Synthesis of 2-Benzyloxy and 2-Benzylthio Analogues of Primaquine as Potential Antimalarials

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A series of 2-benzyloxy and 2-benzylthio analogues of primaquine has been synthesized and evaluated against *Plasmodium berghei* in the mouse and *Plasmodium cynomolgi* in the rhesus monkey. 8-Aminoquinoline toxicity, as measured in the Rane mouse screen, was reduced, and these compounds showed significant blood schizonticidal antimalarial activity in mice. In monkeys, significant tissue-schizonticidal activity was observed.

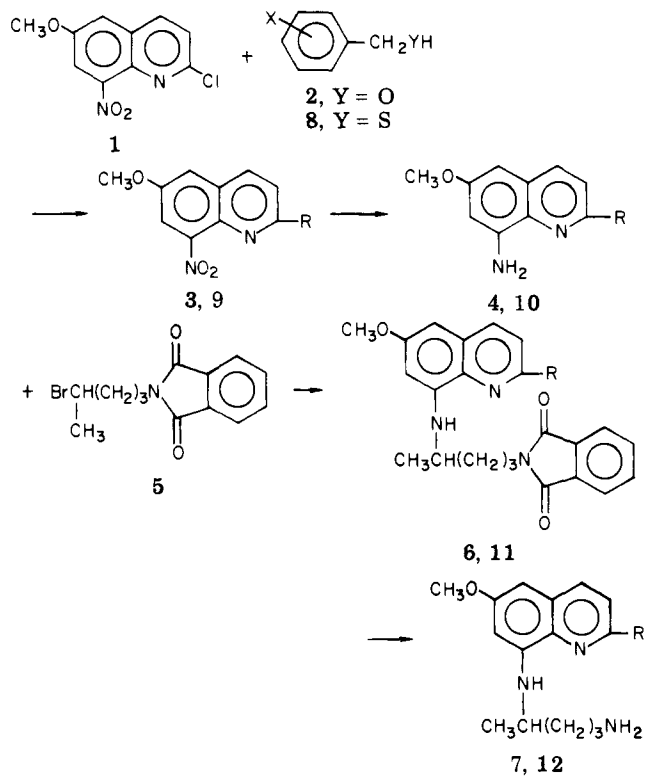
It has been suggested that primaquine and related 8-aminoquinolines (e.g., pamaquine, pentaquine) are active against more of the life cycle stages of plasmodia than any other class of drugs.[1] Primaquine is the tissue schizonticidal drug of choice, and it is used, mainly with a strong blood schizontocide, to achieve radical cure of relapsing malaria and chemoprophylaxis or the interruption of transmission.[1c] Since resistance to the 8-aminoquinolines does not appear to be a problem, we were encouraged to reexamine this class in an effort to synthesize additional derivatives with (1) improved potential for prophylactic action and (2) fewer of the characteristic 8-aminoquinoline toxicities.

Our earlier study[2] had indicated that the 2-benzyloxy analogue (7, X = H) of primaquine was less toxic. At 640 mg/kg all five mice died with primaquine in the *Plasmodium berghei* screen,[3] but none with the 2-benzyloxy analogue (7, X = H). Subsequent investigation established that 2-benzyloxyprimaquine demonstrated radical curative activity[2b] by the Schmidt technique.[4] As a continuation of the study to evaluate various 2-substituted analogues of primaquine, we have now synthesized and evaluated some additional 2-benzyloxy and 2-benzylthio (sulfur isostere) analogues.

Chemistry. For preparation of 2-chloro-6-methoxy-8-nitroquinoline (1), the procedure of Mislow and Koepfli[5] was adopted (Scheme I). The key intermediates, 8-amino-2-benzyloxy-6-methoxyquinolines (4), were prepared by our standard procedure[6] of condensing the appropriate benzyl alcohol 2 with 2-chloro-6-methoxy-8-nitroquinoline (1) in the presence of dimethylformamide and anhydrous potassium carbonate followed by reduction of 3 with Raney nickel-hydrazine hydrate.[7] The analogous benzylthio compounds (9, Scheme I) were prepared by reaction of 1 with the appropriate benzyl mercaptan (toluenethiol) in the presence of dimethylformamide and triethylamine. The amines 10 were prepared by reduction with ethanolic hydrazine hydrate and 10% palladium on carbon[8] or iron-acetic acid.[9] The former method appeared to be favored with the substituted benzylthio compound (9b → 10b). Alkylation of the amines (4 or 10) with 5[10] utilizing the acetate buffer method[10] gave low yields and inconsistent results. This method was abandoned in favor of the triethylamine procedure[11] which effectively improved the overall yield of 6 (7) and 11 (12). It was necessary to use at least 3 equiv of triethylamine and 2 equiv of 5 to ensure a good yield. Elimination competes with alkylation (formation of phthalimidopentene), thus necessitating the use of 2 equiv of 5. The intermediate phthalimidoalkylaminoquinoline derivatives 6 and 11 were not generally isolated, but subjected directly to hydrazinolysis, and the resulting amines 7 and 12 characterized as maleate salts. Pertinent physical and analytical data for all new compounds are presented in Table I.

Biological Results. The antimalarial test results were provided by the Walter Reed Army Institute of Research.

Scheme I



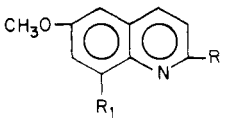
The activity was assessed against *P. berghei* in mice by the method of Rane and co-workers.[3] Of the various substituted and sulfur analogues screened, compounds **7a** and **7e** were "active" as blood schizonticides at dose level of 640 mg/kg, and compound **12b** was "active" at dose levels of 320 and 640 mg/kg.[3] None of these were comparable to the unsubstituted 2-benzyloxy derivative[2a] (7, X = H) in the *P. berghei* screen.

In the "radical curative monkey test"[4] compounds **7a**, **7b**, **7e**, and **12a** demonstrated radical curative activity (see Table II). The radical curative effects of **7a** and **7b** were approximately equal to the activity shown in this model by primaquine. The other two (**7e** and **12a**) were less active than primaquine. However, analogues **7a**, **7b**, **7e**, and **12a** were superior to the unsubstituted compound 7 against *P. cynomolgi*. The lead compound (7, X = H, WR106147)[2a] was curative at 1.5 mg/kg,[2b] whereas primaquine was curative at 1 mg/kg or less.[6b] The activity patterns were not considered adequate to justify expanded testing or further extension of the present series.

Experimental Section

Melting points were determined on a Thomas-Hoover apparatus (capillary method) and are uncorrected. Elemental analyses were performed by Atlantic Microlab, Inc., Atlanta, Ga., and results were within ±0.4% of the calculated values unless otherwise noted. Satisfactory IR (Perkin-Elmer 467 grating spectrophotometer,

Table I. 2-Benzyloxy- and 2-Benzylthioquinoline Derivatives



Compd	R	R_1	Mp, °C	Yield, %	Formula
3a	*p*-$FC_6H_4CH_2O$-	NO_2	142-144	93[a]	$C_{17}H_{13}N_2O_4F$[b]
3b	*p*-$CF_3C_6H_4CH_2O$-	NO_2	126-127	72[a]	$C_{18}H_{13}N_2O_4F_3$[b]
3c	*m*-$CF_3C_6H_4CH_2O$-	NO_2	130-131	91[a]	$C_{18}H_{13}N_2O_4F_3$[b]
3d	*p*-$ClC_6H_4CH_2O$-	NO_2	129.5-133.5	84[a]	$C_{17}H_{13}N_2O_4Cl$[b]
3e	2,4-$Cl_2C_6H_3CH_2O$-	NO_2	163-164.5	80[a]	$C_{17}H_{12}N_2O_4Cl_2$[b]
3f	*p*-$CH_3OC_6H_4CH_2O$-	NO_2	122-123.5	81[a]	$C_{18}H_{16}N_2O_5$[b]
9a	$C_6H_5CH_2S$-	NO_2	132-134	66[c]	$C_{17}H_{14}N_2O_3S$[d]
9b	*p*-$ClC_6H_4CH_2S$-	NO_2	154-157	54[e]	$C_{17}H_{13}N_2O_3SCl$[f]
4a	*p*-$FC_6H_4CH_2O$-	NH_2	111-113	81[g]	$C_{17}H_{15}N_2O_2F$[b]
4b	*p*-$CF_3C_6H_4CH_2O$-	NH_2	133-134.5	77[g]	$C_{18}H_{15}N_2O_2F_3$[b]
4c	*m*-$CF_3C_6H_4CH_2O$-	NH_2	81-83	83[a]	$C_{18}H_{15}N_2O_2F_3$[b]
4d	*p*-$ClC_6H_4CH_2O$-	NH_2	123.5-125	97[a]	$C_{17}H_{15}N_2O_2Cl$[h]
4e	2,4-$Cl_2C_6H_3CH_2O$-	NH_2	125-126.5	93[a]	$C_{17}H_{14}N_2O_2Cl_2$[h]
4f	*p*-$CH_3OC_6H_4CH_2O$-	NH_2	109.5-110.5	90[a]	$C_{18}H_{18}N_2O_3$[b]
10a	$C_6H_5CH_2S$-	NH_2	92-94	98[a]	$C_{17}H_{16}N_2OS$[d]
10b	*p*-$ClC_6H_4CH_2S$-	NH_2	111-112.5	86[i]	$C_{17}H_{15}N_2OSCl$[d]
7a	*p*-$FC_6H_4CH_2O$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	158-160	57[j]	$C_{26}H_{30}N_3O_6F$[b]
7b	*p*-$CF_3C_6H_4CH_2O$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	161.5-163.5	62[k]	$C_{27}H_{30}N_3O_6F_3$[b]
7c	*m*-$CF_3C_6H_4CH_2O$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	74-76	55[k]	$C_{27}H_{30}N_3O_6F_3$[b]
7d	*p*-$ClC_6H_4CH_2O$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	176-177.5	71[l]	$C_{26}H_{30}N_3O_6Cl$[h]
7e	2,4-$Cl_2C_6H_3CH_2O$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	132-134	36[m]	$C_{26}H_{29}N_3O_6Cl_2$[h]
7f	*p*-$CH_3OC_6H_4CH_2O$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	163-164	51[l]	$C_{27}H_{33}N_3O_7$[b]
12a	$C_6H_5CH_2S$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	114-116	25[k]	$C_{26}H_{31}N_3O_5S$[d]
12b	*p*-$ClC_6H_4CH_2S$-	$NHCH(CH_3)(CH_2)_3NH_2$[j]	128-130	50[k]	$C_{26}H_{30}N_3O_5SCl$[f]

[a] 95% ethanol. [b] Analyses for C, H, and N are within ±0.4% of the theoretical value except for 12a (C: calcd. 62.76; found, 62.03). [c] Benzene. [d] Analyses for C, H, N, and S. [e] Acetone. [f] Analyses for C, H, N, S, and Cl. [g] Ether-petroleum ether. [h] Analyses for C, H, N, and Cl. [i] Benzene-hexane. [j] Isolated as the maleate salt. [k] Ethanol-ether. [l] Ethanol. [m] Chloroform.

Table II. Antimalarial Test Results against *P. cynomolgi*

Compd	R	Dose, mg/kg (oral)	Cures[a]	Relapses[b]
7a	*p*-$FC_6H_4CH_2O$	1.0	1/1	
		0.5	1/2	(91)
		0.25	1/3	(5, 16)
7b	*p*-$CF_3C_6H_4CH_2O$	1.0	2/2	
		0.5	1/2	(5)
		0.25		(9)
7c	*m*-$CF_3C_6H_4CH_2O$	1.0		(13)
		0.5		(6)
7d	*p*-$ClC_6H_4CH_2O$	10.0	Toxic	
		1.0		(9)
7e	2,4-$Cl_2C_6H_3CH_2O$	1.0	1/1	
		0.5		(8)
7f	*p*-$CH_3OC_6H_4CH_2O$	10.0		(18)
		1.0		(7)
12a	$C_6H_5CH_2S$	1.0	1/2	(15)
		0.5		(9)
12b	*p*-$ClC_6H_4CH_2S$	10		(7)
		0.5		(7)
7 P[c]	$C_6H_5CH_2O$	1.5	1/1	
		0.75	4/4	
		0.50	10/12	(11, 22)
		0.375	0/2	(11, 14)

[a] Monkeys that do not relapse in 90 days are considered cured. [b] The number represents the relapse day. [c] P = primaquine phosphate, included for comparison.

KBr) and NMR (Hitachi Perkin-Elmer R20A high-resolution NMR spectrophotometer and Me_4Si as internal reference) spectra were obtained for all new compounds ($CDCl_3$, Me_2SO-d_6). TLC were performed on Eastman chromatogram sheets, type 6060 (silica gel).

General Preparation of 2-Benzyloxy-6-methoxy-8-nitroquinolines (3). The preparation of 2-(*p*-trifluoromethylbenzyloxy)-6-methoxy-8-nitroquinoline (**3b**) is presented as an example; the remaining derivatives of **3** were obtained by essentially the same procedure (Table I).

A mixture of 12 g (0.05 mol) of 2-chloro-6-methoxy-8-nitroquinoline (**1**),[6] 12.3 g (0.07 mol) of (*p*-trifluoromethyl)benzyl alcohol (**2**),[13] 6.9 g (0.05 mol) of anhydrous K_2CO_3, and 50 mL of DMF was heated with stirring for 14 h in an oil bath (155–160 °C) under a nitrogen atmosphere. The mixture was poured into cold water, stirred briefly, and filtered. The solid was recrystallized from 95% EtOH to give 13.7 g (72%) of **3b**: mp 126–127 °C. Anal. ($C_{18}H_{13}N_2O_4F_3$) C, H, N.

General Preparation of 2-Benzyloxy-6-methoxy-8-aminoquinolines (4). The preparation of 2-(*p*-trifluoromethylbenzyloxy)-6-methoxy-8-aminoquinoline (**4b**) is presented as an example;[7] the remaining derivatives of **4** were obtained by essentially the same procedure (Table I).

A mixture of 7.6 g (0.02 mol) of **3b**, 100 mL of toluene–95% EtOH (1:1), 10 mL (0.17 mol) of 85% hydrazine hydrate, and 3 g (wet weight, washed with EtOH) of Raney nickel catalyst (W. R. Grace no. 28) was heated under reflux for 5 h. The condenser was removed and the mixture heated until the vapors were faintly alkaline (0.75 h, EtOH added). The mixture was filtered over Celite, charcoaled, and concentrated in vacuo. The solid residue was recrystallized from Et_2O–petroleum ether to give 5.4 g (77%) of **4b**: mp 133–134.5 °C. Anal. ($C_{18}H_{15}N_2O_2F_3$) C, H, N.

General Preparation of 2-Benzyloxy-6-methoxy-8-(4-amino-1-methylbutylamino)quinoline Maleates (7). The preparation of 2-(*p*-trifluoromethylbenzyloxy)-6-methoxy-8-(4-amino-1-methylbutylamino)quinoline maleate (**7b**) is presented as an example; the remaining derivatives of **7** were obtained by essentially the same procedure (Table I).

A mixture of 7.0 g (0.02 mol) of **4b**, 6.0 g (0.02 mol) of 2-bromo-5-phthalimidopentane (**5**), and 2.0 g (0.02 mol) of triethylamine[11] was stirred and heated at 135 °C for 20 h. After 1 h, 2.0 g of triethylamine was added; after 6 h, 6.0 g of **5** and 2.0 g of triethylamine were added. The mixture was diluted with Et_2O, and the insoluble triethylamine hydrobromide was separated by filtration. The Et_2O filtrate was concentrated and the residue was refluxed with 150 mL of 95% EtOH and 15 mL of 85% hydrazine hydrate for 3 h.[6,12] The EtOH was removed in vacuo, and the residual solid was stirred with 40 g of 50% aqueous KOH

and Et_2O for 0.5 h. The Et_2O layer was separated and the aqueous portion was extracted with Et_2O. The combined Et_2O extracts were washed with H_2O and saturated NaCl solution and dried (Na_2SO_4). After concentration in vacuo, the residual oil was redissolved in anhydrous Et_2O and treated with a solution of 3.0 g of maleic acid in MeOH. The light brown solid was collected and recrystallized from EtOH–Et_2O to yield 6.8 g (62%): mp 161.5–163.5 °C. Anal. ($C_{27}H_{30}N_3O_6F_3$) C, H, N.

2-Benzylthio-6-methoxy-8-nitroquinoline (9a). A mixture of 12.4 g (0.1 mol) of benzyl mercaptan (8, α-toluenethiol), 24.0 g (0.1 mol) of **1**, 11.0 g (0.11 mol) of triethylamine, and 100 mL of DMF was heated at 100 °C for 48 h. The dark solution was poured into ice–H_2O. A brownish solid was collected and recrystallized from benzene as a yellow brown material: mp 132–134 °C (66% yield). Anal. ($C_{17}H_{14}N_2O_3S$) C, H, N, S.

Compound **9b** was prepared similarly (Table I).

2-Benzylthio-6-methoxy-8-aminoquinoline (10a). A mixture of 16.3 g (0.05 mol) of **9a**, 150 mL of H_2O, 2.5 mL of HOAc, and 17.5 g of iron fillings was stirred for 24 h at 80–90 °C.[9] The mixture was filtered and washed with warm H_2O and Me_2CO. The aqueous Me_2CO solution was extracted with Et_2O, dried (Na_2SO_4), treated with charcoal, and concentrated in vacuo. The solid residue was covered with petroleum ether, collected and recrystallized from EtOH–H_2O. The product (14.5 g, 98%) melted at 92–94 °C. Anal. ($C_{17}H_{16}N_2OS$) C, H, N, S.

Compound **10b** was obtained by reduction of **9b** with alcoholic hydrazine hydrate and 10% Pd/C[8] (Table I).

2-Benzylthio-6-methoxy-8-(4-amino-1-methylbutylamino)quinoline Maleate (12a). Compound **12a** (and **12b**) was obtained by the alkylation[11] and hydrazinolysis[12] method employed for **7a–f** (Table I).

2-(*p*-Chlorobenzyloxy)-6-methoxy-8-(4-phthalimido-1-methylbutyl)aminoquinoline (6d). In only one case was the intermediate phthalimido derivative **6** isolated.

A mixture of 6.3 g (0.2 mol) of **4d**, 6.0 g (0.02 mol) of **5**, 8.2 g (0.1 mol) of NaOAc, and 150 mL of 66% EtOH–H_2O was refluxed for 96 h.[10] On the third day, 0.03 mol of **5** and 0.1 mol of NaOAc were added to the reaction mixture; on the fourth day 0.1 mol of NaOAc was added. At the end of the reflux period the mixture was saturated with K_2CO_3 and EtOH removed in vacuo. The mixture was diluted with H_2O and extracted with Et_2O. The Et_2O was washed with H_2O and saturated NaCl solution, and the solution was dried ($MgSO_4$) and concentrated in vacuo. The residual oil was dissolved in 200 mL of MeOH and treated with excess 48% HBr. Dilution of this mixture with anhydrous Et_2O (300 mL) precipitated 4.5 g (47%) of salt which proved to be **4d**. Further dilution with Et_2O to a total volume of 1 L, and cooling for 2.5 days, gave 3.4 g (25%) of a salt (**6d**·HBr). The free base **6d** was liberated by aqueous Na_2CO_3 and recrystallized from EtOH: mp 94.5–96 °C. Anal. ($C_{30}H_{28}N_3O_4Cl$) C, H, N, Cl.

Isolation of **6d** in the triethylamine method[11] resulted in a 69% yield of the free base as compared to the 20–25% yields obtained by the NaOAc buffer method.[10]

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Synthesis of 5,6-Dihydro-8(7*H*)-quinolinone Thiosemicarbazones as Potential Antitumor Agents

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5,6-Dihydro-8(7*H*)-quinolinone was synthesized and converted into thiosemicarbazones which could be considered to be semirigid analogues of the 2-formylpyridine thiosemicarbazone class of antitumor agents. The *Z* and *E* isomers were separated and identified by ^1H NMR and UV. Although the compounds showed essentially no inhibitory activity against the enzyme alkaline phosphatase, several of these agents had demonstrable anticancer activity in mice bearing the P388 leukemia. The *E*-configuration analogues in general were slightly more active than their corresponding *Z* isomers.

Since the discovery by Brockman et al.[1] that 2-formylpyridine thiosemicarbazone (PT, 1) had antileukemic activity in mice, a large number of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones have been synthesized