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Original article

Synthesis and bioevaluation of novel 4-aminoquinoline-tetrazole derivatives as potent antimalarial agents

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ABSTRACT

A series of novel tetrazole derivatives of 4-aminoquinoline were synthesized and screened for their antimalarial activities against both chloroquine-senstive (3D7) and chloroquine-resistant (K1) strains of *Plasmodium falciparum* as well as for cytotoxicity against VERO cell lines. Most of the synthesized compounds exhibited potent antimalarial activity as compared to chloroquine against K1-strain. Compounds with significant *in vitro* antimalarial activity were then evaluated for their *in vivo* efficacy in Swiss mice against *Plasmodium yoelii* following both intraperitoneal (ip) and oral administration, wherein compounds **20** and **23** each showed *in vivo* suppression of 99.99% parasitaemia on day 4.

1. Introduction

Parasitic diseases are a global problem, affecting 30% of the world's population. Among parasitic diseases, Malaria is one of the most devastating infectious disease claiming more lives than any other parasitic infections. There were at least 216 million cases of acute malaria reported in 2010 and about 655,000 people died from malaria, 86% of which are children under 5 years of age [1]. It is most common in tropical and subtropical areas and 90% of all cases are found in sub-Saharan Africa. These facts are attributable almost exclusively to *Plasmodium falciparum*, one of the five *Plasmodium* species responsible for malaria in humans.

Since the development of quinine, drugs with quinoline scaffold have immense significance as antimalarial chemotherapeutic agents. Among the quinoline derivatives, 4-aminoquinolines (Fig. 1) especially Chloroquine (CQ, 1) have been extensively utilized for the treatment of malaria [2]. However, the widespread resistance of *P. falciparum* to chloroquine and other existing antimalarial drugs including the recent artemisinin class of antimalarial has hampered the efforts to combat this deadly disease [3–6]. So, there is an urgent need for the development of new antimalarial agents active against drug-resistant malaria strains. However, despite the development of resistance, CQ is still attractive pharmacophore for chemical modification, owing its excellent clinical efficacy, limited host toxicity, ease to use, simple cost-effective synthesis [7]. Furthermore, literature survey on 4-aminoquinoline antimalarials clearly suggested that antimalarial activity, particularly, inhibition of β-hematin formation and accumulation of the drug at the target site, resides in 4-aminoquinoline core [8]. In the past two decades, a number of new 4-aminoquinoline analogues with enhanced activity against CQR strains were developed by synthetic modifications of the CQ side chain [9–12]. Consequently, researches regarding the exploration of novel 4-aminoquinoline analogues that are equally active against chloroquine sensitive (CQ-S) and chloroquine resistant (CQ-R) strains have received much attention during recent years.

4-aminoquinoline hybridization is another quite attractive strategy which has been recently introduced in medicinal chemistry and drug discovery process, in order to circumvent the

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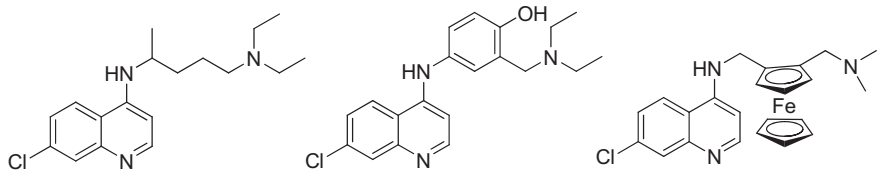


Chloroquine (CQ) **1** **Amodiaquine (AQ)** **2** **Ferroquine** **3**

Fig. 1. Structures of some 4-aminoquinoline derivatives with antimalarial activity.

problem of antimalarial drug resistance [13–17]. Moreover, in recent years, incorporation of other functionalities in the side chain of 4-aminoquinoline emerged as promising strategies to construct the molecules with enhanced activity against drug-resistant *P. falciparum* and also with improved metabolic stability.

Recently, Campiani et al. synthesized Clotrimazole (CLT) analogues [18] and subsequently prepared hybrid of the 4-aminoquinoline with the clotrimazole-like pharmacophore [19] in which imidazole ring of CLT behaves as a Fe(III) axial ligand and inhibit β-hematin formation and reported promising *in vitro* (against both sensitive and resistant strain) and *in vivo* antimalarial activities. On the other hand, tetrazole based compounds have received attention due to their wide range of biological activities [20–24]. Moreover, various tetrazole-based compounds have also shown good coordination properties and are able to form stable complexes with several metal ions inhibitors [25]. Recently, Roman et al. had exploited the ability of the tetrazole ring to coordinate with the iron centre of heme in the design of heme oxygenase inhibitors [26].

Inspired by these encouraging results, we envisaged that incorporation of tetrazole moiety (with ability to coordinate with the heme) in the side chain of 4-aminoquinoline pharmacophore would lead to develop the new antimalarial agents active against chloroquine resistant strains (CQ-R) of *P. falciparum*. On the basis of the above fact and in the continuation of our ongoing programme to develop new potent antimalarial agents [27–30] we herein, report the efficient synthesis of new 4-aminoquinoline analogues containing tetrazole moiety (Fig. 2) and their evaluation as potentially active compounds against *Plasmodium* malaria parasite.

2. Results and discussion

2.1. Chemistry

The targeted compounds were synthesized *via* two-step simple and efficient synthetic protocol. The detailed synthetic route for the synthesis of intermediate (**7**–**10**) and target compounds (**11**–**32**) is outlined in Scheme 1. Reaction of commercially available 4,7-dichloroquinoline with corresponding diaminoalkanes/piperazine/*p*-phenylenediamine *via ipso* nucleophilic substitution reaction resulted in the formation of compounds (**7**–**10**). These intermediate compounds (**7**–**10**) were then utilized as amine input in isocyanide based multicomponent reaction. The target compounds (**11**–**32**) were efficiently prepared by treating commercially available aldehyde with amine (**7**–**10**), corresponding isocyanide and $TMSN_3$ in methanol at room temperature. All compounds were characterized using ^1H NMR, ^{13}C NMR, HRMS and IR spectroscopy.

2.2. Biological assay

All the synthesized target compounds (**11**–**32**) were screened for their *in vitro* antimalarial activity against CQ sensitive 3D7 strain and CQ-resistant K1 strain of *P. falciparum* (Table 1) using a standardized inexpensive assay based on Malaria SYBR Green I nucleic acid staining dye based fluorescence (MSF) assay. The IC_{50} values were calculated from experiments carried out in triplicate. The cytotoxicity of all the synthesized molecules was determined against VERO cell line using MTT assay. The *in vivo* drug responses of selected compounds were evaluated in Swiss mice infected with resistant strain N-67 of *Plasmodium yoelii* which is intrinsically resistant to CQ.

2.2.1. In vitro antimalarial activity

Initially, all the synthesized 4-aminoquinoline-tetrazole derivatives (**11**–**32**) were evaluated for their *in vitro* antimalarial efficacy against CQ-sensitive (3D7) and CQ-resistant (K1) strains of *P. falciparum*. All tested compounds exhibited moderate activity against the CQ-S (3D7) strain, with IC_{50} value ranging from 10.66 to 216 nM. Among these 22 compounds, 3 compounds (**20**, **21** and **23**) showed promising activity 10.66, 11.01, 11.78 nM respectively, 11 compounds displayed moderate activity with the IC_{50} values ranging from 23.89 nM to 81.40 nM. Furthermore, 13 compounds were found to be more active (with IC_{50} values between 73.70 and 233.70 nM) than CQ ($IC_{50} = 254$ nM), when screened against CQ-R (K1) strain of *P. falciparum*. Two compounds **11** and **17** showed IC_{50} values 277.80 and 383.50 nM which was comparable to CQ. Moreover, some of our synthesized compounds were also showing low resistance index (Table 1).

The structure–activity relationship studies on these compounds suggested that the activity of these compounds were greatly influenced by type of linker, substitutions on aromatic ring as well as substitution in the tetrazole ring. The activity data suggested that the type of linker had a remarkable influence on the activity of 4-aminoquinoline tetrazole derivatives. According to our results, compounds with ethylene diamine linker (**11**, **12**) exhibited mild inhibition against 3D7 strain in comparison to standard drug. However, the activity decreases dramatically on increasing the chain length to 3 carbon (**13**–**15**). Interestingly, substitution of the flexible aliphatic linker with rigid aromatic linker *p*-phenylenediamine resulted in manifold increased activity against both the strains (3D7, K1). This increased activity may be due to increase in lipophilicity of phenylene linker. Moreover, among the synthesized series, compound **25**, **27** and **30** with phenylenediamine linker were found to be most active against resistant strain (K1) and were 3.44–3.38 fold more active than chloroquine. These SAR data clearly suggested that aromatic linker have crucial role in activity of the compounds and analogues with aromatic linker probably endowed with new leads that overcome drug resistance.

Another modification performed in the series was substitution of *tert* butyl group in tetrazole ring with cyclohexyl ring. The cyclohexyl substituted analogues **28**–**30** showed manifold decrease in potency than analogues having *tert* butyl **18**, **20** and **23** against 3D7 strain. Nevertheless, against K1 strain, 4-chloro **28** and 4-bromo **29** analogue with cyclohexyl substituted tetrazole ring showed increased activity while 3,4 dimethoxy **30** resulted in

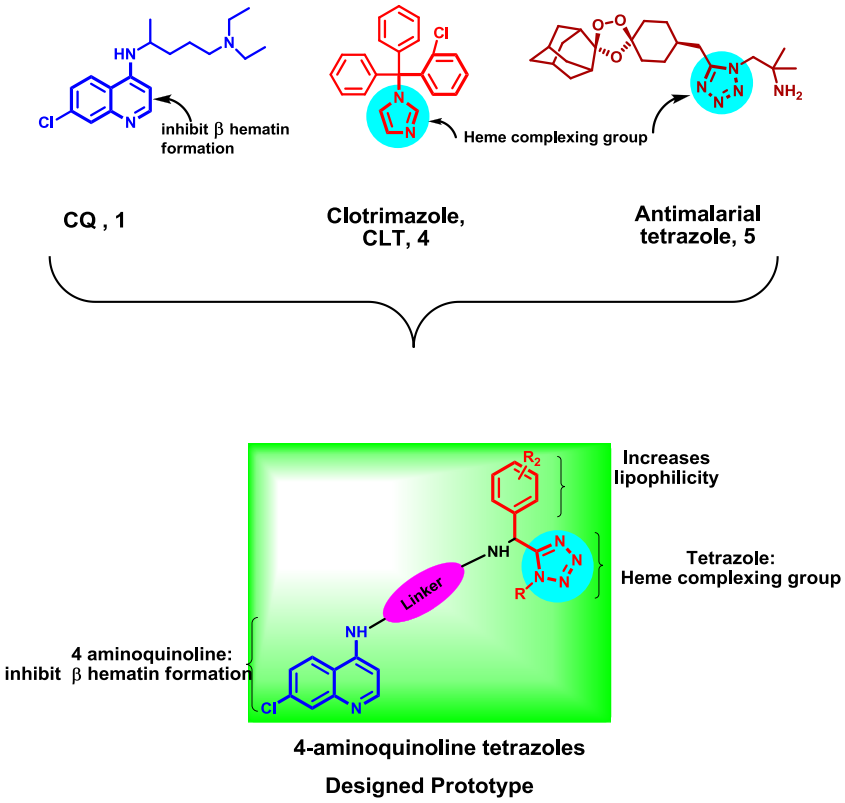
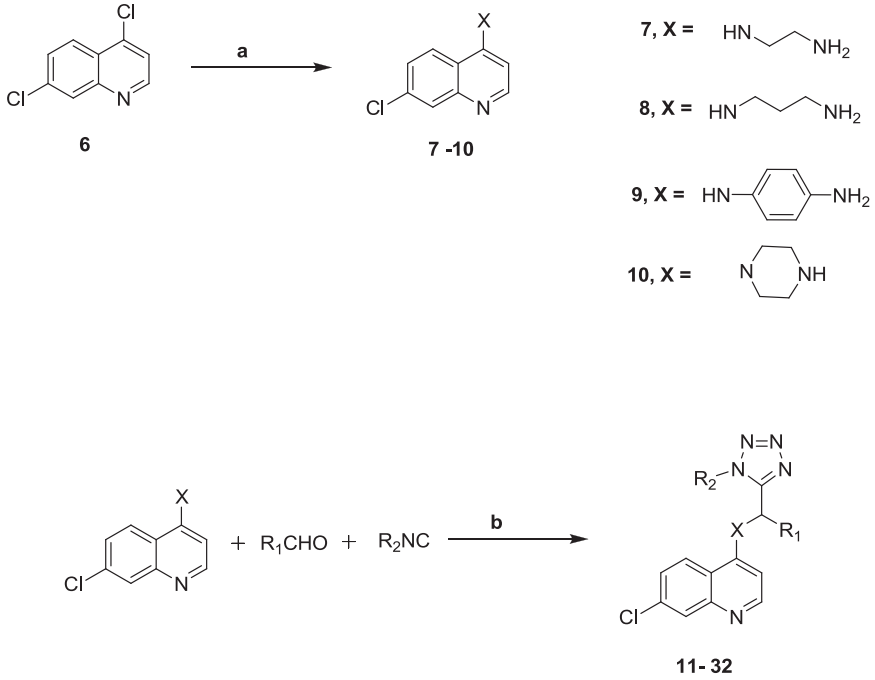


Fig. 2. Basis for the synthesis of tetrazole derivatives of 4-aminoquinoline.

decreased activity as compared to respective analogue with *tert* butyl substituted tetrazole ring.

Furthermore, the activity of compounds also depends on the substitution on phenyl ring however there is no obvious trend of activity with respect to substituted group and strains of parasite. Compounds with unsubstituted phenyl ring **24** and 4-ethyl substituted group **26** were showing mild activity IC_{50} 59.64, 51.35 nM respectively against 3D7 strain but none of compounds



Reagents and Conditions: (a) diamines, 110 °C, neat or ethanol; (b) $TMSN_3$, MeOH, rt, 24 h.

Scheme 1. Synthesis of 4-aminoquinoline-tetrazole derivatives.

Table 1
In vitro antimalarial activity of compounds against 3D7 and K1 strains of *P. falciparum* and their cytotoxicity against VERO cell line.

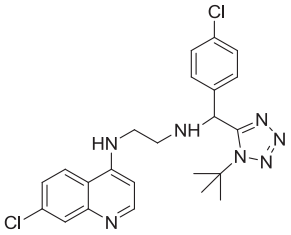
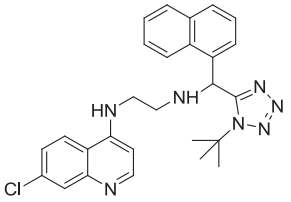
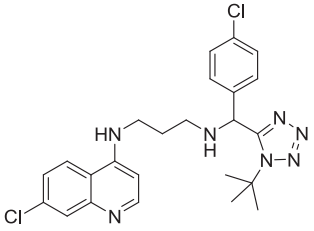
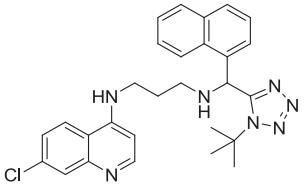
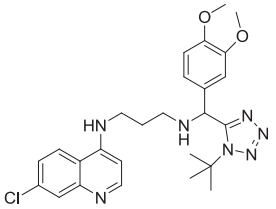
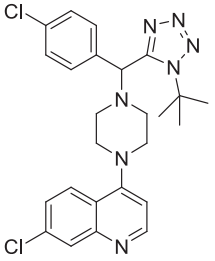
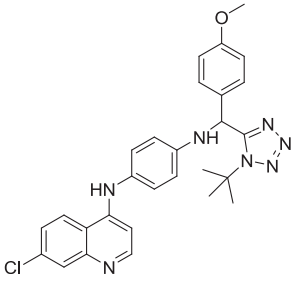
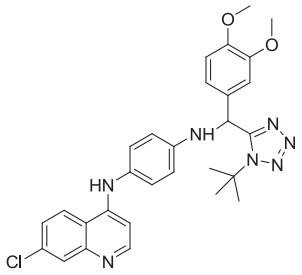
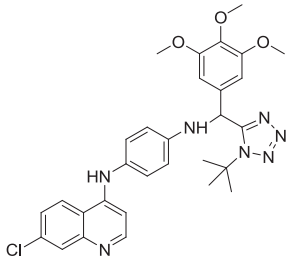
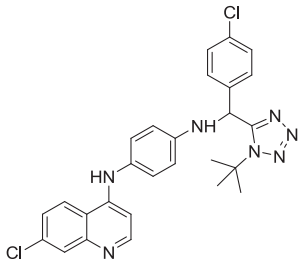
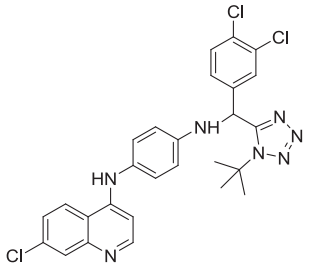
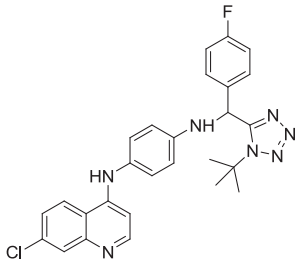
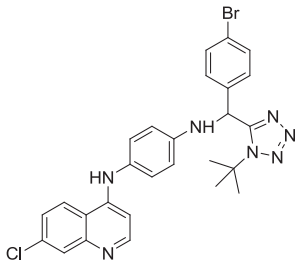
Compound no.	Structure	*In vitro* antimalarial activity IC_{50} (nM)[a]		SI[b]	Log *P*[c]	RI[d]
		3D7	K1			
11		23.89	277.80	839.68	4.99	11.6
12		120.40	184.40	305.32	5.47	1.5
13		81.40	754.50	248.65	5.26	9.2
14		216.10	170.20	58.67	5.75	0.8
15		57.88	137.50	1662.92	4.23	2.3
16		786.9	159.00	182.96	5.34	0.2
17		57.66	383.50	702.34	6.58	6.6

Table 1 (*continued*)

Compound no.	Structure	*In vitro* antimalarial activity IC_{50} (nM)[a]		SI[b]	Log *P*[c]	RI[d]
		3D7	K1			
18		70.58	76.16	193.28	6.17	1.0
19		54.22	572.80	300.57	6.15	10.5
20		10.66	142.9	4616.14	7.02	13.4
21		11.01	141.90	7076.29	7.81	12.8
22		29.52	669.30	3165.31	6.66	22.67
23		11.78	233.7	2332.43	7.33	19.8

(*continued on next page*)

Table 1 (*continued*)

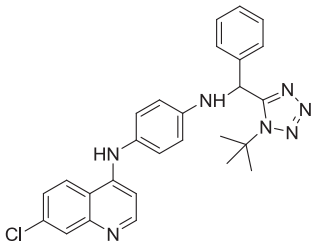
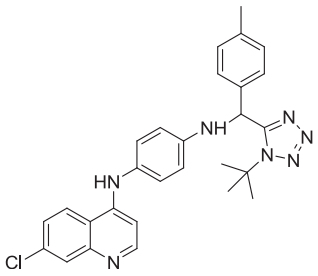
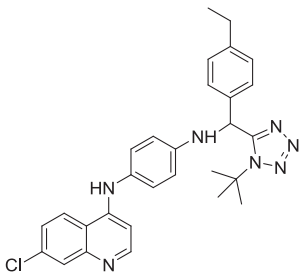
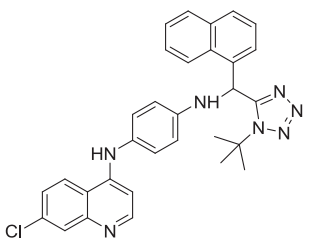
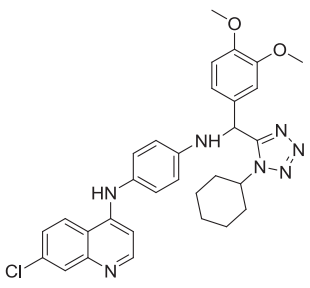
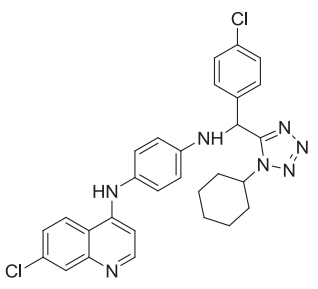
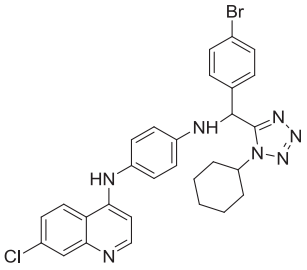
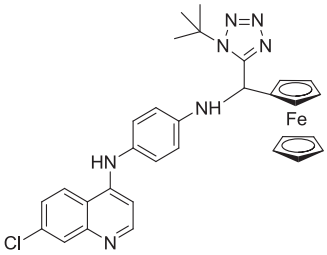
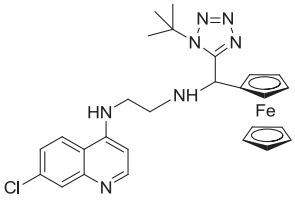
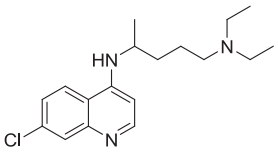
Compound no.	Structure	*In vitro* antimalarial activity IC_{50} (nM)[a]		SI[b]	Log *P*[c]	RI[d]
		3D7	K1			
24		59.64	>1023	2383.47	6.52	–
25		1006	75.39	–	6.97	0.07
26		51.35	688.30	3652.19	7.44	13.4
27		28.00	77.79	3815.38	7.68	2.7
28		853.40	144.40	49.73	6.89	0.1
29		156.40	138.60	694.18	7.92	0.9

Table 1 (*continued*)

Compound no.	Structure	*In vitro* antimalarial activity IC_{50} (nM)[a]		SI[b]	Log *P*[c]	RI[d]
		3D7	K1			
30		92.66	73.70	1191.58	8.05	0.8
31		585.83	>1000	261.70	–	–
32		>1000	>1000	48.77	–	–
CQ		5.4 ± 0.98	254 ± 20.65	8983.00	5.00	47.03

[a] IC_{50} (nM): concentration corresponding to 50% growth inhibition of the parasite.
[b] Selectivity index (SI): (IC_{50} values of cytotoxic activity/IC_{50} values of antimalarial activity).
[c] log *P* values calculated using online software www.molinspiration.com.
[d] Resistance index (IC_{50} − K1/IC_{50} − 3D7).

were active against resistant strain K1, while 4-methyl substituted analogue **25** was found to be inactive against 3D7 strain but found to be most potent in series against resistant strain with IC_{50} value 75.39 nM. Interestingly, despite the type of linker naphthalene substituted derivatives were found to be active against K1 strain wherein compound **27** showed promising activity with IC_{50} 77.79 nM and also showed better activity in 3D7 strain IC_{50} 28.00 nM. In case of methoxy substituted compounds, 4-methoxy, 3,4-dimethoxy and 3,4,5 trimethoxy derivatives were showing good activity in sensitive strain (3D7) and showed mild activity in resistant strain except 3,4 dimethoxy compounds which exhibited promising activity against CQ-R strain with IC_{50} 76.16 nM. Introduction of halogen substituent have immense influence on activity. Compounds substituted with 4-chloro **20** and 4-bromo **23** on phenyl ring were found to be most active against 3D7 strain and have very promising activity against K1 strain while 4-flouro **22** substituted analogue showed mild activity against 3D7 and found to be inactive against K1. Additionally analogue **20** and **23** were also found to be *in vivo* active.

Additionally, ferrocene containing heterocyclic compounds gaining interest because of their wide applicability in organic, organometallic synthesis as well as in medicinal chemistry [14,31–33]. More precisely, the successful development of Ferroquine, Trioxaferroquines and ferrocenyl derivative of Ciprofloxacin as antimalarial drugs having strong antimalarial activity against resistant strain of *plasmodium* as compared to parent drug inspired us to synthesize ferrocene containing analogues **31** and **32**. However, disappointingly in our case these analogues were not found to be active.

2.2.2. *In vitro cytotoxicity*

The cytotoxicity of all the synthesized molecules **11**–**32** was determined against VERO cell line using MTT assay (Table 1). 17 molecules showed high selectivity index (SI) ranging between 182.96 and 7076.29, rest of molecules showed selectivity index <100 but not less than 49.73. Compounds **20**, **21** and **23** which were most potent against 3D7 also showed good selectivity index 4616.14, 7076.29 and 2332.43 respectively (Table 1). Compound **30** which was most potent against resistant strain K1, also showed good selectivity index of 1191.58. In general, most of the compounds of the series exhibited reasonably promising activity against K1 strain, less cytotoxic effect with fairly high selectivity index, and consequently these 4-aminoquinoline tetrazole derivatives are good candidate for further lead optimization.

Table 2
β-Hematin inhibitory activity of selected compounds.[a]

Compound no.	IC_{50} (μg/mL)
11	6.15
12	6.33
13	5.95
14	4.45
15	5.50
16	5.32
17	5.55
18	5.42
19	5.65
20	5.29
21	5.40
22	5.49
23	5.65
24	5.55
25	5.39
26	5.95
27	5.12
28	5.19
29	**3.81**
30	4.38
31	4.55
32	6.32
CQ	**3.95**

The bold values represent compound having best β-hematin inhibitory activity in present series.
[a] The IC_{50} represents the concentration of compound that inhibits β-hematin formation by 50%., Data are the mean of three different experiments in triplicate.

2.2.3. β-Hematin inhibitory activity

To find out the mode of action of the synthesized molecules, the β-hematin inhibitory activity of all the molecules was carried out (Table 2). All of the screened compounds inhibited β-hematin formation in a concentration-dependent manner. All the tested compounds showed IC_{50} values between 3.81 μg/mL and 6.33 μg/mL. Among these, compound **29** showed better activity with IC_{50} values = 3.81 μg/mL than CQ (3.95 μg/mL).

2.2.4. In vivo antimalarial activity

Compounds with significant activity *in vitro* (**16**, **18**, **20**, **21**, **23**, **25**, **27**, **29** and **30**) were selected for *in vivo* efficacy in Swiss mice against with CQR N-67 strain of *P. yoelli*. Initially the *in vivo* activity of selected molecules was determined through intraperitoneal route at the dose of 50 mg/kg administered once daily for four consecutive days and monitored for parasitaemia, and survival of mice until day 28 postinfection (Table 3). The compounds **20** and **23** exhibited 99.99% parasitaemia suppression on day 4, while compounds **16**, **21**, **27** and **30** suppressed parasitaemia by 73.91%, 71.49%, 57.03%, 69.56% respectively. Furthermore, these data clearly, showed that halogen substituted phenyl derivatives have better *in vivo* activity profile. Compound **20**, one of the potent compound of the series, showed 99.99% parasitaemia suppression on day 4 and 60% survival on day 28 of treatment at a dose of 50 mg/kg × 4 days, while compound **23**, showed 99.99% parasite suppression on day 4 and 80% survival on day 28 of treatment. Furthermore, compound **20**, cured 60% of treated mice while compound **23**, cured 40% of treated mice. Compound **20** and **23**, with promising result by intraperitoneal route were further screened at 100 mg/kg by oral route. Both compounds exhibited promising results with 99.99% parasite suppression on day 4, and 60% survival on day 28 of treatment.

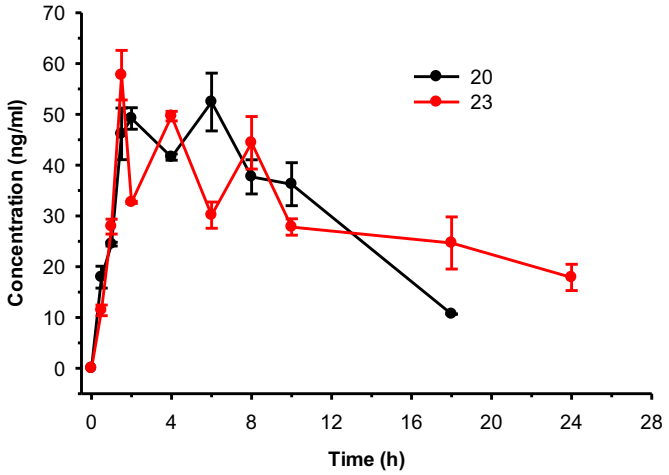


Fig. 3. Concentration–time profile of **20** and **23** after a single oral (10 mg/kg) dose in male Sprague–Dawley rats. Bar represents SEM.

2.2.5. Preliminary pharmacokinetic studies

The compelling *in vitro* and *in vivo* antimalarial activity exhibited by compound **20** and **23** encouraged us to perform *in vivo* oral pharmacokinetic study of compound **20** and **23**. Pharmacokinetic studies revealed that the animals tolerated the treatment as no peculiarities in the animals behaviour were observed. Calculations of the pharmacokinetic data were based on the mean serum concentrations and were performed by the use of noncompartmental approaches using WinNonlin program, version 5.1 (Scientific Consulting Inc.). **20** and **23** were rapidly absorbed and slowly eliminated (Fig. 3 and Table 4). Both compounds showed multiple peaks phenomenon which may be due to the enterohepatic

Table 3
In vivo antimalarial activity of selected compounds against CQ resistant N-67 strain in Swiss mice.

Compound code	Dose (mg/kg × 4 days)	Route of administration	Percent suppression on day 4 postinfection	Survival[a]	MST[b]	Cure[c]
16	50	IP	73.91	0/5	13.00 ± 1.58	0/5
18	50	IP	26.91	0/5	9.80 ± 1.02	0/5
20	**50**	**IP**	**99.99**	**3/5**	**23.60 ± 2.71**	**3/5**
20	**100**	**Oral**	**99.99**	**3/5**	**25.2 ± 1.74**	**3/5**
21	50	IP	71.49	0/5	16.83 ± 1.83	0/5
23	**50**	**IP**	**99.99**	**4/5**	**26.00 ± 2.00**	**2/5**
23	**100**	**Oral**	**99.99**	**3/5**	**25.60 ± 1.47**	**2/5**
25	50	IP	21.30	0/5	11.00 ± 1.23	0/5
27	50	IP	57.03	0/5	12.00 ± 0.55	0/5
29	50	IP	20.43	0/5	10.40 ± 1.21	0/5
30	50	IP	69.56	0/5	12.60 ± 1.5	0/5
Control	–	IP	–	–	9.6 ± 1.20	0/5
CQ	10	Oral	99.99	0/5	16.5 ± 1.40	0/5

The bold values represent compounds with best *in vivo* activity in series.
[a] Number of mice that survived till day 28 postinfection/total mice in the group.
[b] MST, mean survival time (days) ± standard error (SE).
[c] Number of mice without parasitaemia (cured) till day 28 postinfection.

Table 4
Pharmacokinetic parameters of **20**, **23** and CQ in male rats.

Parameters		**20**[a]	**23**[a]	CQ[b]
C_{max} (ng/mL)	1	49.20 ± 2.10	57.73 ± 4.88	5228.7
	2	52.44 ± 5.69	49.65 ± 0.94	
	3	–	44.42 ± 5.16	
t_{max} (h)	1	2	1.5	0.91
	2	6	4	
	3	–	8	
AUC_{0-t} (ng h/mL)		593.3	703.52	30,147
$t_{1/2}$ (h)		5.27	22.64	9.5
V_d (L)		28.16	63.38	1.49
Clearance (L/h)		3.70	1.94	0.15

Abbreviations: AUC_{0-t} = area under the serum concentration–time curve up to last sampling time, C_{max} = serum peak concentration, t_{max} = time to C_{max}, $t_{1/2}$ = elimination half-life, V_d = volume of distribution.
[a] Each value represents the average of three rats dosed orally (10 mg/kg).
[b] Each value represents the average of six rats dosed orally (5 mg/kg); values of C_{max} are mean ± SEM.

recirculation, delayed gastric emptying, and variability of absorption in different regions of gastrointestinal tract [34]. For both compounds **20** and **23**, the volume of distribution (V_d, 28.6 L and 63.38 L, respectively) is greater than the total blood volume (13.5 mL; Davies and Morris, 1993) [35], indicating high extravascular distribution of the compound. Moreover, the systemic clearance of both **20** and **23** was higher than the hepatic blood flow of the rat (Davies and Morris, 1993) which indicates an extrahepatic elimination. On comparison with reported pharmacokinetic profile of CQ [36], compound **23** showed higher terminal half-life than CQ. Both compounds **20** and **23** have higher volume of distribution which indicates their extensive distribution in the body than that of CQ. A 1.9-fold lower clearance, 1.9-fold higher AUC and 3.3-fold higher MRT of **23** than that of **20** suggest that **23** could be a better candidate drug than **20**.

3. Conclusion

In summary, we have synthesized new tetrazole derivatives of 4-aminoquinoline by utilizing highly efficient $TMSN_3$-Ugi multicomponent reaction. The majority of these synthesized molecules exhibited promising *in vitro* antimalarial activity especially against CQ-R strain (K1). Most of the compounds have shown the fairly high selectivity index. In present series, compounds **20** and **23** showed promising *in vitro* activity against both CQ-S as well as CQ-R strain of *P. falciparum* and also excellent *in vivo* antimalarial activity against *P. yoelli*. Taken as a whole, these studies highlights the potential of these new 4-aminoquinolne-tetrazole derivatives especially compounds **20** and **23** for developing novel antimalarials that address CQ resistance as well as able to reduce parasitaemia after oral administration.

4. Experimental methods

4.1. General information

Commercially available reagents and solvents were used without further purification. Thin-layer chromatography (TLC) was carried out with silica gel plates (silica gel 60 F254), that were visualized by exposure to ultraviolet light. IR spectra were recorded on a FTIR spectrophotometer Shimadzu 8201 PC and are reported in terms of frequency of absorption (cm^{-1}). ^1H NMR and ^{13}C NMR spectra were recorded on Bruker Supercon Magnet Avance DRX-400, 300 or DPX 200 FT spectrometers using TMS as an internal reference and the samples were dissolved in suitable deuterated solvents (Chemical shifts (δ) are given in ppm relative to TMS and coupling constants (J) in Hz). HR-DART MS were recorded on JEOL, JMS T100LC Accu TOF. Column chromatography purifications were performed in flash using 60–120 or 100–200 mesh silica gel. The melting points were recorded on an electrically heated melting point apparatus and are uncorrected.

4.2. General procedure for the synthesis of compounds **7** and **8**

A mixture of 4,7-dichloroquinoline (1 equiv.) and 1,2-diaminoethane/1,3-diaminopropane (5 equiv.) were heated for 6–8 h with continued stirring to drive the reaction to completion. After completion of the reaction the excess solvent was removed under vacuum. The reaction mixture was then poured into ice cold water and precipitate was filtered and washed with ethyl acetate to furnish compound **7** and **8** in quantitative yields.

4.3. General procedure for the synthesis of compounds **9** and **10**

A solution of 4,7-dichloroquinoline (1 equiv.) and *p*-phenylenediamine/piperazine (2.0 equiv.) in absolute ethanol was refluxed in presence of catalytic amount of *p*-TSA for 3–5 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 **9** and **10** in excellent yields.

4.4. General procedure for the synthesis of compounds **11**–**32**

To a stirred solution of amine **7**–**10** (1 mmol) in methanol (6 mL) were added successively commercially available benzaldehyde (1.1 mmol), isocyanide (1 mmol) and $TMSN_3$ (1 mmol). The mixture was stirred at room temperature for 24 h. After completion of the reaction, solvent was evaporated at reduced pressure and purified through column chromatography (eluent: $CHCl_3$/MeOH) using 60–120 or 100–200 mesh silica gel to afford the desired products (**11**–**32**).

4.4.1. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(4-chlorophenyl)methyl)-N^2-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**11**)

White semi solid; Yield: 76%; IR (cm^{-1}, KBr): 3269, 2954, 1581, 1434, 1234; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.48 (d, 1H, J = 5.1 Hz), 8.04 (d, 1H, J = 8.8 Hz), 7.94 (s, 1H), 7.44 (d, 1H, J = 8.9 Hz), 7.34 (d, 2H, J = 7.9 Hz), 7.21 (d, 2H, J = 7.9 Hz), 6.37 (br s, 1H), 6.32 (d, 1H, J = 5.1 Hz), 5.37 (s, 1H), 3.34 (br s, 2H), 3.31–2.89 (m, 2H), 2.74 (br s, 1H), 1.60 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.3, 151.4, 150.2, 148.5, 136.8, 134.9, 134.7, 129.3, 127.9, 125.4, 122.2, 117.3, 98.8, 61.8, 58.3, 45.8, 42.5, 29.9; HRMS (ESI TOF (+)) calcd for [$C_{23}H_{25}Cl_2N_7 + H^+$] 470.1621 found 470.1622.

4.4.2. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(naphthalen-1-yl)methyl)-N^2-(7-chloroquinolin-4-yl) ethane-1,2-diamine (**12**)

White solid; Yield: 70%; mp 115–116 °C; IR (cm^{-1}, KBr): 3370, 2977, 1587, 1235; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.49 (d, 1H, J = 5.3 Hz), 8.23 (d, 1H, J = 8.0 Hz), 7.97–7.92 (m, 2H), 7.86 (d, 1H, J = 8.2 Hz), 7.79 (d, 1H, J = 8.9 Hz), 7.63–7.53 (m, 2H), 7.35–7.27 (m, 2H), 6.80 (d, 1H, J = 7.0 Hz), 6.38 (br s, 1H), 6.33 (d, 1H, J = 5.3 Hz), 6.18 (s, 1H), 3.49–3.38 (m, 2H), 3.29–3.13 (m, 2H), 1.51 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.4, 151.7, 150.0, 148.8, 134.8, 134.4, 134.2, 130.6, 129.7, 129.5, 128.2, 127.5, 126.2, 125.4, 125.1, 122.1, 122.0, 117.3, 98.9, 61.8, 55.5, 46.5, 42.3, 29.7; HRMS (ESI TOF (+)) calcd for [$C_{27}H_{28}ClN_7 + H^+$] 486.2167 found 486.2167.

4.4.3. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(4-chlorophenyl)methyl)-N^3-(7-chloroquinolin-4-yl)propane-1,3-diamine (**13**)

White solid; Yield: 72%; mp 130–132 °C; IR (cm^{-1}, KBr): 3432, 2988, 1636, 1455, 1217; ^1H NMR (300 MHz, $CDCl_3$): δ = 8.41 (d, 1H, J = 5.2 Hz), 7.88 (d, 1H, J = 1.7 Hz), 7.37–7.27 (m, 6H), 7.06 (dd, 1H,

J = 1.8 Hz, J = 8.8 Hz), 6.29 (d, 1H, J = 5.5 Hz), 5.32 (s, 1H), 3.44 (br s, 2H), 2.92–2.78 (m, 3H), 1.94 (br s, 2H), 1.51 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 154.9, 150.7, 150.6, 147.7, 136.6, 135.1, 134.8, 129.5, 129.4, 127.2, 125.2, 122.0, 116.9, 98.1, 61.6, 58.6, 47.5, 43.4, 29.9, 27.3; HRMS (ESI TOF (+)) calcd for [$C_{24}H_{27}Cl_2N_7$ + H^+] 484.1778 found 484.1782.

*4.4.4. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(naphthalen-1-yl)methyl)-N^3-(7-chloroquinolin-4-yl)propane-1,3-diamine (**14**)*

Off white solid; Yield: 75%; mp 68–70 °C; IR (cm^{-1}, KBr): 3269, 2830, 1580, 1371; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.37–8.31 (m, 2H), 8.06 (d, 1H, J = 8.9 Hz), 7.97 (d, 1H, J = 8.2 Hz), 7.91 (d, 1H, J = 8.1 Hz), 7.77 (s, 1H), 7.54–7.51 (m, 2H), 7.45–7.39 (m, 2H), 7.29 (d, 1H, J = 9.8 Hz), 6.99 (d, 1H, J = 7.1 Hz), 6.43 (d, 1H, J = 5.3 Hz), 6.19 (s, 1H), 3.34 (br s, 2H), 2.84–2.79 (m, 2H), 1.86 (br s, 2H), 1.51 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 155.1, 151.2, 149.7, 148.3, 134.8, 133.1, 132.8, 130.3, 128.2, 128.1, 126.7, 126.2, 125.4, 124.7, 124.4, 123.4, 123.4, 123.0, 116.8, 98.1, 60.9, 54.1, 45.4, 28.6, 27.7; HRMS (ESI) calcd for HRMS (ESI TOF (+)) calcd for [$C_{28}H_{30}ClN_7$ + H^+] 500.2324 found 500.2326.

*4.4.5. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(3,4-dimethoxyphenyl) methyl)-N^3-(7-chloroquinolin-4-yl)propane-1,3-diamine (**15**)*

White solid; Yield: 68%; mp 146–147 °C; IR (cm^{-1}, KBr): 3356, 2973, 1584, 1216; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.43 (d, 1H, J = 5.8 Hz), 8.18 (d, 1H, J = 9.0 Hz), 7.8 (s, 1H), 7.46 (d, 1H, J = 8.9 Hz), 7.16 (s, 1H), 6.88 (s, 2H), 6.58 (d, 1H, J = 5.9 Hz), 5.42 (s, 1H), 3.71 (s, 3H), 3.68 (s, 3H), 3.39–3.35 (m, 2H), 2.62–2.58 (m, 2H), 1.86–1.83 (m, 2H), 1.62 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 156.0, 152.4, 148.8, 148.6, 147.9, 144.4, 135.4, 131.1, 125.2, 124.7, 123.8, 120.7, 116.5, 112.2, 111.5, 98.6, 61.4, 57.1, 55.6, 55.5, 45.2, 41.6, 29.4, 27.5; HRMS (ESI TOF (+)) calcd for [$C_{26}H_{32}ClN_7O_2$ + H^+] 510.2379 found 510.2379.

*4.4.6. 4-(4-((1-tert-Butyl-1H-tetrazol-5-yl)(4-chlorophenyl)methyl) piperazin-1-yl)-7-chloroquinoline (**16**)*

Off white solid; Yield: 78%; mp 200–210 °C; IR (cm^{-1}, KBr): 3421, 3018, 1575, 1493, 1215; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.67 (d, 1H, J = 4.9 Hz), 8.05 (s, 1H), 7.89 (d, 1H, J = 8.9 Hz), 7.39–7.37 (m, 5H), 6.79 (d, 1H, J = 5.0 Hz), 5.44 (s, 1H), 3.23 (s, 4H), 3.08–3.05 (m, 2H), 2.74–2.71 (s, 2H), 1.72 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 156.6, 153.4, 151.8, 150.0, 134.8, 134.7, 133.4, 130.9, 128.9, 128.7, 126.0, 125.1, 121.7, 108.9, 64.1, 61.5, 52.2, 49.8, 30.2; HRMS (ESI TOF (+)) calcd for [$C_{25}H_{27}Cl_2N_7$ + H^+] 496.1778 found 496.1782.

*4.4.7. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(4-methoxyphenyl)methyl)-N^4-(7-chloroquinolin-4-yl) benzene-1,4-diamine (**17**)*

Yellow solid; Yield: 78%; mp 106–107 °C; IR (cm^{-1}, KBr): 3269, 2954, 1581, 1434, 1234; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.35 (d, 1H, J = 5.3 Hz), 7.92 (s, 1H), 7.79 (d, 1H, J = 8.8 Hz), 7.34 (d, 2H, J = 8.7 Hz), 7.19–7.17 (m, 3H), 7.04 (d, 2H, J = 8.3 Hz), 6.83 (d, 2H, J = 8.5 Hz), 6.64 (d, 2H, J = 8.4 Hz), 6.54 (d, 1H, J = 5.3 Hz), 6.03 (d, 1H, J = 9.2 Hz), 4.84 (d, 1H, J = 9.2 Hz), 3.73 (s, 3H), 1.62 (s, 9H); ^{13}C NMR (50 MHz, $CDCl_3$) δ = 159.8, 155.3, 151.7, 149.3, 143.9, 135.0, 130.4, 129.7, 129.1, 128.5, 126.2, 125.5, 121.6, 117.5, 115.1, 114.5, 101.3, 61.8, 55.3, 54,3, 30.0; HRMS (ESI TOF (+)) calcd for [$C_{28}H_{28}ClN_7O$ + H^+] 514.2117 found 514.2117.

*4.4.8. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(3,4-dimethoxyphenyl) methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**18**)*

Yellow solid; Yield: 80%; mp 80–81 °C; IR (cm^{-1}, KBr): 3412, 3018, 1576, 1422, 1251; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.46 (d, 1H, J = 5.3 Hz), 7.99 (d, 1H, J = 1.83 Hz), 7.82 (d, 1H, J = 8.9 Hz), 7.42 (dd, 1H, J = 1.9 Hz, J = 8.9 Hz), 7.11 (d, 2H, J = 8.5 Hz), 6.93 (s, 1H), 6.85–6.82 (m, 2H), 6.73 (d, 2H, J = 8.6 Hz), 6.62 (d, 1H, J = 5.3 Hz), 6.53 (s, 1H), 6.09 (d, 1H, J = 9.3 Hz), 4.92 (d, 1H, J = 9.2 Hz), 3.87 (s, 3H), 3.85 (s, 3H), 1.71 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 155.1, 151.3, 149.6, 149.4, 149.3, 149.0, 143.9, 135.0, 130.3, 130.1, 128.1, 126.1, 125.5, 121.6, 120.3, 117.4, 115.1, 111.1, 110.7, 101.2, 61.8, 55.9, 55.8, 54.7, 29.9; HRMS (ESI TOF (+)) calcd for [$C_{29}H_{30}ClN_7O_2$ + H^+] 544.2222 found 544.2222.

*4.4.9. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(3,4,5-trimethoxyphenyl) methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**19**)*

Pale Yellow solid; Yield: 74%; mp 168–170 °C; IR (cm^{-1}, KBr): 3428, 3017, 1578, 1373, 1234, 1105, 848; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, 1H, J = 5.3 Hz), 7.98 (d, 1H, J = 2.0 Hz), 7.84 (d, 1H, J = 8.9 Hz), 7.41 (dd, 1H, J = 2.1 Hz, J = 8.9 Hz), 7.11 (d, 2H, J = 8.6 Hz), 6.62 (d, 2H, J = 5.3 Hz), 6.55 (s, 2H), 6.06 (d, 1H, J = 9.3 Hz), 4.91 (d, 1H, J = 9.3 Hz), 3.84 (s, 3H), 3.80 (s, 6H), 1.73 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 154.9, 153.7, 151.7, 149.4, 149.1, 143.9, 138.3, 135.0, 133.2, 130.6, 128.6, 126.1, 125.5, 121.3, 117.5, 115.2, 105.0, 101.3, 61.8, 60.8, 56.2, 55.1, 30.0; HRMS (ESI TOF (+)) calcd for [$C_{30}H_{32}ClN_7O_3$ + H^+] 574.2328 found 574.2328.

*4.4.10. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(4-chlorophenyl)methyl)-N^4-(7-chloroquinolin-4-yl) benzene-1,4-diamine (**20**)*

Yellow solid; Yield: 84%; mp 128–130 °C; IR (cm^{-1}, KBr): 3435, 3019, 2927, 1576, 1374, 1215; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.44 (d, 1H, J = 5.2 Hz), 7.98 (d, 1H, J = 1.6 Hz), 7.84 (d, 1H, J = 8.9 Hz), 7.41–7.30 (m, 5H), 7.12 (d, 2H, J = 8.5 Hz), 6.72 (d, 2H, J = 8.5 Hz), 6.61 (d, 1H, J = 5.3 Hz), 6.13 (d, 1H, J = 8.6 Hz), 4.93 (d, 1H, J = 8.6 Hz), 2.50 (br s, 1H), 1.62 (s, 9H); ^{13}C NMR (75 MHz, $CDCl_3$) δ = 154.7, 151.3, 149.3, 149.0, 143.6, 136.3, 135.2, 134.8, 130.6, 129.4, 129.1, 128.3, 126.2, 125.7, 121.4, 117.4, 115.2, 101.3, 61.9, 54.1, 30.1; HRMS (ESI TOF (+)) calcd for [$C_{27}H_{25}Cl_2N_7$ + H^+] 518.1621 found 518.1622.

*4.4.11. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(3,4-dichlorophenyl) methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**21**)*

Yellow solid; Yield: 82%; mp 200–201 °C; IR (cm^{-1}, KBr): 3412, 3018, 1610, 1515, 1215; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 10.82 (br s, 1H), 8.74 (d, 1H, J = 9.1 Hz), 8.43 (d, 1H, J = 6.9 Hz), 8.09 (s, 1H), 7.86 (s, 1H), 7.83 (d, 1H, J = 9.1 Hz), 7.70 (d, 1H, J = 8.3 Hz), 7.57 (d, 1H, J = 8.2 Hz), 7.35 (d, 1H, J = 8.6 Hz), 7.20 (d, 2H, J = 8.1 Hz), 6.89 (d, 2H, J = 8.2 Hz), 6.63 (d, 1H, J = 6.9 Hz), 6.37 (d, 1H, J = 8.7 Hz), 1.76 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.9, 154.2, 145.4, 143.2, 140.0, 139.2, 138.1, 130.9, 130.5, 130.4, 130.2, 128.4, 127.1, 126.6, 126.5, 125.8, 119.3, 115.6, 113.9, 100.03, 62.4, 50.7, 29.2; HRMS (ESI TOF (+)) calcd for [$C_{27}H_{24}Cl_3N_7$ + H^+] 552.1232 found 552.1231.

*4.4.12. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(4-fluorophenyl)methyl)-N^4-(7-chloroquinolin-4-yl) benzene-1,4-diamine (**22**)*

Yellow solid; Yield: 65%; mp 110–112 °C; IR (cm^{-1}, KBr): 3258, 2989, 1574, 1515, 1230; ^{1}H NMR (300 MHz, $CDCl_3$): δ = 8.45 (d, 1H, J = 5.4 Hz), 7.99 (d, 1H, J = 1.7 Hz), 7.83 (d, 1H, J = 9.0 Hz), 7.42–7.33 (m, 3H), 7.12–7.05 (m, 4H), 6.73 (d, 2H, J = 8.6 Hz), 6.62 (d, 1H, J = 5.4 Hz), 6.14 (d, 1H, J = 8.7 Hz), 4.91 (d, 1H, J = 9.6 Hz), 2.11 (br s, 1H), 1.72 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 158.8, 155.4, 151.5, 149.6, 149.1, 144.4, 133.7, 130.7, 129.3, 129.0, 127.2, 125.7, 124.4, 124.2, 117.5, 113.9, 113.7, 100.3, 61.8, 55.0, 51.7, 29.3; HRMS (ESI TOF (+)) calcd for [$C_{27}H_{25}ClFN_7$ + H^+] 502.1917 found 502.1918.

*4.4.13. N^1-((4-Bromophenyl)(1-tert-butyl-1H-tetrazol-5-yl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**23**)*

Yellow solid; Yield: 84%; mp >240 °C; IR (cm^{-1}, KBr): 3436, 3019, 1571, 1516; ^{1}H NMR (300 MHz, DMSO-d_6) = 10.88 (s, 1H), 8.76 (d, 1H, J = 8.9 Hz), 8.43 (d, 1H, J = 6.9 Hz), 8.11 (s, 1H), 7.84 (d, 1H, J = 9.1 Hz), 7.61–7.48 (m, 4H), 7.25 (d, 1H, J = 8.3 Hz), 7.18 (d, 2H, J = 7.9 Hz), 6.68 (d, 2H, J = 7.9 Hz), 6.62 (d, 1H, J = 6.8 Hz), 6.32 (d,

1H, J = 8.1 Hz), 1.74 (s, 9H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.5, 154.1, 145.2, 142.3, 138.5, 137.7, 137.6, 130.7, 129.8, 126.5, 126.0, 125.7, 125.6, 120.6, 118.5, 115.1, 113.3, 99.4, 61.7, 50.7, 28.7; HRMS (ESI TOF (+)) calcd for [$C_{27}H_{25}$ BrClN$_7$ + H$^+$] 562.1116 found 562.1116.

*4.4.14. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(phenyl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**24**)*

Yellow solid; Yield: 72%; mp 112–114 °C; IR (cm^{-1}, KBr): 3433, 3020, 1575, 1517, 1216; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.44 (d, 1H, J = 5.3 Hz), 7.98 (d, 1H, J = 1.8 Hz), 7.84 (d, 1H, J = 8.9 Hz), 7.39–7.36 (m, 6H), 7.10 (d, 2H, J = 8.5 Hz), 6.72 (d, 2H, J = 8.6 Hz), 6.61 (d, 1H, J = 5.3 Hz), 6.15 (d, 1H, J = 9.3 Hz), 4.95 (d, 1H, J = 9.3 Hz), 1.70 (s, 9H); ^{13}C NMR (75 MHz, CDCl$_3$) δ = 155.0, 151.6, 149.3, 149.2, 143.8, 137.7, 134.9, 130.4, 129.1, 128.8, 128.4, 127.7, 126.1, 125.5, 121.5, 117.5, 115.0, 101.3, 61.8, 54.8, 30.0; HRMS (ESI TOF (+)) calcd for [$C_{27}H_{26}$ClN$_7$ + H$^+$] 484.2011 found 484.2012.

*4.4.15. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(p-tolyl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**25**)*

Yellow solid; Yield: 70%; mp 128–130 °C; IR (cm^{-1}, KBr): 3431, 2921, 1577, 1248; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.45 (d, 1H, J = 4.9 Hz), 7.98 (s, 1H), 7.82 (d, 1H, J = 9.1 Hz), 7.41 (d, 1H, J = 8.6 Hz), 7.22 (d, 4H, J = 5.7 Hz), 7.10 (d, 2H, J = 7.9 Hz), 6.71 (d, 2H, J = 7.8 Hz), 6.62 (d, 1H, J = 4.9 Hz), 6.56 (s, 1H), 6.11 (d, 1H, J = 9.1 Hz), 4.91 (d, 1H, J = 9.5 Hz), 2.34 (s, 3H), 1.69 (s, 9H); ^{13}C NMR (75 MHz, CDCl$_3$) δ = 155.2, 151.6, 149.3, 143.9, 138.7, 134.9, 134.8, 130.3, 129.8, 128.4, 127.6, 126.1, 125.4, 121.5, 117.5, 115.0, 101.2, 61.8, 54.6, 29.9, 21.0; HRMS (ESI TOF (+)) calcd for [$C_{28}H_{28}$ClN$_7$ + H$^+$] 498.2167 found 498.2174.

*4.4.16. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(4-ethylphenyl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**26**)*

Yellow solid; Yield: 74%; mp 136–138 °C; IR (cm^{-1}, KBr): 3455, 2956, 1564, 1248; ^{1}H NMR (300 MHz, DMSO-d_6): δ = 8.42 (d, 1H, J = 5.3 Hz), 8.01 (s, 1H), 7.90 (d, 1H, J = 8.9 Hz), 7.42 (d, 1H, J = 7.4 Hz), 7.28–7.21 (m, 5H), 7.14 (d, 2H, J = 8.3 Hz), 6.73 (d, 2H, J = 8.3 Hz), 6.62 (d, 1H, J = 5.3 Hz), 6.14 (d, 1H, J = 8.3 Hz), 4.94 (d, 1H, J = 8.0 Hz), 2.70 (q, 2H, J = 7.5 Hz), 1.71 (s, 9H), 1.27–1.21 (m, 3H); ^{13}C NMR (50 MHz, CDCl$_3$) δ = 155.2, 150.9, 149.6, 148.6, 145.1, 144.1, 135.4, 134.9, 130.2, 128.7, 127.8, 126.1, 125.7, 121.7, 117.3, 115.1, 101.2, 61.8, 54.6, 30.1, 28.4, 15.3; HRMS (ESI TOF (+) calcd for [$C_{29}H_{30}$ClN$_7$ + H$^+$] 512.2324 found 512.2327.

*4.4.17. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(naphthalen-1-yl)methyl)-N^4-(7-chloroquinolin-4-yl) benzene-1,4-diamine (**27**)*

Yellow solid; Yield: 74%; mp 140–142 °C; IR (cm^{-1}, KBr): 3358, 2933, 1573, 1375; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.49 (d, 1H, J = 5.3 Hz), 8.14 (d, 1H, J = 8.2 Hz), 8.00 (d, 1H, J = 1.7 Hz), 7.97 (d, 1H, J = 7.9 Hz), 7.91 (d, 1H, J = 8.1 Hz), 7.84 (d, 1H, J = 9.0 Hz), 7.67–7.57 (m, 2H), 7.43–7.36 (m, 2H), 7.14 (d, 2H, J = 8.5 Hz), 6.92–6.86 (m, 2H), 6.77 (d, 2H, J = 8.5 Hz), 6.68 (d, 1H, J = 5.3 Hz), 6.54 (s, 1H), 4.79 (d, 1H, J = 9.7 Hz), 1.59 (s, 9H); ^{13}C NMR (50 MHz, DMSO-d_6) δ = 154.9, 151.6, 149.5, 149.2, 144.4, 133.8, 133.7, 133.5, 130.6, 129.1, 128.7, 127.4, 126.8, 125.9, 125.3, 124.4, 124.1, 122.9, 117.6, 113.6, 100.4, 62.3, 49.7, 29.9; HRMS (ESI TOF (+)) calcd for [$C_{31}H_{28}$ClN$_7$ + H$^+$] 534.2167 found 534.2167.

*4.4.18. N^1-(7-Chloroquinolin-4-yl)-N^4-((1-cyclohexyl-1H-tetrazol-5-yl)(3,4-dimethoxyphenyl) methyl)benzene-1,4-diamine (**28**)*

Yellow solid; Yield: 76%; mp 138–140 °C; IR (cm^{-1}, KBr): 3432, 2933, 1634, 1516, 1254; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.43 (d, 1H, J = 5.3 Hz), 7.97 (d, 1H, J = 1.8 Hz), 7.85 (d, 1H J = 8.9 Hz), 7.40 (dd, 1H, J = 1.8 Hz, J = 8.9 Hz), 7.10 (d, 2H, J = 8.5 Hz), 6.97–6.84 (m, 3H), 6.73 (d, 2H, J = 8.6 Hz), 6.59 (d, 1H, J = 5.3 Hz), 5.82 (d, 1H, J = 5.9 Hz), 5.18 (d, 1H, J = 5.9 Hz), 4.30–4.23 (m, 1H), 3.87 (s, 3H), 3.83 (s, 3H), 2.09–1.95 (m, 5H), 1.83–1.72 (m, 3H), 1.37–1.29 (m, 3H); ^{13}C NMR (100 MHz, CDCl$_3$ + DMSO-d_6) δ = 154.2, 151.1, 149.5, 149.3, 149.0, 143.7, 134.5, 130.1, 129.6, 127.7, 125.9, 124.9, 122.4, 119.4, 117.4, 114.4, 110.8, 109.9, 100.6, 57.9, 55.7, 55.6, 53.2, 32.0, 25.0, 24.9, 24.3; HRMS (ESI TOF (+)) calcd for [$C_{31}H_{32}$ClN$_7$O$_2$ + H$^+$] 570.2379 found 570.2372.

*4.4.19. N^1-((4-Chlorophenyl)(1-cyclohexyl-1H-tetrazol-5-yl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**29**)*

Pale yellow solid; Yield: 76%; mp 206–208 °C; IR (cm^{-1}, KBr): 3480, 2933, 1574, 1517, 1247; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.45 (d, 1H, J = 7.2 Hz), 7.98 (s, 1H), 7.84 (d, 1H, J = 10.1 Hz), 7.37 (s, 5H), 7.11 (d, 1H, J = 9.2 Hz), 6.72–6.67 (m, 3H), 6.60 (d, 1H, J = 5.7 Hz), 5.87 (d, 1H, J = 7.5 Hz), 5.18 (d, 1H, J = 6.8 Hz), 4.27–4.20 (m, 1H), 2.07–1.75 (m, 8H), 1.45–1.28 (m, 2H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.7, 151.7, 149.5, 149.4, 144.2, 137.5, 133.6, 132.6, 129.6, 129.4, 128.5, 127.5, 125.8, 124.4, 124.1, 117.6, 114.0, 100.3, 56.9, 50.2, 32.5, 32.4, 24.7, 24.6, 24.4; HRMS (ESI TOF (+)) calcd for [$C_{29}H_{27}Cl_2N_7$ + H$^+$] 544.1778 found 544.1738.

*4.4.20. N^1-((4-Bromophenyl)(1-cyclohexyl-1H-tetrazol-5-yl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**30**)*

Yellow solid; Yield: 80%; mp 208–209 °C; IR (cm^{-1}, KBr): 3358, 2933, 1573, 1315; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.96 (d, 1H, J = 5.1 Hz), 8.68 (d, 1H, J = 8.7 Hz), 8.49 (s, 1H), 8.37 (br s, 1H), 8.11 (d, 2H, J = 7.5 Hz), 7.94–7.90 (m, 3H), 7.69 (d, 2H, J = 8.2 Hz), 7.32 (d, 2H, J = 8.2 Hz), 7.17 (d, 1H, J = 5.6 Hz), 6.53 (d, 1H, J = 6.3 Hz), 6.29 (d, 1H, J = 7.1 Hz), 4.95–4.87 (m, 1H), 2.31–2.16 (m, 4H), 1.86–1.82 (m, 6H); ^{13}C NMR (75 MHz, DMSO-d_6) δ = 154.6, 151.5, 149.6, 149.2, 144.2, 137.9, 133.7, 131.4, 129.9, 129.3, 127.3, 125.8, 124.4, 124.1, 121.2, 117.6, 114.0, 100.3, 56.9, 50.3, 32.5, 32.3, 24.7, 24.6, 24.4; HRMS (ESI TOF (+)) calcd for [$C_{29}H_{27}$ BrClN$_7$ + H$^+$] 583.1273 found 583.1271.

*4.4.21. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(ferrocenyl)methyl)-N^4-(7-chloroquinolin-4-yl)benzene-1,4-diamine (**31**)*

Yellow solid; Yield: 86%; mp 118–120 °C; IR (cm^{-1}, KBr): 3437, 3020, 1610, 1575, 1216; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.47 (d, 1H, J = 5.2 Hz), 7.99 (s, 1H), 7.86 (d, 1H J = 8.9 Hz), 7.41 (d, 1H, J = 8.3 Hz), 7.14 (d, 2H, J = 8.3 Hz), 6.76 (d, 2H, J = 8.3 Hz), 6.67 (br s, 1H), 6.63 (d, 1H, J = 5.2 Hz), 5.83 (d, 1H, J = 9.7 Hz), 5.06 (d, 1H, J = 9.7 Hz), 4.48 (s, 1H), 4.28 (s, 6H), 4.17 (s, 1H), 3.60 (s, 1H), 1.76 (s, 9H); ^{13}C NMR (50 MHz, CDCl$_3$) δ = 155.6, 151.5, 149.4, 149.1, 144.3, 135.2, 130.5, 128.3, 126.3, 125.6, 121.5, 117.5, 115.6, 101.3, 89.8, 69.1, 68.6, 68.1, 67.6, 67.2, 61.9, 49.6, 30.4; HRMS (ESI TOF (+)) calcd for [$C_{31}H_{30}$ClFeN$_7$ + H$^+$] 592.1673 found 592.1677.

*4.4.22. N^1-((1-tert-Butyl-1H-tetrazol-5-yl)(phenyl)methyl)-N^2-(7-chloroquinolin-4-yl)ethane-1,2-diamine (**32**)*

Yellow solid; Yield: 84%; mp 120–121 °C; IR (cm^{-1}, KBr): 3435, 2928, 1636, 1583, 1334; ^{1}H NMR (300 MHz, CDCl$_3$): δ = 8.54 (d, 1H, J = 5.1 Hz), 8.03 (d, 1H, J = 8.9 Hz), 7.96 (s, 1H), 7.47 (d, 1H, J = 8.5 Hz), 6.42 (d, 1H, J = 4.8 Hz), 6.11 (s, 1H), 5.06 (s, 1H), 4.52 (s, 1H), 4.24 (s, 1H), 4.11 (s, 6H), 3.54 (s, 1H), 3.40–3.34 (m, 2H), 3.05–2.85 (m, 2H), 1.73 (s, 9H); ^{13}C NMR (50 MHz, CDCl$_3$) δ = 156.4, 151.9, 150.0, 148.9, 134.9, 128.5, 125.5, 121.6, 117.4, 99.1, 89.2, 68.9, 68.6, 68.2, 67.4, 67.1, 61.6, 52.8, 45.3, 42.5, 30.3; HRMS (ESI) calcd for [$C_{27}H_{30}$ClFeN$_7$ + H$^+$] 544.1673; found 544.1679.

4.5. Bioevaluation methods

4.5.1. In vitro antimalarial assay

The compounds were evaluated for antimalarial activity against 3D7 (CQ-sensitive) and K1 (CQ-resistant) strains of *P. falciparum* using Malaria SYBR Green I nucleic acid staining dye based fluorescence (MSF) assay as mentioned by Singh et al. (2011) [37]. The

stock (5 mg/mL) solution was prepared in DMSO and test dilutions were prepared in culture medium (RPMI-1640-FBS). Chloroquine-diphosphate was used as reference drug.

4.5.1.1. Test technique. 50 μl of culture medium was dispensed in 96 well plate followed by addition of 50 μl of highest concentration of test compounds (in duplicate wells) in row B. Subsequent two-fold serial dilutions were prepared and finally 50 μl of 1.0% parasitized cell suspension containing 0.8% parasitaemia was added to each well except 4 wells in row 'A' received non parasitized erythrocyte suspension. The plates were incubated at 37 °C in CO_2 incubator in an atmosphere of 5% CO_2 and air mixture and 72 h later 100 μl of lysis buffer containing 2× concentration of SYBR Green-I (In Nitrogen) was added to each well and incubated for 1 h at 37 °C. The plates were examined at 485 ± 20 nm of excitation and 530 ± 20 nm of emission for relative fluorescence units (RFUs) per well using the fluorescence plate reader (FLX800, BIOTEK).

4.5.1.2. Statistical analysis. Data was transferred into a graphic programme (EXCEL) and IC_{50} values were obtained by Logit regression analysis of dose response curves using pre-programmed Excel spreadsheet.

4.5.2. In vitro assay for evaluation of cytotoxic activity

Cytotoxicity of the compounds was carried out using Vero cell line (C1008; Monkey kidney fibroblast) following the method as mentioned in Sashidhara et al. (2012) [38]. The cells were incubated with compound-dilutions for 72 h and MTT was used as reagent for detection of cytotoxicity. 50% cytotoxic concentration (CC_{50}) was determined using nonlinear regression analysis of dose response curves using pre-programmed Excel spreadsheet. Selectivity Index (SI) was calculated as

$$SI = CC_{50}/IC_{50}$$

4.5.3. In vitro assay for evaluation of β-hematin inhibition

Inhibition of *in vitro* β-hematin formation was analysed by using the method of Pandey et al. (1999) with some modifications [39]. Male Swiss albino mice, weighing 15–20 g were inoculated with 1×10^5 *P. yoelii* infected RBCs. Blood of infected animal at 50% parasitaemia 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 assay mixture contained 100 mM sodium acetate buffer pH (5.1), 50 μL plasma, 100 μM hemin as the substrate and 1–20 μg compound/drug in a total reaction volume of 1.0 mL. The control tubes contained all reagents except compound. 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 100 mM Tris–HCl buffer pH (7.4) containing 2.5% SDS. The pellet obtained after centrifugation was washed thrice with distilled water (TDW) to remove free heme attached to β-hematin. The pellet was solubilized in 50 μL of 2 N NaOH and volume was made up to 1.0 mL with TDW. Absorbance was measured at 400 nm. The 50% inhibitory concentration (IC_{50}) was determined using non-linear regression analysis of dose response curves.

4.5.4. In vivo antimalarial assay

The *in vivo* drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain) which is innately resistant to CQ [40]. The mice (22 ± 2 g) were inoculated with 1×10^6 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. Parasitaemia 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 parasitaemia with respect to the untreated control group. Mice treated with CQ served as reference controls.

4.5.5. Pharmacokinetic evaluation

The pharmacokinetic studies of compounds **20** and **23** were carried out in young and healthy male Sprague–Dawley rats weighing 250 ± 25 g. The rats were obtained from Laboratory Animal Division of the Institute and were housed in plastic cages under standard laboratory conditions with a regular 12 h day–night cycle. Standard pelleted laboratory chow (Goldmohar Laboratory Animal Feed, Lipton India Ltd, Chandigarh, India) and water were allowed *ad libitum*. The rats were acclimatized to this environment for at least two days before conducting the experiments. In all the studies mentioned below the dose was administered after overnight fasting (12–16 h). The study was conducted in three rats per time point. In all experiments, euthanasia and disposal of carcasses were carried out as per the guidelines of Local Ethics Committee for animal experimentation.

Suspension formulation of the compounds were separately prepared by triturating the compound with gum acacia and water in a pestle with mortar, and a single oral dose of 10 mg/kg was given to conscious rats by oral gavage in a volume of approximately 1 mL/250 g rat and the time of dosing was recorded. Rodent food and water were provided 2 h after the dose. Blood samples were collected at 0.5, 1, 1.5, 2, 4, 6, 8, 10, 18, and 24 h post dose. Two blood samples were withdrawn from each animal. An initial 1.5 mL blood sample was drawn by cardiac puncture under light anaesthesia followed by a sample drawn from the inferior vena cava (terminal sample), from the dosed rats using a 24G needle and a syringe in a clean and dry test tube. The total volume of blood collected from each rat was not more than 3% of the total body volume. The blood was allowed to clot, by keeping the tube on a slant, approximately for 45 min. Then it was centrifuged at 2000 rpm for 10 min and the serum was separated into clean and neatly labelled tubes. All samples were stored at −20 °C until analysis.

Chromatographic separations and quantification of the compounds was achieved by a reverse phase HPLC method on a Discovery HS C-18 column (5 μm, 100 × 4.6 mm id) preceded with a guard column (5 μm, 20 × 4.0 mm, id) packed with the same material under isocratic condition at a flow rate of 1 mL/min. The HPLC system used in this study consisted of a pump (LC-10AT VP with FCV-10AL VP), degasser (DGU-14A) and auto-injector (SIL-HTc, fixed with a 100 μl loop) (Shimadzu, Japan). Eluents were monitored at 253 nm for **20** and 257 nm for **23** with UV–Vis multiple wavelength detector (Shimadzu, Japan) and chromatograms were integrated using Class-VP (version 6.12 SP5) software. The mobile phase composition was 0.01 M aqueous ammonium acetate:acetonitrile (35:65, v/v) and it was degassed by ultrasonication for 15 min before use. The HPLC system was equilibrated for approximately 30 min before commencement of analysis and chromatography was carried out at ambient temperature. The lower limit of quantification for the analytical method was 10 ng/mL of test analyte in serum. The mean and SEM of the serum concentrations of the candidate drug at each time point was calculated using Microsoft Excel for Windows. All pharmacokinetic parameters were calculated by non-compartmental models using WinNonlin program, version 5.1 (Scientific Consulting Inc.).

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Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.ejmech.2013.05.023.

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