4,5-Disubstituted Primaquine Analogues as Potential Antimalarial Agents

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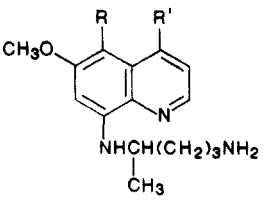
Three 4,5-disubstituted primaquine analogues were synthesized and evaluated for radical curative activity against *Plasmodium cynomolgi* in rhesus monkeys. One of the compounds showed moderate activity; however, none of the three compounds were as active as the lead compounds 5-methoxy-4-methylprimaquine and 4-(methoxymethyl)-5-[*m*-(trifluoromethyl)phenoxy]primaquine.

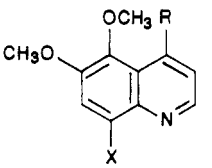
Primaquine (**1a**), which was originally synthesized in 1955, is still the clinical drug of choice for the treatment of relapsing *Plasmodium vivax* and *P. ovale* malaria.[1] However, since **1a** suffers from serious toxic side effects, numerous 8-aminoquinoline derivatives of **1a** have been prepared and evaluated as potential replacements for this drug. In 1983 Schmidt summarized the evaluation of 200 8-aminoquinolines for activity against *P. cynomolgi* in rhesus monkeys.[2] In this study 34 8-aminoquinolines were identified that showed activity equal or superior to that of primaquine. One of the more active compounds was 5-methoxy-4-methylprimaquine (**1b**).[3] In early studies we reported that 4-vinylprimaquine (**1c**) showed activity equal to primaquine but was less toxic, and more recently we reported that 4-(methoxymethyl)-5-[(*m*-trifluoromethyl)-phenoxy]primaquine (**1d**) was both more active and less toxic than **1a**.[4,5] Based on the above along with information reported from other laboratories,[6,7] a study was initiated to prepare and evaluate the antimalarial activity of the primaquine analogues **1e–g**.

Compound **1e** differs from **1b** in that a vinyl group has replaced the methyl group in the 4-position of the quinoline ring. Compound **1f**, which is isomeric with **1d**, has the position of the methoxy and *m*-(trifluoromethyl)-phenoxy groups exchanged. In compound **1g** the methoxy group of **1d** has been replaced by a second *m*-(trifluoromethyl)phenoxy moiety.



1a, R = R' = H
b, R = CH_3O; R' = CH_3
c, R = H; R' = CH_2=CH
d, R = *m*-$CF_3C_6H_4O$; R' = CH_3OCH_2
e, R = CH_3O; R' = CH_2=CH
f, R = CH_3O; R' = *m*-$CF_3C_6H_4OCH_2$
g, R = *m*-$CF_3C_6H_4O$; R' = *m*-$CF_3C_6H_4OCH_2$

Chemistry. Compounds **1e** and **1f** were both prepared from 5,6-dimethoxy-4-methyl-8-nitroquinoline (**2**). Selenium dioxide oxidation of **2** gave the 4-carboxaldehyde **3**. Attempts to reduce **3** to the alcohol **4** with sodium borohydride were unsuccessful; however, when triethylsilane was used as the reducing agent, the conversion of **3** to **4** proceeded smoothly. Treatment of **4** with phosphorus oxychloride gave the 4-chloromethyl compound, which was treated with the potassium salt of *m*-(trifluoromethyl)-phenol to give **5**. Stannous chloride reduction of **5** yielded the 8-aminoquinoline **6**. Alkylation of **6** with 4-iodo-1-phthalimidopentane followed by removal of the phthaloyl protecting group with hydrazine gave the target compound **1f**.



2, R = CH_3; X = NO_2
3, R = CHO; X = NO_2
4, R = CH_2OH, X = NO_2
5, R = *m*-$CF_3C_6H_4OCH_2$; X = NO_2
6, R = *m*-$CF_3C_6H_4OCH_2$; X = NH_2
7, R = CH=CH_2; X = NO_2
8, R = CH=CH_2; X = NH_2
9, R = CH=CH_2; R = $NHC(CH_3)H(CH_2)_3NPht$

Subjection of the aldehyde **3** to the Wittig reaction using methylenetriphenylphosphorane and inverse addition gave the 4-vinyl compound **7**. Stannous chloride reduction of **7** afforded the 8-amino derivative **8** which on alkylation with 4-iodo-1-phthalimidopentane gave the protected product **9**. Attempts to remove the protecting group with hydrazine were unsuccessful. However, **9** was smoothly converted to **1e** when a hydroxylamine sodium methoxide mixture in methanol was used in place of hydrazine.

Compound **1g** was prepared from 4-(hydroxymethyl)-6-methoxy-8-[(4'-phthalimido-1'-methylbutyl)amino]-5-[*m*-(trifluoromethyl)phenoxy]quinoline (**10**).[5] Subjection of **10** to mild treatment with thionyl chloride in methylene chloride gave the chloro compound **11**. Treatment of **11** with the potassium salt of *m*-(trifluoromethyl)phenol followed by removal of the protecting group gave target compound **1g**.

Biological Testing. Compounds **1e–g** were tested for radical curative activity against *P. cynomolgi* in rhesus monkeys.[8,9] The data along with a comparison of the data for primaquine as well as derivatives **1b** and **1d** are shown in Table I. Compound **1e** showed $^1/_2$ cures at 1.0 mg/kg. Compounds **1f** and **1g** were inactive at all dose levels tested.

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(9) The test procedure is described in World Health Organization (1972b); WHO/MAL/72.763 (cyclostyled report), World Health Organization: Geneva.

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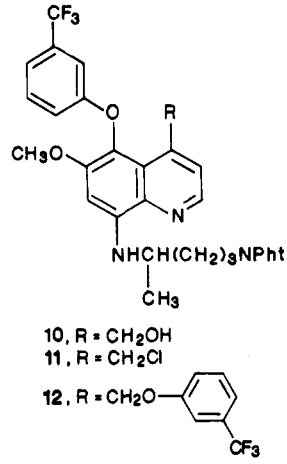

10, R = CH_2OH
11, R = CH_2Cl

The data in Table I show that the replacement of the methyl group of **1b** with a vinyl group causes a reduction in antimalarial activity. Replacement of the methyl group of **1b** by the larger m-$CF_3C_6H_4OCH_2$ moiety results in complete loss of activity. Similarly, replacement of the CH_3OCH_2 group of the highly active **1d** with the larger m-$CF_3C_6H_4OCH_2$ group resulted in loss of activity.

Experimental Section

Melting points were determined on a Kofler hot-stage microscope using a calibrated thermometer. IR spectra were measured with a Perkin-Elmer Model 267 or 467 grating infrared spectrophotometer. ^1H NMR spectra were recorded on a Varian Model HA-100 spectrometer using tetramethylsilane as an internal standard. MS were determined on an AEI-MS 902 spectrometer. Microanalyses were carried out by Micro-Tech Laboratories, Skokie, IL, or Integral Microanalytical Laboratories, Inc., Raleigh, NC.

5,6-Dimethoxy-8-nitroquinoline-4-carboxaldehyde (3). The 4-methylquinoline **2**[3] (5.5 g, 0.022 mol) dissolved in 160 mL of dioxane, 50 mL of acetic acid, and 50 mL of acetic anhydride was refluxed for 2 h with 2.5 g (0.022 mol) of freshly sublimed selenium dioxide. After standing overnight, the suspension was filtered from precipitated selenium and evaporated to dryness. The crude residue (7 g) was dissolved in 50 mL of THF and heated with 10 mL of 3 N hydrochloric acid on the steam bath for 20 min. The solution was concentrated to a small volume, neutralized with sodium bicarbonate solution, and extracted with methylene chloride. The crude concentrated extract was chromatographed on silica gel using first methylene chloride–5% ethyl acetate followed by 10% and 15% ethyl acetate as eluent. This gave 4.5 g (78%) of aldehyde **3** as pure yellow crystals: mp 170–172 °C dec; ^1H NMR ($CDCl_3$) δ 4.04, 4.10 (2 s, OCH_3), 7.54 (d, H_3), 7.99 (s, H_7), 8.96 (d, H_2). Anal. ($C_{12}H_{10}N_2O_5$) C, H, N.

5,6-Dimethoxy-4-(hydroxymethyl)-8-nitroquinoline (4). A mixture of 15 g of phenol, 1.8 g of water, and 4.2 g (0.016 mol) of the aldehyde (**3**) was cooled to 8 °C and mixed thoroughly with 2 g (0.017 mol) of triethylsilane. The stirred mixture was cooled to <10 °C, and 9 mL of trifluoroacetic acid was added over a 50-min period. Stirring was continued at 30 °C overnight. The mixture was concentrated in vacuo to a small volume at low temperature. The remaining syrup was partitioned with chloroform and 20% sodium hydroxide solution. The extraction of the aqueous phase was repeated 3 times, and the combined organic extracts were evaporated to give 4 g (94%) of **4** as a yellow-brown crystalline solid. A sample of 150 mg after recrystallization from 1 mL of methylene chloride–hexane (1:2) had: mp 138–139 °C; ^1H NMR ($CDCl_3$) δ 4.03 (s, 2 OCH_3), 5.13 (s, CH_2OH), 7.57 (d, H_3), 7.80 (s, H_7), 8.81 (d, H_2). Anal. ($C_{12}H_{12}N_2O_5$) C, H, N.

5,6-Dimethoxy-8-nitro-4-[[m-(trifluoromethyl)phenoxy]methyl]quinoline (5). A suspension of 9 g (0.034 mol) of 5,6-dimethoxy-4-(hydroxymethyl)-8-nitroquinoline (**4**) in 80 mL of methylene chloride was heated with 7 g (0.046 mol) of phosphorus oxychloride under reflux for 1 h. The ice-cold methylene chloride solution was then stirred with 2 N sodium hydroxide solution until an aqueous extract of a sample showed pH 5. The mixture was filtered, and the organic layer was isolated and dried (Na_2SO_4).

Table I. Antimalarial Activities against *P. cynomolgi* in Rhesus Monkeys[a,b]

compd	dose,[c] mg/kg	cures[d]	relapses[e]
1a[f]	0.25	0/6	
	0.5	4/6	
	0.75	4/4	
1b[f]	0.0625	0/2	
	0.125	2/2	
	0.25	2/2	
	0.5	1/1	
1d	0.1	0/2	8, 8
	0.316	1/2	28
	1.0	2/2	
1e	0.1	0/1	9
	0.316	0/1	23
	1.0	1/2	27
1f	1.0	0/2	12, 13
1g	0.1	0/1	11
	1.0	0/2	11,14

[a] Data were supplied by H. A. Musallam and B. T. Poon, Walter Reed Army Institute of Research. [b] Tests were carried out by SEATO Medical Research Laboratory, Bangkok (see ref 8 and 9). [c] Dose administered via stomach tube once daily for 7 days with 6.2 mg of base/kg of chloroquine. [d] Monkeys that did not relapse are considered cured (see ref 8). [e] The number given is the days between the end of treatment and relapse. [f] Taken from ref 2.

To this solution a mixture of 5 g (0.025 mol) of potassium m-(trifluoromethyl)phenoxide in 20 mL of dimethylformamide was added. The solution was immediately concentrated and heated at 55–60 °C for 1 h. The solvents were evaporated under vacuum, and the pasty remainder was partitioned with methylene chloride and 10% sodium hydroxide. The organic layer upon concentration in vacuo gave light-brown crystals, which afforded 7.7 g (57%) of **5** after washing with 2-propanol and a little bit of ethyl acetate. A sample recrystallized from 2-propanol had: mp 136–138 °C; ^1H NMR ($CDCl_3$) δ 4.03 (s, 2 OCH_3), 5.68 (s, CH_2O), 7.2 (m, phenoxy), 7.80 (s, H_7), 8.87 (d, H_2). Anal. ($C_{19}H_{13}N_2O_5F_3$) C, H, N.

8-Amino-5,6-dimethoxy-4-[[m-(trifluoromethyl)phenoxy]methyl]quinoline (6). The above nitroquinoline derivative (5.6 g, 0.014 mol) was dissolved in 100 mL of THF and diluted with 40 mL of ethanol. The cooled solution (8 °C) was mixed with 40 mL of 1.3 N hydrochloric acid and 0.2 g of tin powder. Subsequently, 11.5 g (0.013 mol) of stannous chloride was introduced as a solid, and the suspension was stirred at 8 °C for $^1/_2$ h. After this time, TLC analysis of a sample indicated the reaction was completed. The mixture was treated with excess 30% sodium hydroxide solution, which led to the formation of two phases. The organic layer was isolated, concentrated in vacuo, and partitioned with methylene chloride and water. After filtration and evaporation, the organic phase gave 4.0 g (75%) of **6** as a brown semisolid: ^1H NMR ($CDCl_3$) δ 3.78, 3.90 (2 s, 2 OCH_3), 5.66 (s, CH_2O), 6.63 (s, H_7), 7.46 (d, H_3), 8.42 (d, H_2).

The crude aminoquinoline **6** was immediately submitted to the following alkylation procedure.

8-[(4′-Amino-1′-methylbutyl)amino]-5,6-dimethoxy-4-[[m-(trifluoromethyl)phenoxy]methyl]quinoline (1f) Fumarate. The 8-aminoquinoline **6** (4 g, 0.011 mol) and 0.5 mL of dimethylformamide were heated in an oil bath under nitrogen at 85–90 °C. A stirred solution of 9 g (0.027 mol) of 4-iodo-1-phthalimidopentane in 0.6 mL of dimethylformamide and 3 g (0.03 mol) of triethylamine was added dropwise within 5 h. Partitioning of the resulting product with methylene chloride and water gave an organic phase, which was chromatographed on silica gel using chloroform–1% acetone as the eluent. The alkylated product (3.4 g, 54%) was recovered as an orange-yellow syrup: ^1H NMR ($CDCl_3$) δ 1.26 (d, CH_3CH), 3.8 (s, OCH_3), 3.96 (s, OCH_3), 5.70 (s, CH_2O), 6.35 (s, H_7), 7.65 (m, phthaloyl), 8.40 (d, H_2).

For hydrazinolysis, the product was refluxed with excess hydrazine in ethanol for 2 h. The cooled mixture was filtered, and the filtrate was evaporated, vacuum dried, and the residue extracted with ether. Evaporation of the ether gave 2.3 g (87%) of **1f** as a clear yellow oil. This product (0.005 mol) was dissolved in 20 mL of ethanol and combined with an alcoholic solution of 0.6 g (0.005 mol) of fumaric acid. The concentrated solution was

diluted with 2-propanol and cooled to −15 °C. After standing overnight, the fumarate crystallized to give 2.3 g (79%) as a bright-yellow powder: mp 173–174 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.19 (d, $CHCH_3$), 3.72 (s, OCH_3), 3.89 (s, OCH_3), 5.66 (s, CH_2OAr), 6.33 (s, fumarate), 6.51 (s, H_7), 8.46 (d, H_2). Anal. ($C_{28}H_{32}N_3O_7F_3 \cdot 0.5H_2O$) C, H, N.

5,6-Dimethoxy-8-nitro-4-vinylquinoline (7). The quinoline 4-carboxaldehyde (**3**; 5 g, 0.019 mol) was dissolved in 800 mL of hot tetrahydrofuran. The solution was placed under a nitrogen atmosphere and rapidly cooled to −70 °C. A solution of triphenylmethylenephosphorane in 100 mL of ether (prepared from 7.1 g (0.02 mol) of methyltriphenylphosphonium bromide and 0.019 mol of butyllithium added at room temperature) was cooled to −50 °C under nitrogen. This reagent was rapidly introduced with vigorous stirring into the aldehyde solution. The resulting brown solution was allowed to warm to room temperature. After 2 h, 10 mL of ethanol was added and the mixture evaporated to a dark syrup. Methylene chloride (35 mL) was added, and triphenylphosphine oxide complex precipitated with ether (500 mL). The clear supernatant was evaporated and the residue purified by silica gel column chromatography using first methylene chloride–5% ethyl acetate followed by 10% ethyl acetate as eluent. Workup gave 3.5 g (71%) of the vinyl compound **7**: mp 135–136 °C; ^{1}H NMR ($CDCl_3$) δ 3.86 (s, OCH_3), 4.04 (s, OCH_3), 5.43 (m, CH_2=CH), 7.3 (d, H_3), 7.67 (m, CH_2=CH), 7.78 (s, H_7), 8.73 (d, H_2). Anal. ($C_{13}H_{12}N_2O_4$) C, H, N.

8-Amino-5,6-dimethoxy-4-vinylquinoline (8). Compound **8** was prepared in a manner analogous to that described for **6**. Thus, 3.5 g (0.013 mol) of the nitroquinoline (**7**) gave 2.7 g (87%) of the 8-amino compound **8** as a brown solid: ^{1}H NMR ($CDCl_3$) δ 3.64 (s, OCH_3), 3.87 (s, OCH_3), 5.28 (m, CH_2=CH), 6.58 (s, H_7), 7.13 (d, H_3), 7.73 (m, CH=CH), 8.41 (d, H_2).

Because of its instability, the product was immediately submitted to alkylation.

8-[(4′-Amino-1′-methylbutyl)amino]-5,6-dimethoxy-4-vinylquinoline (1e) Fumarate. At an oil bath temperature of 75 °C (higher temperature led to complete decomposition), a mixture of 6.5 g (0.019 mol) of 4-iodo-1-phthalimidopentane and 2 g (0.02 mol) of triethylamine was added under nitrogen to 2.7 g (0.013 mol) of the 8-amino-4-vinylquinoline (**8**). The addition rate was adjusted so that it was complete in 12 h. The cooled dark syrup was partitioned with chloroform–water. The organic phase upon evaporation left a dark oil, which was chromatographed on silica gel using methylene chloride–2% acetone as eluent to give 1.5 g (22%) of alkylated product: ^{1}H NMR ($CDCl_3$) δ 1.23 (d, $CHCH_3$), 3.62 (s, OCH_3), 3.92 (s, OCH_3), 5.34 (m, CH_2=CH), 6.3 (s, H_7), 7.14 (m, CH_2=CH), 7.66 (m, phthaloyl), 8.31 (d, H_2).

The alkylated product **9**, 1.5 g (0.03 mol) in 25 mL of ethanol, was treated with a mixture of 0.5 g (0.0075 mol) of hydroxylamine hydrochloride and 0.7 g (0.013 mol) of sodium methoxide in methanol. After stirring at 20 °C overnight, the suspension was filtered, concentrated in vacuo, and partitioned with methylene chloride and water: δ 1.26 (d, CH_3CH), 3.63 (s, OCH_3), 3.93 (s, OCH_3), 5.37 (m, CH_2=CH), 6.32 (s, H_7), 7.20 (d, H_3), 7.79 (m, CH_2=CH), 8.37 (d, H_2).

For salt formation a solution of 0.6 g (0.006 mol) of fumaric acid in 30 mL of ethanol was added immediately to the methylene chloride extract. Upon concentration and cooling overnight at −15 °C, the bright-yellow salt precipitated and was collected and washed with ice-cold 2-propanol–acetonitrile. The fumarate (0.7 g, 34%) had: mp 153–155 °C dec; ^{1}H NMR (Me_2SO-d_6) δ 1.15 (d, CH_3CH), 3.54 (s, OCH_3), 3.87 (s, OCH_3), 5.46 (m, CH_2=CH), 6.31 (s, fumarate), 6.46 (s, H_7), 7.37 (d, H_3), 8.37 (d, H_2). Anal. ($C_{24}H_{31}N_3O_8 \cdot 0.5H_2O$) C, H, N.

8-[(4′-Amino-1′-methylbutyl)amino]-6-methoxy-5-[*m*-(trifluoromethyl)phenoxy]-4-[[*m*-(trifluoromethyl)phenoxy]methyl]quinoline (1g) Resorcylate. 4-(Hydroxymethyl)-6-methoxy-8-[(4′-phthalimido-1′-methylbutyl)amino]-5-[*m*-(trifluoromethyl)phenoxy]quinoline (**10**)[5] (2.5 g, 0.0044 mol) was dissolved in 100 mL of methylene chloride and cooled to −15 °C. Slowly with stirring 2 mL of thionyl chloride was added over a 15-min period. After 1 h at −15 °C, the brown-red mixture was stored at 0 °C for 20 h, then at 20 °C for $^1/_2$ h. The excess thionyl chloride was decomposed by careful addition of sodium carbonate to the cooled (0 °C) solution. The organic phase was separated and concentrated to 50 mL. A solution of 1.6 g (0.01 mol) of *m*-(trifluoromethyl)phenol and 0.6 g (0.01 mol) of potassium hydroxide in dimethylformamide (3 mL) was added to the methylene chloride solution of **11**. The concentrated mixture was heated at 60 °C for 20 min and evaporated in vacuo. The remaining brown syrup was dissolved in methylene chloride, washed with sodium hydroxide and water, and chromatographed on silica gel using first methylene chloride–2% acetone followed by 5% acetone as eluent. The product **12** was obtained as a yellow syrup 1.5 g (47%): ^{1}H NMR ($CDCl_3$) δ 1.30 (d, CH_3CH), 3.80 (s, OCH_3), 5.44 (s, CH_2O), 6.42 (s, H_7), 8.47 (d, H_2).

The intermediate **12** accumulated from several alkylations (4.1 g, 0.0057 mol) was dissolved in 20 mL of tetrahydrofuran. A solution of 0.3 g (0.01 mol) of hydrazine in 100 mL of ethanol was added and the clear mixture heated at 60 °C for 3 h. After this time, the ^{1}H NMR spectrum of a sample showed hydrazinolysis to be completed. The mixture was then filtered and the filtrate evaporated to a syrup. This was extracted with ether and the extract heated with charcoal for 5 min. Evaporation of the solvent gave 3.2 g (95%) of **1g** as a brown sticky material. An ethereal solution of **1g** was treated with a solution of 1.6 g (0.01 mol) of resorcylic acid in ether. The addition of hexane to incipient turbidity caused the separation of a brown impurity, which was removed after standing for 1 h. The clarified solution (40 mL) was added dropwise with vigorous stirring to 500 mL of hexane. After 12 h, the precipitated salt was collected, washed with hexane, and vacuum dried at 78 °C to give 3.4 g (84%) of **1g**: mp 120–122 °C; ^{1}H NMR (Me_2SO-d_6) δ 1.27 (d, CH_3CH), 3.77 (s, OCH_3), 5.48 (s, CH_2OR), 6.66 (s, H_7), 8.47 (d, H_2). Anal. ($C_{37}H_{35}N_3O_7F_6 \cdot H_2O$) C, H, N.

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Registry No. **1a**, 90-34-6; **1b**, 64992-94-5; **1d**, 98510-03-3; **1e**, 102781-08-8; **1e**·fumarate, 102781-09-9; **1f**, 102781-03-3; **1f** (phthalimidate), 102781-02-2; **1f**·fumarate, 102781-04-4; **1g**, 102781-12-4; **1g**·resorcylate, 102781-14-6; **2**, 64992-96-7; **2** (diacetal), 102780-97-2; **3**, 102780-98-3; **4**, 102780-99-4; **5**, 102781-00-0; **6**, 102781-01-1; **7**, 102781-05-5; **8**, 102781-06-6; **9**, 102781-07-7; **10**, 102781-13-5; **11**, 102781-10-2; **12**, 102781-11-3; 3-$F_3CC_6H_4OK$, 56705-78-3; 3-$F_3CC_6H_4OH$, 98-17-9; 4-iodo-1-phthalimidopentane, 63460-47-9.