In vitro Antimalarial activity against *P. falciparum* (D-10 and K1 strain) and *β*-Haematin inhibition of novel synthesized Quinoline-Triazole analogues

Mukesh Chandra Joshi*[1], Timothy John Egan[2]

[1]*Department of Chemistry, Motilal Nehru College, Benito Juarez Marg, South Campus, University of Delhi, New Delhi-110021, India*

[2]*Department of Chemistry, University of Cape Town, 7700, South Africa*

**Corresponding Author Email: mukeshjoshi21@gmail.com*

ABSTRACT

The side chains of quinoline antimalarials play a very important role in making then potent antimalarial agents as they contain an amino group in the side chain which has a critical role in pH trapping. We have reported two series, the dibemequines that have a dibemethin side chain with excellent antimalarial activity and the quinoline-triazole amide analogues (**38-51**) which contain an amide terminal group and showed moderate to good antimalarial activity and strong *β*-haematin inhibitory activity. Compounds **40**, **45** and **49** were found to be most active compounds in the latter series against the chloroquine sensitive D10 strain of *P. falciparum* with an IC_{50} value in the range of 349-1247 nM while **49** was active against the chloroquine resistant K1 strain of parasite. 7-chloro quinoline triazoles **40** and **44** were potent *β*-haematin inhibitors with an IC_{50} 14.7 and IC_{50} 8.9 μM. Overall the 7-chloro substituted quinoline triazole analogues showed better *β*-haematin inhibitory activity than the 7-cyano quinoline triazole analogues. These studies are reviewed here.

Key words: Chloroquine, dibemethin, quinoline-trazole, *P. falciparum*, antiplasmodial activity, *β*-haematin activity

1. Introduction

Malaria is the one of the most life threatening diseases worldwide which is caused by a lethal human parasitic infection. Malaria threatens more than 50% of the population worldwide and there are an estimated 355 million cases of malaria annually while 297 million cases are at high risk. The Sub-Saharan African region accounts for ~90% of malaria cases, while 7% were in South-East Asia and 3% in the Eastern mediterranean [World Malaria Report 2016]. Malaria is caused by four deadly species of protozoan parasites of the genus *Plasmodium, Plasmodium falciparum, P. vivax, P. ovale* and *P. malariae*. They differ in microscopic, geographical and clinical features and are gradually becoming resistant to the antimalarial drugs which left a wide scope for new discoveries.

Chloroquine is among the most widely studied antimalarials to date due to its low toxicity, easy synthesis, good pharmacokinetic properties and less side-effects [Eicher et al., 2003; Ginsburg, 1992; Foley et al., 1997; O'Neill et al., 1998; Fitch, 2004; Kouznetsov et al., 2009; Solomon et al., 2007; Idan et al., 2005; Gelb, 2007; Wiesner et al., 2003; Biagini et al., 2005]. Yet complications associated with drug resistance necessitate the discovery of new effective antimalarial agents. Use of chloroquine was intensive during the mid 20th century in an attempt to eradicate malaria. Later, numerous quinoline drugs have been synthesized and marketed viz. mefloquine, amodiaquine, primaquine, and piperaquine. These are in addition to the original quinoline drug, quinine (**Fig. 1**).

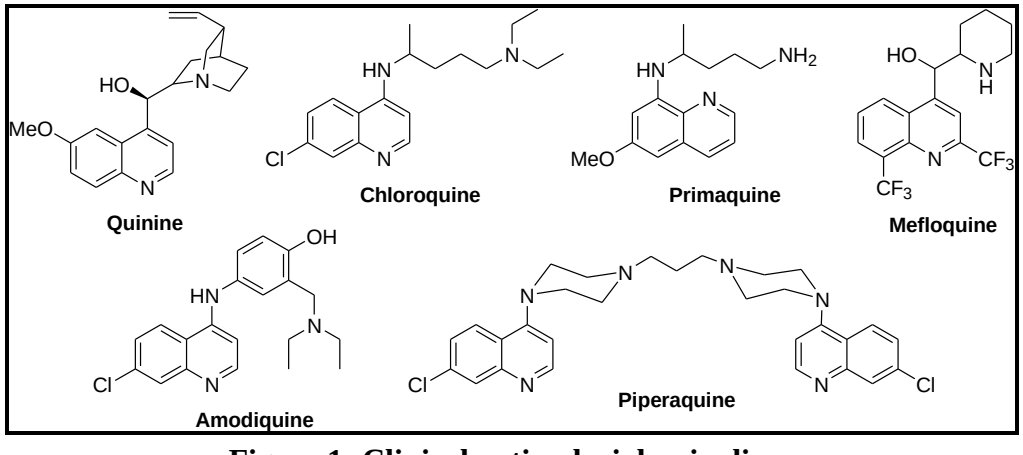


Figure 1: Clinical antimalarial quinolines

The actual mode of action of quinoline analogues has been studied in the recent years, but still remains incomplete [Ginsburg et al., 1992; Foley et al., 1997; Foley et al., 1998; O'Neill et al., 1998; Fitch at al., 2004]. The general understanding of scientists is these drugs mostly act during the blood stages and hepatic stages of the life cycle of the parasite [Baird et al., 2003]. The side chain quinoline analogues have brought a great advance in understanding structure-activity relationships and mechanism of action of this class of drugs which are known to inhibit the crystallisation of Fe(III)-haem to β-haematin, the synthetic counterpart of haemozoin [Hanscheid et al., 2007], an insoluble product formed in the parasite digestive vacuole following digestion of human haemoglobin [Egan, 2008]. Inhibition of haemozoin formation leads to intraparasitic accumulation of free Fe(III)-haem [Combrinck, 2013] which is highly toxic to the parasite.

Recently, numerous side chain modified 4-amino-quinoline analogues have been synthesized and it was anticipated that such hybrid molecules might help to counteract drug resistance of the parasite [Zishiri et al., 2011; Andrews et al., 2010; Burgess et al., 2006; 2010]. Some previous investigations reported that polar side chains may have more susceptibility to drug-resistance accounting for the introduction of more lipophilic groups such as triazoles, triazines, imidazoles and other hetercyclic moieties [Andrews et al., 2010; Peyton et al., 2006]. Thus keeping these ideas in mind we provide here a useful comprehensive antimalarial compilation and also review the synthesis of quinoline-dibemethin and quinoline-triazole amide analogues as well as their antimalarial and β-haematin inhibition activity.

Potent side chain variant quinoline based antimalarial agents

Numerous side chain variant quinoline antimalarial agents have been synthesized and some of them have been found to have potent antimalarial activity. Various studies on hybrid compounds have been conducted which combine antiplasmodial activity with CQR-reversing ability. Burgess et al. first reported that these so-called reversed-CQ molecules (RCQs) are active against both chloroquine

sensitive (CQS) and chloroquine resistant (CQR) parasites [Burgess et al., 2006; Peyton et al., 2006]. Some acridone scaffolds were found with haem-binding and resistance-reversing abilities and they also exhibited additional activity against the CQS strain while synergistic activity was found against the CQR strain of *P. falciparum* [Kelly et al., 2009]. Resistance-reversing side chain containing quinoline antimalarials **1** and **2** (Fig. 2) were found to be active against CQS or CQR strain of *P. falciparum* [Andrews et al., 2010; Bhattacharjee et al., 2002; Peyton et al., 2006]. They contain acridone like moieties and 3^o basic terminial, which provides considerable flexibility available to the drug designer while preserving the pharmacophore features. These studies opened the door for side chain linking reversal-agent-like moieties. Some other dibasic side chain quinoline analogues (**3-8**) were found to have submicromolar antimalarial activity against CQS HB3 and CQR Dd2 strains of *P. falciparum* and low resistance indices were found in most cases. Compounds **3–7** were the most potent against the CQR Dd2 strain in the range of IC_{50} 19.9 - 53.0 nM (standard CQ IC_{50} = 122 nM) and compound **8** was active against both the CQS HB3 and CQR Dd2 strains of *P. falciparum* with IC_{50} 77.5 (standard CQ IC_{50} = 9.8 nM) and 108 nM (standard CQ IC_{50} = 138 nM), respectively [Iwaniuk et al., 2009]. It indicates that side chain branching is one of the crucial requirements for activity against the CQR strain. Some 7-chloro-4-aminoquinolinyl-derived sulphonamides (**9**), amides (**10**, **11**), ureas (**12**, X = O), thioureas (**12**, X = S) have shown submicromolar antimalarial activity against CQS HB3 and CQR Dd2 strains of *P. falciparum* while low resistance indices were obtained in most cases. They showed IC_{50} values in range of 10-1000 nM against CQS HB3 and IC_{50} value in the range of 23-1000 nM against DCQR d2 strains of *P. falciparum* [Ekoue-Kovi et al., 2009]. The *β*-amino alcohol containing 4-aminoquinoline-isatin conjugates and side chain modified analogues have been successfully evaluated for their antimalarial activity (**13-16**). When the side chain of compound **13** is varied with n = 2 and 3, then the IC_{50} values were found to be 11.7 and 13.5 nM, respectively, against the CQR W2 strain of *P. falciparum*. But overall ranges for all the compounds were

found to be 11.7-1960 nM. Compound **14** was found to be most active among piperazine series with an IC_{50} value of 216 nM (when R = H) and it was observed that the activity was reduced with the addition of Cl, F or CH_3 at the R position on the phenyl ring. Chloroquine and artemisinin (IC_{50} of CQ = 36.37 nM and IC_{50} of ART = 4.37 nM, respectively) were used as a standard drug for comparison [Nisha et al., 2014]. Compounds **15** and **16** showed potent antimalarial activity against the CQR W2 strain of *P. falciparum* with IC_{50} values of 5.6 and 17.3 nM respectively, while CQ was used as a standard drug with IC_{50} of 382 nM. These molecules were also tested *in vivo* in mice and found to have biological half-lives and plasma exposure values similar to or higher than those of CQ. This study also opened the door for clinical investigations [Ray et al., 2010].

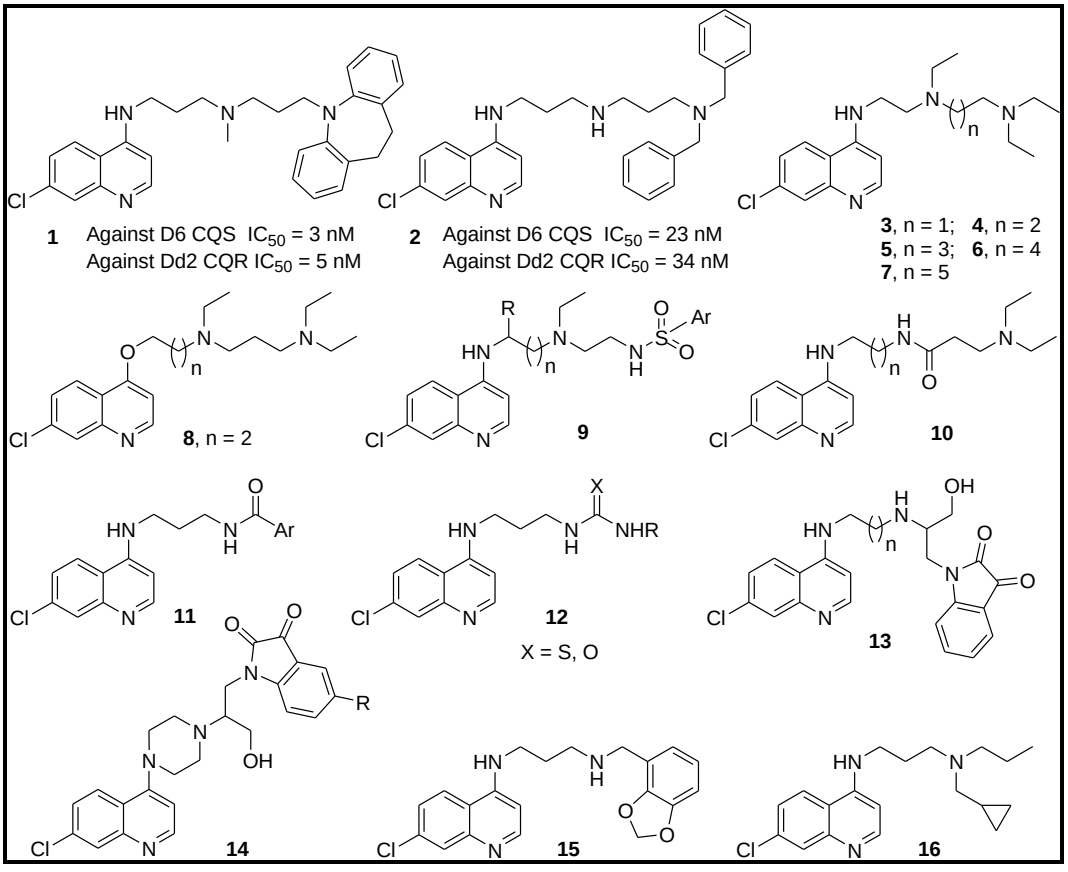


Figure 2: Experimental 4-aminoquinolines with various side chains

Burgess et al., have also reported a number of antimalarial agents having potential antimalarial activity. Some of them (**17-19**) were among the most active *in vitro* of RCQ compounds against CQS D6, CQR Dd2, and 7G8 strains of *P. falciparum* [Burgess et al., 2010]. Compound **18** was found to be a potent *in vivo* antimalarial agent against mouse model (mouse spleen lymphocytes) and had the highest level of hemozoin inhibition, both *in vitro* & *in vivo*. This study indicates that improved reversal side chain drugs are more active as antimalarials. Side-chain modified 4-aminoquinoline compounds **20-24** exhibited *in vitro* activity where chloroquine (IC_{50} = 0.106 μM) was used as standard drug against the NF-54 strain *P. falciparum in vitro* while **21**, **22**, and **24** exhibited significant suppression against N-67 strain of *P. yoelii* in the *in vivo* assay. These compounds also exhibited excellent β-haematin activity with IC_{50} in the range of 0.24-0.76 μM [Solomon et al., 2007].

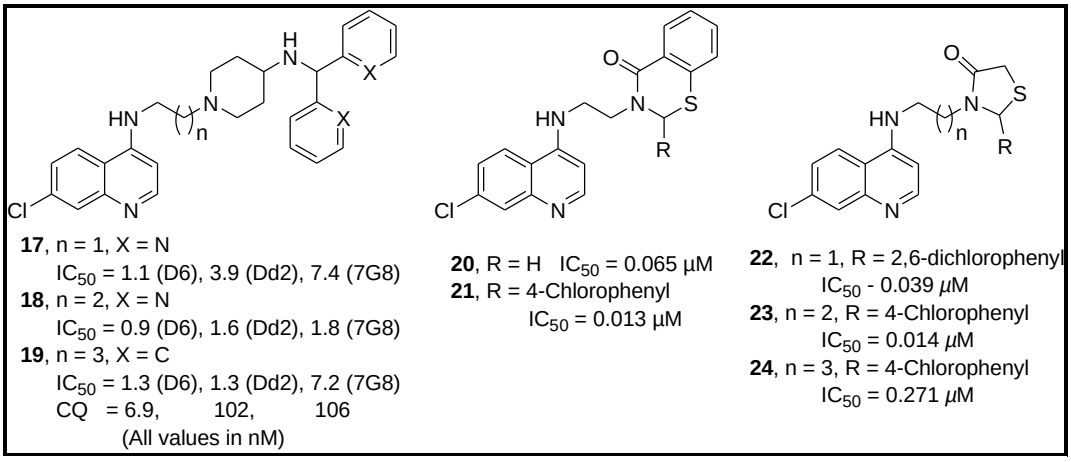


Figure 3: Further RCQs and side chain modified 4-aminoquinolines

Pretorius et al. have reported pyrimidine modified quinoline hybrids and evaluated them for their *in vitro* antimalarial activity as well as cytotoxicity. Some of them **25-28** were screened against the CQS D10 and CQR Dd2 strains of *P. falciparum*. They also exhibited low cytotoxicity against the mammalian Chinese Hamster Ovarian cell line [Pretorius et al., 2013]. Another lipophilic ring, triazine,

has been used in modified quinoline hybrids (**29-35**) which also exhibited potent antimalarial activity against the CQS 3D7 strain of *P. falciparum*, while **29** and **30** were found to be orally active (at a dose of 100 mg/kg 4 days) against CQR strain of *P. yoelii* and they also exhibited low cytotoxicity toward the Vero cell line [Kumar et al., 2011]. Some other lipophilic compounds 4-aminoquinoline-1,2,3-triazole and 4-aminoquinoline-1,2,3-triazole-1,3,5-triazine hybrids (**36**) were evaluated for their antimalarial activity against D6 and W2 strains of *P. falciparum*. Some of the compounds have shown promising antimalarial activity without toxicity against Vero cells [Manohar et al., 2011].

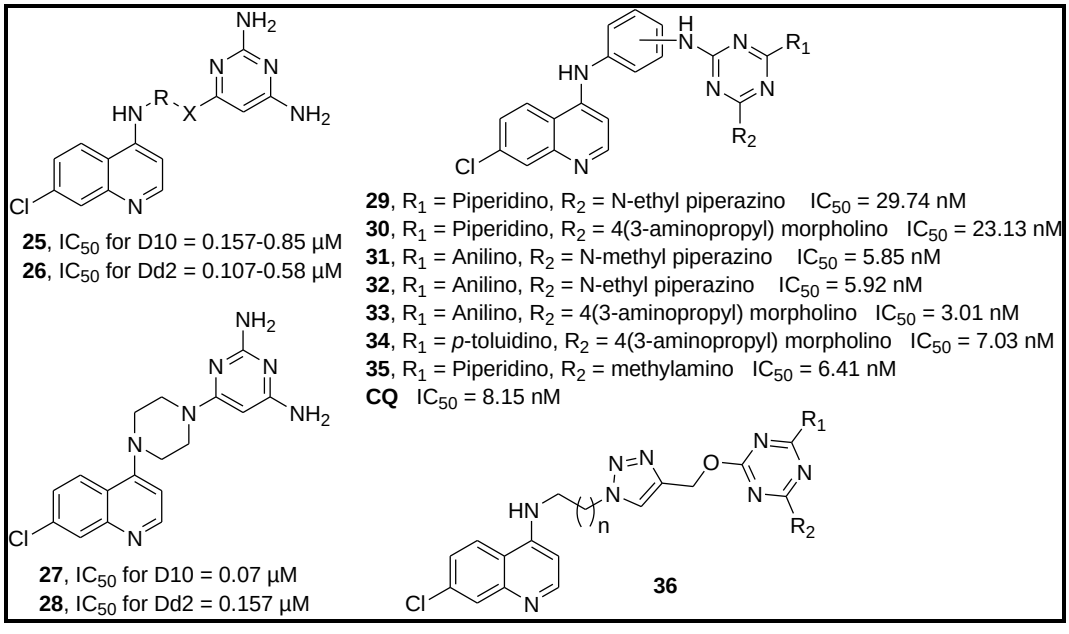
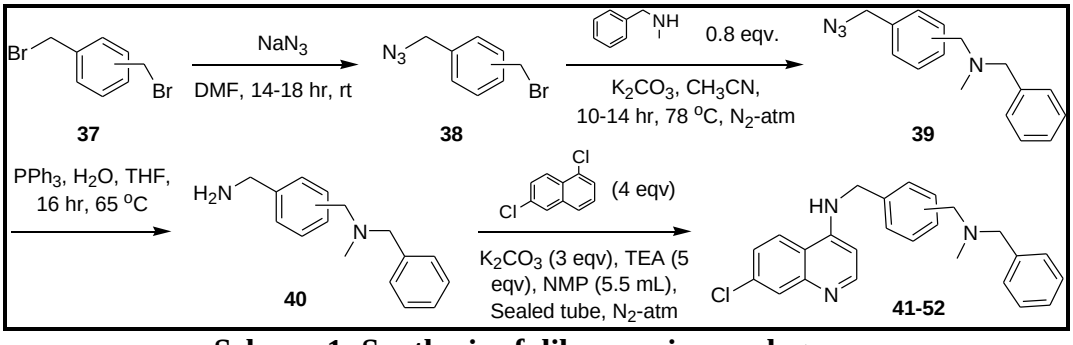


Figure 4: Pyrimdine and triazine side chain modified 4-aminoquinolines

In a more recent study 4-amino-7-chloroquinolines with dibenzyl-methylamine (so called dibemethin) side chains, compounds that have been named the dibemequines (**41-52**, **Scheme-1**, **Fig. 5**) were developed and shown to inhibit synthetic hemozoin formation [Zishiri et al., 2011; 2011]. These dibemethin containing quinoline antimalarial compounds were found to be equally active against the CQS D10 and CQR K1 strains of *P. falciparum* and were

compared with CQ (**Table 1**). Compounds **42**, **43**, and **52** displayed potent *in vitro* antimalarial activity against both CQS D10 and CQR K1 strains of parasite, with IC_{50} values less than 100 nM. Compounds **42**, **43**, **and 52** exhibited little or no cytotoxicity in a mammalian cell line. Compounds **41**, **42**, **and 43** were tested *in vivo* against mouse malaria via oral administration, where two compounds, **41** and **42**, reduced parasitemia by over 99% (with mice treated at 100 mg/kg surviving for 30 days). These three compounds were also shown to inhibit chloroquine transport via the parasite's CQR transporter (PfCRT) in a *Xenopus* oocyte expression system. These compounds were the first example of dual functioning antimalarials for which the ability to inhibit both hemozoin formation and PfCRT together was directly demonstrated. The activity of these compounds was ascribed to their ability to accumulate in the parasite digestive vacuole and inhibit hemozoin formation, a finding which is consistent with previous structure activity studies of other CQ analogues. CQR is caused by mutations in the *P. falciparum* CQ-resistance transporter (PfCRT) which is located in the membrane of the parasite's digestive vacuole, the organelle in which CQ exerts its antimalarial effects by interfering with the formation of hemozoin [Kaschula et al., 2002]. Expression of PfCRT at the surface of *Xenopus laevis* oocytes has allowed the function of the protein to be studied directly, leading to the demonstration that the resistance-conferring form of PfCRT (PfCRT CQR) has the ability to transport CQ out of the digestive vacuole, whereas the CQS form (PfCRT CQS) does not [Martin et al., 2009].



Scheme 1: Synthesis of dibemequine analogues

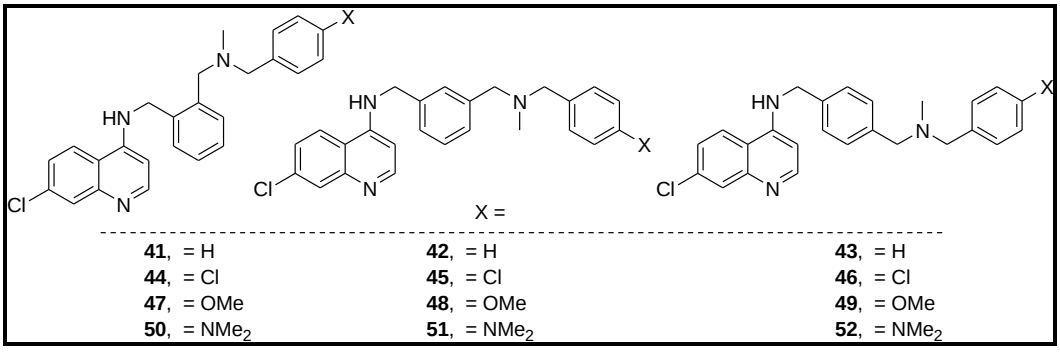


Figure 5: Various dibemequine derivatives

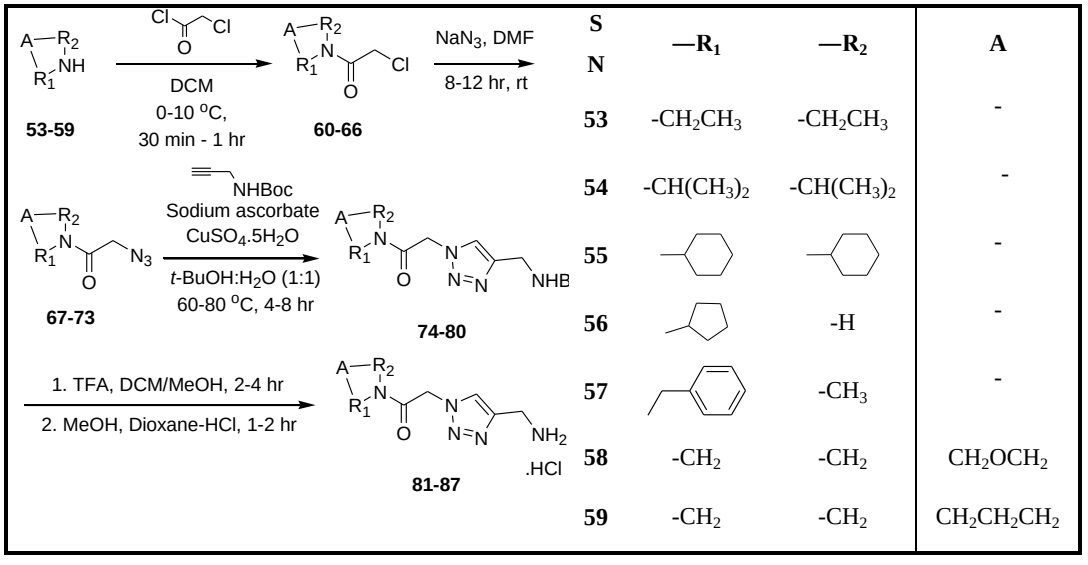
Table 1: Antimalarial and *β*-haematin inhibitory activity of dibemequine derivatives (41-52)

SN	pK_{a1}	pK_{a2}	$BHIA_{50}$	D10 IC_{50} (nM)	K1 IC_{50} (nM)	RI
41	7.57	9.85	1.1	140	122	0.9
42	7.63	9.77	0.62	41	43	1.0
43	7.56	9.90	0.32	22	26	1.2
44	7.44	9.60	1.4	ND	1128	-
45	7.55	9.89	0.66	138	85	0.6
46	7.44	9.74	0.34	ND	ND	-
47	7.47	9.85	0.46	175	134	0.8
48	7.38	9.67	0.52	91	120	1.3
49	7.44	9.70	1.44	130	88	0.7
50	7.40	9.71	0.48	178	149	0.8
51	7.36	9.67	0.5	ND	281	-
52	7.33	9.66	0.4	48	37	0.9
CQ	8.40	10.8	1.9	23	144	6.3

RI = Resistance indices [(IC_{50} (K1)/IC_{50} (D10)], ND = Not determined

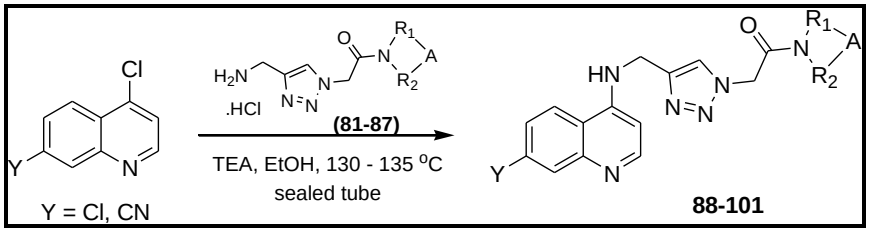
Quinoline-triazole amide conjugates

We also reported a series of quinoline-triazole amide hybrid conjugates prepared using Click chemistry and evaluated for their *in vitro* antimalarial activity (**Scheme-2 & 3; Table-2 & 3**). These compounds proved a valuable addition to antimalarial discovery [Joshi et al., 2013]. Their synthesis is shown in Schemes 2 and 3.



SN	—R_1	—R_2	A
53	-CH_2CH_3	-CH_2CH_3	-
54	-$CH(CH_3)_2$	-$CH(CH_3)_2$	-
55			-
56		-H	-
57		-CH_3	-
58	-CH_2	-CH_2	CH_2OCH_2
59	-CH_2	-CH_2	$CH_2CH_2CH_2$

Scheme 2: Synthesis of Intermediate triazole amides (81-87)



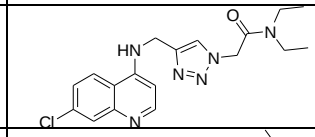
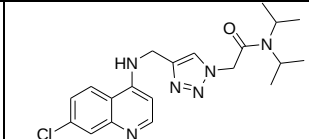
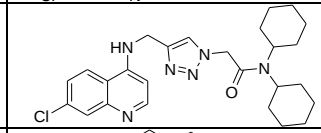
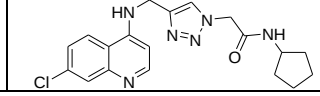
Scheme 3: Synthesis of Quinoline-triazole amide analogues (88-101)

Quinoline-triazole amide hybrid conjugates (**88-101**) were evaluated for their *in vitro* antimalarial activity against the *P. falciparum* CQS D10 and CQR K1 strains of *P. falciparum* (**Table 2**) while chloroquine (CQ) was used as a standard drug. Among all quinoline-triazole amide conjugates, **90, 95** and **99** were found the most active against the D10 strain of *P. falciparum* while compound **95** and **99** (compounds having a 7-CN quinoline core) were less effective than those with the 7-Cl group. Resistance indices (RI) varied from low values (in **90** and **97**, 1.5 and 2.5, respectively) to high (in **91** and **99**, 10.8 and 12.1, respectively). Compounds **95** and **99** were also tested for cytotoxicity in mammalian (Chinese Hamster Ovarian, CHO) cells and found that both the compounds were cytotoxic at much higher concentrations than their antimalarial activity (**Table 3**).

Generally, active 4-aminoquinoline antimalarial contains a basic amino side chain and it is believed that it plays a crucial role in pH trapping [Egan et al., 2000; Kaschula et al., 2002]. In the quinoline triazole conjugates, we incorporated an amide side chain after the triazole which is unable to assist in pH trapping, thus it is impressive that compounds **90** and **95** have parasite IC_{50} values below 1 μM in the CQS D10 strain of the parasite. Compound **90** contains two cyclohexyl groups attached to the amide N, resembling the reversed chloroquine motifs reported by Andrews et al. [Andrews et al., 2010]. Compound **90** was the most active against both the CQS D10 and CQR K1 strains of the parasite, and also exhibited evidence of cross-resistance with CQ.

Compounds **88-101** were evaluated for β-haematin inhibiting properties using an NP-40 detergent mediated assay [Carter et al., 2010; Ncokazi, 2005; Sandlin, 2011]. Chloroquine and amodiaquine were used as standard drugs with IC_{50} values of 18.2 and 6.8 μM, respectively. Among all compound **90** and **94** (7-Cl substituent), and **98** and **99** (7-CN substituent) were found to be the most active (**Table 2**).

Table 2: IC_{50} data against parasite and β-haematin inhibition of compounds (88-101)

SN	Compound Structure	Parasite IC_{50} (nM)		*RI*	β-H IC_{50} (μM)	logP
		D10	K1			
88		1440	10498	7.3	20.3	2.45
89		1592	9518	6.0	32.7	3.27
90		348.8	518.6	1.5	14.7	4.93
91		2473	26681	10.8	30.5	2.70

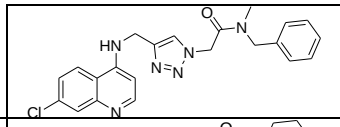
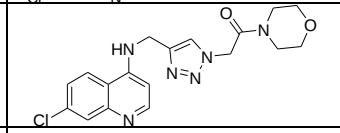
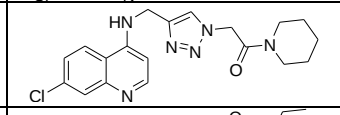
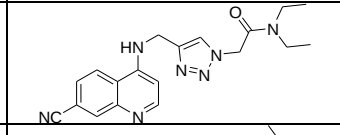
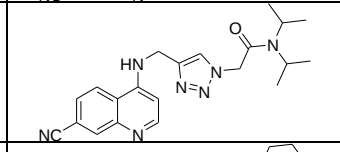
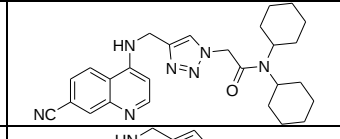
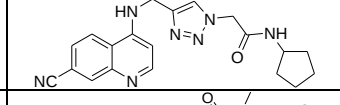
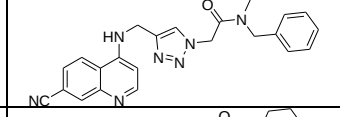
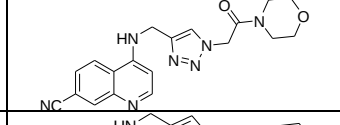
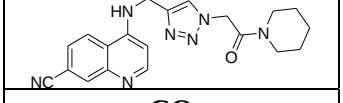
92		1910	9077	4.8	21.7	3.54
93		8387	69624	8.3	28.7	1.42
94		2063	11287	5.5	8.9	2.48
95		584.5	2399	4.1	46.3	1.74
96		3969	14440	3.6	23.7	2.57
97		2599	6563	2.5	26.5	4.23
98		12131	37297	3.1	16.1	2.00
99		1247	15057	12.1	15.9	2.84
100		27518	130625	4.7	44.6	0.72
101		13619	43439	3.2	32.7	1.78
	CQ	17.4	354.7	20.3	18.2	3.81

Table 3: Cytotoxicity and selectivity index values of compound 40 and 45.

Compound	D10 IC_{50} (µM)	K1 IC_{50} (µM)	CHO IC_{50} (µM)	SI (D10)	SI (K1)
90	0.349	0.519	10.7	30.7	20.6
95	0.585	2.40	73.2	125	30.5
Emetine	0.041				

In conclusion, we have highlighted the 4-aminoquinoline side chain analogues and their antimalarial activity. The side chain plays a crucial role in activity and resistance and allows for great variation in these compounds.

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