, C.C. Boehme, A. Mussigbrodt, L. Maes, C. Sergheraert, P. Grellier, R.H. Schirmer, K.A. Becker, J. Med. Chem. 44 (2001) 4268;
(b) W. Friebolin, B. Jannack, N. Wenzel, J. Furrer, T. Oeser, C.P. Sanchez, M. Lanzer, V. Yardley, K. Becker, E. Davioud-charvet, J. Med. Chem. 51 (2008) 1260–1277.
[44] I. Chiyanzu, C. Clarkson, P.J. Smith, J. Gut, P.J. Rosenthal, K. Chibale, Bioorg. Med. Chem. 13 (2005) 3249–3261.
[45] S.J. Burgess, A. Selzer, J.X. Kelly, M.J. Smilkstein, M.K. Riscoe, D.H. A Peyton, J. Med. Chem. 49 (2006) 5623–5625.
[46] J.X. Kelly, M.J. Smilkstein, R. Brun, S. Wittlin, R.A. Cooper, K.D. Lane, A. Janowsky, R.A. Johnson, R.A. Dodean, R. Winter, D.J. Hinrichs, M.K. Riscoe, Nature 459 (2009) 270–273.
[47] L.K. Basco, P.H. Eldin de Pecoulas, C.M. Wilson, J. Le Bras, Mol. Biochem. Parasitol. 69 (1995) 135–138.
[48] D.S. Peterson, W.K. Milhous, T.E. Wellems, Proc. Natl. Acad. Sci. U.S.A. 87 (1990) 3018–3022.
[49] Y. Yuthavong, T. Vilaivan, N. Chareonsethakul, S. Kamchonwongpaisan, W. Sirawaraporn, R. Quarrell, G. Lowe, J. Med. Chem. 43 (2000) 2738–2744.
[50] B. Tarnchompoo, C. Sirichaiwat, W. Phupong, C. Intaraudom, W. Sirawaraporn, S. Kamchonwongpaisan, J. Vanichtanankul, Y. Thebtaranonth, Y. Yuthavong, J. Med. Chem. 45 (2002) 1244–1252.
[51] S. Kamchonwongpaisan, R. Quarrell, N. Charoensetakul, R. Ponsinet, T. Vilaivan, J. Vanichtanankul, B. Tarnchompoo, W. Sirawaraporn, G. Lowe, Y. Yuthavong, J. Med. Chem. 47 (2004) 673–680.
[52] (a) S. Melato, D. Prosperi, P. Coghi, N. Basilico, D. Monti, ChemMedChem 3 (2008) 873–876;
(b) B. Klenke, M.P. Barrett, R. Brun, I.H. Gilbert, J. Antimicrob. Chemother. 52 (2003) 290–293;
(c) L.M. Werbel, E.F. Elslager, C. Hess, M.P. Hutt, J. Med. Chem. 30 (1987) 1943–1948;
(d) D.J. Knight, P. Mamalis, W. Peters, Anna. Trop. Med. Parasitol. 76 (1982) 1–7.
[53] A. Agarwal, K. Srivastava, S.K. Puri, P.M.S. Chauhan, Bioorg. Med. Chem. Lett. 15 (2005) 531–533.
[54] S.B. Katiyar, K. Srivastava, S.K. Puri, P.M.S. Chauhan, Bioorg. Med. Chem. Lett. 15 (2005) 4957–4960.
[55] A. Kumar, K. Srivastava, S. Rajakumar, S.K. Puri, P.M.S. Chauhan, Bioorg. Med. Chem. Lett. 18 (2008) 6530–6533.
[56] A. Kumar, K. Srivastava, S. Rajakumar, S.K. Puri, P.M.S. Chauhan, Bioorg. Med. Chem. Lett. 19 (2009) 6996–6999.
[57] L. Gupta, K. Srivastava, S. Singh, S.K. Puri, P.M.S. Chauhan, Bioorg. Med. Chem. Lett. 18 (2008) 3306–3309.
[58] I.P.J. Hoglund, S. Silver, M.J. Engstrom, H. Salo, A. Tauber, H.K. Kyronem, P. Saarenketo, A.M. Hoffren, K. Kokko, K. Pohjanoksa, J. Sallinen, J.M. Savola, S. Wurster, O.A. Kallatsa, J. Med. Chem. 49 (2006) 6351–6363.
[59] M. Smilkstein, N. Sriwilaijaroen, J.X. Kelly, P. Wilairat, M. Riscoe, Antimicrob. Agents Chemother. 48 (2004) 803–806.
[60] T.J. Mosmann, Immunol. Methods 65 (1983) 55–63.
[61] S.K. Puri, N. Singh, Exp. Parasitol. 94 (2000) 8–14.
[62] A.V. Pandey, N. Singh, B.L. Tekwani, S.K. Puri, V.S. Chauhan, J. Pharm. Biomed. Anal. 20 (1999) 203–207.
[63] C.M. Venkatachalam, X. Jiang, T. Oldfield, M. Waldman, J. Mol. Graph. Model. 21 (2003) 289–307.