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Synthesis of Alkyl-4,7-dioxobenzothiazoles with Prophylactic Antimalarial Activity†

Martin D. Friedman, Philip L. Stotter, Thomas H. Porter, and Karl Folkers*

Department of Chemistry and Institute for Biomedical Research, The University of Texas at Austin, Austin, Texas 78712.
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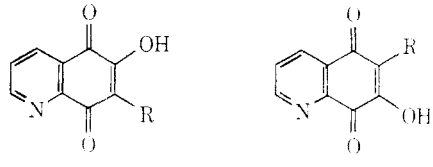
5-*n*-Undecyl- and 5-*n*-pentadecyl-6-hydroxy-4,7-dioxobenzothiazoles have been synthesized as examples of new lipoidal benzothiazoloquinones for study as potential antimetabolites of coenzyme Q. The 5-*n*-undecyl-6-hydroxy-4,7-dioxobenzothiazole showed exemplary and effective prophylactic activity against *Plasmodium gallinaceum* in the chick without toxicity.

Recent studies[1] have shown that a variety of substituted quinones (*e.g.*, structures 1 and 2) function as effective *in vivo* antimalarial agents, presumably as antimetabolites of coenzyme Q_8[2] (CoQ_8) **3**, which is intrinsic to the growth of the malaria-causing protozoan genus, *Plasmodium*. *In vitro* inhibition has been demonstrated by such analogs of coenzyme Q for both the succinoxidase and DPNH-oxidase enzyme systems which need coenzyme Q.[3] On the basis of the effective inhibition observed for such



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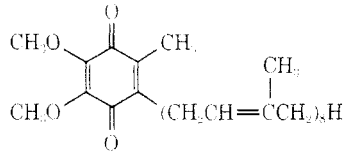

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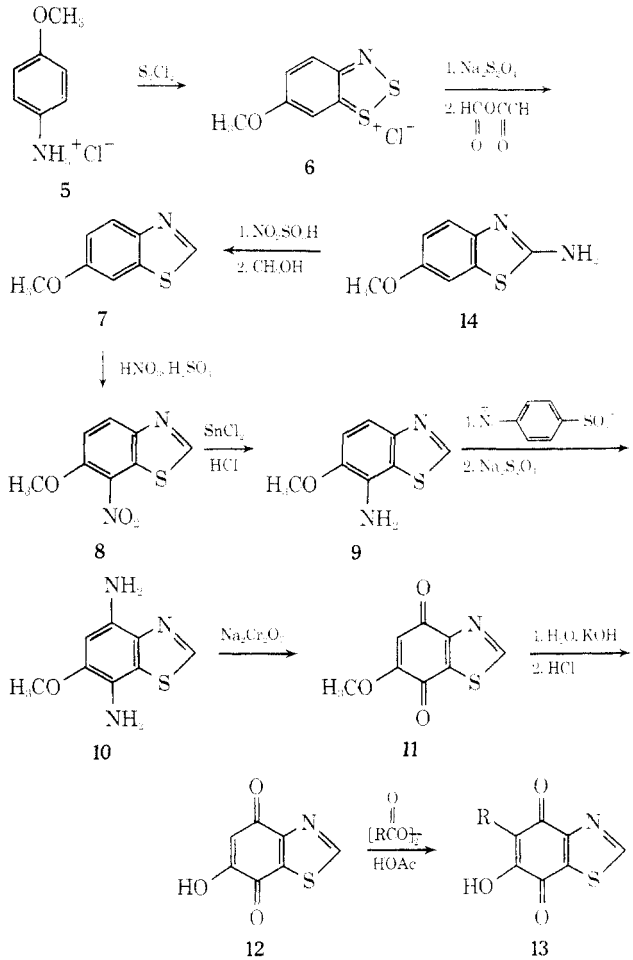
coenzyme Q analogs and the obvious possibility of their use as antimalarial agents, we have been investigating other fused bicycloheterocyclic quinones with the hope of designing even better new antimalarial agents, as well as elucidating the nature of an inhibition mechanism and/or the enzymatic site of CoQ.

The synthesis of two alkyl derivatives of the previously unknown 6-hydroxy-4,7-dioxobenzothiazole (**12**) is described herein (Scheme I), and exemplary assay data for the undecyl derivative are included (Table I).

6-Methoxybenzothiazole (**7**) was prepared by two methods. In 62% yield, by a modification of a known procedure,[4] *p*-anisidine hydrochloride (**5**) was converted to **7** by sequential exposure to sulfur monochloride, aqueous base, sodium dithionite, and formic–acetic anhydride[5] (Scheme I). After distillation and one recrystallization, the 6-methoxybenzothiazole melted at 66–68°. 6-Methoxybenzo-

Scheme I



† Coenzyme Q. 166. Antimetabolites of Coenzyme Q. 21.

Table I. Prophylactic Antimalarial Activity[a] of 5-*n*-Undecyl-4,7-dioxobenzothiazole (**13a**)

Dose, mg/kg	$T - C$[b]	Cures (deaths)
30	2.5	1/5 (0/5)[c]
60	3.0	2/5 (0/5)
120	3.0	4/5 (0/5)
240	5.0	4/5 (0/5)

[a] *In vivo* antimalarial activity against *P. gallinaceum* in the sporozoite-induced chick test. All compounds were administered subcutaneously to groups of five chicks. [b] $T - C$ = Change in survival time, in days, of treated and nontreated (control) chicks. [c] Drug toxicity deaths.

thiazole (**7**) was also prepared *via* a diazotization-reduction[6] sequence from commercially available 2-amino-6-methoxybenzothiazole (**14**) in about 30% yield from **14**. The benzothiazole **7** after distillation and one recrystallization melted at 69.5–71°. The second method of synthesis was preferred because of fewer purification problems. Furthermore, the diazotization–reduction procedure was convenient for the larger scale preparation of **7**.

Nitration of **7** followed by reduction of the resulting 7-nitro compound **8** afforded 6-methoxy-7-aminobenzothiazole (**9**) in about 90% yield. Coupling of **9** with diazotized sulfanilic acid and reduction of the intermediate azo compound with sodium dithionite produced the unstable diamine **10**, which was directly oxidized to 6-methoxy-4,7-dioxobenzothiazole (**11**). Exposure of **11** to aqueous alkali produced the hydroxylated quinone **12**. Free-radical alkylation of **12** yielded two 5-alkyl-6-hydroxy-4,7-dioxobenzothiazoles **13**.

Biological Results. 5-*n*-Undecyl-6-hydroxy-4,7-dioxobenzothiazole (**13a**) was tested for prophylactic antimalarial activity against *P. gallinaceum* in chicks[7] (Table I) and was found to show prophylactic activity (4/5 cures, 0/5 deaths) at 120 mg/kg in this sporozoite-induced malaria. One out of five cures was effected at 30 mg/kg in this routine one-dose (sc) assay.

Experimental Section

All melting points were taken on a Thomas-Hoover melting point apparatus and are uncorrected. Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements were within ±0.4% of the theoretical values. Nmr spectra were taken on a Varian A-60 spectrometer.

6-Methoxybenzothiazole (7). Method A. A mixture of 150 g (0.94 mol) of *p*-anisidine hydrochloride and 1 kg (7.4 mol) of sulfur monochloride was stirred at room temperature for 20 hr and then was stirred at 70° for 5 hr. The mixture was poured into 1 l. of benzene, and the orange precipitate was filtered, washed three times with benzene, and dried *in vacuo* (yield, 215 g). To a solution of 30 g of this product in 3 l. of ice water was added 210 ml of 4 *N* NaOH, followed by 40 g (0.23 mol) of $Na_2S_2O_4$. The mixture became homogeneous after being heated to boiling, except for a small amount of insoluble material, from which the solution was easily decanted. This light green solution was cooled to 4°, and 100 ml of formic–acetic anhydride[5] was added. The temperature was maintained at 4° for 24 hr, at which time a crude tan precipitate was isolated. Vacuum distillation [120–125° (2 mm)] of this solid, followed by recrystallization from hexane, gave 24.5 g (62% based on *p*-anisidine hydrochloride) of colorless solid, mp 66–68° (lit.[4] 72.5°). Further attempts at additional purification were not effective.

Method B.[5] To a solution of 1.38 g (0.016 mol) of $NaNO_2$ in 10 ml of H_2SO_4 cooled to 0° was added 3.6 g (0.02 mol) of 2-amino-6-methoxybenzothiazole dissolved in 20 ml of HOAc; the reaction temperature was maintained below 5°. The solution was stirred 15 min and poured into 100 ml of methanol. Methanol reduction of the diazonium salt was completed by refluxing for 15 min. The solvents were evaporated, and the residue was partitioned between water and ether. The extract was dried and evaporated, and the residue was purified by vacuum distillation and subsequent recrystallization: yield 955 mg (29%); mp 69.5–71°; nmr ($CDCl_3$) δ 3.80 (s, 3 H), 7.09 (d of d, 1 H), 7.36 (d, 1 H), 8.03 (d, 1 H), 8.80 (s, 1 H).

7-Nitro-6-methoxybenzothiazole (8). 6-Methoxybenzothiazole (1.4 g) in 6 cc of concentrated H_2SO_4 and 6 cc of HNO_3 (at 25–30°), followed by recrystallization of the product from $CHCl_3$, gave 1.5 g (87%) of yellow product: mp 198–200° (lit.[4] 202°); nmr ($CDCl_3$) δ 4.12 (s, 3 H), 7.36 (d, 1 H), 8.37 (d, 1 H), 8.99 (s, 1 H).

7-Amino-6-methoxybenzothiazole (9). To 300 mg (0.0016 mol) of $SnCl_2$ dissolved in 1.2 ml of concentrated HCl at 0° was added 100 mg (0.471 mmol) of 7-nitro-6-methoxybenzothiazole.[4] The mixture was stirred at room temperature for 30 min and then at 60° for 1 hr. After dilution with 5 ml of water, this solution was extracted with 15 ml of CH_2Cl_2. The water layer was made basic with K_2CO_3 and extracted three more times with 15 ml of CH_2Cl_2. The combined extract was dried over K_2CO_3, filtered, and evaporated to yield 80 mg (93%) of colorless crystalline product: mp 128–130° (lit.[4] 130.5°); nmr ($CDCl_3$) δ 3.91 (s) superimposed on a broad singlet at 4.04 (5 H), 7.08 (d, 1 H), 7.59 (d, 1 H), 8.78 (s, 1 H).

6-Methoxy-4,7-dioxobenzothiazole (11). A solution of 5.9 g (0.034 mol) of sulfanilic acid and 2.5 g (0.031 mol) of sodium acetate in 75 ml of 50% acetic acid was cooled to 0°, and 2.5 g (0.036 mol) of $NaNO_2$ was added. The orange solution was stirred at 9° for 30 min and 5.6 g (0.031 mol) of 6-methoxy-7-aminobenzothiazole[4] (**9**) in 50% acetic acid was added dropwise. The deep purple suspension was stirred at 0° for 30 min and filtered; the black filter cake was repeatedly washed with 10% acetic acid until the wash was colorless. To a solution of this solid in 100 ml of 5% NaOH was slowly added 100 g (0.575 mol) of $Na_2S_2O_4$; chloroform extracts of the resulting yellow solution yielded the crude red diamine **10** as a solid after removal of solvent.

This red solid was dissolved in a solution of 15 ml of 12 *N* H_2SO_4 and 100 ml of H_2O; oxidation was effected by addition of a chromic acid solution prepared from 20 ml of 10% $Na_2Cr_2O_7$ and 15 ml of 12 *N* H_2SO_4. After 1 min, an additional portion of chromic acid (from 50 ml of 10% $Na_2Cr_2O_7$ and 7 ml of 12 *N* H_2SO_4) was added, and the resulting brown solution was repeatedly extracted for 1.5 hr with chloroform. From the extract was obtained a crude green solid which was recrystallized from ethanol yielding 3.8 g (63%) of yellow crystalline product, mp 243–244°. The substance was leucomethylene blue positive: nmr ($CDCl_3$) δ 3.89 (s, 3 H), 3.51 (s, 1 H), 9.12 (s, 1 H). *Anal.* ($C_8H_5NO_3S$) C, H, N, S.

6-Hydroxy-4,7-dioxobenzothiazole (12). Quantitative conversion of **11** to **12** was observed when 4.0 g (0.021 mol) of **11** was shaken for 2.5 min with 40 ml of 0.5 *N* KOH. Acidification with 10% HCl precipitated 3.8 g of yellow powder.

5-*n*-Undecyl-6-hydroxy-4,7-dioxobenzothiazole (13a). To a solution of 20 g (0.1 mol) of lauric acid in 300 ml of dry benzene was added 45 ml of thionyl chloride, and the green solution was refluxed overnight. The volatile materials were removed *in vacuo*, and residual thionyl chloride was removed by addition and evaporation of three additional portions of benzene. The crude acid chloride was dissolved into 400 ml of ether; at 0°, 17 ml of 30% H_2O_2 and 20 ml of pyridine were successively added dropwise. This mixture was stirred at room temperature for 20 min, and the layers were separated. The organic layer was washed successively with 5% HCl, water, and 2% NaOH and was finally dried over $MgSO_4$, filtered, and evaporated to give lauroyl peroxide.

To a solution of 3.0 g (0.017 mol) of hydroxyquinone **12** in 400 ml of acetic acid initially heated to 90° was added the lauroyl peroxide over a period of 2 hr. The temperature of the reaction was maintained between 80 and 85° during the addition and for an additional 5.5 hr. The reaction mixture was cooled to room temperature, stirred overnight, and filtered, and the filtrate was concentrated at reduced pressure. After the acetic acid was removed, the residue was dissolved in ether. After washing with water, the ether was evaporated, and the residue was chromatographed (silica gel, ether eluent). The orange fractions were combined and recrystallized from hexane–ether: yield 0.85 g (15.8%) of orange solid; mp 131–132°; nmr ($CDCl_3$) δ 0.90, 1.26 (m, 21 H), 2.59 (t, 2 H), 9.14 (s, 1 H). *Anal.* ($C_{18}H_{25}NO_3S$) C, H, N.

5-*n*-Pentadecyl-6-hydroxy-4,7-dioxobenzothiazole (13b). Palmitic acid (75 g, 0.29 mol) and 135 ml of thionyl chloride were dissolved in 900 ml of dry benzene and refluxed for 12 hr. The benzene was evaporated, and residual thionyl chloride was removed by three distillations with 100 ml of benzene. Vacuum distillation gave 49.2 g (61%) of palmitoyl chloride, bp 165–170° (1 mm).

Palmitoyl chloride (49 g, 0.178 mol) was dissolved in 500 ml of ether and cooled to 0°, and 500 ml of 30% H_2O_2 was added dropwise. After the solution was stirred for 20 min, 50 ml of pyridine was added, and the resulting solution was stirred for an additional 30 min at room temperature. The ether layer was washed with 250 ml of 5% HCl, water, and 2% NaOH, dried over $MgSO_4$, and evaporated: yield 30 g (62%).

6-Hydroxy-4,7-dioxobenzothiazole (4.0 g, 0.022 mol) was dissolved in 500 ml of acetic acid. The temperature was raised to 90°, and palmitoyl peroxide (16 g, 0.031 mol) in 300 ml of ether was added over a 4-hr period. The solution was filtered to give 1.7 g of starting material. The filtrate was stirred overnight, and the solvent was evaporated. The remaining oil was placed on a silica gel column and eluted with ether. The yellow fractions were combined, and the solvent was removed. Two recrystallizations from hexane–ether gave 0.32 g of orange solid (6.5%): mp 129–130°; nmr ($CDCl_3$) δ 0.90, 1.26 (m, 29 H), 2.61 (t, 2 H), 9.16 (s, 1 H). *Anal.* $C_{22}H_{33}NO_3S$) C, H, N.

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Acute Oral Toxicity of 2-Alkyl- and 2,6-Dialkylanilines. Correlation with Lipophilicity

John A. Durden, Jr.

Research and Development Department, Chemicals and Plastics Division, Union Carbide Corporation, South Charleston, West Virginia 25303. *Received May 24, 1973*

Jacobson[1] has recently presented data pertaining to the acute rat oral toxicity of a group of 2-alkyl- and 2,6-dialkylaniline derivatives. Also presented was a very brief discussion of the structure–activity relationships which may be operative in this series.

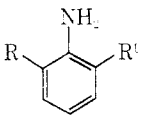
A high correlation (r = 0.926) has now been found between these toxicity data and the partition coefficients (P)[2] of the corresponding anilines. Regression of the acute oral data against log P provided a relationship which accounted for 85.9% of the observed variation in data. Inclusion of a parameter (E_s)[3,4] related to the steric requirement of the ortho substituents(s) did not significantly change the accountability of eq 1. The correlation (r =

$$\log 1/C = 2.551 - 0.274 \log P \qquad (1)$$

$s = 0.0503$; $MR^2 = 0.8586$; $F = 36.45$; $r = 0.926$

0.775, accountability 60.1%) between E_s and log $1/C$ was also significantly less than that observed with P. It should be noted that the correlation between P and E_s for these compounds is quite high (r = 0.894); about 80% of the variation in P is accounted for by the variation in E_s. Although there may be steric effects influencing the activity of these compounds, the high correlation with P suggests that relative lipophilicity (P) is probably the primary toxicity determinative. If other than alkyl substituents were included in the series almost certainly electronic or steric effects would begin to play a role along with lipophilicity. The importance of P may reflect a lipophilicity-related *in vivo* distribution effect[5] and/or a direct relationship between lipophilicity and *in vivo* degradation (metabolism, conjugation)[6] for these materials.

Table I. Structural Parameters and Observed and Calculated Toxicity Values



No.	R	R¹	Log P	E_s, R,R¹	Rat peroral LD_{50} values, g/kg Obsd	Calcd
1	H	H	0.90[a]	2.48	0.44	0.46
2	H	CH_3	1.30[a]	1.24	0.90	0.68
3	CH_3	CH_3	1.70	0	0.84	1.0
4	H	C_2H_5	1.80	1.07	1.26	1.06
5	C_2H_5	C_2H_5	2.70	−0.14	2.69	2.31
6	H	$CH(CH_3)_2$	2.10	0.75	1.18	1.43
7	$CH(CH_3)_2$	$CH(CH_3)_2$	3.30	−0.98	4.27	4.00
8	CH_3	C_2H_5	2.20	−0.07	1.18	1.52

[a] From ref 2; the remainder was calculated by standard methods.

The data employed in this study are recorded in Table I together with the calculated toxicities from eq 1. Log $1/C$ in eq 1 is calculated by eq 2.

$$\log 1/C = -\left[\log \frac{100 \times LD_{50}}{\text{mol wt}}\right] + 2 \qquad (2)$$

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