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SYNTHESIS AND ANTIMALARIAL ACTIVITY OF SOME NEWER -ALKOXY SUBSTITUTED-2-*TERT*-BUTYL-8-QUINOLINAMINES

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Six new 8-quinolinamine analogues **8(a-f)** related to previously reported potent blood-schizontocidal antimalarial agent, 2-tertbutylprimaquine were synthesized and evaluated for *in vivo* antimalarial activities against drug-sensitive *Plasmodium berghei* strain. Acute toxicity studies found that synthesized compounds were less toxic than the parent compound primaquine, while preserving the desired antimalarial activity. Three of the analogues (**8b, 8c** and **8f**) have exhibited curative antimalarial activity at a dose of 25 mg/kg/day×4 and produced suppressive activity at a lower dose of 10 mg/kg/day×4.

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1. Introduction

Malaria is one of the most widespread diseases in the world. According to WHO estimates 40% of the world's population presently lives under malarial threat. Around 300 and 500 million cases of malaria occur annually, leading to 1-3 million deaths. Mortality is particularly high for children under the age of five years, accounting for about 25% of childhood deaths in Africa [1]. Malaria is caused by different species of Plasmodium, of which *P. falciparum* is the most vicious one. Rapid development of resistance by *P. falciparum* to the available conventional antimalarial drugs such as chloroquine (CQ) but also to alternative drugs, such as mefloquine, sulfadoxine-pyrimethamine and even qunine [2-3] necessitates search for new antimalarial drugs and a careful re-examination of the existing drugs. The earlier studies demonstrated that CQ-resistant parasites are not cross-resistant to Primaquine [4-5].

Primaquine (PQ) **1** belonging to 8-quinolinamies class of antimalarials, represents a typical tissue-schizontocide agent, but is ineffective as blood-schizontocidal and can't be used to cure infection caused by *P. falciparum* and PQ is also suffered because of serious side effects including hemolytic anemia (caused by methemoglobin production), pronounced in the patients deficient in 6-GP dehydrogenase [6-7]. PQ is known to be active against more of the life cycle stages of plasmodia than any other class of antimalarial drugs, but has minimal suppressive activity; that is, ineffective as blood-schizontocide.

Despite these drawbacks, in addition to excellent radical curative activity, PQ has broad range of antimalarial activity including efficacy as caused prophylactic, gametocide and sporontocide. These interesting chemical and pharmacological attributes, PQ, is considered an excellent lead prototype for the development of congeners with potent blood-schizontocidal activities. Thus, there is a need to enhance blood-schizonticidal activity of PQ and at the same time reducing the toxicity that is associated with it. 2-*tert*-butyl PQ **2a** was synthesized to eliminate a putative oxidation metabolic pathway of PQ by placement of bulky metabolically stable *tert*-butyl group at the C-2 position of quinoline ring of PQ [8]. 4-ethyl-5-penthoxy PQ **2b** was synthesized to optimize substitution at the C-4 and C-5 positions of PQ (Figure 1), known sites of transport to

inactive/toxic metabolites [9]. Keeping these observations in mind, we report herein, synthesis and antimalarial activity of six newer 2-*tert*-butyl-5-alkoxy-8-quinolinamines.

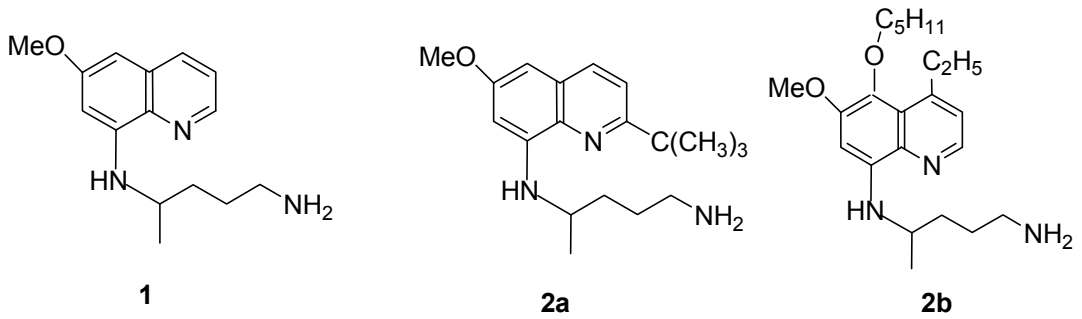


*Fig. 1 Structures of (1) PQ, (**2a**) 2-tert-butyl PQ and (**2b**) 4-ethyl-5-penthoxy PQ.*

2. Experimental

2.1. Materials

Melting points were determined in one end open capillary tubes on a Buchi 530 melting point apparatus and are uncorrected. ^{1}H NMR spectra were recorded for the compounds on Advance bruker (300 MHz) instrument. Chemical shifts are reported in parts per million (ppm) using tetramethylsilane (TMS) as an internal standard. Mass spectra were recorded on HRMS (Finnigan Mat LCQ spectrometer) (APCI/ESI). Elemental analysis was undertaken with Elemental vario EL III Carlo Erba 1108 analyzer. 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 F254, 0.2 mm thickness) sheets. All chemicals were purchased from Aldrich Chemical Ltd (Milwaukee, WI, USA) and Spectrochem Pvt. Ltd (Mumbai, India). All solvents used were of spectral grade or distilled prior to use.

2.2. Methods

The synthesis of the target compounds was accomplished as shown in Scheme 1.

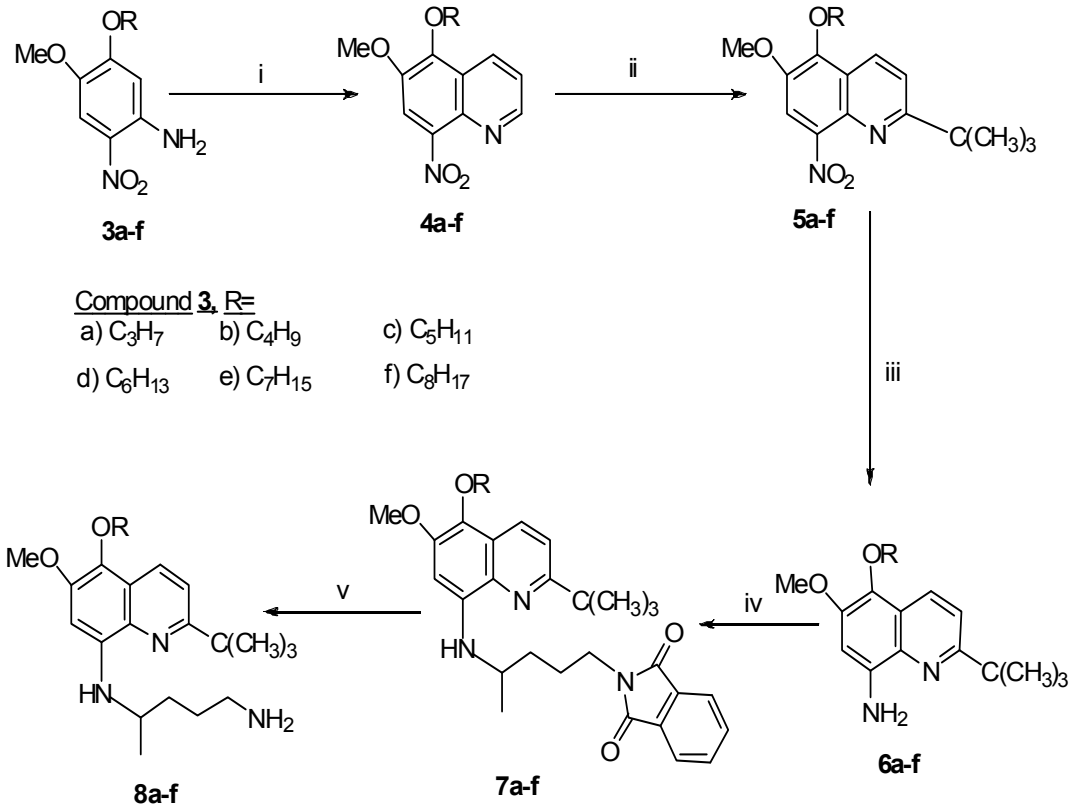
5-Alkoxy-6-methoxy-8-nitroquinolines [9-10] **4a-f** were synthesized by reacting corresponding 4-alkoxy-5-methoxy-2-nitroaniline **3a-f** with glycerol via Skraup synthesis in the presence on arsenic (V) oxide at 90 ^{o}C and then upon direct ring-alkylation via a silver catalyzed radical oxidative decarboxylation of trimethylacetic acid by ammonium persulfate in CH_3CN and 10% H_2SO_4 at 70 ^{o}C efficiently produced 2-*tert*-butyl-5-alkoxy-6-methoxy-8-nitroquinolines **5a-f.** The latter compounds **5a-f** were converted to the requisite 2,5-disubstituted N^8-(4-amino-1-methylbutyl)-6-methoxy-8-quinolinamines **8a-8f** in three steps following previously published procedure [11] (Scheme 1).

*2.2. 1 Synthesis of 5-propoxy-6-methoxy-8-nitroquinolines (**4a**)*

A homogeneous mixture of 3-propoxy-4-methoxy-6-nitroaniline [12] (**3**, 0.037 mol), glycerol (0.020 mol) and concentrated H_2SO_4 (15 mL) was placed in a three necked flask fitted with a thermometer and a dropping funnel. The reaction mixture was heated at 90°C (internal) with mechanical stirring for 10 min. An additional quantity of glycerol (0.020 mol) was added and stirring continued for another 10 min at 90°C. Arsenic (V) oxide (0.029 mol) was then added at once to the reaction mixture and stirring was continued for 2.5 h at 90°C. The dark colored reaction mixture was cooled, diluted with water (100 mL) and filtered. The filtrate was basified

with 25% NH_4OH solution, extracted with dichloromethane (3×100 mL). Combined organic extracts were washed with brine solution (2×20 mL) and water (2×10 mL), and dried over sodium sulfate. The solvent was removed under vacuum to afford brown colored crude product. Purified by flash column chromatography using EtOAc/hexanes (20:80) to provide 5-propoxy-6-methoxy-8-nitroquinolines **4a** as low melting solid. yield 72%; m.p. 55-57 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 8.70 (d, 1H, 2Ar-H J=8.5 Hz), 7.85 (s, 1H, 7Ar-H), 7.28 (d, 1H, 3Ar-H J=8.5 Hz), 6.98 (s, 1H, 4Ar-H), 4.15 (t, 2H, OCH_2), 4.02 (s, 3H, OCH_3), 3.34 (m, 2H, CH_2), 1.12 (t, 3H, CH_3 J=7.4 Hz); APCIMS m/z 263 (M+1); Anal. Calcd. For $C_{13}H_{14}N_2O_4$. C(59.53), H(5.39), N(10.79). Found C(59.45), H(5.31), N(10.67).

The other 5-alkoxy-6-methoxy-8-nitroquinolines **4(b-f)** were prepared by the similar procedure.



Scheme 1. (i) glycerol, H_2SO_4, As_2O_5, 90ºC, 21 h; (ii) $(CH_3)_3CCO_2H$, $AgNO_3$, $(NH_4)_2S_2O_8$, 10% H_2SO_4, CH_3CN, 70 ºC, 15 min; (iii) Raney Ni, H_2, EtOH, 45 psi, 45 min; (iv) 2-(4-bromopentyl)-1,3-isoindolinedione, Et_3N, DCC, stirring, 4 h; (v) $NH_2NH_2.H_2O$, EtOH, reflux, 8 h, ethereal HCl, 50°C, 10 min.

*2.2. 2 Synthesis of 5-propoxy-2-tert-butyl-6-methoxy-8-nitroquinolines (**5a**)*

5-Propoxy-6-methoxy-8-nitroquinoline (**4a**, 1 mmol) in CH_3CN (5 mL) was dissolved 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 drop wise to the pre-heated (70°C) during 10 min. The heating source was then removed and reaction proceeded with the evolution of carbon dioxide. After 15 min, reaction mixture was poured onto ice, and made alkaline by adding 30% aqueous NH_4OH solution. It was extracted with ethylacetate (4×50 mL), and combined extracts were washed with NaCl solution (2×10 mL). Dried over Na_2SO_4 and

solvent removed in vacuo to afford oil, which upon column chromatography over silica gel (230–400 mesh) afforded 5-propoxy-2-*tert*-butyl-6-methoxy-8-nitroquinolines **5a**. Yield 69%; m.p. 95-97 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.96 (d, 1H, 7Ar-H, J=8.6), 7.78 (s, 1H, 3Ar-H J=8.6 Hz), 6.96 (s, 1H, 4Ar-H), 4.14 (t, 2H, OCH_2), 3.82 (s, 3H, OCH_3), 1.89 (m, 2H, CH_2), 1.42 (s, 9H, 3×CH_3), 0.92 (t, 3H, CH_3 J=7.9 Hz); APCIMS m/z 319 (M+1); Anal. Calcd. For $C_{17}H_{22}N_2O_4$. C(64.12), H(6.98), N(8.80). Found C(64.25), H(6.94), N(8.73).

The other 5-alkoxy-2-*tert*-butyl-6-methoxy-8-nitroquinolines **5(b-f)** were prepared by the similar procedure.

*2.2. 3 Synthesis of 5-propoxy-2-tert-butyl-6-methoxy-8-quinolinamines (**6a**)*

A solution of 5-propoxy-2-*tert*-butyl-6-methoxy-8-nitroquinoline (**5a**, 5 mmol) in 95% ethyl alcohol (15 mL) was hydrogenated over wet raney nickel (T1 grade) at 45 psi in a parr hydrogenator for 45 min. Catalyst was removed by filtration, and filtrate was evaporated under vacuum to afford 5-propoxy-2-*tert*-butyl-6-methoxy-8-quinolinamine **6a** as dark colored oil. Yield 82%; oil; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.95 (d, 1H, 7Ar-H, J=8.9 Hz), 7.51 (d, 1H, 3Ar-H, J=8.9 Hz), 6.88 (s, 1H, 4Ar-H), 5.01 (bs, 2H, NH_2), 3.93 (s, 3H, OCH_3), 3.85 (t, 2H, OCH_2), 1.85 (m, 2H, CH_2), 1.51 (s, 9H, 3×CH_3), 0.95 (t, 3H, CH_3 J=6.4 Hz); APCIMS m/z 289 (M+1); Anal. Calcd. For $C_{17}H_{24}N_2O_2$. C(70.79), H(8.40), N(9.71). Found C(70.71), H(8.47), N(9.63).

The other 5-alkoxy-2-*tert*-butyl-6-methoxy-8-quinolinamines **6(b-f)** were prepared by the similar procedure.

*2.2. 4 Synthesis of 2-[4-(5-alkoxy-2-tert-butyl-6-methoxy-8-quinolylamino)-pentyl]-1,3-isoindolinediones (**7a**)*

To an ice cooled stirred solution of 5-propoxy-2-*tert*-butyl-6-methoxy-8-quinolinamine (**6a**, 4 mmol), 2-(4-bromopentyl)-1,3-isoindolinedione (4 mmol) and triethylamine (4 mmol) in dichloromethane (15 mL), 1,3-dicyclohexylcarbodiimide (DCC) (4 mmol) was added [13]. 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 (100 mL) was added to the residue and the additional quantity of separated DCU was again removed by filtration. The solvent was removed under reduced pressure to afford 2-[4-(5-propoxy-2-*tert*-butyl-6-methoxy-8-quinolylamino)-pentyl]-1,3-isoindolinediones **7a** as viscous oil. Yield 72%; oil; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.94 (d, 1H, 7Ar-H, J=8.4 Hz), 7.82 (m, 4H, Ar-H), 7.44 (d, 1H, 3Ar-H J=8.4 Hz), 6.76 (s, 1H, 4Ar-H), 6.04 (bs, 1H, NH), 3.95 (s, 3H, OCH_3), 3.87 (t, 2H, OCH_2), 3.70 (m, 3H, N-CH, and N-CH_2), 1.76 (m, 6H, 3×CH_2), 1.44 (s, 9H, 3×CH_3), 1.32 (t, 3H, CH_3 J=6.9 Hz), 1.02 (t, 3H, CH_3 J=6.9); APCIMS m/z 504 (M+1); Anal. Calcd. For $C_{30}H_{37}N_3O_4$. C(71.53), H(7.42), N(8.34). Found C(71.67), H(7.49), N(8.27).

The other 2-[4-(5-alkoxy-2-tert-butyl-6-methoxy-8-quinolylamino)-pentyl]-1,3-isoindolinediones **7(b-f)** were prepared by the similar procedure.

*2.2. 5 General Procedure for the Synthesis of N^8-(4-amino-1-methylbutyl)-5-alkoxy-2-tert-butyl-6-methoxy -8-quinolinamines (**8a-f**)*

A solution of 2-[4-(5-alkoxy-2-*tert*-butyl-6-methoxy-8-quinolylamino) pentyl]-1,3-isoindolinedione (**7a-f**, 5 mmol) in 95% ethyl alcohol (25 mL), and hydrazine hydrate (100 mmol) was heated under reflux for 8 h. Solvent was removed under reduced pressure, and the residue was diluted with water (25 mL), The reaction mixture was basified with 8N NaOH solution, and extracted with chloroform (3×20 mL). Chloroform extracts were concentrated under reduced pressure and dry residue was warmed to 50°C for 10 minutes in 25 mL of ethereal HCl (2N) and allowed to cool at room temperature. The phthalyl hydrazide (white precipitate) was filtered off. The hydrochloride salts product of N^8-(4-amino-1-methylbutyl)-5-alkoxy-2-*tert*-butyl-6-methoxy-8-quinolinamines **8a-f** was obtained by concentrating the filtrate under reduced pressure.

*N^8-(4-amino-1-methylbutyl)-2-tert-butyl-6-methoxy-5-propoxy-8-quinolinamines (**8a**).*

Yield 71%; m.p. 105-108 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.82 (s, 1H, 7Ar-H, J=9.0 Hz), 7.52 (d, 1H, 3Ar-H J=9.0 Hz), 6.85 (s, 1H, 4Ar-H), 6.04 (bs, 1H, NH), 3.95 (s, 3H, OCH_3), 3.81

(t, 2H, OCH_2), 3.65 (m, 3H, N-CH), 3.20 (m, 2H, N-CH_2), 2.01 (bs, 2H, NH_2), 1.72 (m, 6H, 3×CH_2), 1.49 (s, 9H, 3×CH_3), 1.30 (t, 3H, CH_3 J=6.3 Hz), 1.04 (t, 3H, CH_3 J=7.8); APCIMS m/z 374 (M+1); Anal. Calcd. For $C_{22}H_{37}N_3O_2Cl_2$. C(59.17), H(8.37), N(9.41). Found C(59.23), H(8.22), N(9.48).

*N^8-(4-amino-1-methylbutyl)-5-butoxy-2-tert-butyl-6-methoxy-8-quinolinamines (**8b**).*

Yield 78%; m.p. 110-112 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.88 (s, 1H, 7Ar-H, J=8.9 Hz), 7.68 (d, 1H, 3Ar-H, J=8.9 Hz), 6.74 (s, 1H, 4Ar-H), 6.01 (bs, 1H, NH), 3.92 (s, 3H, OCH_3), 3.80 (t, 2H, OCH_2), 3.63 (m, 1H, N-CH), 2.75 (m, 2H, N-CH_2), 1.98 (bs, 2H, NH_2), 1.74 (m, 8H, 4×CH_2), 1.41 (s, 9H, 3×CH_3), 1.35 (t, 3H, CH_3 J=6.3 Hz), 1.01 (t, 3H, CH_3 J=7.8); APCIMS m/z 388 (M+1); Anal. Calcd. For $C_{23}H_{39}N_3O_2Cl_2$. C(59.98), H(8.55), N(9.13). Found C(59.84), H(8.49), N(9.24).

*N^8-(4-amino-1-methylbutyl)-2-tert-butyl-6-methoxy-5-pentoxy-8-quinolinamines (**8c**).*

Yield 68%; m.p. 125-128 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.80 (s, 1H, 7Ar-H, J=8.7 Hz), 7.65 (d, 1H, 3Ar-H, J=8.7 Hz), 6.89 (s, 1H, 4Ar-H), 5.90 (bs, 1H, NH), 3.91 (s, 3H, OCH_3), 3.84 (t, 2H, OCH_2, J=6.9), 3.61 (m, 1H, N-CH), 2.73 (m, 2H, N-CH_2), 1.95 (bs, 2H, NH_2), 1.70 (m, 10H, 5×CH_2), 1.40 (s, 9H, 3×CH_3), 1.31 (t, 3H, CH_3 J=6.3 Hz), 0.91 (t, 3H, CH_3 J=7.8); APCIMS m/z 402 (M+1); Anal. Calcd. For $C_{24}H_{41}N_3O_2Cl_2$. C(60.74), H(8.73), N(8.86). Found C(60.69), H(8.68), N(8.89).

*N^8-(4-amino-1-methylbutyl)-2-tert-butyl-5-hexoxy-6-methoxy-8-quinolinamines (**8d**).*

Yield 76%; m.p. 116-120 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.76 (s, 1H, 7Ar-H, J=8.9 Hz), 7.41 (d, 1H, 3Ar-H, J=8.9 Hz), 6.93 (s, 1H, 4Ar-H), 6.03 (bs, 1H, NH), 3.85 (s, 3H, OCH_3), 3.70 (t, 2H, OCH_2), 3.65 (m, 1H, N-CH), 2.72 (m, 2H, N-CH_2), 1.90 (bs, 2H, NH_2), 1.66 (m, 12H, 6×CH_2), 1.40 (s, 9H, 3×CH_3), 1.34 (t, 3H, CH_3 J=6.3 Hz), 0.98 (t, 3H, CH_3 J=7.8) APCIMS m/z 416 (M+1); Anal. Calcd. For $C_{25}H_{43}N_3O_2Cl_2$. C(61.45), H(8.89), N(8.60). Found C(61.48), H(8.75), N(8.67).

*N^8-(4-amino-1-methylbutyl)-2-tert-butyl-5-heptoxy-6-methoxy-8-quinolinamines (**8e**).*

Yield 68%; m.p. 121-124 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.71 (s, 1H, 7Ar-H, J=8.4 Hz), 7.22 (d, 1H, 3Ar-H, J=8.4 Hz), 6.89 (s, 1H, 4Ar-H), 6.02 (bs, 1H, NH), 3.93 (s, 3H, OCH_3), 3.82 (t, 2H, OCH_2), 3.63 (m, 1H, N-CH), 2.71 (m, 2H, N-CH_2), 1.87 (bs, 2H, NH_2), 1.72 (m, 14H, 7×CH_2), 1.48 (s, 9H, 3×CH_3), 1.34 (t, 3H, CH_3 J=6.3 Hz), 0.94 (t, 3H, CH_3 J=7.8); APCIMS m/z 430 (M+1); Anal. Calcd. For $C_{26}H_{45}N_3O_2Cl_2$. C(62.12), H(9.04), N(8.36). Found C(62.03), H(9.23), N(8.28).

*N^8-(4-amino-1-methylbutyl)-2-tert-butyl-6-methoxy-5-octoxy-8-quinolinamines (**8f**).*

Yield 63%; m.p. 128-132 ºC; ^{1}H NMR ($CDCl_3$): δ [ppm] 7.68 (s, 1H, 7Ar-H, J=8.6 Hz), 7.28 (d, 1H, 3Ar-H, J=8.6 Hz), 6.85 (s, 1H, 4Ar-H), 6.05 (bs, 1H, NH), 3.97 (s, 3H, OCH_3), 3.85 (t, 2H, OCH_2), 3.60 (m, 1H, N-CH), 2.83 (m, 2H, N-CH_2), 1.91 (bs, 2H, NH_2),1.67 (m, 16H, 8×CH_2), 1.43 (s, 9H, 3×CH_3), 1.31 (t, 3H, CH_3 J=6.3 Hz), 0.97 (t, 3H, CH_3 J=7.8); APCIMS m/z 444 (M+1); Anal. Calcd. For $C_{27}H_{47}N_3O_2Cl_2$. C(62.76), H(9.19), N(8.13). Found C(62.66), H(9.28), N(8.04).

2.3. Protocol for blood-schizontocidal activity evaluation of analogues against p. berghei (sensitive strain)

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* [10]. 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, 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.

3. Results and Discussion

The results acute toxicity studies in Swiss mice revealed that all the synthesized compounds were safe and nontoxic up to 150 mg/kg as compared to 80 mg/kg for PQ. All of the synthesized compounds were tested for *in vivo* blood-schizontocidal activity against *P. berghei* infected mice model (Table 1). The concentrations tested orally were 100, 50, 25, and 10 mg/kg/day×4. The compound was administered on day 0-3 post infection. The results for all analogues were compared to a positive control group of mice treated with CQ at the concentration of 10 mg/kg/day×4, with mice showing negative parasitemia on D+4 and D+7. The results were also compared to a negative control group of mice where no treatment for the disease was administered, and in this case all animals usually died by D+14.

*Table 1. In Vivo Blood-Schizontocidal Antimalrial Activity of the Synthesized Compounds **8a-f** against P. Berghei Infection in Mice (Six Mice per Group)*

Comp No.	**R**	**Dose (mg/kg/day ×4, oral)**			
		10	**25**	**50**	**100**
8a	C_3H_7	-	-	-	Active (3/6)
8b	C_4H_9	Active (2/6)	Curative (6/6)	Curative (6/6)	Curative (6/6)
8c	C_5H_{11}	Active (4/6)	Curative (6/6)	Curative (6/6)	Curative (6/6)
8d	C_6H_{13}	-	-	Active (2/6)	Curative (6/6)
8e	C_7H_{15}	-	Active (4/6)	Curative (6/6)	Curative (6/6)
8f	C_8H_{17}	Active (4/6)	Curative (6/6)	Curative (6/6)	Curative (6/6)
1		-	-	-	Inactive (0/6)
2		Active (3/6)	Curative (6/6)	Curative (6/6)	Curative (6/6)

The term 'curative' indicates complete elimination of malaria parasites from the body, so that relapse cannot occur up to day D+60. The term 'active' or minimum effective dose (MED) indicates that the treated animals show negative parasitaemia up to D+7. However, by D+28, some mice show negative and some mice 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.

All of the 2,5-disubstituted N^8-(4-amino-1-methylbutyl)-6-methoxy-8-quinolinamines **8a-f** were found to be more or equal effective compared to analogue **2** in the *P. berghei* test. The most effective compound **3c** and **3f** produced 100% cures at the preliminary tested dose of 100 mg/kg, but exhibited no cures at 5 mg/kg.

4. Conclusions

In summary, to further explore the most suitable alkyl group at the C-2 position in primaquine, we have synthesized additional analogues **8a-f** of the recently discovered 2-*tert*-butylprimaquine **2**.The results of this study clearly established that the potent antimalarial activity displayed by analogue **2** is attributed to the incorporation of 2-*tert*-butyl group in primaquine, and its 5-alkoxy analogues results in increased antimalarial activity. Furthermore, two synthesized derivatives **8c** and **8f** exhibited promising antimalarial effects similar to that of analogue **2** against drug-sensitive malaria strains.

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