Synthesis of New Alkylamino- and Alkylaminomethyl-5,8-quinolinequinones as Inhibitors of Coenzyme Q and as Antimalarials†

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A series of fifteen 6-alkylamino-5,8-quinolinequinones and 7-*n*-tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone have been synthesized and evaluated for antimalarial activity against *Plasmodium berghei* in the mouse. Representative compounds were tested in mitochondrial NADH- and succinoxidase systems for inhibition of coenzyme Q. The 6-alkylamino-5,8-quinolinequinones, represented in the assay by 7 of the 15 compounds, were highly inhibitory to NADH-oxidase and the inhibition was completely reversed by CoQ_{10}. These 7 quinolinequinones were even more inhibitory to succinoxidase than to NADH-oxidase, but the inhibition was not reversed by CoQ_{10}. The inhibition of 6-alkylamino-5,8-quinolinequinone was, in general, 5-fold less than that by the 7-alkyl-6-hydroxy-5,8-quinolinequinones. Ten of the 16 compounds showed definite antimalarial activity against *P. berghei* in the mouse, and 3 met the arbitrary criterion of effectiveness to be declared "active," and none showed any apparent toxicity at the highest level tested. As representative of another category, 7-*n*-tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone was inactive both as an *in vitro* inhibitor of coenzyme Q and against *P. berghei*.

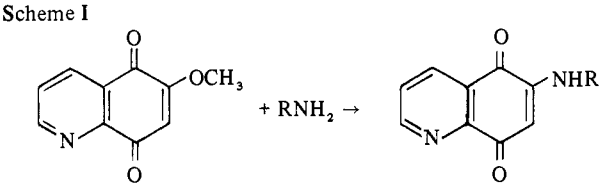
The background research for this work has been described elsewhere.[1] A series of 7-alkyl-6-hydroxy-5,8-quinolinequinones and 6-alkyl-7-hydroxy-5,8-quinolinequinones were synthesized by alkylation of 6-hydroxy-5,8-quinolinequinone and 7-hydroxy-5,8-quinolinequinone, respectively, with the appropriate diacyl peroxide, and tested against *Plasmodium berghei* in the mouse and *P. gallinaceum* in the mosquito.[1] Antimalarial activity accompanied by no observable toxicity at the highest level tested was demonstrated for a number of these lipoidal 5,8-quinolinequinones. An alkyl side chain length of about 15 CH_2 units was apparently necessary for maximum activity.[1] Most of these compounds were evaluated in mitochondrial NADH- and succinoxidase systems for inhibition of coenzyme Q, and these *in vitro* data were compared with the data obtained from the *in vivo* evaluation of these compounds against *P. berghei* in the mouse and *P. gallinaceum* in the mosquito.‡

7-*n*-Hexylaminomethyl-6-hydroxy-5,8-quinolinequinone was synthesized by Pratt and Drake[2] by a Mannich reaction of *n*-hexylamine with 6-hydroxy-5,8-quinolinequinone, and this substance showed significant amebicidal activity against infection in the guinea pig induced by *Entamoeba histolytica*.[3]

The syntheses and biological activities of a number of new 6-alkylamino-5,8-quinolinequinones and one new 7-*n*-alkylaminomethyl-6-hydroxy-5,8-quinolinequinone are described herein. Although several of these two types of 5,8-quinolinequinones were previously prepared by Pratt and Drake,[2,4,5] it is of current interest to prepare new 6-alkylamino-5,8-quinolinequinones with relatively longer alkyl side chains to increase the lipoidal character of the molecule and with alkyl side chains containing varying numbers of heteroatoms in an attempt to design molecules which could function as antimetabolites of the highly lipoidal coenzymes Q. Designing inhibitors based on the structure of coenzyme Q may be more productive in finding new antimalarials than design based on the lipophilic-hydrophilic balance,[6,7] although the latter concept can be relevant to the objective.

Organic Syntheses. The preparation of the 6-alkylamino-5,8-quinolinequinones was accomplished by treating 6-methoxy-5,8-quinolinequinone in EtOH with the appropriate alkylamine as indicated in Scheme I. The MeO group of 6-methoxy-5,8-quinolinequinone, like that of the corresponding naphthoquinone,[8-10] could be replaced by an amino group upon direct interaction with the appropriate alkylamine. Generally, the amines reacted very readily with 6-methoxy-5,8-quinolinequinone, especially the short unbranched aliphatic primary amines. Each of the 6-alkylamino-5,8-quinolinequinones was a red or orange-red cryst substance, which was apparently stable at room temp.

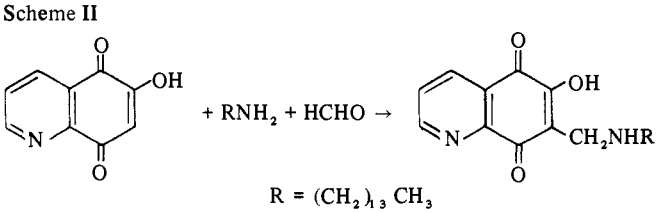
Scheme I



R	R
$(CH_2)_3CH_3$	$(CH_2)_2C_6H_5$
$(CH_2)_6CH_3$	$(CH_2)_2NHCH_2$-4-pyridyl
$(CH_2)_9CH_3$	$(CH_2)_3N[(CH_2)_3CH_3]_2$
$(CH_2)_{11}CH_3$	$(CH_2)_4$-*c*-C_6H_{11}
$(CH_2)_{13}CH_3$	$(CH_2)_2$-morpholino
$(CH_2)_{14}CH_3$	$(CH_2)_3$-piperidino
$(CH_2)_{15}CH_3$	*c*-C_7H_{13}
	c-C_8H_{15}

The 7-alkylaminomethyl-6-hydroxy-5,8-quinolinequinone was prepared[2,11] from 6-hydroxy-5,8-quinolinequinone by a modification of the Mannich reaction with *n*-tetradecylamine as depicted in Scheme II. Most of the starting materia

Scheme II



R = $(CH_2)_{13}CH_3$

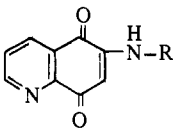
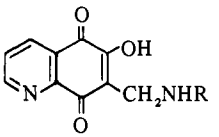
appeared to dissolve before precipitation of the product in high yield occurred. After stirring at room temp, the reaction mixture was filtered, and the product was recrystallized to give a dark red powder.

Antimalarial Test Results. These compounds were tested for antimalarial activity against *P. berghei* in mice[12] and against *P. gallinaceum* in the infected mosquito *(Aedes aegypti)*.[12] A single dose at the desired level is given sc 72 hr after the mice are infected with *P. berghei*. A minimum mean survival time of 13.0 days is required for the com-

†Coenzyme Q. 139.

‡C. M. Bourman, F. S. Skelton, T. H. Porter, and K. Folkers, unpublished results.

Table I. Antimalarial Activity of Certain 6-Alkylamino-5,8-quinolinequinones and 7-Tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone

Compd No.	R	Mp, °C	% yield[c]	*In vivo* antimalarial activity, mouse test[a] (*P. berghei*) $T - C$,[b] mg/kg	Toxicity, mg/kg
					
1	$(CH_2)_3CH_3$	148–150	82[d]	4.3 at 320; 5.9 at 640	3/5 deaths at 640
2	$(CH_2)_6CH_3$	116–117	61[d]	0.5 at 640	
3	$(CH_2)_9CH_3$	118–120	48[g]	0.4 at 640	
4	$(CH_2)_{11}CH_3$	118–120	56[g]	0.3 at 640	
5	$(CH_2)_{13}CH_3$	116–118	33[e,f,g]	0.9 at 640	
6	$(CH_2)_{14}CH_3$	119–120	39[g]	0.3 at 640	
7	$(CH_2)_{15}CH_3$	117–119	28[g]	0.5 at 320	
8	$(CH_2)_2C_6H_5$	195–197	51[g]	4.1 at 320; 7.3 at 640	0/5 deaths at 640
9	$(CH_2)_2NHCH_2$-4-pyridyl	152–154	28[e,g]	0.9 at 160	3/5 deaths at 160
10	$(CH_2)_3N[(CH_2)_3CH_3]_2$	88–89	28[e,g]	5.7 at 160	3/5 deaths at 160
11	$(CH_2)_4$-*c*-C_6H_{11}	161–162	67[g]	4.0 at 640	
12	$(CH_2)_2$-morpholino	183–184	46[d]	1.9 at 160	2/5 deaths at 160
13	$(CH_2)_3$-piperidino	142–146	44[e,g]	1.1 at 40	5/5 deaths at 80
14	-*c*-C_7H_{13}	157–159	35[d,f]	6.5 at 320; 7.9 at 640	0/5 deaths at 640; 0/5 deaths at 640
15	-*c*-C_8H_{15}	135–136	27[d,f]	6.9 at 640	
					
16	$(CH_2)_{13}CH_3$	150 dec 145	66[d]	0.3 at 160	

[a] All compds were administered sc, in graded doses, to groups of 5 mice. [b] $T - C$ = Change in survival time, in days, of treated and non-treated (control) mice. [c] Yields are based on starting quinone. [d] In this reaction, there was a molar excess of starting amine. [e] Yield from pooling product from 2 reactions followed by silica gel chromatog and elution with Et_2O–EtOH. [f] Work-up included silica gel chromatog and then recrystn. [g] In this reaction, there was a molar excess of starting quinone.

pound to be declared active. Mice living 60 days or more after treatment are considered as cured.

Ten of the fifteen 6-alkylamino-5,8-quinolinequinones showed definite activity in the *in vivo* antimalarial test against *P. berghei* in the mouse by procedures devised by Rane. Three of the 10 compounds, 6-phenethylamino-5,8-quinolinequinone ($T - C$ = 7.3 at 640 mg/kg), and 6-cyclooctylamino-5,8-quinolinequinone ($T - C$ = 6.9 at 640 mg/kg), and 6-cycloheptylamino-5,8-quinolinequinone ($T - C$ = 6.5 at 320 mg/kg) were declared "active" by the arbitrary criterion of 100% increase or greater in the survival time for antimalarial activity against *P. berghei*; no toxicity was evident. Two additional compounds, 6-*n*-butylamino-5,8-quinolinequinone ($T - C$ = 5.7 at 160 mg/kg) were nearly "active" by this criterion. However, both of these 2 latter derivatives showed some toxicity at these dose levels (Table I). Apparently, the length of the alkyl side chain and the placement of an additional heteroatom in the side chain contribute to the toxicity of these latter compounds, **1** and **10**, respectively.

Unlike the similar 7-alkyl-6-hydroxy-5,8-quinolinequinones,[1] the tetradecylaminomethyl derivative was inactive against *P. berghei* in mice at 160 mg/kg, the highest level tested. A comparison of the values of $T - C$ for 7-*n*-pentadecyl-6-hydroxy-5,8-quinolinequinone (8.1 at 160 mg/kg)[1] with 7-*n*-tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone (0.3 at 160 mg/kg) demonstrates the inactivating effect of the secondary amino function one methylene group from the ring in the side chain.

Inhibition of CoQ_{10} Enzyme Systems. The methodology for the testing of these 6-alkylamino-5,8-quinolinequinones and the 7-*n*-tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone has been described.§ The data are summarized in Table II for 7 representative 6-alkylamino-5,8-quinolinequinones. The tests were conducted in the Warburg respirometer using mitochondria from beef heart, and coenzyme Q_{10} was used for the tests for reversal of the inhibition. The concn of the inhibitor to cause 50% inhibition was determined, and the specific activity was based on microatoms of O/min per mg of protein.

The 6-alkylamino-5,8-quinolinequinones at 14–41 mμmoles caused approximately 50% inhibition of NADH-oxidase, and reversal of the inhibition was observed after the addition of approximately 5- to 15-fold amount of coenzyme Q_{10}.

These same seven quinolinequinones were a little more inhibitory against succinoxidase than NADH-oxidase, and concns of about 10–15 mμmoles caused 50% inhibition, but up to a 20-fold level of coenzyme Q_{10} on a molar basis failed to cause reversal.

Compared to the 7-alkyl-6-hydroxy-5,8-quinolinequinones,‡ these 6-alkylamino-5,8-quinolinequinones were only

§ Each flask contd 0.2 ml of KOH (20%) in the center well and 0.2 ml of enzyme in the side arm. The order of addn of reagents and quantities used were as follows: Tris-chloride (0.1 *M*; pH 7.5), 1 ml; sucrose (1 *M*), 0.5 ml; mitochondrial phospholipids (12.8 mg/ml), 0.05 ml; CoQ_{10}, 0.05 ml (in abs EtOH); inhibitor, 0.05 ml (in abs EtOH); EDTA (0.8 μ*M*/ml) 0.1 ml; cytochrome *c* (3 μg/ml of H_2O), 0.05 ml; abs EtOH (total vol not to exceed 0.1 ml).[14]

Table II. Inhibition of Coenzyme Q Enzyme Systems by 6-Alkylamino-5,8-quinolinequinones

	In vitro assay systems[a]					
	DPNH-oxidase			Succinoxidase		
Compd No.[b]	Specific activity[c]	Inhibitor concn,[d] mμmoles	% reversal[e]	Specific activity[c]	Inhibitor concn,[d] mμmoles	% reversal[e]
CoQ_{10}	0.582			0.562		
1	0.302	41	90	0.292	15	
3	0.321	17	97	0.288	10	
5	0.320	17	98	0.286	11	
6	0.328	20	95	0.290	10	
7	0.326	20	95	0.288	10	
10	0.318	14	98	0.290	9	
11	0.320	17	98	0.291	10	

[a] *In vitro* assays were conducted by the Warburg method using heavy beef heart mitochondria. § [b] 100 mμmoles of CoQ_{10} was added in each case. [c] Microatoms of O/min per mg of protein. [d] For 50% inhibition. [e] After addn of a further 200 mμmoles of CoQ_{10}.

about 0.2 as active as inhibitors of the NADH-oxidase system, and only about 0.5 as active as inhibitors of the succinoxidase system.

The 7-*n*-tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone was inactive as an inhibitor of both the NADH-oxidase and succinoxidase systems up to a level of 200 mμmoles.

Experimental Section

General Procedures. 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.

6-Alkylamino-5,8-quinolinequinones. Fifteen new 6-alkylamino-5,8-quinolinequinones were prepd by treating the appropriate alkylamine with 6-methoxy-5,8-quinolinequinone in EtOH at room temp as previously described.[4] After stirring at room temp, the reaction mixt was dild with Et_2O or hexane and placed in the freezer. Generally a dark red ppt was then collected and recrystd repeatedly from EtOH-H_2O (charcoal) or occasionally Et_2O-hexane (charcoal) or EtOH-Et_2O (charcoal). When necessary, column chromatography on the crude reaction mixt followed by crystn was used to secure a pure product (Table I).

7-*n*-Tetradecylaminomethyl-6-hydroxy-5,8-quinolinequinone.[4] This new 7-alkylaminomethyl-6-hydroxy-5,8-quinolinequinone was prepd in a manner similar to previously published procedures[2,11] by adding a mixt of *n*-tetradecylamine (1.4 g) and 37% CH_2O soln (0.8 ml) in 5 ml of EtOH to a stirred suspension of 6-hydroxy-5,8-quinolinequinone (1 g) in EtOH at room temp. The 6-hydroxy-5,8-quinolinequinone rapidly dissolved upon addn of the amine soln, and the product pptd from the reaction mixt in about 10 min, although stirring was continued for about 6 hr. The product was recrystd from EtOH to yield the red cryst substance. An analytical sample was recrystd from EtOH-$CHCl_3$.

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Mechanism of Action of Amodiaquine. Synthesis of Its Indoloquinoline Analog†

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The relationship between structural modifications in the antimalarial amodiaquine (**1a**) and the corresponding ability to complex with DNA has been studied. This led to the synthesis of ring-closed amodiaquine or 3-chloro-8-methoxy-9-diethylaminomethyl-11*H*-indolo[3,2-*c*]quinoline (**3b**). Results from DNA binding and RNA polymerase inhibition suggest that the increased binding and activity of **3b** over that of amodiaquine (**1a**) results from the increased planar area afforded by **3b**. Similar considerations also suggest that the mechanism of action of amodiaquine differs from that of chloroquine.

Amodiaquine[1] (**1a**) is, along with chloroquine, one of the most widely used drugs both for the treatment of acute malaria and for suppression.[2,3] Generally, it is pharmacologically not differentiated from chloroquine, and an analogous mechanism of action is assumed because it contains the same number of C atoms between the two side-chain nitrogens. It is our belief, however, that the side chain in amodiaquine confers special steric and electronic properties to the molecule. When ω Hückel MO calculations were performed on chloroquine and amodiaquine models,[4,§] we found a difference between the corresponding energy levels of the highest occupied molecular orbital (HOMO) and the lowest empty molecular orbital (LEMO), as seen in Table I. Amodiaquine and chloroquine differ in chemical reactivity as evidenced by the fact that the former does not form a

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§MO calculations on chloroquine have been performed previously.[5]