Indian Journal of Chemistry
Vol. 50B, February 2011, pp. 233-241

Note

Synthesis and antimalarial activity of novel N-{2-[2-(2-aminoethoxy) ethoxy] ethyl}-7-chloroquinolin-4-amine and its derivatives

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Received 23 September 2009; accepted (revised) 10 January 2011

A number of N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-7-chloroquinolin-4-amine derivatives have been prepared by condensation of N-{2-[2-(2-aminoethoxy) ethoxy] ethyl}-7-chloroquinolin-4-amine with substituted aldehyde. The newly synthesized compounds have been characterized by IR, ^{1}H and ^{13}C NMR, and mass spectral data. These compounds have been screened for *in vitro* antimalarial activity using the Sybr Green assay of *P. falciparum* in culture and the heme detoxification based Heme-HRP assay. Among these, compounds 7-chloro-N-[2-(2-{2-[(2, 4-difluorobenzyl) amino] ethoxy} ethoxy) ethyl] quinolin-4-amine is found to be the most effective with IC_{50} value 60 μM (in Heme-HRP assay) and 48 nM (in *P. falciparum* culture assay). These values compare well with the potency of chloroquine in the respective assays.

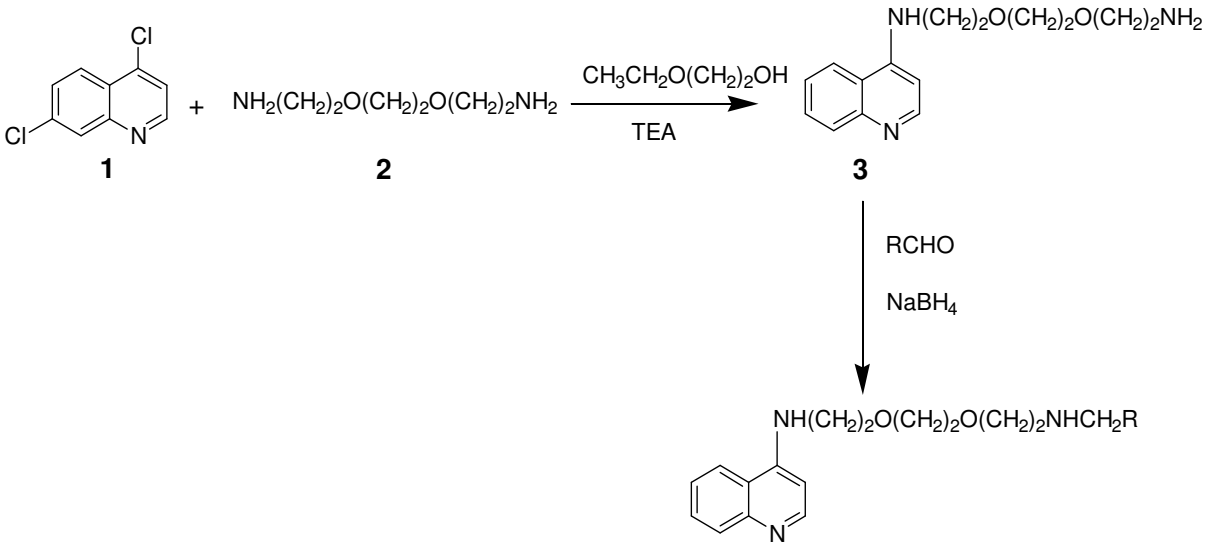
Keywords: Antimalarial, 4-aminoquinoline, heteroalkane-amine-quinolines, alkyl ether substituted quinolines

Malaria is an infectious disease caused by parasitic protozoans of the genus *Plasmodium*. There are four species of *Plasmodium* that infect humans: *falciparum, ovale, malariae*, and *vivax*; of which, *P. falciparum* is the most deadly. Despite over a hundred years of drug development, malaria remains one of the most devastating infectious diseases in the world[1]. Quinoline containing drugs, particularly 4-amino-quinolines, have a long and successful history as antimalarials[2,3]. 4-Aminoquinolines such as chloro-quine have proven to be the most successful class of compounds for the treatment and prophylaxis of malaria[2]. The development of resistance to the potent and affordable drug chloroquine has left many developing countries without sustainable options for antimalarial chemotherapy[4]. It has been reported that bis-4-aminoquinolines with alkyl ether bridge are substantially more effective than the alkylamine bridge containing bisquinolines against *Plasmodium berghei in vivo*[5-8]. Further, there is no relationship between the length of the bisquinoline heteroalkane bridge and antimalarial activity[5]. Keeping this in view it was thought worthwhile to design the synthesis of the title compound and its derivatives wherein the biologically active 7-chloroquinoline moiety of chloroquine is linked to potent alkyl ether side chain.

Results and Discussion

In the present work it was wished to explore the tolerance of various bulky substitutions on the distal amine group of the 4-aminoquinolines having hetero-alkanediamine side chain.

N-{2-[2-(2-aminoethoxy) ethoxy] ethyl}-7-chloro-quinolin-4-amine **3** was synthesized by dissolving 4,7-dichloroquinoline **1** in ethoxyethanol, followed by refluxing with 2,2′-[ethane-1,2-diylbisoxydiethan-amine] **2** in presence of triethylamine (**Figure 1**). The primary amine group of **3** was chosen as a point to introduce the chemical diversity by reductive amina-tion (**Figure 1**), a commonly used reaction due to its reliability, commercial availability of aldehyde building blocks, and process simplicity[9]. Compounds having substituted aldehyde groups (RCHO) were added to form the unstable intermediate imines which were further reduced with sodium borohydride (**Figure 1**). In an attempt to avoid the formation of tertiary amine as side product and also to eliminate the need for purification of the secondary amine, the conditions that gave the best results were found to be (i) formation of the imine with excess aldehyde (R) in anhydrous methanol, followed by (ii) concomitant reduction of the imine using sodium borohydride. By this procedure, primary alcohol, due to reduction of excess aldehyde, was obtained as side product; however this could be easily separated from the desired product by work-up procedure. Additionally, methanol was found to be the optimal solvent of choice, since all reagents were sufficiently soluble in it. Also, while the reaction in methanol gave exclusively the monosubstituted product, the corresponding reaction in dichloroethane gave predominantly the dialkylated product (as observed in



where RCHO is:

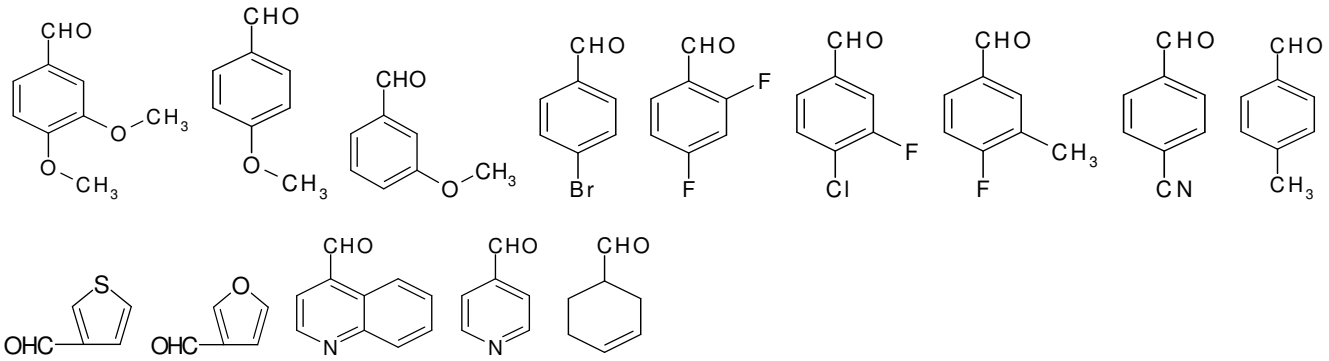


Figure 1 — Synthesis of substituted N-{2-[2-(2-aminoethoxy) ethoxy] ethyl}-7-chloroquinolin-4-amine derivatives

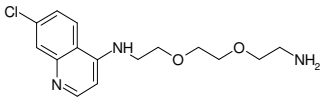
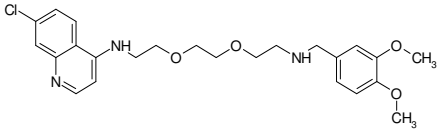
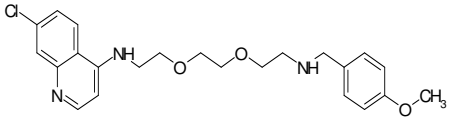
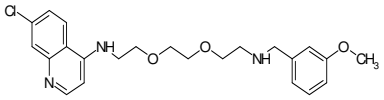
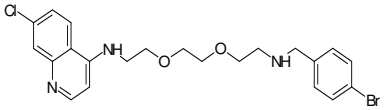
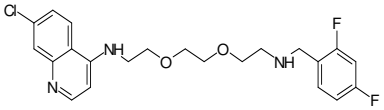
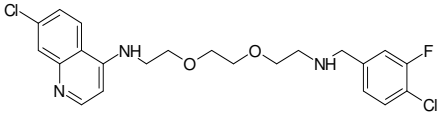
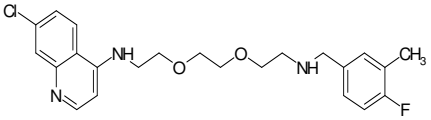
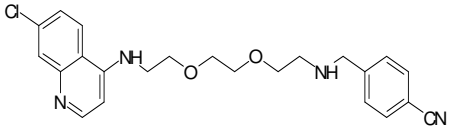
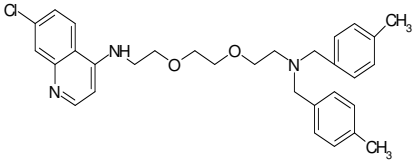
the reductive amination with *p*-tolualdehyde in dichloroethane as solvent, compound **12**). The imine formation step was allowed to go on for 24 hr, since measurements of the kinetics of this reaction show reaction time varying from minutes to several hours[9]. Sodium borohydride was the reducing agent of choice for the reactions of primary amines where dialkylation is a problem[9].

Biological activity

Anti-malarial activity of the above synthesized compounds was carried out *in vitro* by using heme detoxification based Heme-HRP assay and inhibition of *P. falciparum* growth in culture using the SYBR green assay. The results are reported here.

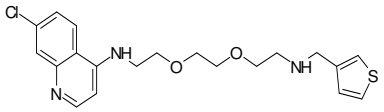
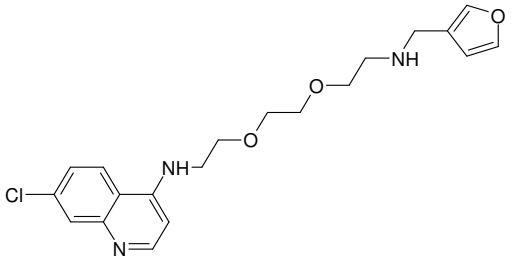
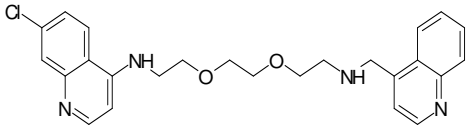
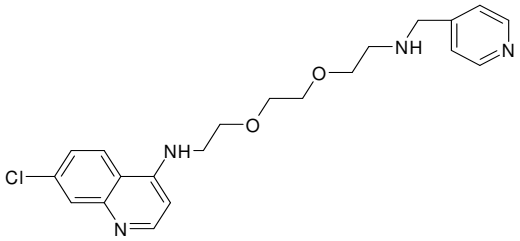
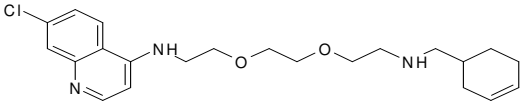
Heme-HRP assay: When the above synthesized compounds were tested by Heme-HRP assay, compound **3** (IC_{50} 48 μM), the base compound of the series was found to act as a disruptor of heme-HRP II interactions (**Table I**). Substitution on the pendant amine group of **3** with 3,4-dimethoxy benzyl group *i.e.* compound **4** reduced the potency reflected in the enhanced IC_{50} of 80 μM whereas compound **5** (4-methoxy benzyl derivative, IC_{50} 50 μM) and **6** (3-methoxybenzyl derivative, IC_{50} 65 μM) were found to be inhibitors of heme-HRP complex. The compounds obtained by substituting 4-bromo **7**, 2,4-difluoro **8**, 3-fluoro-4-chloro **9**, and 3-methyl-4-fluoro **10** on the benzyl group were all active in Heme-HRP assay (**Table I**). The range of IC_{50} values obtained for 7-10 indicates that it is possible to fine tune the potency of these quinolines for their ability to disrupt heme-HRP interactions. Compounds **11** to **16** were found to be inactive (**Table I**), which indicates that

Table I — Antimalarial activity of 4-aminoquinolines having alkyl ether side chain

Compd	Structure	IC_{50} Molecular assay Heme-Hrp (μM)	IC_{50} *P. falciparum* 3D7 (nM)
3		48	>100
	Alaryl series		
4		80	>100
5		50	>100
6		65	66
7		40	>100
8		60	48
9		30	>100
10		40	>100
11		80	88
	Dialaryl series		
12		>80	>100

— *Contd*

Table I — Antimalarial activity of 4-aminoquinolines having alkyl ether side chain — *Contd*

Compd	Structure	IC_{50} Molecular assay Heme-Hrp (μM)	IC_{50} *P. falciparum* 3D7 (nM)
	Alaheteroaryl series		
13		80	104
14		>80	>100
15		>80	>100
16		>80	>100
	Alacycloalkylene series		
17		70	67
Standard	Chloroquine	40	46

the substituents used in these derivatives render them inactive in their ability to disrupt heme-HRP interactions. Compound **17** (having cyclohexene group as substituent) showed moderate activity with an IC_{50} value of 70 μM.

Inhibition of *P. falciparum* growth in culture: Compounds **6** (3-methoxy benzyl), **8** (2,4-difluoro benzyl derivative), **11** (4-cyanobenzyl derivative),**13** (3-thienylmethyl derivative) and **17** (cyclohexene derivative) were found to be active in the culture assay while rest of the compounds **3** to **5, 7, 9, 10, 12, 14** to **16** were found to be inactive (**Table I**).

Materials and Methods

NMR spectrum were recorded on Bruker 400 MHz instrument using tetramethylsilane (TMS) as internal standard and chemical shift (δ values) are expressed in ppm units relative to TMS. Mass spectra were recorded on API-3000 LCMS/MS from P.E. Sciex. IR spectra were recorded on Perkin Elmer Spectrum 100 FT-IR spectrometer. Compounds were routinely checked for their homogeneity on thin layer chromatography (TLC) plates having silica gel G_{60} F_{254} supported on aluminium sheet (Aldrich), and their spots were visible by exposing them to iodine

vapours, by spraying with ninhydrin (2% w/v in absolute ethanol) and under ultraviolet irradiation. All chemicals were purchased from Aldrich Chemicals Ltd. Solvents used for the chemical synthesis were acquired from commercial sources and used as received.

Synthesis of N-{2-[2-(2-aminoethoxy)ethoxy]ethyl}-7-chloroquinolin-4-amine, 3 (Figure 1)

Compound **1** (126.2 mmole) was dissolved in ethoxyethanol (375 mL) in a dry round bottom flask equipped with condenser. Triethylamine (252.46 mmole) was added to it followed by compound **2** (378.6 mmole) under stirring and refluxed at 135°C for 8-10 hr. Reaction was monitored for completion by thin layer chromatography using solvent system 20% methanol in dichloromethane. Reaction mixture was concentrated to thick mass under reduced pressure at 55-60°C. The thick mass thus obtained was dissolved in dichloromethane (375 mL) and organic layer was washed with water (2 × 50 mL). The organic layer was dried over sodium sulphate and solvent was distilled off under reduced pressure at 40°C. The crude product thus obtained was purified by column chromatography eluting with methanol and dichloromethane in different ratios giving a brown semisolid mass. Yield: 64%, Color/state: brown semisolid mass, R_f value = 0.60; ^{1}H NMR ($CDCl_3$): δ 2.22 (bs,NH_2 disappeared on D_2O exchange), 2.86 (t, *J*=8Hz, 2H), 3.47-3.84 [m,10H, Ar-N-CH_2, $(OCH_2)_4$], 5.83 (bs, NH, disappeared on D_2O exchange), 6.36(d, *J* = 8Hz, 1H), 7.33(d, *J* = 4Hz, 1H), 7.80(d, *J* = 8Hz, 1H), 7.92(s,1H), 8.49(d,*J*=4Hz,1H); ^{13}C NMR (Me_3OD): δ 43.71, 45.57, 69.96, 71.57, 99.91, 118.76, 124.31, 126.16, 127.77, 136.45, 148.64, 150.84, 152.52; IR (CH_2Cl_2): 3352.28 (primary and secondary N-H), 1580.77 (C=C) and 1541.81 cm^{-1} (C=N); MS: *m/z* 309.88(M^+), 311.76(M+2), and 312.90. Anal. Calcd for $C_{15}H_{20}ClN_3O_2$: C, 58.16; H, 6.51; N, 13.56. Found: C, 58.48; H, 6.74; N, 13.96%.

Synthesis of compound 4 to 17 (Figure 1): Compound **3** (1 mol equivalent) was dissolved in methanol (20 mL) in a dry round bottom flask equipped with a calcium chloride guard tube. Molecular sieves powder activated in microwave oven was added to it and the reaction mixture was stirred for 30 min. Substituted aldehyde compounds (R) was added to it and stirring was continued for another 24 hr. Sodium borohydride (5 mol equivalent) was added to it and stirring was further continued for 12-24 hr. Reaction was monitored by TLC using 5% methanol in dichloromethane and few drops of ammonia as solvent system and visualizing by spraying with ninhydrin solution and charring the TLC with hot gun. The crude reaction mixture was filtered to remove molecular sieves and any remaining inorganic salts; the solvent was distilled *in vacuo* at 35-40°C. The thick mass obtained was dissolved in dichloromethane (20 mL) and organic layer was washed with water (10 mL) followed by brine solution (10 mL). The solvent was concentrated under reduced pressure to get a thick light brown mass. The crude product was purified by column chromatography using methanol and dichloromethane as eluting solvent to give thick brownish mass which was again purified for a second time by preparatory thin layer chromatography using dichloromethane and methanol as solvent system with trace amount of ammonia. By the above procedure, compounds **4** to **17** were synthesized (**Figure 1**) and characterized as follows.

7-Chloro-N-[2-(2-{2-[(3,4-dimethoxybenzyl) amino] ethoxy} ethoxy) ethyl] quinolin-4-amine, 4

Yield: 35%, Color/state: brown viscous mass; R_f value = 0.79; ^{1}H NMR ($CDCl_3$): δ 2.81 (t, *J*=12Hz, 2H, CH_2-N), 3.42-3.84 (m,18H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N, $(OCH_3)_2$), 5.76 (bs, NH, disappeared on D_2O exchange), 6.37 (d, *J*=4Hz,1H), 6.77 (d, *J*=8Hz, 2H), 6.88 (s,1H), 7.31 (d, *J*=12Hz,1H), 7.76 (d, *J*=12Hz, 1H), 7.89 (s,1H), 8.51 (d, *J*=8Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 42.65, 48.72, 53.79, 55.83, 55.91, 68.60, 70.18, 70.33, 70.67, 99.11, 111.02, 111.47, 117.44, 120.27, 121.41, 125.27, 128.73, 132.10, 134.61, 148.10, 148.98, 150.84, 152.01; IR (CH_2Cl_2): 3322.07 (primary and secondary N-H), 1581.17(C=C) and 1540.81 cm^{-1} (C=N); MS: *m/z* 460.39(M+1), 462.23(M+3), 463.24. Anal. Calcd for $C_{24}H_{30}ClN_3O_4$: C, 62.67; H, 6.57; N, 9.14. Found: C, 62.89; H, 6.91; N, 9.48%.

7-Chloro-N-[2-(2-{2-[(4-methoxybenzyl)amino]-ethoxy} ethoxy)ethyl] quinolin -4-amine, 5

Yield: 30%, Color/state: brown viscous mass; R_f value = 0.72; ^{1}H NMR ($CDCl_3$): δ 2.80(t, *J*=8Hz, 2H, CH_2-N), 3.44 - 3.82(m,15H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N, OCH_3), 5.76 (bs, NH, disappeared on D_2O exchange), 6.36 (d, *J*=8Hz, 1H), 6.80 (dd, J_1=8Hz and J_2=4Hz, 2H), 7.23(dd, J_1=8Hz and J_2=4Hz, 2H), 7.32(d, *J*=4Hz, 1H), 7.75(d, *J*=8Hz, 1H), 7.95(s,1H), 8.51(d, *J*=4Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 42.67,

48.64, 53.37, 55.24, 68.61, 70.20, 70.31, 70.66, 99.10, 113.79, 117.45, 121.44, 125.26, 128.70, 129.32, 132.11, 134.86, 149.19, 149.85, 151.99, 158.67; IR (CH_2Cl_2): 3304.24(primary and secondary N-H), 1581.00(C=C) and 1538.97 cm^{-1} (C=N); MS: *m/z* 430.40 (M+1), 432.24 (M+3), 433.44. Anal. Calcd for $C_{23}H_{28}ClN_3O_3$: C, 64.25; H, 6.56; N, 9.77. Found: C, 64.65; H, 6.91; N, 9.46%.

7-Chloro-N-[2-(2-{2-[(3-methoxybenzyl)amino]-ethoxy}ethoxy)ethyl]quinolin-4-amine, 6

Yield: 22%, Color/state: brown viscous mass; R_f value = 0.70; ^1H NMR ($CDCl_3$): δ 2.81(t, *J*=12Hz, 2H, CH_2-N), 3.43-3.83(m,15H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N, OCH_3), 5.69 (bs,NH,disappeared on D_2O exchange), 6.37(d, *J*=8Hz, 1H), 6.75(d, *J*=4Hz, 1H), 6.85(d, *J*=4Hz, 1H), 6.87(s,1H), 7.18(d, *J*=8Hz, 1H), 7.31 (d, *J*=8Hz, 1H), 7.73 (d, *J*=8Hz, 1H), 7.94(s,1H), 8.52(d, *J*=8Hz, 1H); ^{13}C NMR (MeOD): δ 43.83, 48.36, 54.18, 55.59, 69.91, 70.79, 71.34, 71.50, 99.85, 113.67, 115.02, 118.7, 121.66, 124.25, 127.65, 130.46, 136.37, 141.89, 149.69, 152.43, 152.73, 161.33; IR (CH_2Cl_2): 3309.66(primary and secondary N-H),1582.60(C=C) and 1538.42 cm^{-1} (C=N); MS: *m/z* 430.40(M+1), 432.24(M+3), 433.44. Anal. Calcd for $C_{23}H_{28}ClN_3O_3$: C, 64.25; H, 6.56; N, 9.77. Found: C, 63.98; H, 6.25; N, 9.44%.

N-[2-(2-{2-[(4-Bromobenzyl)amino]ethoxy}etho-xy)ethyl]-7-chloroquinolin-4-amine, 7

Yield: 30%, Color/state: Thick brown viscous mass; R_f value = 0.65; ^1H NMR ($CDCl_3$): δ 2.78 (t, *J*=8Hz, 2H, CH_2-NH), 3.44-3.83(m,12H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N), 5.66(bs, NH, disappeared on D_2O exchange), 6.38(d, *J*=4Hz, 1H), 7.11(dd, J_1=8Hz and J_2=12Hz, 2H), 7.30(dd, J_1=8Hz and J_2=12Hz, 2H), 7.35(d, *J*=8Hz, 1H), 7.72 (d, *J*=8Hz, 1H), 7.95(s,1H), 8.51(d, *J*=8Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 42.85, 48.83, 53.44, 68.84, 70.44, 70.50, 70.83, 99.36, 117.60, 121.49, 122.73, 125.46, 126.84, 130.12, 130.24, 131.22, 135.07, 142.79, 149.35, 149.99, 152.19; IR (CH_2Cl_2): 3299.86 (primary and secondary N-H), 1579.887(C=C), 1539.02 (C=N), 848 cm^{-1} (C-Br); MS: *m/z* 478.24(M^+), 480.20(M+2), 482.10, 483.30. Anal. Calcd for $C_{22}H_{25}BrClN_3O_2$: C, 55.19; H, 5.26; N, 8.78. Found: C, 55.39; H, 4.95; N, 8.48%.

7-Chloro-N-[2-(2-{2-[(2,4-difluorobenzyl)amino]-ethoxy}ethoxy)ethyl] quinolin-4-amine, 8

Yield: 38%, Color/state: light-brown viscous mass; R_f value = 0.77; ^1H NMR ($CDCl_3$): δ 2.79(t, *J*=8Hz, 2H, CH_2-N), 3.44-3.84(m,12H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N), 5.66 (bs, NH, disappeared on D_2O exchange), 6.38(d, *J*=8Hz, 1H), 6.72(m,2H), 7.22(d, *J*=8Hz,1H), 7.34(d, *J*=12Hz, 1H), 7.72(d, *J*=12Hz, 1H), 7.95(s,1H), 8.52(d, *J*=8Hz, 1H); ^{13}C NMR (Me_3OD): δ 43.85, 46.81, 48.37, 69.92, 70.88, 71.37, 71.52, 99.85, 104.44, 111.19, 115.34, 121.36, 124.28, 126.08, 127.62, 130.60, 136.41, 149.65, 152.39, 152.78, 159.57, 162.39; IR (CH_2Cl_2): 3297.85 (primary and secondary N-H), 1581.03(C=C), 1538.81 (C=N), 768 cm^{-1} (C-F); MS: *m/z* 436.48 (M+1), 438.19(M+3), 439.39. Anal. Calcd for $C_{22}H_{24}ClF_2N_3O_2$: C, 60.62; H, 5.55; N, 9.64. Found: C, 60.36; H, 5.89; N, 9.42%.

7-Chloro-N-[2-(2-{2-[(4-chloro-3-fluorobenzyl)-amino]ethoxy}ethoxy)ethyl] quinolin-4-amine, 9

Yield: 28%, Color/state: brown viscous mass; R_f value = 0.74; ^1H NMR ($CDCl_3$): δ 2.76(t, *J*=12Hz, 2H, CH_2-N), 3.45-3.84(m,12H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N), 5.60 (bs, NH, disappeared on D_2O exchange), 6.39(d, *J*=4Hz, 1H), 6.94(s,1H), 7.10(d, *J*=8Hz, 1H), 7.29(d, *J*=12Hz, 1H), 7.33(d, *J*=8Hz, 1H), 7.71(d, *J*=12Hz, 1H), 7.95(s,1H), 8.53(d, *J*=4Hz, 1H); ^{13}C NMR (Me_3OD): δ 43.86, 50.55, 53.07, 69.93, 70.98, 71.35, 71.51, 99.85, 115.34, 117.29, 118.76, 124.27, 126.08, 127.60, 131.51, 140.65, 146.93, 149.61, 152.36, 152.78; IR (CH_2Cl_2): 3310.11 (primary and secondary N-H), 1581.03(C=C) and 1538.76 (C=N), 880 (C-Cl), 811 cm^{-1} (C-F); MS: *m/z* 452.30(M^+), 454.19(M+2), 456.16, 457.29. Anal. Calcd for $C_{22}H_{24}Cl_2FN_3O_2$: C, 58.41; H, 5.35; N, 9.29. Found: C, 58.63; H, 5.10; N, 9.11%.

7-Chloro-N-[2-(2-{2-[(4-fluoro-3-methylbenzyl)-amino]ethoxy}ethoxy)ethyl] quinolin- 4–amine, 10

Yield: 34%, Color/state: Thick brown viscous mass; R_f value = 0.65; ^1H NMR ($CDCl_3$): δ 2.2(s,3H), 2.79 (t, *J*=8Hz, 2H, CH_2-N), 3.43-3.83 (m,12H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N), 5.66 (bs, NH, disappeared on D_2O exchange), 6.38(d, *J*=4Hz, 1H), 6.87(d, *J*=8Hz, 2H), 7.07(d, *J*=8Hz, 1H), 7.33 (d, *J*=4Hz, 1H), 7.73(d, *J*=8Hz, 1H), 7.95(s,1H), 8.52 (d, *J*=8Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 15.41, 42.64, 48.69, 53.26, 68.61, 70.20, 70.31, 70.63, 99.12, 114.66, 117.41, 121.41, 124.59, 125.28, 126.94, 127.95, 128.68, 134.91, 135.33, 149.12, 149.85, 151.97, 161.66; IR (CH_2Cl_2): 3295.46 (primary and secondary N-H), 1580.98(C=C) and 1538.50(C=N), 813 cm^{-1} (C-F); MS: *m/z* 432.49 (M+1), 434.26,

435.47. Anal. Calcd for $C_{23}H_{27}ClFN_3O_2$: C, 63.96; H, 6.30; N, 9.73. Found: C, 63.67; H, 6.47; N, 9.52%.

4-({[2-(2-{2-[(7-chloroquinolin-4-yl)amino]ethoxy}-ethoxy) ethyl] amino} methyl) benzonitrile, 11

Yield: 29%, Color/state: light-brown viscous mass; R_f value = 0.75; ^{1}H NMR($CDCl_3$): δ 2.79 (t, *J*=12Hz, 2H, CH_2-N), 3.44-3.84(m,12H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N), 5.60 (bs,NH,disappeared on D_2O exchange), 6.38(d, *J*=8Hz, 1H), 7.31 (dd, J_1=12Hz and J_2=16Hz, 2H), 7.37(dd, J_1=12Hz and J_2=8Hz, 2H), 7.55(d, *J*=8Hz,1H), 7.72 (d, *J*=8Hz, 1H), 7.95(s,1H), 8.52 (d, *J*=8Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 42.63, 48.77, 53.37, 68.65, 70.20, 70.66, 99.23, 110.66, 116.75, 117.38, 121.18, 125.29, 128.54, 128.85, 132.17, 134.89, 145.79, 149.19, 152.06; IR (CH_2Cl_2): 3341.49 (primary and secondary N-H), 2228.33(HC≡N), 1581.36 (C=C) and 1539.73 cm^{-1} (C=N); MS: *m/z* 423.32, 425.41(M+1), 427.24. Anal. Calcd for $C_{23}H_{25}ClN_4O_2$: C, 65.01; H, 5.93; N, 13.19. Found: C, 64.86; H, 6.14; N, 12.93%.

N-[2-(2-{2-[bis(4-methylbenzyl)amino]ethoxy}-ethoxy)ethyl]-7-chloroquinolin -4-amine, 12

Yield: 19%, Color/state: brown viscous mass; R_f value = 0.88; ^{1}H NMR ($CDCl_3$): δ 2.30(s,6H), 2.65 (t, *J*=12Hz, 2H, CH_2-N), 3.42-3.62 (m,10H), 3.79 (d, *J*=4Hz, 4H), 5.54(bs,NH, disappeared on D_2O exchange), 6.36 (d, *J*=4Hz, 1H), 7.07 (dd, *J*=4 and 8Hz, 4H), 7.21 (m,5H), 7.64 (d, *J*=8Hz, 1H), 7.95(s,1H), 8.52 (d, *J*=4Hz, 1H); MS: *m/z* 518.54(M^+), 520.43(M+2), 521.57, 522.58. Anal. Calcd for $C_{31}H_{36}ClN_3O_2$: C, 71.87; H, 7.00; N, 8.11. Found: C, 71.67; H, 6.89; N, 7.97%.

7-Chloro-N-[2-(2-{2-[(3-thienylmethyl)amino]-ethoxy}ethoxy)ethyl]quinolin-4-amine, 13

Yield: 34%, Color/state: brown viscous mass; R_f value = 0.69; ^{1}H NMR ($CDCl_3$): δ 2.82(t, *J*=8Hz, 2H, CH_2-N), 3.44-3.83 (m,12H, $(OCH_2)_4$, Ar-N-CH_2, -CH_2-N), 5.74 (bs,NH,disappeared on D_2O exchange), 6.37(d, *J*=8Hz, 1H), 7.00 (d, *J*=8Hz, 1H), 7.11(s,1H), 7.23 (d, *J*=8Hz, 1H), 7.32(d, *J*=8Hz, 1H), 7.75(d, *J*=12Hz, 1H), 7.95(s,1H), 8.52 (d, *J*=8Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 42.67, 48.73, 48.82, 68.62, 70.20, 70.33, 70.59, 99.12, 117.43, 121.40, 121.69, 125.29, 125.79, 127.55, 128.70, 134.90, 141.01, 149.14, 149.86, 151.95, IR (CH_2Cl_2): 3337.42 (primary and secondary N-H), 1580.97(C=C) and 1540.58 cm^{-1} (C=N); MS: *m/z* 406.36(M+1), 408.20, 410.40. Anal. Calcd for $C_{20}H_{24}ClN_3O_2S$: C, 59.17; H, 5.96; N, 10.35. Found: C, 59.33; H, 5.82; N, 10.55%.

7-Chloro-N-[2-(2-{2-[(3-furylmethyl)amino]-ethoxy}ethoxy)ethyl]quinolin-4-amine, 14

Yield: 36%, Color/state: brown viscous mass; R_f value = 0.72; ^{1}H NMR ($CDCl_3$): δ 2.81(t, *J*=8Hz, 2H, CH_2-N), 3.45-3.83(m,12H, $(OCH_2)_4$, Ar-N-CH_2, -CH_2-N), 5.71 (bs, NH, disappeared on D_2O exchange), 6.35(s,1H), 6.38(d, *J*=8Hz, 1H), 7.33(d, *J*=8Hz, 3H), 7.75(d, *J*=8Hz, 1H), 7.95 (s,1H), 8.53 (d, *J*=4Hz, 1H); IR (CH_2Cl_2): 3337.42 (primary and secondary N-H), 1580.97(C=C) and 1540.58 cm^{-1} (C=N); MS: *m/z* 405.36, 407.20, 406.13. Anal. Calcd for $C_{20}H_{24}ClN_3O_3$: C, 61.61; H, 6.20; N, 10.78. Found: C, 61.97; H, 6.48; N, 10.46%.

7-Chloro-N-[2-(2-{2-[(quinolin-4-ylmethyl)amino]-ethoxy}ethoxy)ethyl] quinolin-4-amine, 15

Yield: 34%, Color/state: Thick brown viscous mass; R_f value = 0.77; ^{1}H NMR ($CDCl_3$): δ 2.91(t, *J*=12Hz, 2H, CH_2-NH), 3.42-3.81(m,10H, $(OCH_2)_4$, Ar-N-CH_2), 3.95 (s,2H), 5.30 (bs, NH, disappeared on D_2O exchange), 6.34(d, *J*=8Hz, 1H), 7.31(d, *J*=4Hz, 1H), 7.51 (d, *J*=28Hz, 2H), 7.62 (d, *J*=24Hz, 2H), 7.90(s,1H), 8.0 (d, *J*=32Hz, 2H), 8.58 (d, *J*=24Hz, 1H), 8.81(d, *J*=30Hz, 1H); IR (CH_2Cl_2): 3302.09 (primary and secondary N-H),1580.67(C=C) and 1541.57 cm^{-1} (C=N); MS: *m/z* 449.32(M^+), 451.28, 453.31. Anal. Calcd for $C_{25}H_{27}ClN_4O_2$: C, 66.58; H, 6.03; N, 12.42. Found: C, 66.78; H, 6.32; N, 12.56%.

7-Chloro-N-[2-(2-{2-[(pyridin-4-ylmethyl) amino] ethoxy} ethoxy) ethyl] quinolin-4-amine, 16

Yield: 37%, Color/state: Thick brown viscous mass; R_f value = 0.68; ^{1}H NMR ($CDCl_3$): δ 2.78(t, *J*=8Hz, 2H, CH_2-NH), 3.44-3.84(m,12H, $(OCH_2)_4$, Ar-N-CH_2, Ar-CH_2-N), 5.61(bs, NH, disappeared on D_2O exchange), 6.38 (d, *J*=8Hz, 1H), 7.20 (d, *J*=12Hz, 2H), 7.35(d, *J*=12Hz, 1H), 7.73 (d, *J*=8Hz, 1H), 7.95 (d, *J*=8Hz, 1H), 8.50 (m,3H); ^{13}C NMR ($CDCl_3$): δ 42.67, 48.78, 52.63, 68.79, 70.38,70.42, 70.69, 99.29, 117.51, 121.53, 123.08, 125.44, 128.86, 134.61, 147.89, 148.64, 149.28, 149.93, 152.16; IR (CH_2Cl_2): 3313.59 (primary and secondary N-H), 1581.85(C=C) and 1541.54 cm^{-1} (C=N); MS: *m/z* 401.43(M+1), 403.26. Anal. Calcd for $C_{21}H_{25}ClN_4O_2$: C, 62.91; H, 6.29; N, 13.98. Found: C, 62.73; H, 6.58; N, 13.67%.

7-Chloro-N-[2-(2-{2-[(cyclohex-3-en-1-ylmethyl)-amino]ethoxy}ethoxy)ethyl] quinolin -4-amine, 17

Yield: 38%, Color/state: brown viscous mass; R_f value = 0.63; ^{1}H NMR: ($CDCl_3$) δ 1.22(q, *J*=12Hz, 2H), 1.65(m,1H), 2.00(m,4H), 2.50(d, *J*= 4Hz, 2H), 2.78(t, *J*=8Hz, 2H), 3.47(m,10H, $(OCH_2)_4$, Ar-N-CH_2), 5.60(q,2H), 5.83 (bs, NH, disappeared on D_2O exchange), 6.38(d, *J*=8Hz, 1H), 7.34(d, *J*=8Hz, 1H), 7.79(d, *J*=8Hz, 1H), 7.95 (s,1H), 8.50(d, *J*=12Hz, 1H); ^{13}C NMR ($CDCl_3$): δ 24.83, 26.95,30.00, 33.86, 42.70, 49.52, 55.99, 68.65, 70.20, 70.35, 70.70, 99.11, 117.47, 121.49, 125.28, 126.01, 127.12, 128.72, 134.88, 149.21, 149.88, 152.02; IR (CH_2Cl_2): 3292.73 (primary and secondary N-H), 3020.55(=C-H) 1580.78(C=C) and 1540.78 cm^{-1} (C=N); MS: *m/z* 404.47(M+1), 406.30, 407.50. Anal. Calcd for $C_{22}H_{30}ClN_3O_2$: C, 65.41; H, 7.49; N, 10.40. Found: C, 65.79; H, 7.10; N, 10.66%.

Antimalarial screening: Antimalarial activity was screened using following two methods.

Heme-HRP assay: Hematin polymerization experiments were performed as described by Sahal *et al*.[10] using microtiter plate based screening of antimalarial drug potential by heme-HRP II based colorimetric system. In this method different concentrations of each drug in total volume of 50 µL of 100 mM HEPES buffer *p*H 7 (buffer A) were first transferred to the respective wells. 80 µg HRP II in 100 µL of 20 mM acetate buffer, *p*H 5.5 was mixed with 400 µL of 1 mM heme in buffer A. The mixture was incubated (37°C, 1 hr) before diluting to 10 mL in buffer A. Aliquots (5.2 µg heme + 1.6 µ g HRP II/200 µL) were transferred to the respective wells using multipipette. The plate, covered with a lid, was incubated on shaker (120 rpm, 37°C, and 1 hr). Absorbance was read at 415 nm on a microtiter plate reader. Unlike the greenish colour of heme, the colour of heme-HRPII solution is red. Drugs like chloroquine, which interfere with heme binding, cause a reversal in colour from red to green. A plot of the data from the plate is obtained after subtracting the corresponding heme + drug absorbances from the respective heme + HRP II + drug.

Inhibition of *P. falciparum* growth in culture

Chloroquine sensitive *P. falciparum* strain 3D7 was used in culture. Culture was diluted with uninfected erythrocytes and complete medium (RPMI – 1640 with 0.5% Albumax II) to achieve 1% parasitemia and 2% hematocrit. In 96-well micro-plates, chloroquine (positive control) or compounds diluted in complete medium from 10 mM stock in DMSO were added to the cell mixture to yield triplicate wells with drug concentrations ranging from 0 to 10^{-4}M in a final well volume of 100 µL. After 72 hr of incubation under standard culture conditions, plates were harvested and read by the SYBR Green I fluorescence-based method[11], using a 96-well fluorescence plate reader (Victor, Perkin Elmer), with excitation and emission wavelengths at 497 and 520 nm, respectively. The fluorescence readings were plotted against [drug], and IC_{50} values obtained by (Workout software, Victor, Perkin Elmer) visual matching of the drug concentration giving 50% inhibition of growth.

Conclusion

The scope of the present work towards discovery of an antimalarial agent with better profile than the gold standard chloroquine led to the discovery of few new series of compounds *i.e.* **6, 8, 11, 13** and **17** which were found to be active against *P. falciparum* in culture. Out of these, compound **8** *viz.* 7-chloro-N-[2-(2-{2-[(2,4-difluorobenzyl)amino]ethoxy}ethoxy) ethyl]quinolin-4-amine showed potent activity in both Heme-HRP assay as well as *P. falciparum* culture assay. Compounds **7**, **9** and **10** while potent in Heme-HRP assay failed to antagonize the growth of the parasite in culture. This may be so because the altered structures in these molecules may render them incapable to cross the membrane permeability barriers which protect the red cell resident malaria parasite from exogenously added drugs. In conclusion these compounds can be considered as promising lead molecules for further structural optimization of this class of pharmacophores.

Acknowledgment

The authors are thankful to Ranbaxy Laboratories Limited for providing laboratory facility and analytical support.

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