Discovery of a Bulky 2-*tert*-Butyl Group Containing Primaquine Analogue That Exhibits Potent Blood-Schizontocidal Antimalarial Activities and Complete Elimination of Methemoglobin Toxicity

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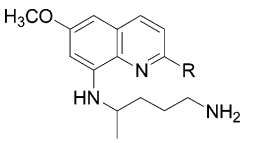
Abstract: To eliminate an unwarranted metabolic pathway of the quinoline ring, a set of two compounds, where C-2 position of the antimalarial drug primaquine is blocked by metabolically stable bulky alkyl group are synthesized. Compound **2** [R = $C(CH_3)_3$] of the series has produced excellent antimalarial efficacy against *P. berghei* and highly virulent multidrug-resistant *P. yoelii nigeriensis* strain in vivo. Compound **2** was also evaluated for methemoglobin (MetHb) toxicity. This study describes the discovery of a highly potent blood-schizontocidal antimalarial analogue **2**, completely devoid of MetHb toxicity.

Parasitic infections are still one of the major reasons of mortality in the third world countries. Parasitic protozoan belonging to genus *Plasmodium* causes malaria, one of the most severe tropical diseases. The infections caused by *Plasmodium falciparum* are highly pathogenic and accounts for almost all deaths attributed to malaria. Malaria is reemerging as the biggest infectious killer and currently is first priority tropical disease of the World Health Organization. The severity of the disease is illustrated by the fact that malaria kills approximately one child every 30 s, and 3000 per day under the age of 5 years.[1] Unfortunately, no vaccine is available for the effective protection against malaria, and treatment is increasingly becoming difficult due to the widespread resistance of the *P. falciparum* malaria parasite to mainstay antimalarial drug chloroquine. This resistance problem has prompted search for new classes of antimalarial agents, and a reexamination of the existing antimalarial drugs that may be effective against resistant strains.

Primaquine (PQ, **1**, Figure 1) over the years is the clinical drug of choice for the radical cure of relapsing *P. vivax* and *P. ovale* malaria.[2] The drug is effective in clearing tissue parasites (erythrocytic stages of malaria parasite life cycle), but has minimal suppressive activity, i.e., is ineffective as blood-schizontocide, and therefore is hazardous for the treatment of infections caused by *P. falciparum*. The usefulness of **1** is also restricted by toxic side effects including hemolytic lesions (caused by methemoglobin production), pronounced in the patients deficient in glucose-6-phosphate dehydrogenase.



1. R= H (primaquine, PQ)
2. R= $C(CH_3)_3$
3. R= 1-adamantyl

Figure 1.

Although toxic, it is known that primaquine is active against more of the life cycle stages of plasmodia than any other class of antimalarial drugs. Research efforts over the years are directed toward finding analogues, which retains the tissue-schizontocidal activity of **1** with improved blood-schizontocidal activity and/or reduced methemoglobin (MetHb) toxicity, and few derivatives with improved therapeutic profiles were synthesized.[3−5] However, scientific endeavors to eliminate MetHb toxic effects of **1** proved to be unsuccessful after 40 years of research.[6]

One of the main metabolic degradation pathways known for the quinoline moiety results in oxidative biotransformation at the C-2 position and converts it to 1*H*-2-oxoquinoline.[7] This pathway is supported by the recent studies conducted by Mirghani and co-workers that identified 2-quininone (known to display phototoxic side-effects) as one of the major metabolite of quinine.[8] Furthermore, high antimalarial activity associated with mefloquine and related compounds is known to be derived by the placement of a trifluoromethyl group at the C-2 position of the quinoline ring, which prevents the biotransformation to inactive and phototoxic 1*H*-2-oxoquinolines.[9] To our surprise, no such metabolic pathway is known for primaquine; though, introduction of 2-alkoxy (OCH_3, $OCH_2C_6H_5$)[5] and 2-alkyl (CH_3, C_2H_5, CF_3),[10] substituents in the primaquine led to an overall increase in therapeutic efficacy. Whether these compounds show improved efficacy by blocking the proposed metabolic pathway is yet uncertain. Upon the basis of these observations, we hypothesize that the placement of a metabolically stable bulky alkyl group at the C-2 position of the quinoline ring in **1** may produce analogues with improved therapeutic efficacies due to their inability to undergo C-2 position metabolic pathway described for the quinoline ring. Thus, our research efforts were focused toward developing a direct synthetic route for the previously inaccessible bulky 2-alkyl group containing primaquine analogues. We report herein, synthesis, blood-schizontocidal antimalarial activities, and MetHb toxicity studies of primaquine derivatives (**2** and **3**) containing *tert*-butyl and 1-adamantyl groups at the C-2 position of the quinoline ring.

Commercially available 6-methoxy-8-nitroquinoline (**4**) upon direct ring-alkylation via a silver-catalyzed radical oxidative decarboxylation of appropriate alkylcarboxylic acid by ammonium persulfate in CH_3CN and 10% H_2SO_4 efficiently produced 2-alkyl-6-methoxy-8-nitroquinolines (**5** and **6**) in good yield (Scheme 1).[11] The reaction is highly regiospecific in nature, with no apparent alkylation observed at other positions on the quinoline ring. The latter compounds (**5** and **6**) were

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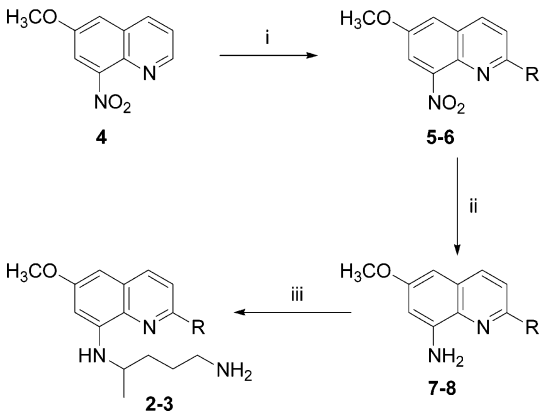
Table 1. In Vitro (*P. falciparum* infection), in Vivo (*P. berghei* and *P. yoelii nigeriensis* infection) Studies of the 2-Alkylprimaquine Analogues (**2** and **3**)[a]

	P. berghei (mg/kg/day × 4, oral)				*P. yoelii nigeriensis* (mg/kg/day × 4, oral)		
no.	10	25	50	100	50	100	*P. falciparum* (IC_{50})[b]
2	(4/6) active	(6/6) curative	(6/6) curative	(6/6) curative	(4/6) active	(6/6) curative	39.06 ng
3	-	-	-	(0/6) inactive	-	(0/6) inactive	>500 μg
PQ	-	-	-	(0/6) inactive	-	(0/6) inactive	-

[a] The term "curative" indicates complete elimination of malaria parasites from the body, and animals survive up to day D+60. The term "active" or "suppressive" indicates that all of the treated animals show negative parasitaemia up to D+7. However, by D+60, some mice die, and some survive with complete elimination of parasitaemia as indicated by numbers given in parentheses. The term "inactive" indicates that the treated animals show positive parasitaemia either on D+4 or D+7 and usually die by D+14. [b] Chloroquine: IC_{50} = 113 ng/mL.

Scheme 1[a]



[a] Reagents and conditions: (i) RCO_2H, $AgNO_3$, $(NH_4)_2S_2O_8$, 10% H_2SO_4, CH_3CN, 70 °C, 60−70%; (ii) Raney Ni, EtOH, H_2, 45 psi, 45 min, 86−94%; (iii) a. 2-(4-bromopentyl)-1,3-isoindolinedione, Et_3N, 120 °C, 24 h, 56−83%; b. $NH_2NH_2 \cdot H_2O$, EtOH, reflux, 8 h, 88−90%.

converted to the requisite N^8-(4-amino-1-methylbutyl)-2-alkyl-6-methoxy-8-quinolinamines (**2** and **3**) in three steps following previously published procedure.[12]

In vitro antimalarial activities of analogues **2** and **3** as IC_{50} values for the inhibition of chloroquine-sensitive *P. falciparum* strain are determined (Table 1). 2-*tert*-Butylprimaquine (**2**) exhibited potent antimalarial activity (IC_{50} = 39 ng/mL), superior to that of chloroquine (IC_{50} = 113 ng/mL). At the same time, to our surprise, 2-(1-adamantyl)primaquine (**3**) was found to be inactive in the test model.

The target compounds were also evaluated for the blood-schizontocidal antimalarial activity against *P. berghei* (sensitive strain) and *P. yoelii nigeriensis* (multidrug-resistant strain) in a rodent model (Table 1). The details of the biological procedures are reported elsewhere.[12] Briefly; testing of compounds **2** and **3** was conducted at various concentrations, orally, in mice (six mice per group). The concentrations tested were 100, 50, 25, and 10 mg/kg/day × 4 (oral). The compounds were administered on days 0−3 postinfection. The results for synthesized analogues are compared to a positive control group of mice treated with chloroquine at the suppressive dose of 10 mg/kg/day × 4 (oral). The results are also compared to a negative control group of mice where no treatment for the infection was administered, and in this case 100% mortality is observed within 6−8 days, with a mean survival time of 6.2 days.

Introduction of the 2-*tert*-butyl group in the primaquine produced a very pronounced increase in blood-schizontocidal antimalarial activity in the *P. berghei* infected mice test. Analogue **2** [R = $C(CH_3)_3$] produced 100% cures at the primary tested dose of 100 mg/kg. Upon evaluation at the subsequent lower doses of 50 and 25 mg/kg, compound **2** again produced 100% cures with all treated animals surviving on day 60 (termination of experiment). Further antimalarial activity evaluation at a dose of 10 mg/kg produced suppressive activity with 4/6 mice surviving on day 60. Thus, 2-*tert*-butylprimaquine (**2**) is equipotent to CQ and is much superior to **1** as a blood-schizontocide. In contrast, 2-(1-adamantyl)primaquine (**3**) was found to be completely devoid of antimalarial activity at the primary tested dose of 100 mg/kg. 2-*tert*-Butylprimaquine (**2**), when subjected to antimalarial activity evaluation in the multidrug-resistant *P. yoelii nigeriensis*-infected mice model, cured all animals at a primary tested dose of 100 mg/kg. Subsequently, at the lower tested dose of 50 mg/kg, analogue **2** produced suppressive activity and cured 4/6 animals. It is important to note that chloroquine and mefloquine are completely inactive against *P. yoelii nigeriensis* strain, and 100% mortality is observed due to parasitic infection.

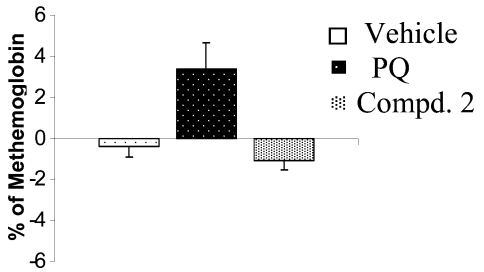


Figure 2. In vivo methemoglobin (MetHb) toxicity estimation of 2-*tert*-butylprimaquine (**2**) in Mastomys coucha. Compound was administered at a dose of 15 mg/kg per day for 3 days. Data are mean ± SEM for each group of animals (set of three experiments).

As mentioned in the introduction, the objective of this investigation was also to prepare less toxic analogue of **1**. It has been established that PQ and related compounds are responsible for enhanced conversion of oxyhemoglobin to MetHb, a side effect that represents the limiting factor in the unrestricted use of PQ.[13] Thus, in vivo MetHb-inducing properties estimation of compound **2** (Figure 2) was carried out in *Mastomys coucha*, a rapid rodent animal model using protocols reported earlier.[14] Briefly, six animals per group of both sexes, 2−3 months old, and body weight between 40 and 50 g were used for the present investigation. Drug was administered intraperitoneally (ip) at a dosage of 15 mg/kg/day for three consecutive days. Primaquine is used as the standard drug for the test. It is known that **1** is readily absorbed from the gastrointestinal tract and

peak plasma concentration reaches within 3 h and falls within an apparent elimination half time of 6 h. Keeping the above pharmacokinetic profile in mind, the blood samples were collected after 4 h of drug administration and % increase in MetHb was calculated. As evident from the results, primaquine induced 3.38% increase in MetHb, whereas compound **2** did not show any increase in MetHb during this investigation. Similar results were also obtained with 50 mg/kg/ip single dose experiment, and oral dose experiment of three day (15 mg/kg/day) and oral single dose experiment (50 mg/kg). This extremely encouraging data, therefore, suggests that compound **2** is completely free of traditional MetHb-inducing properties associated with **1**.

The results obtained established that 2-*tert*-butylprimaquine (**2**) has potent in vitro and in vivo antimalarial activities against both sensitive and multidrug-resistant malaria strains. These findings validate the proposed hypothesis, and blocking of the C-2 position metabolic pathway of the quinoline ring with a *tert*-butyl group results in exceptional increase in the bioefficacy of primaquine. Furthermore, analogue **2** was also found to be completely devoid of MetHb-inducing properties. To conclude, the promising biological data indicate the importance that 2-*tert*-butylprimaquine (**2**) has in the treatment of malaria infections. Analogue **2**, therefore, deserves further preclinical evaluation to determine its suitability as a potential drug candidate for chemotherapy of malaria. Radical curative (tissue-schizontocidal) antimalarial activity evaluation in rhesus monkeys and assessment of ADME properties of **2** are currently underway in our laboratory.

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Supporting Information Available: Detailed experimental procedures and spectral data of compounds **2** and **3** and **5**−**8**. This material is available free of charge via the Internet at http://pubs.acs.org.

References

(1) Butcher, G. A.; Sinden, R. E.; Curtis, C. Malaria: New Ideas, Old Problems, New Technologies. *Parasitol. Today* **2000**, *16*, 43−44.
(2) Carson, P. E. In *Antimalarial Drugs II*; Peters, W., Richards, W. H. G., Eds.; Springer-Verlag: New York, 1984; pp 83−121.
(3) LaMontagane, M. P.; Blumbergs, P.; Strube, R. E. 5-(Aryloxy)-4-methylprimaquine Analogues. A Highly Effective Series of Blood and Tissue Schizontocidal Agents. *J. Med. Chem.* **1982**, *25*, 1094−1097.
(4) Carroll, F. I.; Berrang, B.; Linn, C. P. 4-Substituted 5-[*m*-(Trifluoromethyl)-phenoxy]primaquine Analogues as Potential Antimalarial Agents. *J. Med. Chem.* **1985**, *28*, 1564−1567.
(5) LaMontagane, M. P.; Blumberg, P.; Smith, D. C. Antimalarials 16. Synthesis of 2-Substituted Analogues of 8-[(4-Amino-1-methylbutyl)amino]-6-methoxy-4-methyl-5-[3-(trifluoromethyl)-phenoxy]quinoline as Candidate Antimalarials. *J. Med. Chem.* **1989**, *32*, 1728−1732.
(6) Nodiff, E. A.; Chatterjee, A.; Musallam, H. A. Antimalarial Activities of the 8-Aminoquinolines. *Prog. Med. Chem.* **1991**, *28*, 1−40.
(7) Fetzner, S.; Tshisuaka, B.; Lingens, F.; Kappl, R.; Hüttermann, J. Bacterial Degradation of Quinoline and Derivatives − Pathways and Their Biocatalyst. *Angew. Chem., Int. Ed. Engl.* **1998**, *37*, 576−597.
(8) Mirghani, R. A.; Ericsson, O.; Cook, J.; Yu, P.; Gustafsson, L. L. Simultaneous Determination of Quinine and Four Metabolites in Plasma and Urine by High-Performance Liquid Chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **2001**, *754*, 57−64.
(9) Pinder, R. M.; Burger, A. Antimalarials II. α-(2-Piperidyl)- and α-(2-pyridyl)-2-trifluoromethyl-4-quinolinemethanols. *J. Med. Chem.* **1968**, *11*, 267−269.
(10) Schmidt, L. H. Relationship Between Chemical Structure of 8-Aminoquinolines and Their Capabilities for Radical Cure of Infection With Plasmodium Cynomolgi in Rhesus Monkeys. *Antimicrob. Agents Chemother.* **1983**, *24*, 615−652.
(11) Jain, R.; Vaitilingam, B.; Nayyar, A.; Palde, P. B. Substituted 4-Methylquinolines as a New Class of Anti-Tuberculosis Agents. *Bioorg. Med. Chem. Lett.* **2003**, *13*, 1051−1054.
(12) Vangapandu, S.; Sachedva, S.; Jain, M.; Singh, S.; Singh, P. P.; Kaul, C. L.; Jain, R. 8-Quinolinamines and Their Pro Prodrug Conjugates as Potent Blood-Schizontocidal Antimalarial Agents. *Bioorg. Med. Chem.* **2003**, *11*, 4557−4568.
(13) McChesney, J. D. Considerations About the Structure−Activity Relationships of 8-Aminoquinoline Antimalarial Drugs. *Bull. World. Health Org.* **1981**, *59*, 459−462.
(14) Srivastava, P.; Singh, S.; Jain, G. K.; Puri, S. K.; Pandey, V. C. A simple and Rapid Evaluation of Methemoglobin Toxicity of 8-Aminoquinolines and Related Compounds. *Ecotox. Environ. Safety.* **2000**, *45*, 236−239.

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