Available online at www.sciencedirect.com
SCIENCE DIRECT®
Bioorganic & Medicinal Chemistry 13 (2005) 4458–4466
Bioorganic & Medicinal Chemistry

Synthesis, antimalarial, antileishmanial, and antimicrobial activities of some 8-quinolinamine analogues

Meenakshi Jain,[a] Shabana I. Khan,[b] Babu L. Tekwani,[b] Melissa R. Jacob,[b] Savita Singh,[c] Prati Pal Singh[c] and Rahul Jain[a,*]

[a]*Department of Medicinal Chemistry, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India*
[b]*National Center for Natural Products Research, School of Pharmacy, University of Mississippi, MS 38677, USA*
[c]*Department of Pharmacology and Toxicology, National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India*

Received 1 April 2005; revised 15 April 2005; accepted 15 April 2005
Available online 5 May 2005

Abstract—In the present communication, newly synthesized 8-quinolinamines (**25**–**27**) related to previously reported 2-*tert*-butylprimaquine (**2**) were evaluated for their in vitro antimalarial activity against chloroquine sensitive and resistant *Plasmodium falciparum* strains, in vivo antimalarial activity against *P. berghei* infected mice, in vitro antileishmanial activity against *Leishmania donovani*, in vitro antimicrobial activity against various fungi and bacteria, and cytotoxicity in a panel of mammalian cell lines. No promising cytotoxicities were observed for compounds reported herein. Analogue **25** was found to exhibit curative antimalarial activity at a dose of 25 mg/kg/day × 4 in a *P. berghei* infected mice model, and produced suppressive activity at a lower dose of 10 mg/kg/day × 4. In vitro antileishmanial activities (IC_{50} and IC_{90}) comparable to standard drug pentamidine were exhibited by all synthesized 8-quinolinamines **25**–**27**. At the same time, promising antibacterial and antifungal activities were also observed for synthesized compounds against a panel consisting of several bacteria and fungi.

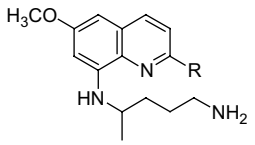
1. Introduction

Protozoal infections such as malaria and leishmaniasis remain major public health problem in large areas of the world. Malaria is responsible for the death of approximately three million people annually, mostly youngsters in more than 100 countries.[1] Majority of these mortality cases occur in sub-Saharan Africa, where malaria accounts for one in five of all childhood deaths. Leishmaniasis is a tropical disease caused by protozoal parasites of the genus *Leishmania* and it remains a significant health issue in large part due to the lack of effective and affordable drugs and increasing resistance against existing drugs.[2] Similarly, increasing number of multidrug-resistant microbial pathogens have become a serious problem particularly during the last decade and provide impetus for the search and discovery of novel antibacterial and antifungal agents active against these pathogens.[3,4]

Rapid development of resistance by *Plasmodium falciparum* to the conventional drugs like chloroquine necessitates search for new antimalarial drugs, and a careful re-examination of the existing drugs. 8-Quinolinamines constitute an interesting class of compounds because of their versatile biological and pharmacological activities, such as antimalarial,[5] antileishmanial,[6] and anticoccidial.[7] Primaquine (PQ, **1**) (Fig. 1) is the only 8-quinolinamine available to treat the malaria parasites in the infections due to *P. vivax* and *P. ovale*. It has been suggested that PQ has various degrees of activities against more life cycle stages of plasmodia than any other drug currently employed for treating malaria infection.[8] However, drawbacks like toxicity, ineffectiveness as blood-schizontocide (infection caused by *P. falciparum*), and rapid metabolism have limited PQ's usefulness. Despite these drawbacks, in addition to excellent radical curative activity, PQ has broad-range of antimalarial activities including efficacy as causal prophylactic, gametocytocide, and sporontocide. These interesting pharmacological

Keywords: 8-Quinolinamine; 2-*tert*-Butylprimaquine; Malaria; Leishmaniasis; Antibacterial; Antifungal; Cytotoxicity.
* Corresponding author. Tel.: +91 0172 221 4682; fax: +91 0172 221 4692; e-mail: rahuljain@niper.ac.in

0968-0896/$ - see front matter © 2005 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2005.04.034



1. Primaquine (PQ), R = H
2. 2-*tert*-Butylprimaquine, R = $C(CH_3)_3$

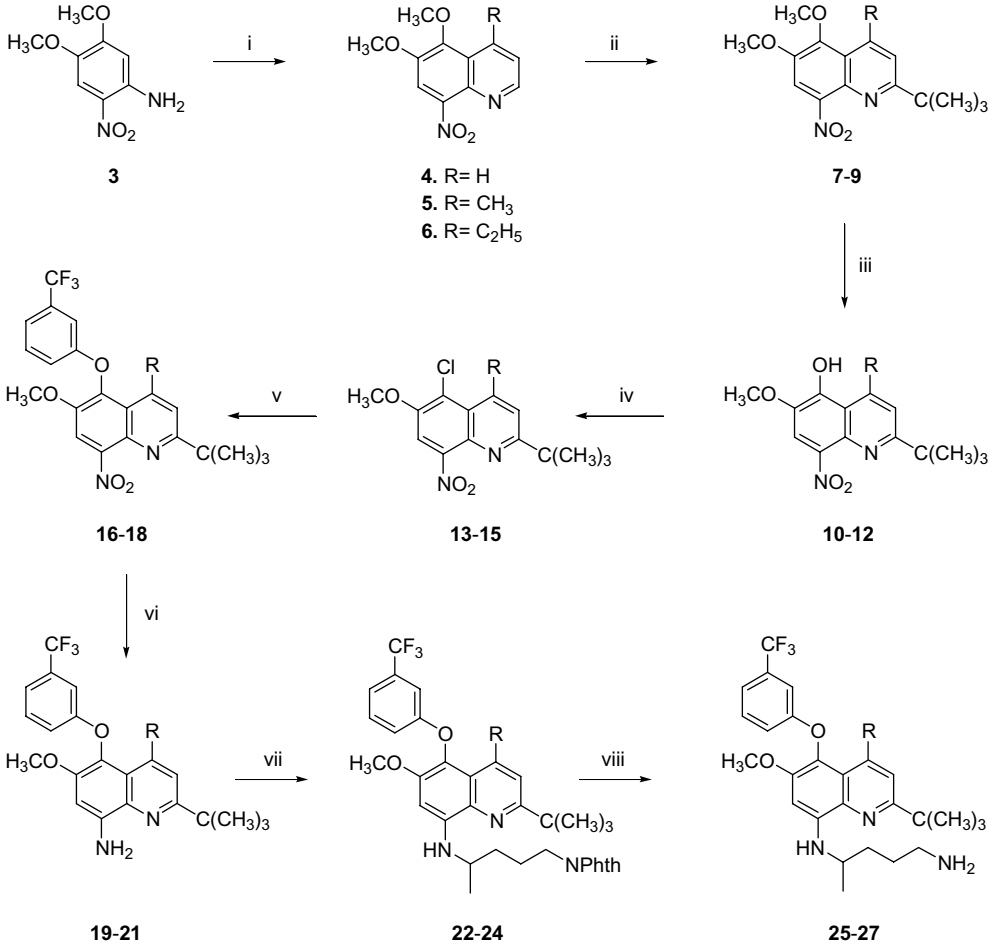
Figure 1.

attributes make PQ an ideal drug to emulate while designing new antimalarials with improved activity.

We have recently reported that the placement of a bulky metabolically stable *tert*-butyl group at the C-2 position of quinoline ring in **1** results in tremendous improvement in the blood-schizontocidal antimalarial activity.[9,10] Antimalarial compound, 2-*tert*-butylprimaquine (**2**) (Fig. 1), exhibits potent in vivo blood-schizontocidal antimalarial activities against both drug-sensitive strain (*P. berghei*) and multi-drug resistant strain (*P. yoelii nige- riensis*). Furthermore, **2** also represents first reported 8-quinolinamine completely devoid of methemoglobin (MetHb) toxicity associated with **1**. In continuation of our research on structural modification of 8-quinolinamines in general,[11,12] and 2-*tert*-butylprimaquine (**2**) in particular, we report herein synthesis, antiprotozoal, and antimicrobial activities of analogues **25**–**27**, in which quinoline ring is substituted with a 5-(3-trifluoromethylphenoxy) group, while C-4 position of the quinoline ring is substituted with a methyl or an ethyl group or is unsubstituted. It is important to note that 5-(3-trifluoromethylphenoxy) substituent has been an integral part of several promising antimalarial compounds and appears to be responsible for enhancement in antimalarial activities. One such compound tafenoquine is presently undergoing clinical trial studies.[13]

2. Chemistry

4-Alkyl-5,6-dimethoxy-8-nitroquinolines (**4**–**6**) synthesized using procedures reported earlier[14,15] starting from 4,5-dimethoxy-2-nitroaniline (**3**) upon direct ring-alkylation via a silver catalyzed radical oxidative decarboxylation of trimethylacetic acid by ammonium persulfate in CH_3CN and 10% H_2SO_4 at 80 °C efficiently produced 4-alkyl-2-*tert*-butyl-5,6-dimethoxy-8-nitroquinolines (**7**–**9**)



Scheme 1. Reagents and conditions: (i) glycerol, H_2SO_4, As_2O_5, 110 °C, 21 h or methylvinylketone, 85% *o*-H_3PO_4, As_2O_5, 100 °C, 3 h or 1-chloro-3-pentanone, 85% *o*-H_3PO_4, As_2O_5, 80 °C, 3 h; (ii) $(CH_3)_3CCO_2H$, $AgNO_3$, $(NH_4)_2S_2O_8$, 10% H_2SO_4, CH_3CN, 70 °C, 15 min; (iii) concd HCl, 95% EtOH, 100 °C, 2 h; (iv) $POCl_3$, 80 °C, 2 h; (v) 3-(trifluoromethyl)phenol, KOH, *p*-xylene, 12 h, 150 °C; (vi) raney nickel, H_2, EtOH, 45 psi, 45 min; (vii) 2-(4-bromopentyl)-1,3-isoindolinedione, Et_3N, 120 °C, 24 h; (viii) $NH_2NH_2{\cdot}H_2O$, EtOH, reflux, 8 h.

in good yields (Scheme 1).[9] Selective demethylation of the 5-methoxy group with concd hydrochloric acid in 95% ethyl alcohol at 100 °C for 2 h easily afforded 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-quinolinols (**10**–**12**) in excellent yields. Reaction of the latter compounds **10**–**12** with phosphorous oxychloride at 80 °C for 2 h produced 4-alkyl-2-*tert*-butyl-5-chloro-6-methoxy-8-nitroquinolines (**13**–**15**) in quantitative yields. Chloro derivatives (**13**–**15**) upon reaction with 3-(trifluoromethyl)phenol in the presence of potassium hydroxide pallets in *p*-xylene at 150 °C for 12 h afforded 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-(3-trifluoromethylphenoxy)-quinolines (**16**–**18**) in good yields. The latter compounds **16**–**18** were then efficiently converted to requisite N^8-(4-amino-1-methylbutyl)-4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamines (**25**–**27**) in three steps using the procedure reported earlier,[9] and finally isolated as hydrochloride salts for their biological evaluation by treatment with ethereal hydrochloric acid solution.

3. Biological activity

Determination of in vitro antimalarial activity was based on the determination of plasmodial LDH activity.[16] As shown in Table 1, antimalarial activities of analogues **25**–**27** are reported as IC_{50} values for the inhibition of growth of chloroquine-sensitive (D6) and chloroquine-resistant (W2) strains of *P. falciparum*. Among the analogues, the most effective was N^8-(4-amino-1-methylbutyl)-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamine (**25**), which exhibited moderate antimalarial activity with IC_{50} of 1000 ng/mL for D6 and 1800 ng/mL for W2 clone (Table 1).

The target compounds were also evaluated for the blood-schizontocidal antimalarial activity against *P. berghei* (sensitive strain) in a rodent model (Table 1). Briefly, testing was conducted at various concentrations, orally, in mice (6 mice per group). The concentrations tested were 100, 50, 25, and 10 mg/kg/day × 4 (oral). The compounds were administered on days 0–3 post infection. The results were compared to a positive control group of mice treated with chloroquine (CQ) at the suppressive dose of 10 mg/kg/day × 4 (oral) and a negative control group of mice where no treatment for the infection was administered, and in this case 100% mortality is observed within 6–8 days, with a mean survival time of 6.2 days.

Analogues **25**–**27** produced 100% cure at the primary tested dose of 100 mg/kg. Upon evaluation at the subsequent lower doses of 50 and 25 mg/kg, compound **25** again produced 100% cure with all treated animals surviving on day 60 (termination of experiment). Further antimalarial activity evaluation of **25** at a dose of 10 mg/kg produced suppressive activity with 2/6 mice surviving on day 60. Thus, compound **25** is equipotent to CQ and **2**, and is much superior to **1** as a blood-schizontocide. In contrast, analogue **26** (curative at 50 mg/kg) and analogue **27** (active at 50 mg/kg) were less effective. As evident from the results, placement of a 5-(3-trifluoromethylphenoxy) group (compound **25**) in 2-*tert*-butylprimaquine (**2**) essentially retains the in vivo antimalarial activity. On the other hand, simultaneous placement of a 5-(3-trifluoromethylphenoxy) and a methyl or an ethyl group in **2** led to reduced antimalarial activity as evident from results obtained for analogues **26** and **27**.

Antileishmanial activity against *Leishmania donovani* promastigotes was determined by Alamar Blue assay as described earlier.[17,18] It was interesting to note that 8-quinolinamines **25**–**27** exhibited potent antileishmanial activities, as shown in Table 2, with IC_{50} values of 3.0, 3.4, and 2.9 μg/mL, respectively, and were comparable to the activity of standard drug pentamidine (IC_{50} = 3.4 μg/mL) used as positive control. Their IC_{90} values ranged from 6.5–6.7 μg/mL as compared to

Table 1. In vitro (*P. falciparum*) and in vivo (*P. berghei*) antimalarial activities of the 8-quinolinamine analogues (**25**–**27**)

No.	*P. berghei* (mg/kg/day × 4, oral)				*P. falciparum* (D6 clone) IC_{50} (ng/mL)	*P. falciparum* (W2 clone) IC_{50} (ng/mL)
	10	25	50	100		
25	(2/6) Active	(6/6) Curative	(6/6) Curative	(6/6) Curative	1800	1000
26	—	(0/6) Inactive	(6/6) Curative	(6/6) Curative	>4760	2800
27	—	—	(3/6) Active	(6/6) Curative	NA	NA
2	(4/6) Active	(6/6) Curative	(6/6) Curative	(6/6) Curative	39	—
PQ	—	—	—	(0/6) Inactive	—	—

The term 'curative' indicates complete elimination of malaria parasites from the body, and animals survive up to day D+60. The term 'active' or 'suppressive' indicates that all of the treated animals show negative parasitaemia up to D+7. However, by D+60, some mice die, and some survive with complete elimination of parasitaemia as indicated by numbers given in parentheses. The term 'inactive' indicates that the treated animals show positive parasitaemia either on D+4 or D+7 and usually die by D+14.
Chloroquine: IC_{50} = 18 ng/mL (D6 strain), 135 ng/mL (W2 strain).
Artemisinin: IC_{50} = 11.5 ng/mL (D6 strain), 15 ng/mL (W2 strain).
NA, Not active.

Table 2. In vitro antileishmanial (*L. donovani*) studies of the 8-quinolinamine analogues (**25**–**27**)

No.	IC_{50} (μg/mL)[a]	IC_{90} (μg/mL)[a]
25	3.0	6.5
26	3.4	6.7
27	2.9	6.5
Pentamidine	3.4	8.0
Amphotericin B	0.17	1.7

[a] IC_{50} and IC_{90} are the sample concentrations that kill 50% and 90% cells compared to solvent controls.

IC_{90} of 8.0 μg/mL for pentamidine. However, they were less potent than amphotericin B (IC_{50} = 0.17 and IC_{90} = 1.7 μg/mL).

The antibacterial activities of 8-quinolinamines **25**–**27** against *Staphylococcus aureus*, methicillin-resistant *S. aureus*, and *Mycobacterium intracellulare* are reported as IC_{50}, MIC, and MBC in Table 3. Ciprofloxacin is included as positive control for comparison. Susceptibility of *S. aureus* and methicillin-resistant *S. aureus* to test compounds was determined according to the procedure as described by the National Committee for Clinical Laboratory Standards (NCCLS).[19–21] Susceptibility of *M. intracellulare* was done using the modified Alamar Blue procedure of Franzblau et al.[22] The IC_{50} was defined as the concentration that affords 50% growth of bacteria, whereas MIC was defined as the lowest concentration resulting in inhibition of visible bacterial growth after incubation at 37 °C for 18–24 h, and MBC was the minimum bactericidal concentration that kills 100% of the organism.

Compounds **25** and **26** exhibited promising antibacterial activities with IC_{50} value in the range of 1.5–3.0 μg/mL against all three organisms and MICs and MBCs in the range of 2.5–10 μg/mL. In contrast, analogue **27** was inactive against *M. intracellulare* and *S. aureus* but showed moderate activity against methicillin-resistant *S. aureus* with an IC_{50} value of 8.5 μg/mL (Table 3).

The antifungal activities of the target compounds against pathogenic fungi associated with opportunistic infections, *Candida albicans*, *Cryptococcus neoformans*, and *Aspergillus fumigatus*, are summarized in Table 4. Amphotericin B was included, as a standard drug, for comparison. IC_{50}, MICs, and MFCs were determined according to NCCLS methods.[19–21]

Compounds **25** and **26** produced promising antifungal effects with IC_{50} values in the range of 1.5–6.5 μg/mL against all three organisms under investigation. Both analogues also exhibited MICs of 5.0 μg/mL against *C. neoformans* and *A. fumigatus*, however MIC for *C. albicans* was $\geqslant$20 μg/mL. At the same time, analogue **27** was effective only against *C. neoformans* with IC_{50} of 3.5 μg/mL, and MIC and MFC of 10 μg/mL (Table 4).

Cytotoxicity of these compounds was tested against four human cancer cell lines (SK-MEL, KB, BT-549, and SK-OV-3) and two noncancerous mammalian cells (VERO and LLC-PK_1) up to a highest concentration of 10 μg/mL using neutral red assay procedure as described earlier.[23,24] None of the analogues showed any cytotoxic effects (data not shown).

Table 3. In vitro antibacterial activities of the 8-quinolinamine analogues (**25**–**27**)

No.	Methicillin-resistant *S. aureus* (MRS)			*M. intracellulare*			*S. aureus*		
	IC_{50}[a] (μg/mL)	MIC[b] (μg/mL)	MBC[c] (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	MBC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	MBC (μg/mL)
25	1.5	2.5	2.5	1.5	5.0	5.0	1.5	10	10.0
26	3.0	5.0	5.0	3.0	5.0	5.0	3.0	5.0	5.0
27	8.5	20	20	20	NA	NA	NA	NT	NT
Ciprofloxacin	0.06	0.31	NA	0.15	0.63	1.25	0.06	0.63	0.63

NA, Not active; NT, not tested.
[a] IC_{50}, The concentration that affords 50% inhibition of bacterial growth.
[b] MIC, (Minimum inhibitory concentration) is the lowest test concentration that allows detectable growth of bacteria.
[c] MBC, Minimum bactericidal concentration (the lowest test concentration that kills 100% of the organism).

Table 4. In vitro antifungal activities of the 8-quinolinamine analogues (**25**–**27**)

No.	*C. albicans*			*C. neoformans*			*A. fumigatus*		
	IC_{50}[a] (μg/mL)	MIC[b] (μg/mL)	MFC[c] (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	MFC (μg/mL)	IC_{50} (μg/mL)	MIC (μg/mL)	MFC (μg/mL)
25	6.5	>20	>20	1.5	5.0	5.0	4.0	5.0	5.0
26	6.5	20	>20	2.0	5.0	5.0	4.0	5.0	5.0
27	NA	NA	NA	3.5	10	10	NA	>20	>20
Amphotericin B	0.40	0.63	1.25	0.70	1.25	1.25	NT	0.63	NT

NA, Not active; NT, not tested.
[a] IC_{50}, The concentration that affords 50% inhibition of bacterial growth.
[b] MIC, (Minimum inhibitory concentration) is the lowest test concentration that allows detectable growth of bacteria.
[c] MFC, Minimum fungicidal concentration (the lowest test concentration that kills 100% of the organism).

4. Conclusions

In conclusion, new 8-quinolinamines related to 2-*tert*-butylprimaquine (**2**) have been synthesized in eight steps from 4,5-dimethoxy-2-nitroaniline. None of the tested compounds showed cytotoxic activity. Compound **25** was the most effective in antimalarial assay and was as potent as 2-*tert*-butylprimaquine against drug-sensitive *P. berghei* in mice model. Most interestingly, all three compounds **25**–**27** exhibited potent in vitro antileishmanial activities against *L. donovani* promastigotes. At the same time, target analogues have also exhibited promising in vitro antibacterial and antifungal activities against a panel consisting of various bacteria and fungi. These results clearly indicate that newly synthesized 8-quinolinamines reported herein are promising compounds and provide useful model for further structural and biological optimization. It can be concluded that 8-quinolinamines provide interesting lead in our search for newer antimicrobial and antileishmanial agents in addition to their proven usefulness in malaria chemotherapy.

5. Experimental

Melting points were recorded on Mettler DSC 851 or capillary melting point apparatus and are uncorrected. ^{1}H spectra were recorded on 300 MHz Bruker FT-NMR (Avance DPX300) spectrometer using tetramethylsilane as internal standard and the chemical shifts are reported in δ units. Mass spectra were recorded on either GCMS (Shimadzu QP 5000 spectrometer) auto sampler/direct injection (EI/CI) or HRMS (Finnigan Mat LCQ spectrometer) (APCI/ESI). Elemental analyses were recorded on Elementar Vario EL spectrometer. All chromatographic purification was performed with silica gel 60 (230–400 mesh), whereas all TLC (silica gel) development was performed on silica gel coated (Merck Kiesel 60 F_{254}, 0.2 mm thickness) sheets. All chemicals were purchased from Aldrich Chemical Ltd. (Milwaukee, WI, USA). Solvents used for the chemical synthesis acquired from commercial sources were of analytical grade and were used without further purification unless otherwise stated.

5.1. General method for the synthesis of 4-alkyl-2-*tert*-butyl-5,6-dimethoxy-8-nitroquinolines (7–9)

4-Alkyl-5,6-methoxy-8-nitroquinoline[14,15] (**4**–**6**, 1 mmol) was dissolved in CH_3CN (5 mL) and the reaction mixture was heated to 70 °C. Silver nitrate (0.6 mmol), trimethylacetic acid (2.5 mmol), and 10% H_2SO_4 (10 mL) were then added to the reaction mixture. A freshly prepared solution of ammonium persulfate (3 mmol) in water (10 mL) was added dropwise to the pre-heated (70 °C) mixture during 10 min. The heating source was then removed and reaction proceeded with the evolution of carbon dioxide. After 15 min, the reaction mixture was poured onto ice. The resulting mixture was made alkaline with addition of 25% aqueous NH_4OH solution. It was extracted with ethyl acetate (4×50 mL), and the combined extracts were washed with NaCl solution (2×10 mL). It was then dried over Na_2SO_4, and the solvent removed under reduce pressure to afford oil, which upon column chromatography over silica gel (230–400 mesh) afforded 4-alkyl-2-*tert*-butyl-5,6-dimethoxy-8-nitroquinolines (**7**–**9**).

5.1.1. 2-*tert*-Butyl-5,6-dimethoxy-8-nitroquinoline (7). Yield: 65%; mp 79–80 °C; ^{1}H NMR ($CDCl_3$): δ 8.41 (d, 1H, $J = 9.0$ Hz), 7.89 (s, 1H), 7.60 (d, 1H, $J = 9.0$ Hz), 4.07 (s, 3H), 4.02 (s, 3H), 1.42 (s, 9H); ESI MS *m*/*z* 291 (M+1); Anal. Calcd for $C_{15}H_{18}N_2O_4$ (290.3): C, 62.06; H, 6.25; N, 9.65. Found: C, 62.11; H, 6.51; N, 9.78.

5.1.2. 2-*tert*-Butyl-5,6-dimethoxy-4-methyl-8-nitroquinoline (8). Yield: 47%; mp 72–73 °C; ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H), 7.29 (s, 1H), 4.00 (s, 3H), 3.98 (s, 3H), 2.87 (s, 3H), 1.38 (s, 9H); ESI MS *m*/*z* 305 (M+1); Anal. Calcd for $C_{16}H_{20}N_2O_4$ (304.3): C, 63.14; H, 6.62; N, 9.20. Found: C, 63.11; H, 6.67; N, 9.07.

5.1.3. 2-*tert*-Butyl-5,6-dimethoxy-4-ethyl-8-nitroquinoline (9). Yield: 32%; mp 75–76 °C; ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H), 7.34 (s, 1H), 4.01 (s, 3H), 3.98 (s, 3H), 3.25 (q, 2H, $J = 15.0$ Hz), 1.39 (s, 9H), 1.31 (t, 3H, $J = 15.0$ Hz); ESI MS *m*/*z* 319 (M+1); Anal. Calcd for $C_{17}H_{22}N_2O_4$ (318.4): C, 64.13; H, 6.97; N, 8.80. Found: C, 64.09; H, 6.89; N, 8.73.

5.2. General method for the synthesis of 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-quinolinols (10–12)

The dimethoxyquinoline (**7**–**9**, 0.69 mmol) was dissolved in 95% ethyl alcohol (4 mL) and concd HCl (0.2 mL) was added. The reaction mixture was stirred at 100 °C for 2 h. Upon cooling, brownish crystals of 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-quinolinols (**10**–**12**) separated out and used without further purification for the next reaction.

5.2.1. 2-*tert*-Butyl-6-methoxy-8-nitro-5-quinolinol (10). Yield: 82%; mp 184–185 °C; ^{1}H NMR ($CDCl_3$): δ 9.20 (d, 1H, $J = 8.4$ Hz), 7.82 (s, 1H), 7.45 (d, 1H, $J = 8.4$ Hz), 3.93 (s, 3H), 3.49 (br s, 1H, exchangeable with D_2O), 1.60 (s, 9H); APCI MS *m*/*z* 277 (M+1); Anal. Calcd for $C_{14}H_{16}N_2O_4$ (276.3): C, 60.86; H, 5.84; N, 10.14. Found: C, 61.08; H, 5.93; N, 9.92.

5.2.2. 2-*tert*-Butyl-6-methoxy-4-methyl-8-nitro-5-quinolinol (11). Yield: 94%; mp 162–163 °C; ^{1}H NMR ($CDCl_3$): δ 7.74 (s, 1H), 7.15 (s, 1H), 3.90 (s, 3H), 3.13 (s, 3H), 1.57 (s, 9H); ESI MS *m*/*z* 291 (M+1); Anal. Calcd for $C_{15}H_{18}N_2O_4$ (290.1): C, 62.06; H, 6.25; N, 9.65. Found: C, 62.17; H, 6.09; N, 9.43.

5.2.3. 2-*tert*-Butyl-4-ethyl-6-methoxy-8-nitro-5-quinolinol (12). Yield: 99%; mp 186–187 °C; ^{1}H NMR ($CDCl_3$): δ 7.76 (s, 1H), 7.18 (s, 1H), 3.91 (s, 3H), 3.59 (q, 2H, $J = 15.0$ Hz), 1.57 (s, 9H), 1.37 (t, 3H, $J = 15.0$ Hz); APCI MS *m*/*z* 305 (M+1); Anal. Calcd for $C_{16}H_{20}N_2O_4$ (304.1): C, 63.14; H, 6.62; N, 9.20. Found: C, 63.37; H, 6.41; N, 9.08.

5.3. General method for the synthesis of 4-alkyl-2-*tert*-butyl-5-chloro-6-methoxy-8-nitroquinolines (13–15)

$POCl_3$ (2.5 mL) was added to 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-quinolinol (**10**–**12**, 0.45 mmol) and the reaction mixture was heated at 80 °C for 2 h. The reaction mixture was poured onto crushed ice, basified with 25% NH_4OH, and extracted with $CHCl_3$ (3×25 mL). Combined organic layer was dried over Na_2SO_4 and removal of solvent under reduced pressure provided 4-alkyl-2-*tert*-butyl-5-chloro-6-methoxy-8-nitroquinolines (**13**–**15**) in excellent yield.

5.3.1. 2-*tert*-Butyl-5-chloro-6-methoxy-8-nitroquinoline (13). Yield: 100%; mp 230–231 °C; ^{1}H NMR ($CDCl_3$): δ 8.50 (d, 1H, $J = 9.0$ Hz), 7.79 (s, 1H), 7.71 (d, 1H, $J = 9.0$ Hz), 4.07 (s, 3H), 1.42 (s, 9H); APCI MS *m*/*z* 295 (M+1); Anal. Calcd for $C_{14}H_{15}ClN_2O_3$ (294.7): C, 57.05; H, 5.13; N, 9.50. Found: C, 56.79; H, 4.85; N, 9.78.

5.3.2. 2-*tert*-Butyl-5-chloro-6-methoxy-4-methyl-8-nitroquinoline (14). Yield: 95%; mp 137–138 °C; ^{1}H NMR ($CDCl_3$): δ 7.65 (s, 1H), 7.37 (s, 1H), 4.04 (s, 3H), 3.06 (s, 3H), 1.38 (s, 9H); ESI MS *m*/*z* 309 (M+1); Anal. Calcd for $C_{15}H_{17}ClN_2O_3$ (308.8): C, 58.35; H, 5.55; N, 9.07. Found: C, 58.67; H, 5.41; N, 9.18.

5.3.3. 2-*tert*-Butyl-5-chloro-4-ethyl-6-methoxy-8-nitroquinoline (15). Yield: 100%; oil; ^{1}H NMR ($CDCl_3$): δ 7.65 (s, 1H), 7.43 (s, 1H), 4.05 (s, 3H), 3.50 (q, 2H, $J = 15.0$ Hz), 1.39 (s, 9H), 1.35 (t, 3H, $J = 15.0$ Hz); APCI MS *m*/*z* 323 (M+1); Anal. Calcd for $C_{16}H_{19}ClN_2O_3$ (322.8): C, 59.54; H, 5.93; N, 8.68. Found: C, 59.74; H, 5.86; N, 8.86.

5.4. General method for the synthesis of 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-(3-trifluoromethylphenoxy)-quinolines (16–18)

To a mixture of 3-(trifluoromethyl)phenol (2.49 mmol) in *p*-xylene (10 mL) containing KOH pallets (2.42 mmol) was added 4-alkyl-2-*tert*-butyl-5-chloro-6-methoxy-8-nitroquinoline (**13**–**15**, 2.20 mmol). The reaction mixture was heated at 150 °C for 12 h. The solvent was removed under reduced pressure and crude product was purified by chromatography on silica gel using EtOAc–hexanes (1:1) as eluant.

5.4.1. 2-*tert*-Butyl-6-methoxy-8-nitro-5-(3-trifluoromethylphenoxy)quinoline (16). Yield: 78%; mp 185–186 °C; ^{1}H NMR ($CDCl_3$): δ 8.21 (d, 1H, $J = 9.0$ Hz), 7.91 (s, 1H), 7.61 (d, 1H, $J = 9.0$ Hz), 7.39 (m, 1H), 7.33 (m, 1H), 7.16 (s, 1H), 6.98 (m, 1H), 3.91 (s, 3H), 1.43 (s, 9H); APCI MS *m*/*z* 421 (M+1); Anal. Calcd for $C_{21}H_{19}F_3N_2O_4$ (420.4): C, 60.00; H, 4.56; N, 6.66. Found: C, 60.09; H, 4.37; N, 6.73.

5.4.2. 2-*tert*-Butyl-6-methoxy-4-methyl-8-nitro-5-(3-trifluoromethylphenoxy)quinoline (17). Yield: 68%; mp 152–153 °C; ^{1}H NMR ($CDCl_3$): δ 7.78 (s, 1H), 7.40 (m, 1H), 7.33 (s, 1H, 3), 7.30 (s, 1H), 7.09 (s, 1H), 6.93 (m, 1H), 3.84 (s, 3H), 2.69 (s, 3H), 1.40 (s, 9H); APCI MS *m*/*z* 435 (M+1); Anal. Calcd for $C_{22}H_{21}F_3N_2O_4$ (434.4): C, 60.83; H, 4.87; N, 6.45. Found: C, 61.14; H, 5.11; N, 6.37.

5.4.3. 2-*tert*-Butyl-4-ethyl-6-methoxy-8-nitro-5-(3-trifluoromethylphenoxy)quinoline (18). Yield: 62%; mp 176–177 °C; ^{1}H NMR ($CDCl_3$): δ 7.77 (s, 1H), 7.38 (m, 1H), 7.32 (s, 1H), 7.30 (m, 1H), 7.09 (s, 1H), 6.94 (m, 1H), 3.82 (s, 3H), 3.03 (q, 2H, $J = 14.3$ Hz), 1.41 (s, 9H), 1.27 (t, 3H, $J = 14.3$ Hz); APCI MS *m*/*z* 449 (M+1); Anal. Calcd for $C_{23}H_{23}F_3N_2O_4$ (448.4): C, 61.60; H, 5.17; N, 6.25. Found: C, 61.72; H, 5.23; N, 6.47.

5.5. General method for the synthesis of 4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamines (19–21)

A solution of 4-alkyl-2-*tert*-butyl-6-methoxy-8-nitro-5-(3-trifluoromethylphenoxy)quinoline (**16**–**18**, 10 mmol) in 95% ethyl alcohol (30 mL) was hydrogenated over raney nickel (T_1 grade) at 45 psi in a parr hydrogenator for 45 min. The catalyst was removed by filtration and the filtrate was evaporated under reduced pressure to afford 4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamines (**19**–**21**) as dark colored oil.

5.5.1. 2-*tert*-Butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamine (19). Yield: 100%; oil; ^{1}H NMR ($CDCl_3$): δ 7.97 (d, 1H, $J = 8.7$ Hz), 7.44 (d, 1H, $J = 8.7$ Hz), 7.34 (m, 1H), 7.25 (m, 1H), 7.14 (s, 1H), 6.99 (m, 1H), 6.76 (s, 1H), 5.07 (br s, 2H, exchangeable with D_2O), 3.84 (s, 3H), 1.43 (s, 9H); APCI MS *m*/*z* 391 (M+1); Anal. Calcd for $C_{21}H_{21}F_3N_2O_2$ (390.4): C, 64.61; H, 5.42; N, 7.18. Found: C, 64.97; H, 5.16; N, 7.43.

5.5.2. 2-*tert*-Butyl-6-methoxy-4-methyl-5-(3-trifluoromethylphenoxy)-8-quinolinamine (20). Yield: 98%; oil; ^{1}H NMR ($CDCl_3$): δ 7.78 (s, 1H), 7.42 (m, 1H), 7.33 (s, 1H), 7.30 (s, 1H), 7.09 (m, 1H), 6.93 (m, 1H), 4.98 (br s, 2H, exchangeable with D_2O), 3.91 (s, 3H), 2.87 (s, 3H), 1.40 (s, 9H); APCI MS *m*/*z* 405 (M+1); Anal. Calcd for $C_{22}H_{23}F_3N_2O_2$ (404.4): C, 65.34; H, 5.73; N, 6.93. Found: C, 65.76; H, 5.92; N, 7.28.

5.5.3. 2-*tert*-Butyl-4-ethyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamine (21). Yield: 100%; oil; ^{1}H NMR ($CDCl_3$): δ 7.35 (s, 1H), 7.33 (m, 1H), 7.34 (m, 1H), 7.22 (m, 1H), 7.05 (s, 1H), 6.77 (s, 1H), 5.15 (br s, 2H, exchangeable with D_2O), 3.80 (s, 3H), 2.95 (q, 2H, $J = 14.7$ Hz), 1.43 (s, 9H), 1.25 (t, 3H, $J = 14.7$ Hz); APCI MS *m*/*z* 419 (M+1); Anal. Calcd for $C_{23}H_{25}F_3N_2O_2$ (418.5): C, 66.02; H, 6.02; N, 6.69. Found: C, 66.38; H, 5.71; N, 6.87.

5.6. General method for the synthesis of 2-{4-[4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolylamino]pentyl}-1,3-isoindolinediones (22–24)

A mixture of 4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamine (**19**–**21**, 2.7 mmol), 2-(4-bromopentyl)-1,3-isoindolinedione (2.7 mmol), and

triethylamine (2.7 mmol) was heated at 120 °C with stirring for 4 h. An additional quantity of 2-(4-bromopentyl)-1,3-isoindolinedione (2.7 mmol) and triethylamine (2.7 mmol) was added, and stirring continued with heating for another 4 h. A third aliquot of 2-(4-bromopentyl)-1,3-isoindolinedione (2.7 mmol) and triethylamine (2.7 mmol) was added, and the reaction mixture stirred at 120 °C for additional 16 h. The dark brown reaction mixture was diluted with ethyl acetate (50 mL) and filtered. The filtrate was basified with 2 N NaOH solution and extracted with ethyl acetate (3 × 30 mL). The combined extracts were washed with water (10 mL), dried over Na_2SO_4, and concentrated to afford dark colored residue. Flash column chromatography over silica using EtOAc–hexanes (25:75) as eluant provided 2-{4-[4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolylamino]pentyl}-1,3-isoindolinediones (**22**–**24**) as colorless or pale yellow viscous oil.

5.6.1. 2-{4-[2-*tert*-Butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolylamino]-pentyl}-1,3-isoindolinedione (22). Yield: 84%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 7.98 (d, 1H, J = 8.7 Hz), 7.82 (m, 4H), 7.70 (d, 1H, J = 8.7 Hz), 7.45 (m, 1H), 7.23 (m, 1H), 7.17 (m, 1H), 6.98 (m, 1H), 6.76 (s, 1H), 6.43 (br s, 1H, exchangeable with D_2O), 3.86 (s, 3H), 3.76 (m, 1H), 3.72 (m, 2H), 1.89 (m, 4H), 1.43 (s, 9H), 1.36 (d, 3H, J = 6.3 Hz); APCI MS *m*/*z* 606 (M+1); Anal. Calcd for $C_{34}H_{34}F_3N_3O_4$ (605.7): C, 67.43; H, 5.66; N, 6.94. Found: C, 67.74; H, 5.88; N, 6.62.

5.6.2. 2-{4-[2-*tert*-Butyl-6-methoxy-4-methyl-5-(3-trifluoromethylphenoxy)-8-quinolyl-amino]pentyl}-1,3-isoindolinedione (23). Yield: 81%; colorless oil; ^{1}H NMR ($CDCl_3$): δ 7.79 (m, 4H), 7.34 (m, 1H), 7.28 (s, 1H), 7.18 (m, 1H), 7.08 (s, 1H), 6.93 (m, 1H), 6.43 (s, 1H), 6.25 (br s, 1H, exchangeable with D_2O), 3.88 (m, 1H), 3.79 (s, 3H), 3.76 (m, 2H), 2.57 (s, 3H), 1.61 (m, 4H), 1.39 (s, 9H), 1.35 (d, 3H, J = 6.3 Hz); APCI MS *m*/*z* 620 (M+1); Anal. Calcd for $C_{35}H_{36}F_3N_3O_4$ (619.7): C, 67.84; H, 5.86; N, 6.78. Found: C, 68.15; H, 6.17; N, 6.49.

5.6.3. 2-{4-[2-*tert*-Butyl-4-ethyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolyl-amino]pentyl}-1,3-isoindolinedione (24). Yield: 89%; pale yellow oil; ^{1}H NMR ($CDCl_3$): δ 7.83 (m, 4H), 7.35 (m, 1H), 7.32 (s, 1H), 7.22 (m, 1H), 7.19 (s, 1H), 7.09 (s, 1H), 6.95 (m, 1H), 6.45 (s, 1H), 4.18 (m, 1H), 3.80 (s, 3H), 3.72 (m, 2H), 2.92 (q, 2H, J = 7.5 Hz), 1.84 (m, 4H), 1.41 (s, 9H), 1.35 (d, 3H, J = 6.3 Hz), 1.25 (m, 3H); APCI MS *m*/*z* 634 (M+1); Anal. Calcd for $C_{36}H_{38}F_3N_3O_4$ (633.7): C, 68.23; H, 6.04; N, 6.63. Found: C, 68.02; H, 6.37; N, 6.41.

5.7. General method for the synthesis of N^8-(4-amino-1-methylbutyl)-4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamines (25–27)

To a solution of 2-{4-[4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolylamino]pentyl}-1,3-isoindolinedione (**22**–**24**, 5 mmol) in 95% ethyl alcohol (25 mL) was added hydrazine hydrate (50 mmol), and the reaction mixture was heated under reflux for 8 h. The solvent was removed under reduced pressure and the residue was diluted with water (25 mL). The reaction mixture was basified with 8 N NaOH solution, extracted with $CHCl_3$ (3 × 25 mL), and washed with water (15 mL). Combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to yield N^8-(4-amino-1-methylbutyl)-4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamines (**25**–**27**) as oil. Treatment with ethereal hydrochloric acid solution provided the requisite N^8-(4-amino-1-methylbutyl)-4-alkyl-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamines as their hydrochloride salts.

5.7.1. N^8-(4-Amino-1-methylbutyl)-2-*tert*-butyl-6-methoxy-5-(3-trifluoromethyl-phenoxy)-8-quinolinamine (25). Yield: 99%; mp 125–126 °C; ^{1}H NMR (free base, $CDCl_3$): δ 7.94 (d, 1H, J = 8.7 Hz), 7.43 (d, 1H, J = 8.7 Hz), 7.34 (m, 1H), 7.22 (m, 1H), 7.20 (m, 1H), 7.01 (m, 1H), 6.43 (s, 1H), 6.23 (br s, 1H, exchangeable with D_2O), 3.88 (s, 3H), 3.68 (m, 1H), 2.81 (m, 2H), 2.03 (m, 4H), 1.42 (s, 9H), 1.38 (d, 3H, J = 6.0 Hz); APCI MS *m*/*z* 476 (M+1); Anal. Calcd for $C_{26}H_{34}Cl_2F_3N_3O_2$ (548.5): C, 56.94; H, 6.25; N, 7.66. Found: C, 56.87; H, 6.13; N, 7.88.

5.7.2. N^8-(4-Amino-1-methylbutyl)-2-*tert*-butyl-6-methoxy-4-methyl-5-(3-trifluoromethylphenoxy)-8-quinolinamine (26). Yield: 90%; mp 115–116 °C; ^{1}H NMR (free base, $CDCl_3$): δ 7.84 (s, 1H), 7.44 (m, 1H), 7.29 (m, 1H), 6.99 (m, 1H), 6.95 (s, 1H), 6.60 (s, 1H), 4.93 (br s, 1H, exchangeable with D_2O), 3.88 (m, 1H), 3.81 (s, 3H), 2.80 (m, 2H), 2.58 (s, 3H), 1.74 (m, 4H), 1.42 (s, 9H), 1.37 (d, 3H, J = 6.3 Hz); APCI MS *m*/*z* 490 (M+1); Anal. Calcd for $C_{27}H_{36}Cl_2F_3N_3O_2$ (562.5): C, 57.65; H, 6.45; N, 7.47. Found: C, 57.77; H, 6.51; N, 7.52.

5.7.3. N^8-(4-Amino-1-methylbutyl)-2-*tert*-butyl-4-ethyl-6-methoxy-5-(3-trifluoromethylphenoxy)-8-quinolinamine (27). Yield: 94%; mp 112–113 °C; ^{1}H NMR (free base, $CDCl_3$): δ 7.60 (s, 1H), 7.26 (m, 1H), 7.08 (m, 1H), 6.92 (m, 1H), 6.90 (m, 1H), 6.45 (s, 1H), 3.80 (s, 3H), 3.82 (m, 1H), 2.93 (q, 2H, J = 6.0 Hz), 2.80 (m, 2H), 1.76 (m, 4H), 1.42 (s, 9H), 1.34 (d, 3H, CH_3, J = 6.0 Hz) 0.88 (t, 3H, J = 7.2 Hz); APCI MS *m*/*z* 505 (M+1); Anal. Calcd for $C_{28}H_{38}Cl_2F_3N_3O_2$ (576.5): C, 58.33; H, 6.64; N, 7.29. Found: C, 58.37; H, 6.75; N, 7.43.

5.8. Assay for in vitro antimalarial activity

The assay is based on the determination of plasmodial LDH activity.[16] For the assay, a suspension of red blood cells infected with D6 or W2 strains of *P. falciparum* (200 μL, with 2% parasitemia and 2% hematocrit in RPMI 1640 medium supplemented with 10% human serum and 60 μg/mL amikacin) is added to the wells of a 96-well plate containing 10 μL of test samples diluted in medium at various concentrations. The plate is placed in a modular incubation chamber (Billups-Rothenberg,

CA) and flushed with a gas mixture of 90% N_2, 5% O_2, and 5% CO_2 and incubated at 37 °C, for 72 h. Parasitic LDH activity is determined by using Malstat™ reagent (Flow Inc., Portland, OR) according to the procedure of Makler and Hinrichs.[16] Briefly, 20 μL of the incubation mixture is mixed with 100 μL of the Malstat™ reagent and incubated at room temperature for 30 min. Twenty microliters of a 1:1 mixture of NBT/PES (Sigma, St. Louis, MO) is then added and the plate is further incubated in the dark for 1 h. The reaction is then stopped by the addition of 100 μL of a 5% acetic acid solution. The plate is read at 650 nm using the EL-340 Biokinetics Reader (Bio-Tek Instruments, Vermont). IC_{50} values are computed from the dose response curves. Artemisinin and chloroquine are included in each assay as the drug controls. DMSO (0.25%) is used as vehicle control.

5.9. Assay for blood-schizontocidal activity evaluation against *P. berghei* infection in mice

The method used for screening of the synthesized compounds for their blood-schizontocidal activity is based on a comparison of responses by groups of treated and control mice, six in each group, after infection with *P. berghei*. Using a standard inoculum of *P. berghei*, it is possible to produce a uniform disease that is fatal to 100% of untreated animals, within 6–8 days, with a mean survival time of 6.2 days. Test animals (Swiss mice of either sex, approximately 15–20 g and same age) were housed in metal-topped cages, given a standard laboratory diet and water ad libitum. In order to check factors such as changes in the infectivity of the strain or in the susceptibility of the host or to detect technical errors, a group of infected animals treated with chloroquine diphosphate at dose levels (10 mg/kg/day × 4), producing definite increases in survival time, is included as a positive control in every experiment. In each experiment, the test compounds were administered in graded doses of 100, 50, 25, and 10 mg/kg. On day '0', groups of 6 mice each were inoculated intraperitoneally with 1×10^7 infected-erythrocytes from a donor mouse. Four h later, mice were administered test compounds/chloroquine/vehicle, orally. A total of four doses were given orally on days D '0', D+1, D+2, and D+3. The tail blood smears were made on day D+4 and D+7, stained with Giemsa and examined microscopically. The minimum dose that completely suppressed parasitaemia on days D+4 and D+7 was termed as minimum effective dose (MED), and the minimum dose that cleared the parasitaemia for up to 60 days was termed as curative dose (CD). The terms 'curative', 'active', and 'inactive' are used to describe the biological activities exhibited by the tested compounds. The term 'curative' indicates complete elimination of malaria parasites from the body, so that relapse cannot occur up to day D+60 and all mice survived. The term 'active' indicates that the treated animals show negative parasitaemia up to D+7. However, by D+28, 50% or more mice show negative and remaining mice may show positive test result for parasitaemia. The term 'inactive' indicates that the treated animals show positive test result for parasitaemia either on D+4 or D+7 or on both D+4 and D+7 and the animal usually dies by D+14.

5.10. Assay for in vitro antileishmanial activity

Antileishmanial activity of the compounds was tested in vitro against a culture of *L. donovani* promastigotes. They were grown in RPMI 1640 medium supplemented with 10% fetal calf serum (Gibco Chem. Co.) at 26 °C. A 3 day-old culture was diluted to 5×10^5 promastigotes/mL. Drug dilutions (50–3.1 μg/mL) were prepared directly in cell suspension in 96-well plates. Plates were incubated at 26 °C for 48 h and growth of leishmania promastigotes was determined by Alamar Blue assay as described earlier.[17,18] Standard fluorescence was measured on a Fluostar Galaxy plate reader (BMG Lab Technologies) at excitation wavelength of 544 nm and emission wavelength of 590 nm. Pentamidine and amphotericin B were used as the standard antileishmanial agents. IC_{50} and IC_{90} values were computed from dose curves generated by plotting percent growth versus drug concentration.

5.11. Assay for in vitro antimicrobial activity

Susceptibility testing against *C. albicans*, *C. neoformans*, *S. aureus*, methicillin-resistant *S. aureus* (MRS), and *A. fumigatus* was performed using a modified version of the NCCLS methods.[19–21] Susceptibility testing against *M. intracellulare* was done using the modified Alamar Blue procedure of Franzblau et al.[22] All organisms were obtained from the American Type Culture Collection (ATCC), Manassas, VA. Samples (dissolved in DMSO) were serially diluted using 0.9% saline and transferred in duplicate to 96-well microplates. Microbial inocula were prepared after comparison of the absorbance at 630 nm of cell suspensions to the 0.5 McFarland standard and diluting the suspensions in broth [Sabouraud Dextrose and cation-adjusted Mueller–Hinton (Difco) for the fungi and bacteria, respectively, and 5% Alamar Blue (BioSource International) in Middlebrook 7H9 broth with OADC enrichment for *M. intracellulare*] to afford recommended inocula. Microbial inocula were added to the diluted samples to achieve a final volume of 200 μL. Growth, solvent, and media controls were included in each assay. Plates are read at either 630 nm or 544ex/590em (*M. intracellulare*) prior to and after incubation. Percent growth was plotted versus test concentration to afford the IC_{50}. The minimum bactericidal/fungicidal concentrations (MBC/MFCs) were determined by removing 5 μL from each clear well, transferring to agar and incubating until growth is seen.

5.12. Cytotoxicity assay

The in vitro cytotoxicity was determined against a panel of cell lines that included SK-MEL, KB, BT-549, SK-OV-3, VERO, and LCC-PK_1 (obtained from ATCC). The assay is performed in 96-well tissue culture-treated microplates and compounds were tested up to a highest concentration of 10 μg/mL as described earlier.[24] Briefly, cells (25,000 cells/well) were seeded to the wells of the plate and incubated for 24 h. Samples were added and plates were again incubated for 48 h. The number of viable cells was determined according to a modified version of neutral red assay procedure.[23] IC_{50} values were

determined from logarithmic graphs of growth inhibition versus concentration. Doxorubicin was used as a positive control, while DMSO was used as the negative (vehicle) control.

Acknowledgment

Financial support from the Council of Scientific and Industrial Research (CSIR), New Delhi, India (Grant no. 01(1555)/98/EMR-II) for the synthesis of primaquine derivatives is gratefully acknowledged.

References and notes

1. (a) Frédérich, M.; Dongé, J.-M.; Angenot, L.; De Mol, P. *Curr. Med. Chem.* **2002**, *9*, 1435; (b) Boss, C.; Richard-Bildstein, S.; Weller, T.; Fischli, W.; Meyers, S.; Binkert, C. *Curr. Med. Chem.* **2003**, *10*, 883.
2. Davis, A. J.; Murray, H. W.; Handman, E. *Trends Parasitol.* **2004**, *20*, 73.
3. Bren, L. *FDA Consum.* **2002**, *36*, 28.
4. Liu, J.; Balasubramanian, M. K. *Curr. Drug Targets—Infect. Disorders* **2001**, *1*, 159.
5. Carson, P. E. In *Antimalarial Drugs II*; Peters, W., Richards, W. H. G., Eds.; Springer: New York, 1984; p 83.
6. Yeates, C. *Curr. Opin. Invest. Drugs* **2002**, *3*, 1446.
7. Armer, R. E.; Barlow, J. S.; Dutton, C. J.; Greenway, D. H. J.; Greenwood, S. D. W.; Lad, N.; Tommasini, I. *Bioorg. Med. Chem. Lett.* **1997**, *7*, 2585.
8. Nodiff, E. A. *Prog. Med. Chem.* **1991**, *28*, 1.
9. Jain, M.; Vangapandu, S.; Sachdeva, S.; Singh, S.; Singh, P. P.; Jena, G. B.; Tikoo, K.; Ramarao, P.; Kaul, C. L.; Jain, R. *J. Med. Chem.* **2004**, *47*, 285.
10. Jain, M.; Vangapandu, S.; Sachdeva, S.; Jain, R. *Bioorg. Med. Chem.* **2004**, *12*, 1003.
11. Vangapandu, S.; Sachdeva, S.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2004**, *12*, 239.
12. Vangapandu, S.; Sachdeva, S.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2003**, *11*, 4557.
13. LaMontagne, M. P.; Blumbergs, P.; Smith, D. C. *J. Med. Chem.* **1989**, *32*, 1728.
14. Jain, R.; Jain, S.; Gupta, R. C.; Anand, N.; Dutta, G. P.; Puri, S. K. *Indian J. Chem.* **1994**, *33B*, 251.
15. Carroll, F. I.; Berrang, B.; Linn, C. P. *J. Med. Chem.* **1985**, *28*, 1564.
16. Makler, M. T.; Hinrichs, D. J. *Am. J. Trop. Med. Hyg.* **1993**, *48*, 205.
17. Mikus, J.; Steverding, D. *Parasitol. Int.* **2000**, *48*, 265.
18. Ma, G.; Khan, S. I.; Jacob, M. R.; Tekwani, B. L.; Li, Z.; Pasco, D. S.; Walker, L. A.; Khan, I. A. *Antimicrob. Agents Chemother.* **2004**, *48*, 4450.
19. NCCLS, In *Reference Method for Broth Dilution Antifungal Susceptibility Testing of Yeasts; Approved Standard M27-A*, National Committee on Clinical Laboratory Standards, 1997; Vol. 17, p 9.
20. NCCLS, In *Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria that Grow Aerobically M7-A5*, National Committee on Clinical Laboratory Standards, 2000; Vol. 20, p 2.
21. NCCLS, In *Susceptibility Testing of Mycobacteria, Nocardia, and Other Aerobic Actinomycetes; Tentative Standard—Second Edition, M24-T2*, National Committee on Clinical Laboratory Standards, 2000; Vol. 20, p 26.
22. Franzblau, S. G.; Witzig, R. S.; McLaughlin, J. C.; Torres, P.; Madico, G.; Hernandez, A.; Degnan, M. T.; Cook, M. B.; Quenzer, V. K.; Ferguson, R. M.; Gilman, R. H. *J. Clin. Microbiol.* **1998**, *36*, 362.
23. Borenfreund, E.; Babich, H.; Martin-Alguacil, N. *In Vitro Cell Dev. Biol.* **1990**, *26*, 1030.
24. Mustafa, J.; Khan, S. I.; Ma, G.; Walker, L. A.; Khan, I. A. *Lipids* **2004**, *39*, 167.