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Synthesis of new 4-aminoquinolines and quinoline–acridine hybrids as antimalarial agents

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ABSTRACT

Despite emergence of resistance to CQ and other 4-aminoquinoline drugs in most of the endemic regions, research findings provide considerable support that there is still significant potential to discover new affordable, safe, and efficacious 4-aminoquinoline antimalarials. In present study, new side chain modified 4-aminoquinoline derivatives and quinoline–acridine hybrids were synthesized and evaluated in vitro against NF 54 strain of *Plasmodium falciparum*. Among the evaluated compounds, compound **17** (MIC = 0.125 μg/mL) was equipotent to standard drug CQ (MIC = 0.125 μg/mL) and compound **21** (MIC = 0.031 μg/mL) was four times more potent than CQ. Compound **17** showed the curative response to all the treated swiss mice infected with CQ-resistant N-67 strain of *Plasmodium yoelii* at the doses 50 mg/kg and 25 mg/kg for four days by intraperitoneal route and was found to be orally active at the dose of 100 mg/kg for four days. The promising antimalarial potency of compound **17** highlights the significance of exploring the privileged 4-aminoquinoline class for new antimalarials.

Despite the extensive research efforts, malaria continues to exert the tremendous burden on the health and economies of developing countries. It is estimated that 40% population of the world is exposed to the malaria, killing more than 2 million people every year.[1] Malaria caused by *Plasmodium falciparum* is the most fatal and accounts for 95% mortality.[2] Since the discovery of natural product quinine, structural modifications of its quinoline pharmacophore led to the development of most effective antimalarial agents namely chloroquine (CQ, **1**), amodiaquine (AQ, **2**), and mefloquine (**3**) (Fig. 1).[3,4] The wide-spread resistance to 4-aminoquinolines and antifolates has seriously limited the therapeutic options. Therefore, there is urgency to develop new affordable, safe, and efficacious antimalarilas.[5] Although the resistance to CQ and related 4-aminoquinoline antimalarial drugs has emerged; designing new antimalarial based on the quinoline pharmacophore has distinct advantages due to unique pharmacological effect of 4-aminoquinoline drugs.[6]

During the erythrocyte stage, malaria parasite invade the red blood cell of human host, digest and degrade a huge amount of hemoglobin as a source of amino acids, consequently releasing toxic heme as a by product.[7] Heme detoxification crucial for parasite survival is a unique non-enzymatic efficient process characterized by conversion of free heme into non-toxic crystalline pigment hemozoin.[8] CQ and other related drugs block the heme detoxification process, thus substantial build up of heme lead to the parasite death.[9] Despite the persistent heavy drug pressure of CQ for several decades, the delayed emergence of resistance to CQ is considered due to the complexity of digestive vacuole environment and the immutable nature of heme target. Multiple point mutations in *P. falciparum* chloroquine resistance transporter protein (*pfcrt*) conferred resistance to CQ characterized by the substantially reduced accumulation of CQ level in food vacuole. Interaction of CQ with *pfcrt* induces resistance very slowly to *P. falciparum* owing to the complexity in amino acid substitutions in pfcrt.[10]

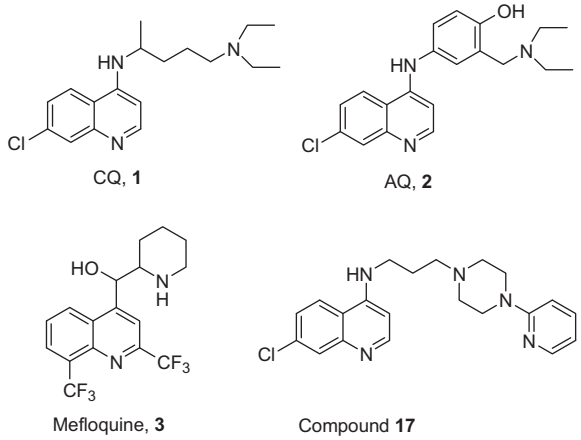

Figure 1. Structures of CQ (**1**), AQ (**2**), mefloquine (**3**), and target compound **17**.

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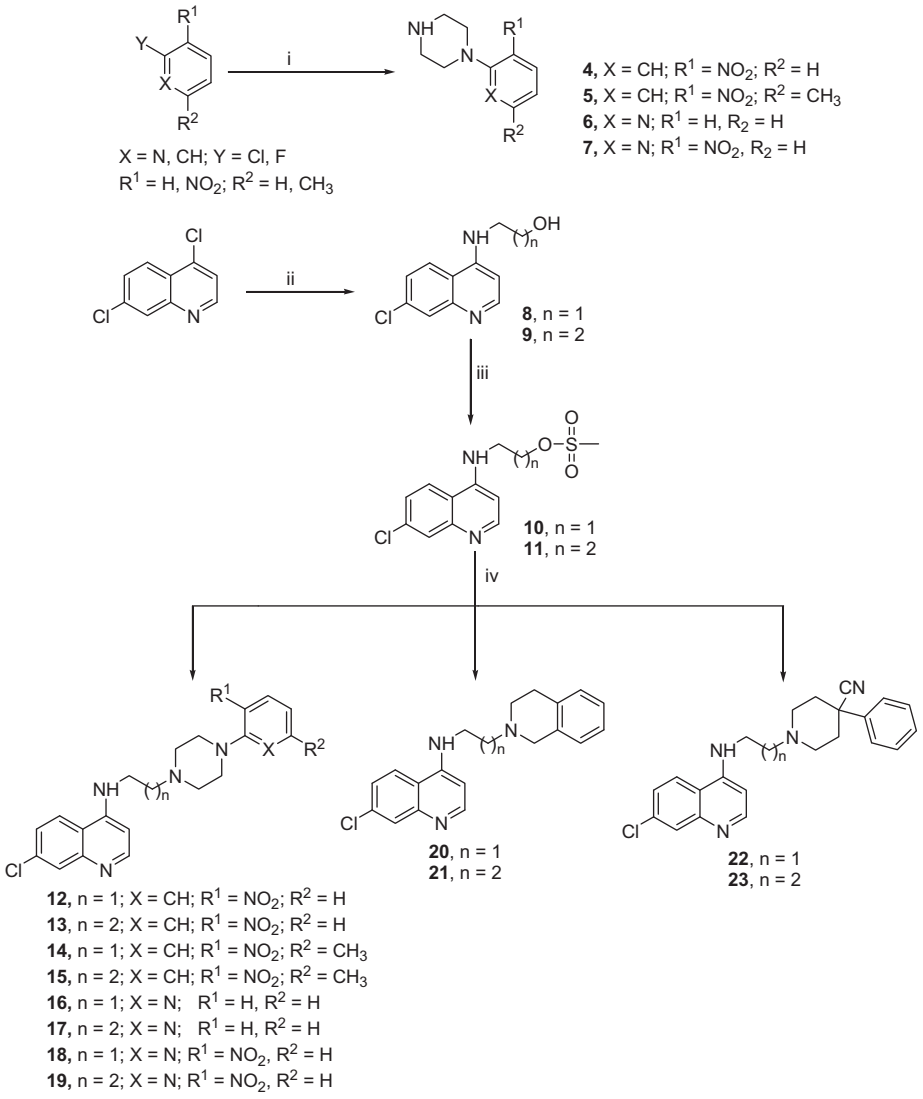
The structure–activity relationship studies on CQ–Heme binding have been explored in order to identify the key structural requirement for designing the new antimalarial agents. It was well established that the 7-chloro-4-aminoquinoline nucleus is quintessential for the inhibition of heme polymerization and parasite growth.[11] Moreover, the basic nature of the side chain is crucial for accumulation of drug within the acidic food vacuole of the parasite.[12] Several studies demonstrated that various structurally diverse modifications in the side chain of CQ were well tolerated for the antimalarial activity. Systematic variation of the branching and basicity of the side chain of CQ yielded the new 4-aminoquinoline derivatives exhibiting excellent potency against CQ-sensitive and CQ-resistant strains.[13] Replacement of diethylamino function of CQ with *tert*-butyl or cyclic amines such as piperidine, morpholine furnished the metabolically stable potent antimalarials.[14,15] Incorporation of guanidines,[16] and intramolecular hydrogen-bonding motif like α-aminocresols[17] in the side chain produced the new potential antimalarials. More recently, the 4-aminoquinoline carrying dimethylaminomethyl substituted phenyl ring, phenylequine (PQ) has been identified as potent antimalarial.[18]

To overcome the resistance, the bulky bisquinolines were designed embodying the hypothesis that steric hindrance would not allow drug efflux by the proteinaceous transporter.[19,20] Though bisquinolines such as Ro 47-7737[20] and other piperaquine, hydroxypiperaquine and dichloroquinazine[21] exhibited promising antimalarial efficacy but toxic liabilities ruled out their development as drug candidate. In addition, bisacridine derivatives with di-, tri-, and tetramine linker have been investigated for their effect on the antimalarial activity.[22] Hybridization of the 4-aminoquinoline and 9-amino acridine with 1,2,4-trioxanes produced the antimalarials exhibiting the low nM potency.[23]

As part of research program devoted to the synthesis of nitrogen heterocycles as antimalarials, our group has identified potential quinoline-based antimalarials.[24] In view of this background, we have synthesized the side chain modified 4-aminoquinolines and quinoline–acridine hybrids and screened in vitro against NF 54 strain of *P. falciparum*. Selected compounds were also subjected to in vivo study against CQ-resistant strain of *Plasmodium yoelii*.

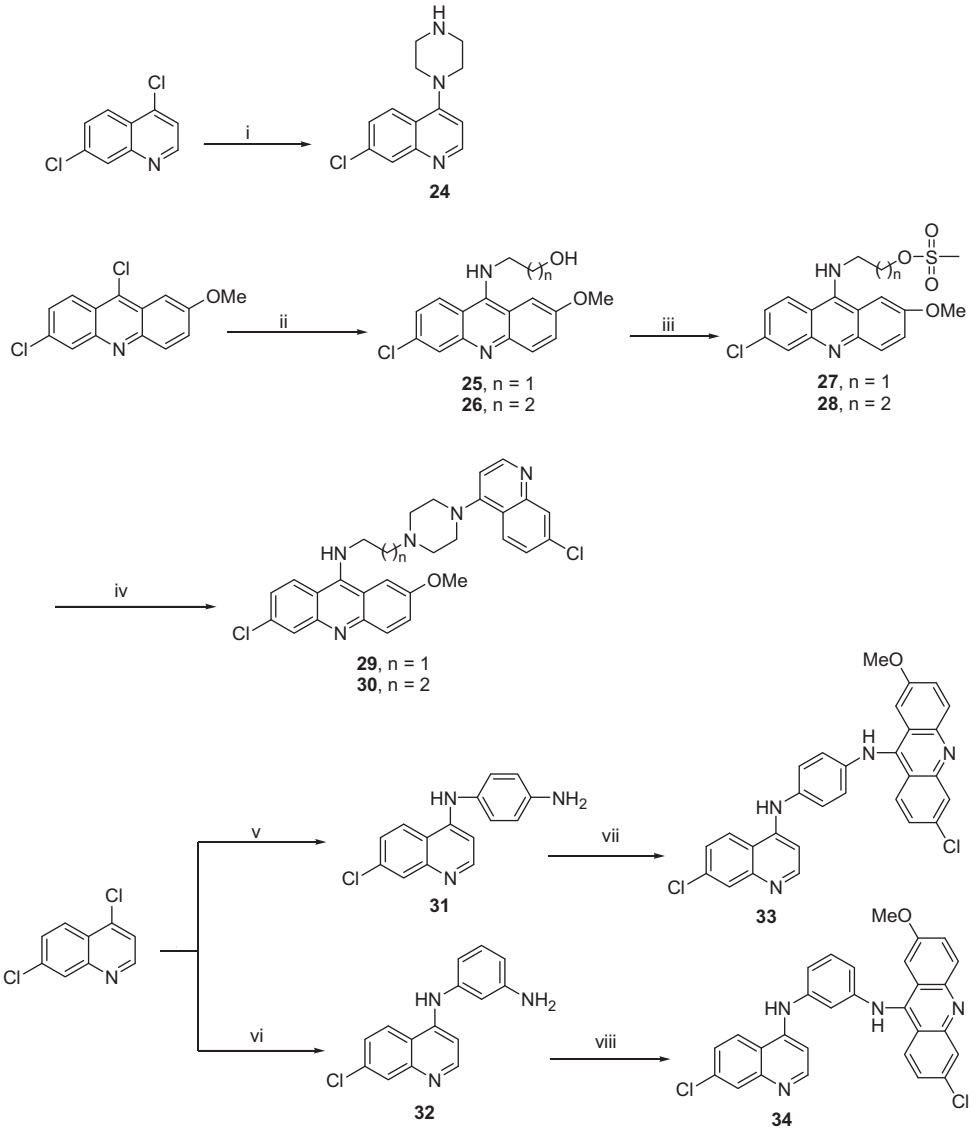
New side chain modified 4-aminoquinoline derivatives (**12–23**) were synthesized by a synthetic route as described in Scheme 1. Starting compounds, substituted aryl/heteroaryl piperazines (**4–7**) were synthesized by aromatic nucleophilic substitution reaction of aryl/heteroaryl substrates with piperazine under appropriate reaction conditions, depending upon the nature of substrates.



Scheme 1. Reagents and conditions: (i) piperazine, THF, 0 °C to rt, 3 h; (ii) 2-amino ethanol or 3-amino-propan-1-ol, *n*-butanol, 100 °C, 8 h; (iii) methanesulfonyl chloride, dry pyridine, 0 °C, 3 h; (iv) amines, NMP, MW, 30 s.

Condensation of 4,7-dichloroquinoline with 2-amino-ethanol and 3-amino-propan-1-ol in presence of Et_3N afforded the 2-(7-chloro-quinolin-4-ylamino)-ethanol **8** and 3-(7-chloro-quinolin-4-ylamino)-propan-1-ol **9**.[25] The chemoselective *o*-mesylation of **8** and **9** was done in pyridine at 0 °C for 5 h to achieve the methanesulfonic acid 2-(7-chloro-quinolin-4-ylamino)-ethyl ester **10** and methanesulfonic acid 3-(7-chloro-quinolin-4-ylamino)-propyl ester **11**, respectively.[26] Compounds **10** and **11** were subjected to nucleophilic substitution with substituted aryl/heteroaryl piperazines, tetrahydroisoquinoline and 4-cyano-4-aryl-piperidine under microwave condition to yield the targeted new 4-amino-quinolines (**12–23**).

The synthetic strategy for the synthesis of hybrid quinoline–acridine derivatives (**29**, **30**, **33**, **34**) is outlined in Scheme 2. Amination of 4,7-dichloroquinoline with piperazine (6 equiv) in *n*-butanol at 100 °C for 10 h furnished the 7-chloro-4-piperazin-1-yl-quinoline **24**. Condensation of 6,9-dichloro-2-methoxy-acridine with 2-amino-ethanol and 3-amino-propan-1-ol (10 equiv) was carried out in *n*-butanol to afford the 2-(6-chloro-2-methoxy-acridin-9-ylamino)-ethanol **25** and 3-(6-chloro-2-methoxy-acridin-9-ylamino)-propan-1-ol **26**, respectively.[25] Compounds **25** and **26** were further underwent chemoselective *o*-mesylation at 0 °C in THF to obtain the corresponding methanesulfonic acid 2-(6-chloro-2-methoxy-acridin-9-ylamino)-ethyl ester **27** and methanesulfonic acid 3-(6-chloro-2-methoxy-acridin-9-ylamino)-propyl ester **28**. Compounds **27** and **28** were subjected to nucleophilic substitution with the 7-chloro-4-piperazin-1-yl-quinoline **24** to yield the hybrid quinoline–acridine (6-chloro-2-methoxy-acridin-9-yl)-{2-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]-ethyl}-amine **29** and (6-chloro-2-methoxy-acridin-9-yl)-{2-[4-(7-chloro-quinolin-4-yl)-piperazin-1-yl]-propyl}-amine **30**, respectively. Coupling of 4,7-dichloroquinoline with *p*-phenylenediamine and *m*-phenylenediamine in presence of catalyst *p*-TSA in absolute ethanol furnished the *N*-(7-chloro-quinolin-4-yl)-benzene-1,4-diamine **31** and *N*-(7-chloro-quinolin-4-yl)-benzene-1,3-diamine **32**, respectively.[27] Compounds **31** and **32** were further subjected to nucleophilic substitution with 6,9-dichloro-2-methoxy-acridine in absolute ethanol using catalyst *p*-TSA to yield the hybrids



Scheme 2. Reagents and conditions: (i) piperazine, *n*-butanol, 100 °C, 10 h; (ii) 2-amino-ethanol or 3-amino-propan-1-ol, *n*-butanol, 100 °C, 8 h; (iii) methanesulfonyl chloride, dry THF, 0 °C, 3 h; (iv) 7-chloro-4-piperazin-1-yl-quinoline, NMP, MW, 30 s; (v) *p*-phenylenediamine, EtOH, *p*-TSA, 3 h; (vi) *m*-phenylenediamine, EtOH, *p*-TSA, 3 h; (vii) 6,9-dichloro-2-methoxy-acridine, EtOH, *p*-TSA, 4 h.

quinoline–acridine *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-(7-chloro-quinolin-4-yl)-benzene-1,4-diamine **33** and *N*-(6-chloro-2-methoxy-acridin-9-yl)-*N*′-(7-chloro-quinolin-4-yl)-benzene-1,3-diamine **34**, respectively. All the synthesized compounds were well characterized by IR, mass, NMR, and elemental analysis.[28]

The in vitro antimalarial assay was carried out in 96 well microtiter plates according to the micro assay of Rieckmann.[29] The culture of *P. falciparum* NF-54 strain is routinely being maintained in medium RPMI-1640 supplemented with 25 mM HEPES, 1% D-glucose, 0.23% sodium bicarbonate and 10% heat inactivated human serum.[30] The asynchronous parasite of *P. falciparum* was synchronized after 5% D-Sorbitol treatment to obtain parasitized cells harboring only the ring stage.[31] For carrying out the assay, an initial ring stage parasitemia of 1% at 3% hematocrit in total volume of 200 μL of medium RPMI-1640 was uniformly maintained. The test compound in 20 μL volume at the required concentration (ranging between 0.25 μg and 50 μg/mL) in duplicate wells, were incubated with parasitized cell preparation at 37 °C in candle jar. After 36–40 h incubation, the blood smears from each well were prepared and stained with giemsa stain. The slides were microscopically observed to record maturation of ring stage parasites into trophozoites and schizonts in presence of different concentrations of compounds. The tested concentration, which inhibits the complete maturation into schizonts, was recorded as the minimum inhibitory concentration (MIC). Chloroquine was used as the standard reference drug. Activity of all the tested compounds is shown in Table 1.

All the synthesized compounds were evaluated in vitro for their antimalarial activity against NF 54 strain of *P. falciparum*. 4-Aminoquinoline derivatives **12** and **13** containing ethyl and propyl chain attached to the 2-nitrophenyl substituted piperazine exhibited MIC value of 10 μg/mL. Further, substitution of methyl at the 4-position of phenyl ring of the side chain of compound **12** and **13** resulting in corresponding compounds **14** and **15** had no effect on the antimalarial activity (**14, 15**, MIC = 10 μg/mL). 4-Aminoquinoline derivative **16** carrying ethyl linker attached to the 1-pyridin-2-yl-piperazine group showed MIC value of 10 μg/mL while replacement of ethyl linker (**16**) with propyl linker (**17**) produced the remarkable improvement in the antimalarial potency with MIC value of 0.125 μg/mL. Introduction of nitro group at the 3-position (**18**) of the pyridine ring of compound **16** showed no effect on the activity. Interestingly, substitution of nitro group at the 3-position (**19**) of the pyridine ring of compound **17** significantly reduced the antimalarial activity from 0.125 to 10 μg/mL. Compound **20** containing ethyl linker attached to the tetrahydroisoquinoline exhibited MIC value of 2 μg/mL while replacement of ethyl linker (**20**) with propyl linker (**21**) led the excellent improvement in the antimalarial potency with MIC value of 0.031 μg/mL. Compound **22** having ethyl linker attached to the 4-phenyl-piperidine-4-carbonitrile displayed MIC value of 10 μg/mL. Introduction of additional methylene unit (**23**) into the side chain of compound **22** improved the potency five times (**20**, MIC = 2 μg/mL). Comparison of antimalarial effects of compounds **16**, **17**, **20**, **21**, **22**, and **23** clearly demonstrated that propyl linker was favorable for the antimalarial activity.

Quinoline–acridine hybrids **29** and **30** attached with ethylene and propylene functionalized piperazine had MIC values of 10 μg/mL. Compound **33** exhibiting quinoline linked to acridine with *p*-phenylenediamine expressed MIC value of 0.5 μg/mL while replacing the *p*-phenyenediamine (**33**) with *m*-phenylenediamine (**34**) resulted in two times improvement in the antimalarial activity.

Representative compounds (**17**, **21**, **34**) characterized by good in vitro antimalarial activity were tested in vivo in swiss mice infected with CQ-resistant N-67 strain of *P. yoelii* parasite (Table 2).[32] Compound **17** provided 100% protection to the treated mice at the doses of 50 and 25 mg/kg × 4 days once daily by intraperitoneal route (ip). Further, compound **17** exhibited the curative response to all the treated mice (5/5) when administered by oral route at the dose of 100 mg/kg × 4 days. Compounds **21** and **34** displayed the complete clearance of parasitemia on day 4 at the dose of 50 mg/kg × 4 days by ip route but none of mice survived beyond day 28. The promising in vivo activity of compound **17** highlights the importance of investigating the privileged 4-aminoquinoline class to deliver the new antimalarials. Modification around the pyridine nucleus of compound **17** might lead to the more potent molecules.

In conclusion, while the most of quinoline-based drugs targeting heme have lost their efficacy due to emergence of resistance, modification in the side chain of CQ still can provide new affordable, safe, efficacious antimalarials. In present study, the side chain modified 4-aminoquinolines and quinoline–acridine hybrids were synthesized and screened in vitro against NF 54 strain of *P. falciparum*. Compounds **17** and **21** exhibited the good antimalarial potency with MIC values of 0.125 and 0.031 μg/mL, respectively. Compound **17** containing pyridine nucleus in the side chain showed the curative response in swiss mice infected with CQ-resistant strain of *P. yoelii* at the doses of 50 mg/kg and 25 mg/kg for four days by ip route as well as orally active at the dose of 100 mg/kg for four days. Considering the promising antimalarial activity of compound **17**, modifications around the pyridine nucleus can guide to the development of more potent compounds.

Table 1
In vitro antimalarial activity of compounds against NF 54 strain of *P. falciparum*

Compound	MIC[a] (μg/mL)
12	10
13	10
14	10
15	10
16	10
17	0.125
18	10
19	10
20	2
21	0.031
22	10
23	2
29	1
30	1
33	0.5
34	0.25
CQ	0.125

[a] MIC = Minimum inhibiting concentration for the development of ring stage parasite into the schizont stage during 40 h incubation.

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

Compound	Dose (mg/kg × 4 days)	% Suppression on day 4[a]	Mice alive on day 28
17	50 (ip)	100	5/5
	25 (ip)	100	5/5
	100 (oral)	100	5/5
21	50 (ip)	100	0/5
34	50 (ip)	100	0/5

[a] Percent suppression = [(C − T)/C] × 100; where C = parasitemia in control group and T = parasitemia in treated group.

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References and notes

1. Maitland, K.; Makanga, M.; Williams, T. N. *Curr. Opin. Infect. Dis.* **2004**, *17*, 405.
2. Frevert, U. *Trend Parasitol.* **2004**, *20*, 417.
3. Gelb, M. H. *Curr. Opin. Chem. Biol.* **2007**, *11*, 440.
4. Wiesner, J.; Ortmann, R.; Jomaa, H.; Schlitzer, M. *Angew. Chem., Int. Ed.* **2003**, *42*, 5274.
5. Hay, S. I.; Guerra, C. A.; Tatem, A. J.; Noor, A. M.; Snow, R. W. *Lancet Infect. Dis.* **2004**, *4*, 327.
6. (a) Payne, D. *Parasitol. Today* **1988**, *4*, 112; (b) Ursos, L. M. B.; Roepe, P. D. *Med. Res. Rev.* **2002**, *22*, 465.
7. Krugliak, M.; Zhang, J.; Ginsburg, H. *Mol. Biochem. Parasitol.* **2002**, *119*, 249.
8. Pagola, S.; Stephens, P. W.; Bohle, D. S.; Kosar, A. D.; Madsen, S. K. *Nature* **2000**, *404*, 307.
9. Leed, A.; DuBay, K.; Ursos, L. M.; Sears, D.; De Dios, A. C.; Roepe, P. D. *Biochemistry* **2002**, *41*, 10245.
10. Bennett, T. N.; Kosar, A. D.; Ursos, L. M.; Dzekunov, S.; Singh Sidhu, A. B.; Fidock, D. A.; Roepe, P. D. *Mol. Biochem. Parasitol.* **2004**, *133*, 99.
11. Kaschula, C. H.; Egan, T. J.; Hunter, R.; Basilico, N.; Parapani, S.; Tarameli, D.; Pasini, E.; Monti, D. *J. Med. Chem.* **2002**, *45*, 3531.
12. Egan, T. J.; Hunter, R.; Kaschula, C. H.; Marques, H. M.; Misplon, A.; Walden, J. C. *J. Med. Chem.* **2000**, *43*, 283.
13. Yearick, K.; Ekoue-Kovi, K.; Iwaniuk, D. P.; Natarajan, J. K.; Alumasa, J.; DeDios, A. C.; Roepe, P. D.; Wolf, C. *J. Med. Chem.* **2008**, *51*, 1995.
14. Madrid, P. B.; Wilson, N. T.; DeRisi, J. L.; Guy, R. K. *J. Comb. Chem.* **2004**, *6*, 437.
15. Stocks, P. A.; Raynes, K. J.; Bray, P. G.; Park, B. K.; O'Neill, P. M.; Ward, S. A. *J. Med. Chem.* **2002**, *45*, 4975.
16. Solomon, V. R.; Puri, S. K.; Srivastava, K.; Katti, S. B. *Biorg. Med. Chem.* **2005**, *13*, 2157.
17. Madrid, P. B.; Liou, A. P.; DeRisi, J. L.; Guy, R. K. *J. Med. Chem.* **2006**, *49*, 4535.
18. Blackie, M. A. L.; Yardley, V.; Chibale, K. *Biorg. Med. Chem. Lett.* **2010**, *20*, 1078.
19. Vennerstrom, J. L.; Ellis, W. Y.; Ager, A. L.; Andersen, S. L.; Gerena, L.; Milhous, W. K. *J. Med. Chem.* **1992**, *35*, 2129.
20. Ridley, R. G.; Matile, H.; Jaquet, C.; Dorn, A.; Hofheinz, W.; Leupin, W.; Masciadri, R.; Theil, F. P.; Richter, W. F.; Girometta, M. A.; Guenzi, A.; Urwyler, H.; Gocke, E.; Potthast, J. M.; Csato, M.; Thomas, A.; Peters, W. *Antimicrob. Agents Chemother.* **1997**, *41*, 677.
21. Basco, L. K.; Ruggeri, C.; Le Bras, J. *Molecules Antipaludiques*; Masson: Paris, 1994. pp 115–120.
22. Girault, S.; Grellier, P.; Berecibar, A.; Maes, L.; Mouray, E.; Lemiere, P.; Debreu, M. A.; Davioud-Charvet, E.; Sergheraert, C. *J. Med. Chem.* **2000**, *43*, 2646.
23. Araujo, N. C. P.; Barton, V.; Jones, M.; Stocks, P. A.; Ward, S. A.; Davies, J.; Bray, P. G.; Shone, A. E.; Cristiano, M. L. S.; O'Neill, P. M. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 2038.
24. (a) Kumar, A.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 6530; (b) Kumar, A.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2009**, *19*, 6996; (c) Naresh, S.; Sharma, M.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Saxena, J. K.; Chauhan, P. M. S. *Bioorg. Med. Chem.* **2009**, *17*, 6451; (d) Naresh, S.; Srivastava, K.; Rajakumar, S.; Puri, S. K.; Saxena, J. K.; Chauhan, P. M. S. *Biorg. Med. Chem. Lett.* **2009**, *19*, 2570; (e) Gupta, L.; Srivastava, K.; Singh, S.; Puri, S. K.; Chauhan, P. M. S. *Bioorg. Med. Chem. Lett.* **2008**, *18*, 3306.
25. De, D.; Krogstad, F. M.; Byers, L. D.; Krogstad, D. J. *J. Med. Chem.* **1988**, *41*, 4918.
26. Lundt, L.; Madsen, R. *Synthesis* **1992**, 1129.
27. Hoglund, I. P. J.; Silver, S.; Engstrom, M. J.; Salo, H.; Tauber, A.; Kyronem, H. K.; Saarenketo, P.; Hoffren, A. M.; Kokko, K.; Pohjanoksa, K.; Sallinen, J.; Savola, J. M.; Wurster, S.; Kallatsa, O. A. *J. Med. Chem.* **2006**, *49*, 6351.
28. Spectroscopic data for **17**: MS: 382 (M+1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.54 (d, 1H, J = 5.34 Hz, Ar-H), 8.26 (dd, 1H, J = 1.77, 5.22 Hz, Ar-H), 7.95 (d, 1H, J = 1.98 Hz, Ar-H), 7.83 (d, 1H, J = 8.97 Hz, Ar-H), 7.56 (dt, 1H, J = 1.8, 8.67 Hz, Ar-H), 7.38 (br s, 1H, NH), 7.27 (dd, 1H, J = 1.98, 8.67 Hz, Ar-H), 6.73–6.69 (m, 2H, Ar-H), 6.38 (d, 1H, J = 5.34 Hz, Ar-H), 3.69 (t, 4H, J = 5.07 Hz, NCH_2), 3.44 (t, 2H, J = 5.67 Hz, CH_2), 2.73–2.68 (m, 6H, NCH_2), 2.02 (quint, 2H, J = 5.43 Hz, CH_2); ^{13}C NMR (75 MHz, DMSO-d_6): δ 164.28, 157.11, 155.38, 154.25, 152.76, 142.86, 138.60, 132.69, 129.23, 122.67, 118.17, 112.25, 103.85, 60.87, 57.87, 49.90, 46.04, 30.23. Anal. Calcd for $C_{21}H_{24}ClN_5$: C, 66.04; H, 6.33; N, 18.34. Found: C, 65.81; H: 6.46, N: 18.53. Compound **21**: MS: 352 (M+1); ^{1}H NMR (300 MHz, $CDCl_3$): δ 8.47 (d, 1H, J = 5.37 Hz, Ar-H), 8.35 (br s, 1H, NH), 7.83 (d, 1H, J = 2.07 Hz, Ar-H), 7.33–7.23 (m, 4H, Ar-H), 7.10 (d, 1H, J = 8.76 Hz, Ar-H), 6.47 (dd, 1H, J = 2.07, 8.91 Hz, Ar-H), 6.29 (d, 1H, J = 5.37 Hz, Ar-H), 3.79 (s, 2H, CH_2), 3.46 (t, 2H, J = 4.44 Hz, CH_2), 3.07 (t, 2H, J = 5.79 Hz, CH_2), 2.92–2.86 (m, 4H, CH_2), 2.07 (quint, 2H, J = 5.31 Hz, CH_2); ^{13}C NMR (75 MHz, $CDCl_3$): δ 154.25, 151.52, 148.73, 141.28, 136.21, 134.68, 129.17, 128.36, 127.13, 126.65, 125.72, 125.24, 122.04, 117.26, 101.07, 58.52, 55.98, 51.28, 49.98, 32.82, 28.46. Anal. Calcd for $C_{21}H_{22}ClN_3$: C, 71.68; H, 6.30; N, 11.94. Found: C, 71.87; H, 6.11; N, 12.18.
29. Rieckmann, K. H.; Sax, L. J.; Campbell, G. H.; Mrema, J. E. *Lancet* **1978**, 1.
30. Trager, W.; Jensen, J. B. *Science* **1979**, *193*, 673.
31. Lambros, C.; Vanderberg, J. P. *J. Parasitol.* **1979**, *65*, 418.
32. *In vivo activity assay.* The in vivo drug response was evaluated in Swiss mice infected with *P. yoelii* (N-67 strain) which is innately resistant to CQ. The mice (22 ± 2 g) were inoculated with 1×106 parasitized RBC on day 0 and treatment was administered to a group of five mice from day 0 to 3, once daily. The aqueous suspensions of compounds were prepared with a few drops of Tween 80. Initially, the efficacy of test compounds was evaluated at 50.0 mg/kg/day and required daily dose was administered in 0.2 mL volume via intraperitoneal route. The efficacy of test compounds was evaluated at 100 mg/kg/day and required daily dose was administered in 0.1 mL volume via oral route. Parasitemia levels were recorded from thin blood smears between days 4 and 6. The mean value determined for a group of 5 mice was used to calculate the percent suppression of parasitaemia with respect to the untreated control group. Mice treated with CQ served as reference controls. Puri, S. K.; Singh, N. *Exp. Parasitol.* **2000**, *94*, 8.