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Synthesis, antimalarial activity and cytotoxicity of 4-aminoquinoline–triazine conjugates

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ABSTRACT

A series of 4-aminoquinoline–triazine conjugates with different substitution pattern have been synthesized and evaluated for their in vitro antimalarial activity against chloroquine-sensitive and resistant strains of *Plasmodium falciparum*. Compounds **16**, **19**, **28** and **35** exhibited promising antimalarial activity against both strains of *P. falciparum*. Cytotoxicity of these compounds was tested against three cell lines. Several compounds did not show any cytotoxicity up to a high concentration (48 μM), others exhibited mild toxicities but selective index for antimalarial activity was high for most of these conjugates.

Malaria remains one of the most devastating parasitic diseases in the developing countries. More than 300 million people in Africa, Southeast Asia and South America are infected with an estimated death of 1–3 million people every year.[1] Malaria is caused by protozoan parasite of the genus *Plasmodium* and *Plasmodium vivax*, *Plasmodium malariae*, *Plasmodium ovale*, and *Plasmodium falciparum* species cause infection in humans. Of these, *P. falciparum* accounts for half of all clinical cases of malaria.[2] Quinine, a natural product isolated from Cinchona bark was used as an antimalarial agent for a long period of time. Further chemical modification of this nucleus led to the development of other therapeutic agents such as pamaquine,[3] mepaquine[4] and chloroquine.[5] Chloroquine has been the compound of choice that has emerged from these studies and it has remained a front line drug for the malaria treatment.[5] However, *P. falciparum* has developed resistance against chloroquine[6] as well as drugs such as sulfadoxine-pyrimethamine[7] and mefloquine[8] resulting into a limited choice of antimalarial drugs. In spite of these problems, there are some positive sites as well which counterbalance the drug resistance problem. The drug resistance of chloroquine is not due to the change in the structure of the drug target, but the resistance problem is due to the fact that the concentration of the drug is reduced at the target site via a specific mechanism.[9] Due to the increasing resistance of parasite to currently used drugs development of new chemotherapeutic agent for the treatment of malaria is always desirable. Many synthetic derivatives have been designed in order to overcome the drug resistance and some of the 4-aminoquinolines have shown promising antimalarial activity against chloroquine resistant strains of parasite.[10] Hence there is still scope for structural manipulation of chloroquine molecule in such a way that problem of drug resistance can be sorted out.

The SAR studies on 4-aminoquinolines suggest that 7-chloro and 4-amino groups in quinoline nucleus are essential for antimalarial activity. Both of these inhibit the β-hematin formation and help the drug to accumulate in the acidic food vacuole of the parasite.[11] Substitution of 7-chloro group by electron donor or electron withdrawing groups reduces antimalarial activity of the resulting 4-aminoquinolines, which suggests that 7-chloro group is important for antimalarial activity of 4-aminoquinolines.[12] 4-Aminopyridine substructure of 4-aminoquinoline is essential as it helps in binding with the heme.[13] The presence of alkyl side chain is essential and experimental data show that both shortening (2–3 carbon atoms) and lengthening (10–12 carbon atoms) of the side chain in chloroquine lead to compounds with retained antimalarial activity against chloroquine resistant strains of *P. falciparum*.[14] Substitution of diethyl group by metabolically stable side chain of *tert*-butyl group as well as the heterocyclic functionality such as piperidyl, pyrrolidinyl and morpholinyl functionality led to an increase in antimalarial activity.[15] These observations gave an impetus to further modify the side chain of the 4-aminoquinolines in such a way that basicity of the resulting molecule can be

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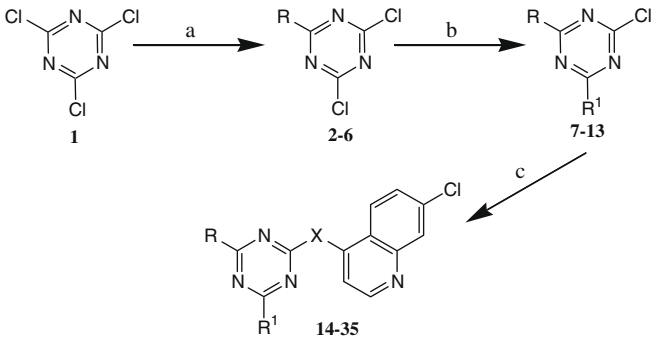
increased and hence concentration of 4-aminoquinolines can be increased in the acidic food vacuole of the parasite.[16] To overcome the problem of drug resistance, concept of hybrid compounds in which two pharmacophores are covalently linked to each other, was developed by Meunier and co-workers[17] in malaria chemotherapy. Some of the hybrid compounds such as artemisinin or trioxane aminoquinolines,[17,18] 4-aminoquinoline-isatin derivatives,[19] 4-aminoquinoline-based β-carbolines,[20] ferrocene-chloroquine analogues,[21] and 4-aminoquinoline–piperazine–triazine based compounds[22] have shown good promises. As a part of our ongoing programmes towards the synthesis of antimalarial compounds,[23] we report here in synthesis and antimalarial activity of 4-aminoquinoline–triazine conjugates.

The target compounds (**14**–**35**) were prepared by the consecutive nucleophilic substitution of cyanuric chloride with different nucleophiles as shown in Scheme 1. To start with, first chlorine of cyanuric chloride was substituted with morpholine, aryl amines and piperidine functionality at 0 °C to yield monosubstituted triazines in 80–85% yield (**2**–**6**). The monosubstituted triazine (**2**–**6**) on reaction with various amines such as 3,5-dimethoxy aniline, morpholine, cyclohexyl amine and toluidine at room temperature provides disubstituted triazines in good yield (**7**–**13**). Third chlorine of disubstituted triazine was substituted by 4-aminoquinolines having different alkyl chain lengths and reaction was carried out at reflux temperature in acetonitrile (Table 1). All of the compounds were purified over silica gel column and characterized spectroscopically.[24]



Scheme 1. Reagents and conditions: (a) Aromatic or aliphatic amines, K_2CO_3, THF, 0 °C, 3 h, 80–85%; (b) aromatic or aliphatic amines, K_2CO_3, THF, rt, 3 h, 80–85%; (c) 4-aminoquinolines, K_2CO_3, CH_3CN, 82 °C, 12–14 h, 49–65%.

In vitro antimalarial activity and mammalian cells cytotoxicity of 4-aminoquinoline–triazine conjugates was determined as described earlier.[25] Although the cytotoxicity of all the analogs was determined against a panel of three cell lines (HepG2, LLC-PK_{11} and Vero), selectivity index of antimalarial activity was calculated based on the cytotoxicity to vero cells.[23,25]

Variation of substitution pattern at second and fourth position of triazine nucleus has been explored in order to identify the best combination of substitution pattern for improvement of antimalarial activity. Among the series of 22 aminoquinoline–triazine conjugates (**14**–**35**), compounds **16**, **19**, **28** and **35** were most active against both chloroquine-sensitive (D6) and chloroquine resistant strains (W2) of *Plasmodium falciparum* with IC_{50} ranging from 0.21 to 0.48 μM. The selectivity index of **16**, **19** and **35** was much higher than the selectivity index of **28** as shown in Table 2. All the compounds with morpholine at second and 3,5-dimethoxy aniline at fourth position and aminoquinoline with different substituent pattern at sixth position of the triazine ring (entries **14**–**19**) exhibited antimalarial activity, except compound **18**. Out of these, compounds with ethylenediamine and propylinediamine linker (entries **14**–**16**) were very potent with IC_{50} values ranging from 0.22 to 0.58 μM (Table 2). Activity profile clearly showed that activity of these conjugates (entries **14**–**19**) depends on the length of the linker that connects 4-aminoquinoline with disubstituted triazine nucleus. With a chain length of C2–C3, the activity against both the strains is retained (entries **14**–**16**) while on increasing the length to C4 or C5 the activity is lost (entries **17** and **18**). At higher chain length (C8), the activity is retained (entry **19**). Interestingly, disubstituted triazine with morpholine at second and aminoquinoline with C2 linker at fourth position and a free Cl at sixth position of triazine nucleus resulted in a decrease in antimalarial activity (entry **20**). Substitution of R^1 in compounds **14** and **15** with morpholine functionality also resulted in a decrease of antimalarial activity (entries **21** and **22**). Compounds with piperidine at second, cyclohexylamine at fourth and 4-aminoquinoline at sixth position with C2 to C4 linker also showed antimalarial activity to a variable extent (entries **23**–**26**). Activity of compounds **27** and **28** was very promising against both strains with IC_{50} ranging from 0.21 to

Table 1
Synthesis of 4-aminoquinoline–triazine conjugates

Entry	R	R^1	X
14	Morpholine	3,5-Dimethoxy aniline	1,2-Ethylenediamine
15	Morpholine	3,5-Dimethoxy aniline	1,2-Propanediamine
16	Morpholine	3,5-Dimethoxy aniline	1,3-Propanediamine
17	Morpholine	3,5-Dimethoxy aniline	1,4-Butanediamine
18	Morpholine	3,5-Dimethoxy aniline	1,6-Hexanediamine
19	Morpholine	3,5-Dimethoxy aniline	1,8-Octanediamine
20	Morpholine	Chloro	1,4-Butanediamine
21	Morpholine	Morpholine	1,2-Ethylenediamine
22	Morpholine	Morpholine	1,2-Propanediamine
23	Piperidine	Cyclohexylamine	1,3-Propanediamine
24	Piperidine	Cyclohexylamine	1,4-Butanediamine
25	Piperidine	Cyclohexylamine	1,2-Ethylenediamine
26	Piperidine	N^1-(7-Chloro-quinolin-4-yl)-propane-1,3-diamine	1,3-Propanediamine
27	Aniline	Chloro	1,2-Ethylenediamine
28	Aniline	Aniline	1,2-Ethylenediamine
29	Aniline	Aniline	1,4-Butanediamine
30	*o*-Toludine	*o*-Toludine	1,2-Ethylenediamine
31	*o*-Toludine	*o*-Toludine	1,2-Propanediamine
32	*o*-Toludine	*o*-Toludine	1,3-Propanediamine
33	*o*-Toludine	*o*-Toludine	1,6-Hexanediamine
34	Diethylamino	Chloro	1,2-Ethylenediamine
35	N^1-(7-Chloro-quinolin-4-yl)-ethane-1,2-diamine	Chloro	1,2-Ethylenediamine

Table 2
In vitro antimalarial activity of 4-aminoquinoline–triazine conjugates

Entry	*P. falciparum* (D6 clone)		*P. falciparum* (W2 clone)	
	IC_{50} (μM)	Selectivity index	IC_{50} (μM)	Selectivity index
14	0.56	53.2	0.58	51.4
15	0.42	69.0	0.56	51.8
16	0.25	130.8	0.22	148.6
17	3.17	>15.1	3.53	>13.6
18	NA		NA	
19	0.48	>100	0.35	>137.1
20	2.45	>19.6	4.91	>9.8
21	1.59	>30.2	2.02	>23.8
22	0.97	>49.5	0.70	>68.6
23	1.74	19.7	2.22	15.4
24	0.67	46.9	0.71	44.2
25	1.46	24.2	1.81	19.5
26	1.27	>37.8	3.16	>15.2
27	0.56	>85.7	0.87	>55.2
28	0.21	49.5	0.25	41.6
29	2.54	11.6	4.91	6.0
30	0.39	75.4	0.72	40.8
31	0.42	59.0	0.61	40.6
32	0.79	33.8	0.69	38.7
33	0.62	36.9	0.58	39.5
34	1.58	30.4	1.89	25.4
35	0.31	>154.8	0.38	>126.3
CQ	0.042	>300	0.42	>30
Art	0.03	>500	0.025	>670

NA: no activity up to 9 μM; selectivity index (IC_{50} for cytotoxicity to vero cells/IC_{50} for antimalarial activity); CQ: chloroquine; Art: artemisinin.

Table 3
In vitro cytotoxicity of 4-aminoquinoline–triazine conjugates to mammalian cells

Entry	IC_{50} (μM)		
	$LLC\text{-}PK_{11}$	HepG2	Vero
14	9.5	29.8	29.8
15	11.6	8.9	29.0
16	8.3	25.4	32.7
17	9.5	38.8	NC
18	28.7	NC	NC
19	NC	NC	NC
20	33.5	12.9	NC
21	NC	NC	NC
22	>48	NC	NC
23	10.5	8.3	34.3
24	8.4	9.6	31.4
25	27.0	10.4	35.3
26	>48	14.1	NC
27	32.8	30.5	NC
28	11.2	3.9	10.4
29	8.8	9.8	29.4
30	8.6	9.8	29.4
31	8.0	9.3	24.8
32	9.0	8.8	26.7
33	6.3	14.6	22.9
34	32.0	12.6	48.0
35	28.8	2.0	NC
Doxorubicin	0.85	0.42	20

NC: No cytotoxicity up to 48 μM; $LLC\text{-}PK_{11}$: pig kidney epithelial cells; HepG2: human hepatoma cells; Vero: monkey kidney fibroblasts.

0.87 μM with high selectivity index. However, upon increasing the length of the linker to C4 the activity diminished (entry **29**).

Compounds having *o*-toludine and aminoquinolines with different linker (entries **30**–**33**) exhibited antimalarial activity with IC_{50} values in the range of 0.31–1.58 μM against D6 clone and 0.38–1.89 μM against W2 strain of *P. falciparum*.

Among the most active compounds (**16**, **19**, **28** and **35**), although **28** was more cytotoxic to Vero cells than others (Table 3) but still exhibited a high selectivity index of antimalarial activity (Table 2). Among the series of conjugates, compounds **28**–**33** were more cytotoxic to all three cell lines than others. In general the cytotoxicity of the most of the conjugates appeared at much higher concentrations than the concentrations responsible for their antimalarial activity. Some of the conjugates were not cytotoxic at all up to the highest tested concentration of 48 μM indicating their safety in the mammalian system.

In conclusion, series of 4-aminoquinoline–triazine conjugates have been prepared in three steps and these conjugates have shown promising antimalarial activity against both D6 and W2 strains of *P. falciparum*. Further chemical modification of selected compounds is under progress, and results will be published in due course of time.

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24. *Spectral data of selected compounds*. *N*-[2-(7-Chloro-quinolin-4-ylamino)-ethyl]-*N*′-(3,5-dimethoxy-phenyl)-6-morpholin-4-yl-[1,3,5] triazine-2,4-diamine (**14**): yield: 55%; mp 148–150 °C; IR (cm^{-1}, Nujol): 3312, 2923, 2854, 1583, 1455, 1378, 1153, 873, 807; ^{1}H NMR (300 MHz, DMSO-d_6): 3.53–3.68 (m, 18H), 6.07 (s, 1H), 6.51–6.60 (m, 1H), 7.02–7.05 (m, 3H), 7.34–7.43 (m, 2H), 7.78 (s, 1H), 8.15–8.18 (m, 1H), 8.36–8.37 (m, 1H), 8.91–9.13 (m, 1H); ESI-MS (*m*/*z*): 537.79 (M^++1), 539.65 (M^++2); Anal. Calcd for $C_{26}H_{29}ClN_8O_3$: C, 58.15; H, 5.44; N, 20.87. Found: C, 58.33; H, 5.69; N, 20.59. N^1-(7-Chloro-quinolin-4-yl)-N^2-(4,6-di-o-toludine-4-yl-[1,3,5]triazin-2-yl)-hexane-1,6-diamine (**33**): yield: 49%; mp 214–216 °C; IR (cm^{-1}, KBr): 3428, 3232, 2931, 1581, 1500, 1453, 1367, 1136, 808; ^{1}H NMR (300 MHz, DMSO-d_6): 1.19–1.64 (m, 8H), 2.18 (s, 6H), 3.13–3.22 (m, 4H), 6.42 (d, 1H, *J* = 5.7 Hz), 6.80–7.10 (m, 7H), 7.29–7.53 (m, 4H), 7.76 (s, 1H), 8.01–8.10 (m, 2H), 8.25 (d, 1H, *J* = 9 Hz), 8.36 (d, 1H, *J* = 5.7 Hz); ^{13}C NMR (75.5 MHz, DMSO-d_6): 24.48, 49.97, 50.15, 104.34, 104.40, 104.67, 108.58, 108.68, 115.73, 116.03, 116.16, 117.77, 120.58, 122.13, 122.25, 122.47, 122.80, 123.06, 123.13, 123.16, 123.28, 123.39, 123.58, 124.37, 125.04, 126.21, 129.82, 131.03, 132.74, 133.80, 153.94, 154.98; ESI-MS (*m*/*z*): 566.96 (M^++1), 568.86 (M^++2); Anal. Calcd for $C_{32}H_{35}ClN_8$: C, 67.77; H, 6.22; N, 19.76. Found: C, 67.89; H, 6.38; N, 19.63.
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