Available online at www.sciencedirect.com
SCIENCE DIRECT
ELSEVIER
Bioorganic & Medicinal Chemistry 12 (2004) 239–247
Bioorganic & Medicinal Chemistry

8-Quinolinamines conjugated with amino acids are exhibiting potent blood-schizontocidal antimalarial activities☆

Suryanarayana Vangapandu†, Sandeep Sachdeva, Meenakshi Jain, Savita Singh, Prati Pal Singh, Chaman Lal Kaul and Rahul Jain*

National Institute of Pharmaceutical Education and Research, Sector 67, S.A.S. Nagar, Punjab 160 062, India

Received 6 August 2003; accepted 3 October 2003

Abstract—Alanine, lysine, ornithine and valine conjugated to primaquine and other 8-quinolinamine antimalarials were prepared for blood-schizontocidal antimalarial activity evaluation. The analogues were examined in vivo against *Plasmodium berghei* (drug-sensitive strain) and *Plasmodium yoelii nigeriensis* (highly virulent multi-drug-resistant strain) infected mice models. N^1-[4-(5-Butoxy-4-ethyl-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide (**20**) which showed curative activity at 5 mg/kg in the *P. berghei* test emerged as the most effective compound. N^1-[4-(4-Ethyl-5-hexoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide (**22**) exhibited curative activity at 50 mg/kg against *P. yoelii nigeriensis* in mice and emerged as the most potent analogue against multi-drug resistant strain. The results of this study represent development of highly potent 8-quinolinamines for antimalarial drug development.

1. Introduction

About one third of the world's population are currently living with a serious risk of contracting malaria.[1] It is estimated that there are approximately 2–3 million deaths every year from malaria,[2] and in sub-Saharan part of Africa alone about 1/2 million children below the age of 5 years die of malaria every year.[3] Despite continuous research efforts of more than two decades no vaccine is yet discovered for effective control of malaria. Treatment of malaria is becoming more difficult due to the spreading resistance of the parasite to standard antimalarials drugs, in particular to chloroquine (CQ), which had been the affordable and effective antimalarial mainstay for more than 50 years,[4,5] to the resistance of mosquitoes to insecticides, and due to climatic changes that have enlarged areas of disease transmission. It is commonly known that infection caused by *Plasmodium falciparum* is frequently more fatal in children than in adults, producing respiratory distress, neurological problems and severe anemia, and leading to death in 5–35% of severe infections. The proliferation of drug-resistant *P. falciparum* has highlighted more than ever the need to develop new antimalarial drugs, by preference inexpensive, and affordable to the developing countries where the disease is prevalent. The phenomenon of acute resistance associated with malaria has prompted researchers around the world to carefully reexamine alternative and classical antimalarial drugs that may be effective against resistant strains of *P. falciparum*.

Primaquine (**1**) belonging to 8-quinolinamine class of antimalarials, over the years is the clinical drug of choice for the radical cure of relapsing *Plasmodium vivax* and *Plasmodium ovale* malaria.[6] The drug is effective in clearing tissue parasites but has almost no suppressive activity; that is, is ineffective as blood-schizontocide, and therefore can not be used for the treatment of infections caused by *P. falciparum*. The usefulness of **1** has been further restricted by toxic side effects, especially with patients who are deficient in glucose-6-phosphate dehydrogenase.[7] Also troublesome is the need to administer primaquine in divided doses over an extended period, since, in addition to its toxicity, it is rapidly absorbed, metabolized and eliminated. Despite these drawbacks, in addition to excellent radical curative activity, primaquine has a broad-range of antimalarial activities including efficacy as causal prophylactic, gametocytocide and sporontocide, and can be produced at low cost. Furthermore, primaquine

☆NIPER Communication no. 230.

* Corresponding author. Tel.: +91-172-2214682; fax: +91-172-2214692; e-mail: rahuljain@niper.ac.in

† *Current address*: Research Institute of Pharmaceutical Sciences, University of Mississippi, MS, USA.

0968-0896/$ - see front matter © 2003 Elsevier Ltd. All rights reserved.
doi:10.1016/j.bmc.2003.10.028

has been found relatively slow to select resistant strains, and studies conducted earlier have demonstrated that chloroquine-resistant parasites are not cross-resistant to primaquine.[8–10] With these encouraging chemical and pharmacological attributes, primaquine in many ways, is an ideal drug to emulate when designing new antimalarial, and is considered an excellent lead prototype for the development of congeners with potent blood-schizontocidal activities that could be effective against drug-resistant and drug sensitive malaria strains.

2. Results and discussion

As part of earlier attempts to improve primaquine (**1**), encapsulating **1** in liposome reduced its lethal toxicity 3.6-fold but left the efficacy unchanged.[11] Thus, Hofsteenge and co-workers considered that toxicity of **1** can be further reduced by linking the drug to a macromolecular carrier protein. A thiol group containing primaquine derivative (**2**) when linked via a disulfide bond to a carrier protein was found to have substantially reduced toxicity.[12] Modifications on the primaquine side-chain were further extended to show that linking **1** to small peptides (**3**) and dihydro-furan-2-one moiety (**4**) marginally enhanced activity with slightly reduced toxicity (Fig. 1).[13–15]

The reason why the side-chain modified peptide derivatives of primaquine have better therapeutic index is not clear; however, following observations can be made: (a) the attachment of peptide possibly reduces the penetration of primaquine into the red cells because of steric hindrance that does not allow destabilization of the red cell membrane, inducing hemolysis, which is the source of main toxicity, (b) approximately 35–83% of primaquine is known to get metabolized to 4-(6-methoxy-quinolin-8-ylamino)-pentanoic acid in a primate model;[16] therefore, peptide residue may serve to protect the primary amino function of **1** against metabolic process,[14] (c) the conjugates of **1** with peptides are slowly hydrolyzed in the presence of serum; thus these analogues are possibly acting as prodrugs of primaquine,[13] (d) Trouet et al. observed that leucylprimaquine is not hydrolyzed in the serum, albeit was found equipotent to **1**, thus, it is either active itself or hydrolyzed to primaquine inside the cell.[13] These observations suggested that in addition to peptide analogues, side-chain amino acid analogues of primaquine and its derivatives should also lead to improvement in the chemotherapeutic properties of **1**, and further research on the modification of the side chain is desired. Thus, we propose that a careful selection of amino acids and their covalently formed conjugates with 8-quinolinamines should also show superior therapeutic profiles.

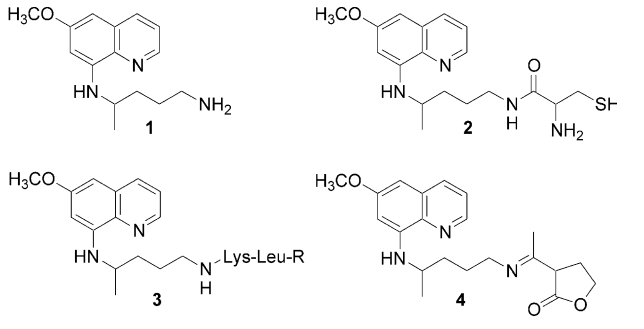


Figure 1.

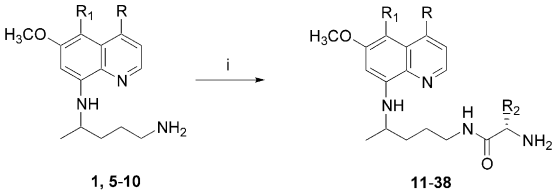
In an earlier study, it was reported that placement of 4-ethyl group at the quinoline ring of primaquine decreases the toxicity while retaining the activity.[17] In order to gain information about optimal substitution pattern for 8-quinolinamines, we have recently reported synthesis and potent blood-schizontocidal activities of analogues **5–10** having straight-chain 5-alkoxy and 4-ethyl groups in the quinoline ring.[18] In the logical extension of this work, we report herein the synthesis and antimalarial activities of the twenty eight conjugated 8-quinolinamine analogues (**11–38**).

As discussed earlier, the mechanism of action of peptide derivatives of primaquine is still not clear, though it can be speculated that these analogues are possibly prodrugs of primaquine. Thus, only L-amino acids are chosen for this study with expectation that incorporation of L-amino acid would allow the conjugates to undergo enzymatic proteolytic process to release the parent drug; and provide legitimacy to the prodrug mechanism. Amino acids chosen for this study includes alanine (ala), leucine (leu), lysine (lys) and ornithine (orn). Decision on the selection of the chosen amino acids was based upon following rationale and observations: (a) ala and leu present in the original sequence synthesized by Trouet et al.[13] are simple hydrophobic natural amino acids, and we were interested to study their individual effect on the efficacy; (b) peptides, peptoids and homopolymers containing a high percentage of cationic amino acids have been shown to possess a unique ability to cross the plasma membrane of the cells, and consequently have been used to facilitate the delivery of a variety of covalently linked small molecules.[19–21] Presence of ε-amino group on the side-chain attributes to the cationic and hydrophilic character of lysine, and conjugates of 8-quinolinamines with a cationic lysine residue should have improved therapeutic properties due to their supposedly improved cell penetration properties; (c) similarly, ornithine being an unnatural amino acid found in numerous naturally occurring peptides, is homologous to lysine in its physicochemical properties, and should increase efficacy of the analogues. Moreover, the ornithine conjugates of 8-quinolinamines may not undergo rapid enzymatic proteolysis as expected for other three chosen amino acids, and could provide inputs to the observations made earlier for the peptides[13] that these analogues may be active themselves and are not operating through a prodrug mechanism.

2.1. Chemistry

8-Quinolinamines (**1**, **5–10**) upon condensation reaction with *N*-carbobenzyloxy (Cbz) protected L-amino acids in the presence of DCC in dichloromethane for 4 h gave the protected amino acid derivatives in excellent yields. Hydrogenolysis of *N*-Cbz group of the protected amino

acid derivatives using 10% Pd/C catalyst proceeded smoothly to afford the free amino group containing compounds that upon treatment with ethereal hydrogen chloride solution provided the 8-quinolinamine-amino acid conjugates (**11–38**)[22] as their hydrochloride salts as outlined in Scheme 1.



Scheme 1. Reagent and condition: (i) (a) Cbz-L-AA-OH, DCC, $CHCl_2$, rt; (b) H_2/Pd/C, 1 h, rt.

2.2. Biological activities

The 28 target compounds were evaluated for the blood schizontocidal test against *Plasmodium berghei* infection in mice (Table 1). In vivo testing of compounds **11–38** was conducted at various concentrations, orally, in mice infected with *P. berghei*. The concentrations tested orally were 100, 50, 25, 10 and 5 mg/kg/day×4. The compound was administered on day 0–3 post infection. The results for all analogues are compared to a positive control group of mice treated with chloroquine at the concentration of 10 mg/kg/day×4, with mice showing negative parasitemia on D + 4 and D + 7. The results are also compared to a negative control group of mice where no treatment for the disease was administered, and in this case all animals usually die by D + 14. Compounds exhibiting 100% efficacy (i.e., curative) at 10 mg/kg were further selected for evaluation of efficacy in *Plasmodium yoelii nigeriensis* infection (a highly virulent strain and naturally resistant to chloroquine and mefloquine) in mice (Table 2).

2.2.1. Blood schizontocidal activity (*P. berghei* mice test). In general, 8-quinolinamines conjugated to alanine are not found to exhibit interesting biological activities, and analogues (**12–16**) were found to be ineffective at the initial tested dose of 100 mg/kg. However, compound **11** ($R = R_1 = H$, $R_2 = CH_3$) of the series was found to have curative activity at a dose of 25 mg/kg and suppressive activity at a dose of 10 mg/kg/day. At the same time, compound **17** ($R = C_2H_5$, $R_1 = OC_8H_{17}$, $R_2 = CH_3$) produced curative activity at a dose of 10 mg/kg and suppressive activity at 5 mg/kg.

Introduction to the 8-quinolinamines of the lysine amino acid produced a very pronounced increase in the biological activity. The lysine derivatives (**18–24**) with the exception of 5-propoxy analogue (**19**) of 8-quinolinamines were found to be 100% curative at the primary

Table 1. In vivo blood-schizontocidal antimalarial activity of the 8-quinolinamines-amino acid conjugates (**11–38**) against *P. berghei* infection in mice (six mice per group)

No.	R	R_1	R_2	Dose (mg/kg/day×4, oral)				
				5	10	25	50	100
11	H	H	CH_3	—	Active	Curative	Curative	Curative
12	C_2H_5	OC_3H_7	CH_3	—	—	—	—	Inactive
13	C_2H_5	OC_4H_9	CH_3	—	—	—	—	Inactive
14	C_2H_5	OC_5H_{11}	CH_3	—	—	—	—	Inactive
15	C_2H_5	OC_6H_{13}	CH_3	—	—	—	—	Inactive
16	C_2H_5	OC_7H_{15}	CH_3	—	—	—	—	Inactive
17	C_2H_5	OC_8H_{17}	CH_3	Active	Curative	Curative	Curative	Curative
18	H	H	$(CH_2)_4NH_2$	—	Active	Curative	Curative	Curative
19	C_2H_5	OC_3H_7	$(CH_2)_4NH_2$	—	—	—	—	Inactive
20	C_2H_5	OC_4H_9	$(CH_2)_4NH_2$	Curative	Curative	Curative	Curative	Curative
21	C_2H_5	OC_5H_{11}	$(CH_2)_4NH_2$	Active	Curative	Curative	Curative	Curative
22	C_2H_5	OC_6H_{13}	$(CH_2)_4NH_2$	Active	Curative	Curative	Curative	Curative
23	C_2H_5	OC_7H_{15}	$(CH_2)_4NH_2$	Inactive	Curative	Curative	Curative	Curative
24	C_2H_5	OC_8H_{17}	$(CH_2)_4NH_2$	—	Active	Curative	Curative	Curative
25	H	H	$(CH_2)_3NH_2$	—	—	—	—	Inactive
26	C_2H_5	OC_3H_7	$(CH_2)_3NH_2$	—	Active	Curative	Curative	Curative
27	C_2H_5	OC_4H_9	$(CH_2)_3NH_2$	Inactive	Curative	Curative	Curative	Curative
28	C_2H_5	OC_5H_{11}	$(CH_2)_3NH_2$	Inactive	Curative	Curative	Curative	Curative
29	C_2H_5	OC_6H_{13}	$(CH_2)_3NH_2$	Active	Curative	Curative	Curative	Curative
30	C_2H_5	OC_7H_{15}	$(CH_2)_3NH_2$	Inactive	Curative	Curative	Curative	Curative
31	C_2H_5	OC_8H_{17}	$(CH_2)_3NH_2$	—	—	—	—	Inactive
32	H	H	$CH(CH_3)_2$	—	—	Inactive	Curative	Curative
33	C_2H_5	OC_3H_7	$CH(CH_3)_2$	—	—	—	—	Inactive
34	C_2H_5	OC_4H_9	$CH(CH_3)_2$	—	—	—	—	Inactive
35	C_2H_5	OC_5H_{11}	$CH(CH_3)_2$	—	—	—	—	Inactive
36	C_2H_5	OC_6H_{13}	$CH(CH_3)_2$	—	—	Inactive	Curative	Curative
37	C_2H_5	OC_7H_{15}	$CH(CH_3)_2$	—	—	—	—	Inactive
38	C_2H_5	OC_8H_{17}	$CH(CH_3)_2$	—	Active	Curative	Curative	Curative
Chloroquine					Active	Curative	Curative	Curative
Primaquine				—	—	—	—	Inactive

The term 'curative' indicates complete elimination of malaria parasites, and animals survive up to day D + 60. The term 'active' indicates that treated animals show negative parasitaemia up to D + 7. However, by D + 60, some mice die, and some survive with complete elimination of parasitaemia. The term 'inactive' indicates that the treated animals show positive parasitaemia either on D + 4 or D + 7 and usually die by D + 14.

Table 2. In vivo blood-schizontocidal antimalarial activity of the 8-quinolinamines-amino acid conjugates against drug resistant *P. yoelii nigeriensis* infection in mice (six mice per group)

No.	R	R_1	R_2	Dose (mg/kg/day×4, oral)	
				50	100
17	C_2H_5	OC_8H_{17}	CH_3	—	Inactive
20	C_2H_5	OC_4H_9	$(CH_2)_4NH_2$	—	Inactive
21	C_2H_5	OC_5H_{11}	$(CH_2)_4NH_2$	—	Active
22	C_2H_5	OC_6H_{13}	$(CH_2)_4NH_2$	Curative	Curative
23	C_2H_5	OC_7H_{15}	$(CH_2)_4NH_2$	—	Active
27	C_2H_5	OC_4H_9	$(CH_2)_3NH_2$	—	Active
28	C_2H_5	OC_5H_{11}	$(CH_2)_3NH_2$	Active	Curative
29	C_2H_5	OC_6H_{13}	$(CH_2)_3NH_2$	—	Inactive
30	C_2H_5	OC_7H_{15}	$(CH_2)_3NH_2$	Active	Curative
Chloroquine				—	Inactive
Primaquine				—	Inactive

The term 'curative' indicates complete elimination of malaria parasites, and animals survive up to day D + 60. The term 'active' indicates that treated animals show negative parasitaemia up to D + 7. However, by D + 60, some mice die, and some survive with complete elimination of parasitaemia. The term 'inactive' indicates that the treated animals show positive parasitaemia either on D + 4 or D + 7 and usually die by D + 14.

tested dose of 100 mg/kg. The propoxy analogue (**19**) was found to be inactive at the dose of 100 mg/kg in the *P. berghei* infected mice model. Furthermore, the 5-butoxy, 5-pentoxy and 5-heptoxy derivatives when tested at lower doses showed 100% curative activity up to a dose of 10 mg/kg, whereas the 5-octoxy derivative was found to be suppressive at this dose level. These observations suggest that the introduction of the lysine amino acid at the side chain of 8-quinolinamines generally produced a substantial increase in the blood-schizontocidal activity. However, presence of the 5-alkoxy group bearing more than seven carbons are found to diminish the activity as indicated by compound (**24**, $R = C_2H_5$, $R_1 = OC_8H_{17}$) at the lower tested dose of 10 mg/kg. The most effective compound (**20**, $R = C_2H_5$, $R_1 = C_4H_9$) produced exceptional antimalarial activity with 100% cures at a dose of 5 mg/kg. At the same time, compounds (**21**, $R = C_2H_5$, $R_1 = C_5H_{11}$) and (**22**, $R = C_2H_5$, $R_1 = OC_6H_{13}$) were slightly less effective and produced only suppressive activity at 5 mg/kg/day.

Similarly, introduction of the ornithine amino acid also produced analogues with pronounced increase in activity. Ornithine-conjugated derivatives (**27–30**) of 8-quinolinamines were found to be curative at 10 mg/kg. As seen in the case of lysine conjugates, not to our surprise, the octoxy analogue (**31**) was found to be inactive at a maximum tested dose of 100 mg/kg. These findings indicate that incorporation of cationic amino acids to 8-quinolinamines results in dramatically improved efficacy possibly due to the improved cell penetration properties. Regardless of the mechanism of action, these extremely encouraging results suggest that synthesis of cationic amino acid conjugates of 8-quinolinamines is a powerful technique to improve chemotherapeutic properties. The promising results obtained with lysine and ornithine opens up avenues for further analogue design with other cationic and even D-amino acids.

Finally, biological evaluation of valine adducts (**32–38**) of 8-quinolinamines did not produce any significant activity. The most effective conjugate **38** ($R = C_2H_5$, $R_1 = OC_8H_{17}$) showed curative activity at 25 mg/kg, and was found to produce suppressive activity at 10 mg/kg. As discussed earlier for alanine conjugates, these observations suggest that attachment of a hydrophobic amino acid to the terminal amino group of 8-quinolinamines results in decreased antimalarial activity.

2.2.2. Blood schizontocidal activity (*P. yoelii nigeriensis* mice test). Analogues (**17**, **20–23** and **27–30**) found to produce curative activities at 10 mg/kg in *P. berghei* test, were further evaluated in *P. yoelii nigeriensis* mice test for their efficacy against drug-resistant strains. In general, selected compounds produced activity at the initial tested dose of 100 mg/kg. Exceptions were the analogues (**17**, **20**, and **29**) with all mice dead within D + 14 because of the infection. Conjugated analogues **22** [$R = C_2H_5$, $R_1 = OC_6H_{13}$, $R_2 = (CH_2)_4NH_2$], **28** [$R = OC_2H_5$, $R_1 = C_5H_{11}$, $R_2 = (CH_2)_3NH_2$] and **30** [$R = OC_2H_5$, $R_1 = C_7H_{15}$, $R_2 = (CH_2)_3NH_2$] were the most effective from the series, and have produced potent efficacy with 100% cures at 100 mg/kg/day.

When tested at a lower dose of 50 mg/kg, analogues **22** produced 100% cures, whereas analogues **28** and **30** produced only partial cures. It is important to note that *P. yoelii nigeriensis* infection is naturally resistant to chloroquine and mefloquine and thus, these analogues hold great promise as future antimalarial agents.

3. Conclusions

In summary, the reported amino acid conjugated 8-quinolinamines have exhibited potent in vivo antimalarial activities much superior when compared to peptide analogues reported earlier. Furthermore, in general, antimalarial activities of amino acid conjugated 8-quinolinamines are superior compared to the activities of respective 8-quinolinamines. Particularly, interesting and highly efficacious compounds are produced by the incorporation of lysine and ornithine residues on the side chain of 8-quinolinamines. To conclude, the pronounced in vivo efficacy of the reported analogues against drug-sensitive *P. berghei* strain and drug-resistant *P. yoelii nigeriensis* strain is indicative of the potential usefulness of these compounds in the therapy of malaria infection caused by *P. falciparum*. Although, these 8-quinolinamines conjugates themselves are clinically useful blood-schizontocides, the rationale for their use as eventual broad-spectrum antimalarial agents can be justified provided they also display excellent tissue-schizontocidal activities. As observed from the results, it is still not clear that whether these analogues produce antimalarial effects by themselves or activities of these analogues emanates from the hydrolysis of putative prodrug forms. Therefore, to get better insights into the mechanism of action of amino acid analogues further investigations are warranted. Research efforts towards design and synthesis of other cationic amino acid and

D-amino acid conjugated analogues of 8-quinolinamines are currently underway, and results of this study will be reported in due course of time.

4. Experimental

Melting points were recorded on Mettler DSC 851 or capillary melting point apparatus and are uncorrected. Both ^{1}H and ^{13}C NMR 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.

4.1. General method for the synthesis of N^1-[4-(5-alkoxy-4-ethyl-6-methoxy-8-quinolylamino/6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino/diamino alkanamides (11–38)

To an ice cooled stirred solution of N^8-(4-amino-1-methylbutyl)-6-methoxy-8-quinolinamine (**1**, 1 mmol) or N^8-(4-amino-1-methylbutyl)-5-alkoxy-4-ethyl-6-methoxy-8-quinolinamine (**5–10**, 1 mmol) and N–Cbz protected L-amino acid (1.1 mmol) in dichloromethane (15 mL), 1,3-dicyclohexylcarbodiimide (1.1 mmol) was added. Reaction mixture was allowed to attain room temperature and stirring was continued for another 4 h. The reaction mixture was kept in refrigerator overnight and the separated 1,3-dicyclohexylurea (DCU) filtered. Filtrate was concentrated under reduced pressure. Ethyl acetate (50 mL) was added to the residue and the additional quantity of separated DCU was again removed by filtration. The filtrate was washed with saturated sodium bicarbonate solution (3×10 mL) followed by water (2×10 mL), and dried over Na_2SO_4. The solvent was removed under reduced pressure to afford the crude product, which was purified by flash column chromatography on silica gel (230–400 mesh) using CH_3OH/$CHCl_3$ (2:98) to afford the N–Cbz protected amino acid conjugates of 8-quinolinamines.

To a mixture of protected amino acid linked quinoline derivatives (0.5 mmol), glacial acetic acid (1 mL) and 10% Pd/C (0.1 g) in methanol (20 mL) was bubbled a slow stream of hydrogen gas for 1 h. The catalyst was removed by filtration, and filtrate was concentrated in vacuum to afford the product as oil, which upon treatment with a solution of ethereal hydrogen chloride provided the corresponding hydrochloride salt derivatives. Recrystallized from methanol/diethyl ether.

4.1.1. N^1-[4-(6-Methoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride (11). Yield: 94%; mp (salt) 121–125 °C (dec.); IR (KBr) 3397, 1623 cm^{-1}; ^{1}H NMR (free base, $CDCl_3$) δ 8.53 (d, 1H, J = 4.2 Hz), 7.93 (d, 1H, J = 8.3 Hz), 7.28 (m, 1H), 6.34 (s, 1H), 6.28 (s, 1H), 6.22 (bs, 1H), 5.50 (bs, 1H), 3.89 (s, 3H), 3.62 (s, 1H), 3.48 (bs, 2H), 3.26 (t, 2H, J = 6.2 Hz), 1.67 (m, 4H), 1.30 (d, 3H, J = 5.6 Hz), 1.20 (d, 3H, J = 5.7 Hz); ^{13}C NMR (free base, $CDCl_3$) δ 175.4, 168.5, 159.2, 152.9, 144.4, 134.9, 129.8, 121.9, 118.2, 55.2, 47.8, 39.0, 33.8, 26.2, 24.9, 21.2, 20.5; APCIMS m/z 331 (M + 1); analysis for (salt) $C_{18}H_{28}Cl_2N_4O_2$ (403.3), calcd, C, 50.60; H, 6.49; N, 13.89; found, C, 50.87; H, 6.56; N, 13.81.

4.1.2. N^1-[4-(4-Ethyl-6-methoxy-5-propoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride (12). Yield: 100%; mp (salt) 104–111 °C (dec.); IR (KBr): 3434, 1665 cm^{-1}; ^{1}H NMR (free base, $CDCl_3$) δ 8.40 (d, 1H, J = 4.2 Hz), 7.13 (d, 1H, J = 4.2 Hz), 6.47 (s, 1H), 4.42 (bs, 1H), 4.22 (m, 1H), 3.97 (s, 3H), 3.88 (t, 2H, J = 6.9 Hz), 3.63 (m, 1H), 3.25 (m, 4H), 2.09 (bs, 2H), 1.71–1.44 (m, 6H), 1.32 (m, 9H), 0.93 (t, 3H, J = 7.8 Hz); ^{13}C NMR (free base, $CDCl_3$) δ 176.1, 172.3, 152.2, 149.0, 144.5, 142.2, 134.2, 132.8, 123.1, 122.9, 120.0, 94.5, 74.8, 66.2, 57.1, 49.5, 39.0, 40.4, 32.4, 29.5, 28.2, 26.0, 15.6, 14.1, 11.5; EIMS m/z 416 (M$^+$), 373, 341, 217, 149, 44 (100%); analysis for (salt) $C_{23}H_{38}Cl_2N_4O_3$ (489.5), calcd, C, 56.43; H, 7.41; N, 11.44; found, C, 56.39; H, 7.48; N, 11.40.

4.1.3. N^1-[4-(5-Butoxy-4-ethyl-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride (13). Yield: 95%; mp (salt) 93–97 °C (dec.); IR (KBr): 3427, 1642 cm^{-1}; ^{1}H NMR (free base, $CDCl_3$) δ 8.39 (d, 1H, J = 4.3 Hz), 7.11 (d, 1H, J = 4.3 Hz), 6.52 (s, 1H), 4.51 (bs, 1H), 4.18 (m, 1H), 3.95 (s, 3H), 3.88 (t, 2H, J = 6.8 Hz), 3.60 (m, 1H), 3.23 (m, 4H), 2.08 (bs, 2H), 1.86–1.48 (m, 8H), 1.29 (m, 9H), 0.98 (t, 3H, J = 7.6 Hz); ^{13}C NMR (free base, $CDCl_3$) δ 175.2, 172.8, 151.6, 149.1, 144.7, 141.8, 134.0, 133.1, 123.6, 122.2, 120.1, 94.7, 74.1, 65.9, 57.8, 50.1, 39.4, 40.1, 32.1, 29.8, 28.2, 26.3, 21.2, 15.5, 14.2, 11.8; EIMS m/z 430 (M$^+$), 373, 341, 217, 149, 44 (100%); analysis for (salt) $C_{24}H_{40}Cl_2N_4O_3$ (503.5), calcd, C, 57.24; H, 7.60; N, 11.12; found, C, 57.28; H, 7.81; N, 11.03.

4.1.4. N^1-[4-(4-Ethyl-6-methoxy-5-pentoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride (14). Yield: 98%; mp (salt) 82–86 °C (dec.); IR (KBr): 3412, 1664 cm^{-1}; ^{1}H NMR (free base, $CDCl_3$) δ 8.40 (d, 1H, J = 4.2 Hz), 7.75 (bs, 1H), 7.14 (d, 1H, J = 4.2 Hz), 6.44 (s, 1H), 4.10 (m, 1H), 3.96 (s, 3H), 3.87 (t, 2H, J = 6.8 Hz), 3.63 (m, 1H), 3.22 (m, 4H), 2.05 (bs, 2H), 1.90–1.62 (m, 10H), 1.30 (m, 9H), 0.89 (t, 3H, J = 7.9 Hz); ^{13}C NMR (free base, $CDCl_3$) δ 176.5, 173.0, 151.9, 149.5, 145.1, 142.0, 134.4, 133.2, 124.2, 122.5, 119.9, 94.5, 74.3, 66.1, 57.0, 50.2, 39.6, 34.9, 32.0, 30.1, 28.5, 26.0, 24.4, 21.4, 15.8, 14.5, 11.0; CIMS (CH_4) m/z 445 (M + 1); analysis for (salt) $C_{25}H_{42}Cl_2N_4O_3$ (517.6), calcd, C, 58.01; H, 7.79; N, 10.82; found, C, 58.22; H, 7.85; N, 10.94.

4.1.5. N^1-[4-(4-Ethyl-5-hexoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride

(15). Yield: 96%; mp (salt) 77–79 °C (dec.); IR (KBr): 3427, 1642 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.40 (d, 1H, $J = 4.3$ Hz), 7.76 (bs, 1H), 7.12 (d, 1H, $J = 4.3$ Hz), 6.45 (s, 1H), 4.18 (m, 1H), 3.96 (s, 3H), 3.87 (t, 2H, $J = 6.9$ Hz), 3.64 (m, 1H), 3.22 (m, 4H), 2.07 (bs, 2H), 1.95–1.67 (m, 12H), 1.29 (m, 9H), 0.87 (t, 3H, $J = 7.9$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 176.9, 173.4, 151.4, 150.1, 144.7, 142.1, 134.1, 132.6, 124.0, 122.8, 119.2, 94.8, 74.6, 66.1, 57.1, 50.3, 39.8, 34.4, 32.8, 30.3, 28.9, 26.4, 25.2, 21.4, 20.9, 15.8, 14.4, 11.7; APCIMS *m/z* 459 (M + 1); analysis for (salt) $C_{26}H_{44}Cl_2N_4O_3$ (531.6), calcd, C, 58.74; H, 7.96; N, 10.54; found, C, 58.86; H, 7.69; N, 10.61.

4.1.6. N^1-[4-(4-Ethyl-5-heptoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride (16). Yield: 93%; mp (salt) 75–77 °C (dec.); IR (KBr): 3438, 1663 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.38 (d, 1H, $J = 4.2$ Hz), 7.61 (bs, 1H), 7.12 (d, 1H, $J = 4.2$ Hz), 6.45 (s, 1H), 4.18 (m, 1H), 3.96 (s, 3H), 3.90 (t, 2H, $J = 6.6$ Hz), 3.63 (m, 1H), 3.25 (m, 4H), 2.06 (m, 2H), 1.94–1.59 (m, 14H), 1.29 (m, 9H), 0.89 (t, 3H, $J = 7.6$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 172.6, 158.2, 151.5, 150.2, 144.7, 142.2, 134.3, 132.8, 124.1, 122.9, 119.2, 95.0, 74.7, 57.3, 50.2, 48.5, 40.0, 34.1, 32.3, 30.4, 29.6, 26.4, 25.2, 21.6, 21.0, 19.3, 15.9, 14.5; CIMS (CH_4) *m/z* 473 (M + 1), 374, 342; analysis for (salt) $C_{27}H_{46}Cl_2N_4O_3$ (545.6), calcd, C, 59.43; H, 8.12; N, 10.26; found, C, 59.06; H, 8.25; N, 10.20.

4.1.7. N^1-[4-(4-Ethyl-6-methoxy-5-octoxy-8-quinolylamino)pentyl]-(2*S*)-2-aminopropanamide dihydrochloride (17). Yield, 95%; mp (salt) 72–74 °C (dec.); IR (KBr): 3360, 1641 cm^{-1}; NMR (free base, $CDCl_3$) δ 8.38 (d, 1H, $J = 4.3$ Hz), 7.60 (bs, 1H), 7.12 (d, 1H, $J = 4.3$ Hz), 6.45 (s, 1H), 3.96 (s, 3H), 3.87 (t, 2H, $J = 6.9$ Hz), 3.63 (m, 1H), 3.23 (m, 4H), 2.07 (bs, 2H), 1.95–1.37 (m, 16H), 1.29 (m, 9H), 0.89 (t, 3H, $J = 6.7$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 173.4, 158.0, 151.4, 150.1, 144.7, 142.1, 134.1, 132.6, 124.0, 122.8, 119.2, 94.8, 74.6, 66.1, 57.1, 50.3, 48.4, 39.8, 34.4, 32.2, 30.3, 29.6, 26.4, 25.2, 23.0, 21.4, 20.9, 19.9, 15.8, 14.4; CIMS (CH_4) *m/z* 491 (M + 1), 374, 342; analysis for (salt) $C_{28}H_{48}Cl_2N_4O_3$ (559.6), calcd, C, 60.09; H, 8.28; N, 10.01; found, C, 60.15; H, 8.33; N, 10.11.

4.1.8. N^1-[4-(6-Methoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (18). Yield: 99%; mp (salt) 147–149 °C (dec.); IR (KBr) 3330, 1624 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.51 (d, 1H, $J = 3.8$ Hz), 8.12 (bs, 1H), 7.92 (d, 1H, $J = 8.1$ Hz), 7.30 (m, 1H), 6.33 (s, 1H), 6.27 (s, 1H), 3.98 (bs, 1H), 3.87 (s, 3H), 3.59 (m, 1H), 3.44 (bs, 2H), 3.24 (t, 2H, $J = 6.0$ Hz), 2.92 (t, 2H, $J = 5.6$ Hz), 1.79–1.50 (m, 12H), 1.26 (d, 3H, $J = 6.0$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.3, 169.7, 168.5, 159.4, 157.9, 144.9, 144.3, 135.2, 130.0, 121.9, 97.0, 91.8, 55.2, 52.9, 47.8, 42.9, 41.9, 39.8, 38.9, 33.9, 25.5, 21.8, 18.7; APCIMS *m/z* 388 (M + 1); analysis for (salt) $C_{21}H_{36}Cl_3N_5O_2$ (496.9), calcd, C, 50.76; H, 6.69; N, 14.09; found, C, 50.65; H, 6.76; N, 14.11.

4.1.9. N^1-[4-(4-Ethyl-6-methoxy-5-propoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (19). Yield: 94%; mp (salt) 126–129 °C (dec.); IR (KBr): 3425, 1667 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 4.3$ Hz), 8.26 (bs, 1H), 7.11 (d, 1H, $J = 4.3$ Hz), 6.44 (s, 1H), 3.95 (s, 3H), 3.84 (t, 2H, $J = 6.7$ Hz), 3.62 (m, 1H), 3.39 (m, 2H), 3.23 (m, 6H), 2.91 (bs, 2H), 1.85–1.35 (m, 14H), 1.26 (d, 3H, $J = 5.2$ Hz), 1.18 (t, 3H, $J = 6.2$ Hz); 1.04 (t, 3H, $J = 7.9$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 170.4, 157.7, 151.2, 149.6, 144.5, 141.9, 134.0, 123.8, 122.4, 94.7, 75.8, 56.9, 53.0, 49.4, 48.1, 39.7, 33.7, 28.6, 25.6, 24.9, 22.2, 20.5, 15.5, 10.6; ESIMS *m/z* 474 (M + 1), 261; analysis for (salt) $C_{26}H_{46}Cl_3N_5O_3$ (583.0), calcd, C, 53.56; H, 7.43; N, 12.01; found, C, 53.42; H, 7.45; N, 12.25.

4.1.10. N^1-[4-(5-Butoxy-4-ethyl-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (20). Yield: 92%; mp (salt) 120–124 °C (dec.); IR (KBr): 3332, 1665 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 4.2$ Hz), 7.84 (bs, 1H), 7.11 (d, 1H, $J = 4.2$ Hz), 6.44 (s, 1H), 3.95 (s, 3H), 3.88 (t, 2H, $J = 5.8$ Hz), 3.62 (s, 1H), 3.23 (m, 4H), 2.91 (bs, 2H), 1.80–1.31 (m, 14H), 1.28 (m, 6H), 0.99 (t, 3H, $J = 7.6$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.3, 170.2, 151.1, 149.7, 144.4, 141.9, 133.9, 132.3, 123.7, 122.2, 94.6, 74.0, 56.9, 53.1, 48.1, 39.8, 38.8, 34.3, 32.1, 28.6, 26.0, 21.4, 20.5, 19.3, 18.7, 15.5, 14.0; APCIMS *m/z* 488 (M + 1); analysis for (salt) $C_{27}H_{48}Cl_3N_5O_3$ (597.1), calcd, C, 54.31; H, 7.59; N, 11.72; found, C, 54.40; H, 7.85; N, 11.71.

4.1.11. N^1-[4-(4-Ethyl-6-methoxy-5-pentoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (21). Yield: 99%; mp (salt) 118–121 °C (dec.); IR (KBr): 3358, 1651 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.36 (d, 1H, $J = 3.6$ Hz), 8.2 (bs, 1H), 7.10 (d, 1H, $J = 3.6$ Hz), 6.44 (s, 1H), 3.95 (s, 3H), 3.87 (t, 2H, $J = 6.6$ Hz), 3.62 (m, 1H), 3.47 (bs, 2H), 3.24 (m, 4H), 2.92 (m, 2H), 1.82–1.43 (m, 18H), 1.26 (m, 6H), 0.94 (t, 3H, $J = 6.4$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.1, 170.2, 150.9, 149.5, 144.3, 141.8, 133.7, 123.5, 122.2, 94.4, 74.0, 57.0, 53.0, 47.8, 39.6, 38.7, 34.2, 33.5, 31.1, 29.6, 28.1, 25.5, 24.8, 22.7, 21.7, 21.2, 20.4, 15.4, 14.0; APCIMS *m/z* 502 (M + 1); analysis for (salt) $C_{28}H_{50}Cl_3N_5O_3$ (611.1), calcd, C, 55.03; H, 7.75; N, 11.45; found, C, 55.27; H, 7.84; N, 11.41.

4.1.12. N^1-[4-(4-Ethyl-5-hexoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (22). Yield: 90%; mp (salt) 114–116 °C (dec.); IR (KBr): 3357, 1650 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 4.3$ Hz), 7.47 (bs, 1H), 7.10 (d, 1H, $J = 4.3$ Hz), 6.44 (s, 1H), 4.18 (m, 1H), 3.95 (s, 3H), 3.87 (t, 2H, $J = 6.9$ Hz), 3.71 (m, 1H), 3.47 (s, 4H), 3.24 (m, 4H), 2.92 (s, 2H), 1.8–1.3 (m, 18H), 1.24 (m, 6H), 0.94 (t, 3H, $J = 7.9$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.0, 170.3, 151.2, 149.7, 144.4, 141.9, 133.9, 123.7, 122.5, 94.5, 74.2, 56.9, 53.1, 48.1, 39.8, 38.9, 34.3, 33.7, 31.2, 29.7, 28.2, 25.6, 24.9, 22.6, 21.8, 21.5, 20.5, 15.5, 14.1; APCIMS *m/z* 516 (M + 1); analysis for (salt) $C_{29}H_{52}Cl_3N_5O_3$ (625.1), calcd, C, 55.72; H, 7.90; N, 11.20; found, C, 55.65; H, 7.93; N, 11.28.

4.1.13. N^1-[4-(4-Ethyl-5-heptoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (23). Yield: 100%; mp (salt) 102–105 °C (dec.); IR (KBr): 3410, 1712 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 3.6$ Hz), 8.11 (bs, 1H), 7.11 (d, 1H, $J = 3.6$ Hz), 6.44 (s, 1H), 3.96 (s, 3H), 3.84 (t, 2H, $J = 6.8$ Hz), 3.73 (m, 1H), 3.48 (m, 1H), 3.27 (m, 4H), 2.89 (m, 2H), 1.88–1.48 (m, 22H), 1.24 (m, 6H), 1.04 (t, 3H, $J = 6.6$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.7, 157.2, 151.1, 149.7, 144.4, 141.7, 132.5, 128.5, 123.7, 122.5, 94.5, 74.0, 60.1, 53.8, 49.2, 47.9, 39.9, 38.9, 34.1, 33.8, 28.6, 25.6, 24.9, 23.3, 22.6, 21.5, 20.3, 17.8, 15.5, 10.6; APCIMS m/z 530 (M + 1); analysis for (salt) $C_{30}H_{54}Cl_3N_5O_3$ (639.2), calcd, C, 56.37; H, 8.04; N, 10.95; found, C, 56.45; H, 8.09; N, 10.89.

4.1.14. N^1-[4-(4-Ethyl-6-methoxy-5-octoxy-8-quinolylamino)pentyl]-(2*S*)-2,6-diaminohexanamide trihydrochloride (24). Yield: 99%; mp (salt) 99–101 °C (dec.); IR (KBr): 3409, 1583 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.36 (d, 1H, $J = 3.5$ Hz), 7.10 (d, 1H, $J = 3.5$ Hz), 6.62 (bs, 1H), 6.43 (s, 1H), 3.94 (s, 3H), 3.86 (t, 2H, $J = 7.3$ Hz), 3.61 (m, 1H), 3.48 (m, 1H), 3.23 (m, 4H), 2.90 (m, 2H), 1.81–1.46 (m, 24H), 1.28 (m, 6H), 0.88 (t, 3H, $J = 6.8$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.4, 170.6, 151.1, 149.6, 144.4, 141.9, 133.9, 132.3, 123.7, 122.5, 94.6, 74.3, 56.9, 53.1, 49.4, 48.1, 39.8, 38.8, 34.3, 33.8, 31.4, 29.3, 28.6, 26.1, 24.9, 22.7, 22.3, 20.5, 17.8, 15.5, 14.1; ESIMS m/z 544 (M + 1); analysis for (salt) $C_{31}H_{56}Cl_3N_5O_3$ (653.2), calcd, C, 57.00; H, 8.17; N, 10.72; found, C, 57.26; H, 8.15; N, 10.85.

4.1.15. N^1-[4-(6-Methoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (25). Yield: 99%; mp (salt) 140–142 °C (dec.); IR (KBr): 3419, 1709 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.50 (d, 1H, $J = 4.1$ Hz), 8.10 (bs, 1H), 7.90 (d, 1H, $J = 8.2$ Hz), 7.26 (m, 1H), 6.31 (s, 1H), 6.25 (s, 1H), 3.85 (s, 3H), 3.57 (m, 1H), 3.45 (m, 4H), 1.93–1.62 (m, 12H), 1.25 (d, 3H, $J = 6.2$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.0, 170.5, 169.2, 159.0, 157.4, 144.5, 137.3, 135.0, 130.5, 121.1, 96.6, 91.1, 55.0, 52.7, 47.4, 42.2, 40.6, 39.2, 38.1, 33.2, 25.5, 21.6, 17.2; ESIMS m/z 374 (M + 1); analysis for (salt) $C_{20}H_{34}Cl_3N_5O_2$ (482.9), calcd, C, 49.78; H, 6.47; N, 14.51; found, C, 49.55; H, 6.40; N, 14.09.

4.1.16. N^1-[4-(4-Ethyl-6-methoxy-5-propoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (26). Yield: 90%; mp (salt) 125–127 °C (dec.); IR (KBr): 3460, 1667 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 4.1$ Hz), 7.61 (bs, 1H), 7.11 (d, 1H, $J = 4.1$ Hz), 6.44 (s, 1H), 3.95 (s, 3H), 3.85 (t, 2H, $J = 6.3$ Hz), 3.62 (m, 1H), 3.23 (m, 6H), 2.96 (bs, 2H), 1.85–1.31 (m, 12H), 1.28 (d, 3H, $J = 5.0$ Hz), 1.15 (t, 3H, $J = 6.4$ Hz); 1.04 (t, 3H, $J = 7.5$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.3, 157.5, 151.2, 149.7, 144.4, 141.9, 134.1, 128.5, 123.7, 122.5, 94.7, 75.8, 56.9, 49.4, 48.1, 33.8, 28.6, 26.1, 25.6, 24.9, 23.3, 22.0, 20.6, 15.5, 10.6; ESIMS m/z 460 (M + 1), 261; analysis for (salt) $C_{25}H_{44}Cl_3N_5O_3$ (569), calcd, C, 52.77; H, 7.26; N, 12.38; found, C, 52.62; H, 7.02; N, 12.21.

4.1.17. N^1-[4-(5-Butoxy-4-ethyl-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (27). Yield: 100%; mp (salt) 121–123 °C (dec.); IR (KBr): 3439, 1720 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 4.4$ Hz), 7.11 (d, 1H, $J = 4.4$ Hz), 6.43 (s, 1H), 6.34 (bs, 1H), 3.94 (s, 3H), 3.88 (t, 2H, $J = 6.3$ Hz), 3.61 (m, 1H), 3.41 (bs, 2H), 3.21 (m, 4H), 2.94 (m, 2H), 1.80–1.30 (m, 14H), 1.28 (m, 6H), 0.88 (t, 3H, $J = 6.8$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 176.8, 151.1, 149.6, 144.4, 141.9, 134.0, 123.7, 122.5, 95.0, 94.5, 74.0, 58.9, 48.0, 34.2, 33.8, 32.1, 29.7, 28.6, 25.6, 24.9, 23.8, 21.2, 20.6, 19.3, 15.5, 14.0; ESIMS m/z 474 (M + 1); analysis for (salt) $C_{26}H_{46}Cl_3N_5O_3$ (583), calcd, C, 53.56; H, 7.43; N, 12.01; found, C, 53.04; H, 7.45; N, 12.25.

4.1.18. N^1-[4-(4-Ethyl-6-methoxy-5-pentoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (28). Yield: 100%; mp (salt) 116–118 °C (dec.); IR (KBr) 3378, 1643 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.36 (d, 1H, $J = 4.2$ Hz), 8.2 (bs, 1H), 7.11 (d, 1H, $J = 4.2$ Hz), 6.44 (s, 1H), 3.95 (s, 3H), 3.88 (t, 2H, $J = 6.9$ Hz), 3.70 (m, 1H), 3.61 (s, 1H), 3.46 (bs, 2H), 3.22 (m, 4H), 2.97 (m, 2H), 1.90–1.41 (m, 16H), 1.29 (m, 6H), 0.94 (t, 3H, $J = 6.9$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.2, 170.4, 151.2, 149.8, 144.5, 141.7, 134.0, 123.7, 122.5, 95.1, 94.7, 74.3, 56.9, 50.6, 48.1, 39.4, 34.3, 33.8, 29.7, 28.6, 28.2, 24.9, 22.6, 21.8, 20.8, 15.5, 14.1; APCIMS m/z 488 (M + 1); analysis for (salt) $C_{27}H_{48}Cl_3N_5O_3$ (597.1), calcd, C, 54.31; H, 7.59; N, 11.72; found, C, 54.20; H, 7.76; N, 11.46.

4.1.19. N^1-[4-(4-Ethyl-5-hexoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (29). Yield: 80%; mp (salt) 109–112 °C (dec.); IR (KBr) 3372, 1645 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 4.3$ Hz), 8.16 (bs, 1H), 7.11 (d, 1H, $J = 4.3$ Hz), 6.44 (s, 1H), 4.18 (m, 1H), 3.96 (s, 3H), 3.87 (t, 2H, $J = 5.4$ Hz), 3.62 (m, 1H), 3.49 (bs, 2H =), 3.24 (m, 4H), 2.96 (s, 2H), 1.79–1.35 (m, 18H), 1.26 (m, 6H), 0.92 (t, 3H, $J = 7.6$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.1, 170.0, 151.1, 149.9, 144.5, 141.2, 133.2, 123.1, 122.2, 94.4, 74.8, 57.2, 53.0, 48.5, 39.4, 38.7, 34.2, 33.5, 31.6, 29.7, 28.6, 25.8, 24.9, 22.6, 21.9, 20.6, 15.6, 14.1; APCIMS m/z 502 (M + 1); analysis for (salt) $C_{28}H_{50}Cl_3N_5O_3$ (611.1), calcd, C, 55.03; H, 7.75; N, 11.45; found, C, 55.14; H, 7.82; N, 11.40.

4.1.20. N^1-[4-(4-Ethyl-5-heptoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (30). Yield: 91%; mp (salt) 98–101 °C (dec.); IR (KBr) 3396, 1623 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.37 (d, 1H, $J = 3.8$ Hz), 8.18 (bs, 1H), 7.12 (d, 1H, $J = 3.8$ Hz), 6.45 (s, 1H), 3.96 (s, 3H), 3.85 (t, 2H, $J = 6.9$ Hz), 3.61 (m, 1H), 3.45 (bs, 4H), 3.24 (m, 4H), 2.97 (m, 2H), 1.90–1.67 (m, 18H), 1.23 (m, 6H), 1.04 (t, 3H, $J = 6.4$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.4, 169.9, 151.1, 149.8, 144.3, 141.9, 133.9, 132.3, 123.7, 122.4, 94.6, 75.8, 56.9, 52.7, 50.4, 49.4, 48.0, 39.8, 38.8, 34.2, 33.6, 28.6, 25.9, 25.5, 24.8, 23.2, 21.8, 20.5, 15.5, 10.5; APCIMS m/z 516 (M + 1); analysis for (salt) $C_{29}H_{52}Cl_3N_5O_3$ (625.1), calcd, C,

55.72; H, 7.90; N, 11.20; found, C, 55.68; H, 7.95; N, 11.21.

4.1.21. N^1-[4-(4-Ethyl-6-methoxy-5-octoxy-8-quinolylamino)pentyl]-(2*S*)-2,5-diaminopentanamide trihydrochloride (31). Yield: 99%; mp (salt) 98–103 °C (dec.); IR (KBr) 3410, 1591 cm^{-1}, ^1H NMR (free base, $CDCl_3$) δ 8.36 (d, 1H, $J = 3.5$ Hz), 7.10 (d, 1H, $J = 3.5$ Hz), 6.46 (bs, 1H), 6.43 (s, 1H), 3.94 (s, 3H), 3.88 (t, 2H, $J = 6.1$ Hz), 3.62 (m, 1H), 3.23 (m, 4H), 2.94 (bs, 2H), 2.17 (bs, 2H), 1.81–1.46 (m, 20H), 1.28 (m, 6H), 0.89 (t, 3H, $J = 6.6$ Hz); ^{13}C NMR (free base, $CDCl_3$) δ 177.3, 170.6, 151.1, 149.7, 144.3, 141.9, 133.9, 132.3, 123.7, 122.5, 95.0, 74.2, 56.8, 52.9, 49.4, 48.1, 39.9, 38.9, 34.3, 33.8, 30.9, 29.5, 28.6, 26.1, 24.9, 22.7, 22.2, 20.5, 15.5, 14.1; ESIMS *m*/*z* 530 (M + 1); analysis for (salt) $C_{30}H_{54}Cl_3N_5O_3$ (639.2), calcd, C, 56.37; H, 8.04; N, 10.95; found, C, 56.42; H, 8.25; N, 10.78.

4.1.22. N^1-[4-(6-Methoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (32). Yield: 99%; mp (salt) 138–141 °C (dec.); IR (KBr): 3411, 1641 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.54 (d, 1H, $J = 4.1$ Hz), 7.93 (d, 1H, $J = 8.3$ Hz), 7.30 (m, 1H), 6.34 (s, 1H), 6.29 (s, 1H), 4.86 (bs, 1H), 3.94 (s, 3H), 3.74 (s, 2H), 3.30 (t, 2H, $J = 6.9$ Hz), 2.05 (bs, 2H), 1.68 (m, 4H), 1.24 (m, 4H), 0.95 (m, 6H); ^{13}C NMR (free base, $CDCl_3$) δ 176.4, 171.3, 159.4, 152.9, 144.8, 144.2, 143.5, 135.2, 134.8, 129.8, 121.8, 96.8, 91.7, 55.1, 47.5, 39.5, 33.8, 26.2, 25.3, 22.2, 21.8, 20.5; APCIMS *m*/*z* 359 (M + 1); analysis for (salt) $C_{20}H_{32}Cl_2N_4O_2$ (431.4), calcd, C, 55.68; H, 7.01; N, 12.98; found, C, 55.88; H, 7.24; N, 12.83.

4.1.23. N^1-[4-(4-Ethyl-6-methoxy-5-propoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (33). Yield: 100%; mp (salt) 135–137 °C (dec.); IR (KBr) 3456, 1583 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.39 (d, 1H, $J = 4.1$ Hz), 7.94 (bs, 1H), 7.12 (d, 1H, $J = 4.1$ Hz), 6.46 (s, 1H), 3.96 (s, 3H), 3.85 (t, 2H, $J = 6.9$ Hz), 3.62 (m, 1H), 3.46–3.23 (m, 4H), 2.01 (bs, 2H), 1.89–1.68 (m, 6H), 1.30 (m, 6H), 1.05 (m, 4H), 0.94 (m, 6H); ^{13}C NMR (free base, $CDCl_3$) δ 177.3, 171.8, 157.7, 151.2, 149.8, 144.4, 141.9, 134.1, 123.7, 122.5, 94.8, 75.8, 56.9, 49.4, 48.2, 38.4, 33.7, 28.6, 26.4, 24.7, 23.3, 21.8, 20.6, 13.5, 10.6; ESIMS *m*/*z* 445 (M + 1) (100%); analysis for (salt) $C_{25}H_{42}Cl_2N_4O_3$ (517.5), calcd, C, 58.02; H, 7.79; N, 10.82; found, C, 58.25; H, 7.81; N, 10.69.

4.1.24. N^1-[4-(5-Butoxy-4-ethyl-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (34). Yield: 95%; mp (salt) 125–127 °C (dec.); IR (KBr) 3434, 1673 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.39 (d, 1H, $J = 4.0$ Hz), 7.34 (bs, 1H), 7.12 (d, 1H, $J = 4.0$ Hz), 6.45 (s, 1H), 3.97 (s, 3H), 3.89 (t, 2H, $J = 6.8$ Hz), 3.66 (m, 1H), 3.49 (bs, 1H), 3.25 (m, 4H), 2.08 (bs, 2H), 1.95–1.36 (m, 8H), 1.30 (m, 6H), 1.01 (m, 4H), 0.85 (m, 6H); ^{13}C NMR (free base, $CDCl_3$) δ 176.2, 173.8, 157.5, 151.1, 149.7, 144.4, 142.0, 134.0, 123.7, 122.5, 94.5, 74.0, 60.1, 56.9, 49.4, 48.0, 39.1, 33.8, 31.9, 29.4, 25.6 20.7, 19.3, 15.5, 14.0; APCIMS *m*/*z* 459 (M + 1) (100%); analysis for (salt) $C_{26}H_{44}Cl_2N_4O_3$ (531.6), calcd, C, 58.74; H, 7.96; N, 10.54; found, C, 58.79; H, 7.99; N, 10.44.

4.1.25. N^1-[4-(4-Ethyl-6-methoxy-5-pentoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (35). Yield: 92%; mp (salt) 118–122 °C (dec.); IR (KBr) 3379, 1643 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.39 (d, 1H, $J = 3.3$ Hz), 7.44 (bs, 1H), 7.12 (d, 1H, $J = 3.3$ Hz), 6.45 (s, 1H), 3.96 (s, 3H), 3.88 (t, 2H, $J = 6.7$ Hz), 3.72 (m, 2H), 3.48 (bs, 1H), 3.24 (m, 4H), 2.06 (bs, 2H), 1.94–1.35 (m, 10H), 1.31 (m, 6H), 0.95 (m, 7H), 0.88 (m, 6H); ^{13}C NMR (free base, $CDCl_3$) δ 176.6, 173.2, 151.2, 149.7, 144.4, 142.0, 134.1, 132.4, 123.7, 122.5, 94.6, 74.3, 60.0, 56.9, 48.1, 39.3, 34.2, 30.8, 29.7, 28.3, 26.3, 24.9, 22.6, 20.7, 19.4, 16.7, 15.5, 14.1; APCIMS *m*/*z* 473 (M + 1) (100%); analysis for (salt) $C_{27}H_{46}Cl_2N_4O_3$ (545.6), calcd, C, 59.43; H, 8.12; N, 10.26; found, C, 59.27; H, 8.37; N, 10.21.

4.1.26. N^1-[4-(4-Ethyl-5-hexoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (36). Yield: 100%; mp (salt) 98–99 °C (dec.); IR (KBr) 3432, 1589 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.40 (d, 1H, $J = 3.6$ Hz), 7.45 (bs, 1H), 7.13 (d, 1H, $J = 3.6$ Hz), 6.45 (s, 1H), 3.96 (s, 3H), 3.90 (t, 2H, $J = 6.9$ Hz), 3.72 (m, 2H), 3.47 (bs, 1H), 3.25 (m, 4H), 2.2 (bs, 2H), 1.95–1.59 (m, 12H), 1.35 (m, 6H), 1.15 (m, 7H), 0.93 (m, 6H); ^{13}C NMR (free base, $CDCl_3$) δ 177.4, 172.3, 151.1, 149.6, 144.3, 141.6, 133.2, 132.0, 125.2, 122.9, 94.8, 74.1, 59.7, 56.2, 48.5, 39.0, 33.1, 30.1, 29.2, 28.3, 25.8, 24.8, 23.2, 22.8, 20.1, 18.1, 16.4, 15.4, 14.2; ESIMS *m*/*z* 487 (M + 1) (100%); analysis for (salt) $C_{28}H_{48}Cl_2N_4O_3$ (559.6), calcd, C, 60.09; H, 8.28; N, 10.01; found, C, 60.22; H, 8.30; N, 10.12.

4.1.27. N^1-[4-(4-Ethyl-5-heptoxy-6-methoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (37). Yield, 99%; mp (salt) 87–89 °C (dec.); IR (KBr) 3435, 1666 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.39 (d, 1H, $J = 4.3$ Hz), 7.38 (bs, 1H), 7.12 (d, 1H, $J = 4.3$ Hz), 6.45 (s, 1H), 3.96 (s, 3H), 3.88 (t, 2H, $J = 7.0$ Hz), 3.65 (m, 1H), 3.48 (bs, 1H), 3.36 (m, 1H), 3.24 (m, 4H), 2.04 (bs, 2H), 1.94–1.47 (m, 14H), 1.30 (m, 6H), 1.16–0.84 (m, 10H); ^{13}C NMR (free base, $CDCl_3$) δ 176.9, 172.9, 151.1, 149.7, 144.4, 141.9, 134.0, 132.4, 123.7, 122.5, 94.5, 75.3, 59.8, 56.9, 49.4, 48.1, 39.3, 34.2, 31.9, 30.0, 29.2, 26.2, 23.7, 21.4, 20.7, 19.3, 16.8, 15.5, 14.1; APCIMS *m*/*z* 501 (M + 1) (100%); analysis for (salt) $C_{29}H_{50}Cl_2N_4O_3$ (573.7), calcd, C, 60.71; H, 8.43; N, 9.76; found, C, 60.65; H, 8.32; N, 9.90.

4.1.28. N^1-[4-(4-Ethyl-6-methoxy-5-octoxy-8-quinolylamino)pentyl]-(2*S*)-2-amino-3-methylbutanamide dihydrochloride (38). Yield, 97%; mp (salt) 76–78 °C (dec.); IR (KBr) 3414, 1589 cm^{-1}; ^1H NMR (free base, $CDCl_3$) δ 8.40 (d, 1H, $J = 4.3$ Hz), 7.13 (d, 1H, $J = 4.3$ Hz), 6.76 (bs, 1H), 6.45 (s, 1H), 3.96 (s, 3H), 3.88 (t, 2H, $J = 6.9$ Hz), 3.65 (m, 2H), 3.49 (bs, 1H), 3.39 (m, 2H), 3.25 (m, 4H), 2.22 (bs, 2H), 1.95–1.48 (m, 16H), 1.29 (m, 6H), 1.19 (m, 4H), 0.88 (m, 6H); ^{13}C NMR (free base, $CDCl_3$) δ 176.8, 172.5, 151.2,

149.6, 144.3, 141.9, 134.2, 132.6, 123.6, 122.5, 94.5, 74.3, 59.9, 56.9, 49.5, 48.1, 39.3, 34.3, 29.5, 26.1, 25.6, 24.9, 22.7, 21.2, 20.7, 19.3, 16.8, 15.6, 14.1; ESIMS *m/z* 515 (M + 1) (100%); analysis for (salt) $C_{30}H_{52}Cl_2N_4O_3$ (587.7), calcd, C, 61.31; H, 8.57; N, 9.53; found, C, 61.25; H, 8.29; N, 9.43.

4.2. Protocol for blood-schizontocidal activity evaluation of analogues against *P. berghei* (sensitive strain) and *P. yoelii nigeriensis* (resistant strain) 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* or *P. yoelii nigeriensis*. Using a standard inoculum of *P. berghei* or *P. yoelii nigeriensis*, 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, 10 mg/kg. The compounds showing curative activity at 10 mg/kg were further selected for screening at lower doses. 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 animal usually die by D + 14.

References and notes

1. Rogers, D. J.; Randolph, S. E. *Science* **2000**, *289*, 1763.
2. *World Health Report 1999*; World Health Organization: Geneva, Switzerland, 1999.
3. Snow, R. W.; Craig, M. H.; Deichman, U.; LeSueur, D. *Parasitol. Today* **1999**, *15*, 99.
4. Foley, M.; Tilley, L. *Int. J. Parasitol.* **1997**, *27*, 231.
5. Warhurst, D. C. *J. Pharm. Pharmcol.* **1997**, *49*, 3.
6. Carson, P. E. In *Antimalarial Drugs II*; Peters, W., Richards, W. H. G., Eds.; Springer: New York, 1984; p 83.
7. Beutler, E.; Dern, R. J.; Alving, A. S. J. *Lab. Clin. Med.* **1954**, *44*, 177.
8. Sweeney, T. R. In *Antimalarial Drugs II*; Peters, W., Richards, W. H. G., Eds.; Springer: Heidelberg, 1984, p 325.
9. Black, R. H.; Canfield, C. J.; Clyde, D. F.; Peters, W.; Wernsdorfer, W. H. In *Chemotherapy of Malaria Monograph Series No. 27*, revised 2nd ed.; Bruce-Chwatt, L. J., Ed.; WHO: Geneva, 1986, p 102.
10. Peters, W. In *Chemotherapy and Drug Resistance in Malaria*, 2nd ed.; Academic: London, 1987, p 581.
11. Pirson, P.; Steiger, R. F.; Trouet, A.; Gillet, J.; Herman, F. *Ann. Trop. Med. Parasitol.* **1980**, *74*, 383.
12. Hofsteenge, J.; Capuano, A.; Altszuler, R.; Moore, S. *J. Med. Chem.* **1986**, *29*, 1765.
13. Trouet, A.; Pirson, P.; Steiger, R. F.; Masquelier, M.; Baurain, R.; Gillet, J. *Bull. World Health Org.* **1981**, *59*, 449.
14. Philip, A.; Kepler, J. A.; Johnson, B. H.; Carroll, F. I. *J. Med. Chem.* **1988**, *31*, 870.
15. Kar, K.; Patnaik, G. K.; Puri, S. K.; Dutta, G. P. *Ind. J. Parasitol.* **1988**, *12*, 259.
16. Baker, J. K.; Clark, A. M.; McChesney, J. D. *J. Pharm. Res.* **1984**, *73*, 502.
17. Carroll, F. I.; Berrang, B.; Linn, C. P.; Twine, C. P., Jr. *J. Med. Chem.* **1979**, *22*, 694.
18. Vangapandu, S.; Sachedva, S.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. *Bioorg. Med. Chem.* **2003**, *11*, 4557.
19. Hawiger, J. *Curr. Opin. Chem. Biol.* **1999**, *3*, 89.
20. Mitchell, D. J.; Kim, D. T.; Steinman, L.; Fathman, C. G.; Rothbard, J. B. *J. Pep. Res.* **2000**, *56*, 318.
21. Garcia-Echeverria, C.; Ruetz, S. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 247.
22. The resolution studies on primaquine (Carroll, F. I.; Berrang, B. Linn, C. P. *J. Med. Chem.* **1978**, *21*, 326) indicate that both enantiomers possess similar antimalarial activities, and drug is used clinically as racemate. Thus, in this study, attempts were not made to separate (*R*, *S*) and (*S*, *S*) diastereoisomers of synthesized 8-quinolinamine conjugates (**11**–**38**).