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Synthesis, Enzyme Inhibition, and Antitumor Activity of New 1,4-Benzoquinone Analogs of Coenzyme Q_{10}[1]

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A rationale based upon coenzyme Q_{10} (CoQ_{10}, ubiquinone) for the synthesis of potential antitumor agents constitutes a new approach in the search toward chemotherapy of cancer. The antitumor activities of 38 alkyl-1,4-benzoquinones, analogs of coenzyme Q, 24 of which are new compounds, are described. The 10 best antitumor analogs of CoQ all showed long-term cures of Walker carcinosarcoma 256 in rats. Particularly impressive were the 6-*n*-octylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 252188), which cured six out of six rats with % T/C = 584 at 3.13 mg/kg, 6-phytyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone (NSC 277818) (four out of four cures, % T/C = 923 at 50 mg/kg), and 5-phytyl-2,3-dimethoxy-1,4-benzoquinone (NSC 276371) (three out of six cures, % T/C = 789 at 0.78 mg/kg). In general, a 5-chloro or 5-hydroxy group on the quinone nucleus or a side chain with unsaturation and branching, such as the phytyl side chain of NSC 277818 and NSC 276371, seemed to increase antitumor activity. Although a perfect correlation was not to be expected, many of the most potent antitumor analogs were also among the best *in vitro* inhibitors of the mitochondrial CoQ_{10}-enzymes, succinoxidase, and NAD oxidase.

INTRODUCTION

Coenzyme Q (or ubiquinone) is an essential component of the respiratory chain with active sites in succinoxidase (*1*), NADH-oxidase (*1*), and α-glycerophosphate oxidase (*2*) of mitochondria. Nyquist *et al.* (*3*) and Fleischer *et al.* (*4*) reported the presence of coenzyme Q_{10} in the Golgi complex of the cell. Ernster *et al.* (*5*) have indicated that CoQ_{10} may act as a regulator of the interaction of NADH and succinate with the cytochrome system. The vitamin-like activity of coenzyme Q has been observed for several mammalian species on deficiency diets including the rabbit (*6*), monkey (*7*), rat (*8*), chicken and turkey (*9*), and hamster (*10*). The biochemical role of coenzyme Q in electron-transfer mechanisms and the vitamin-like activity in several mammalian species constituted the basis of our synthesis of these analogs of coenzyme Q as potential antitumor agents. Selective toxicity for tumor cells may be based on the differential inhibition of pathways.

MATERIALS AND METHODS

Antitumor Evaluation

The methods used in studying the effects of new compounds on the survival of rats with Walker carcinosarcoma 256 are described by the Drug Research and

[1] It is a pleasure to recognize the extraordinarily innovative chemistry of William S. Johnson and his leadership in the profession.

[2] Coenzyme Q 236.

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Development of the National Cancer Institute (*11*). The NSC numbers of the compounds were assigned at NCI and allow a tie-in to the records there.

In Vitro *Screening Against Walker Carcinosarcoma 256.*

An inoculum (10^5 cells) was administered intraperitoneally to Fisher 344 rats on Day 0. Test compounds were suspended in saline with Tween-80, and test suspensions were injected intraperitoneally daily for 5 days. The experiment was terminated on Day 45 or 60. Cures are the number of animals surviving on the day of evaluation in the survival system. Percentage T/C is the ratio of test (T) evaluation to control (C) evaluation, expressed as a percentage. In general, a minimal increase in survival of treated animals over controls resulting in a T/C $\geqslant$ 125% is a baseline for positive activity.

In Vitro *Enzyme Assays*

Effects of the CoQ analogs on the activity of succinoxidase and NADH-oxidase were measured and compared with the *in vivo* antitumor activity of the corresponding compounds. Beef heart mitochondria were prepared essentially as described (*12*). The final mitochondrial pellet, which was a mixture of heavy and light particles, was suspended in 0.25 *M* sucrose and was used immediately or kept in a frozen state until used. Phospholipid micelles were prepared by sonication of commercial soybean phospholipids (Asolectin) (*13*) and used instead of mitochondrial phospholipids. Protein was determined by the method of Lowry *et al.* (*14*). Succinoxidase and NADH-oxidase activities were determined manometrically in a Gilson differential respirometer. The background for assay of succinoxidase and NADH-oxidase has been described (*15*). A total of 2.6 ml of the reaction mixture, in the main compartment of each 15-ml flask, contained 1.0 ml of 0.1 *M* Tris–HCl buffer, pH 7.6; 0.5 ml of 1 *M* sucrose; 0.1 ml of 0.8 m*M* EDTA; 0.05 ml of Asolectin solution (20 mg/ml); 0.01 ml of the inhibitor or the standard dissolved in ethanol; 0.1 ml of 0.2% cytochrome *c* solution; and 0.08 ml of mitochondrial enzyme (0.65–0.8 mg of protein for succinoxidase assay and 0.52–0.68 mg of protein for NADH-oxidase assay). Then, 0.2 ml of 0.75 *M* succinate or 0.07 *M* NADH was put into the side arm, and 0.2 ml of 6 *N* KOH was put into the center well. The reaction was initiated by addition of the substrate from the side arm into the reaction mixture, and the activity was determined at 30°C.

6-ω-Cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone served as a standard inhibitor, because of its potent and stable inhibitory activity to succinoxidase and NADH-oxidase of beef heart mitochondria, as reported (*16*). To compare the inhibitory activities of the test quinones, the inhibitory activities are expressed as antimetabolite–CoQ_{10} indices (*17*) for approximately 50% inhibition of enzyme activity. This index is calculated on the basis of the nanomoles of inhibitor per nanomole of CoQ_{10}. The amount of CoQ_{10} in the mitochondrial preparation was determined by the modified Craven's assay (*18*) after extraction with pentane (*15*). The mitochondria contained approximately 3.46 nmol of CoQ_{10}/mg of mitochondrial protein.

Removal of CoQ from the mitochondria was carried out according to Szarkowska's method with slight modification (*15*). The thawed suspension of mitochondria was diluted three to four times with 0.15 *M* KCl and was centrifuged at 20,000 *g* for 20 min. The residue was washed once with 0.15 *M* KCl by a centrifugation of 20,000 *g* for 2 min, resuspended in a small volume of 0.15 *M* KCl, and lyophilized. The lyophilized

mitochondria were suspended in *n*-pentane and homogenized in a glass homogenizer loosely fitted with a Teflon pestle for a few minutes. After a centrifugation at 3000 rpm for 5 min, the pentane extract was decanted, and the residue was collected. The extraction of CoQ was performed four times in this manner. Finally, the residue was dried completely under high vacuum and then was suspended homogeneously in 0.25 *M* sucrose at a concentration of 8 to 10 mg of protein per milliliter. The suspension of pentane-extracted mitochondria was used immediately or kept in a frozen state until used. The suspension was prepared again in about 2 weeks because of the decrease in activity.

ORGANIC SYNTHESES

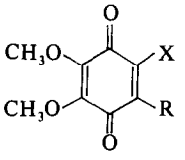
Twenty-four new alkyl-1,4-benzoquinones were synthesized by reactions which were previously used (*19–21*), and the relevant chemical data for these new quinones are in Table 2. The following two examples show general procedures as modified and currently being used.

Experimental Examples

(*1*) *2,3-Dimethoxy-5-p-dodecylbenzylmercapto-1,4-benzoquinone.* A mixture of 2,3-dimethoxy-1,4-benzoquinone (3.3 g, 20 mmol) in EtOH (75 ml) and *p*-dodecylbenzyl

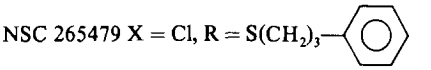
TABLE 1

BEST 10 ANTITUMOR BENZOQUINONES AGAINST WALKER CARCINOSARCOMA 256 IN RATS

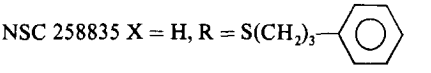


NSC 252188 X = Cl, R = $S(CH_2)_7CH_3$
NSC 220334 X = Cl, R = $S(CH_2)_{11}CH_3$
NSC 259277 X = Cl, R = $S(CH_2)_{17}CH_3$

NSC 265469 X = Cl, R = $S\,CH_2CH_2\,CH\,CH_3$

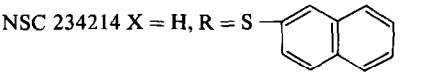


NSC 265479 X = Cl, R = $S(CH_2)_3$—



NSC 258835 X = H, R = $S(CH_2)_3$—

NSC 276371 X = H, R = S-phytyl

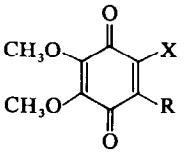


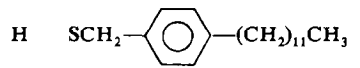
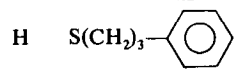
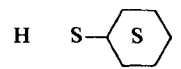
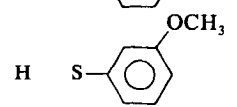
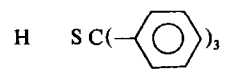
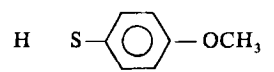
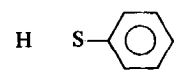
NSC 234214 X = H, R = S—

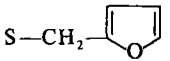
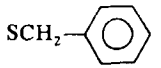
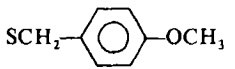
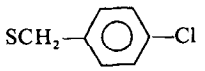
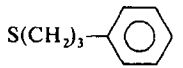
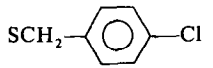
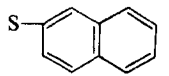
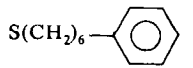
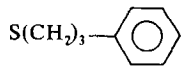
NSC 186889 X = H, R = $S(CH_2)_{11}CH_3$
NSC 277818 X = OH, R = phytyl

TABLE 2

CHEMICAL DATA FOR NEW 1,4-BENZOQUINONES



NSC No.	X	R	Yield (%)	mp (°C)	Recryst. solvent	Reaction Time (hr)	Formula
—	H	$S(CH_2)_6CH_3$	37	42–43	MeOH	~3	$C_{15}H_{22}O_4S$: C,H,S
254674	H	$S(CH_2)_9CH_3$	80	47–49	EtOH	3	$C_{18}H_{28}O_4S$: C,H,S
260616	H			Oil	Column[a]	3	$C_{27}H_{38}O_4S$: C,H,S
258835	H		75	62–64	EtOH–MeOH–Et_2O–hexane	3	$C_{17}H_{18}O_4S$: C,H,S
290503	H		50	127–128	MeOH	1.5	$C_{14}H_{18}O_4S$: C,H,S
290814	H		84	95–96	MeOH	3	$C_{15}H_{14}O_5S$: C,H,S
290819	H		30	148–150	EtOH–acetone	3	$C_{27}H_{22}O_4S$: C,H,S
277807	H		65	87–89	EtOH–acetone	~3	$C_{15}H_{14}O_5S$: C,H,S
270037	H		54	112–114	EtOH	3	$C_{14}H_{12}O_4S$: C,H,S

270038	H		77	80.5–82.5	EtOH	3	$C_{13}H_{12}O_5S$: C,H,S
274233	H		30	88.5–90.5	EtOH–H_2O–acetone	2	$C_{15}H_{14}O_4S$: C,H,S
276022	H		63	111.5–113	EtOH–acetone	2.5	$C_{10}H_{16}O_5S$: C,H,S,
265478	H		59	102–104	EtOH	3	$C_{15}H_{13}ClO_4S$: C,H,S
252188	Cl	$S(CH_2)_7CH_3$	16	Oil	EtOH[b]	—	$C_{16}H_{23}ClO_4S$: C,H,Cl
254673	Cl	$S(CH_2)_{13}CH_3$	12	51–53	EtOH–acetone	24	$C_{22}H_{35}ClO_4C$: C,H,S
259277	Cl	$S(CH_2)_{17}CH_3$	16	Oil	EtOH[b]	2.5	$C_{16}H_{23}ClO_4S$: C,H,Cl
264705	Cl	$S(CH_2)_9CH_3$	19	35–36	Et_2O, EtOH	3	$C_{18}H_{27}ClO_4S$: C,H,S
265469	Cl	$SCH_2CH_2CHCH_3$ (CH$_3$ above CHCH$_3$)	~25	Oil	Column,[a] tlc	—	$C_{13}H_{17}ClO_4S$: C,H,S
265479	Cl		~29	Oil	Column,[a] tlc	—	$C_{17}H_{17}ClO_4S$: C,H,S
266761	Cl		19	99–105	Et_2O	—	$C_{15}H_{12}Cl_2O_4S$: C,H,S
247512	CH_3	$S(CH_2)_{15}CH_3$	34	74–76	EtOH, column[a]	48	$C_{25}H_{42}O_4S$: C,H,S
249318	CH_3		—	99.5–101	Et_2O–acetone	24	$C_{19}H_{16}O_4S$: C,H,S
249319	CH_3		30	Oil	Column[a]	24	$C_{21}H_{32}O_4S$: C 65.41,H,S
274531	CH_3		—	Oil	Column[a]	2.5	$C_{18}H_{20}O_4S$: C,H,S

[a] Silica gel column.
[b] Collected crystals by filtration in cold room.

mercaptan (2.9 g, 10 mmol) in *n*-hexane (25 ml) was stirred at room temperature for 3 hr. Afterward, the reaction mixture was placed into the refrigerator overnight. The oil was separated. A 1.0-g portion of the oil was purified by thin-layer chromatography to give 0.4 g of analytically pure product: nmr ($CDCl_3$), δ ppm 0.7–2.0 (m, 23H), 3.5–3.6 (d, 2H), 3.7–3.85 (d, 2H), 4.0–4.1 (d, 6H), 6.3 (s, 1H), and 7.05–7.50 (m, 4H). *Anal.* $C_{27}H_{38}O_4S$: C, H, S.

(3) 2,3-Dimethoxy-5-n-octylmercapto-6-chloro-1,4-benzoquinone. 2,3-Dimethoxy-5-*n*-octylmercapto-1,4-benzoquinone (1.6 g, 5.1 mmol) was dissolved in EtOH (50 ml). Dry HCl gas was bubbled into the EtOH solution at 5 to 10°C for 2.5 hr. The solvent was evaporated, and the thick oil was redissolved in EtOH (30 ml) and oxidized with $FeCl_3$ (5 g) in H_2O (10 ml). The oily product was extracted three times with ether, and the extract was dried over $MgSO_4$. After filtration, the filtrate was reduced in volume to give an oily product, which was recrystallized in the cold room from EtOH to give 0.3 g (16%). The melting point of the product is lower than room temperature; nmr ($CDCl_3$) δ ppm 0.8–1.9 (m, 15H), 3.1–3.45 (t, 2H), and 4.0 (d, 6H). *Anal.* $C_{16}H_{23}O_4ClS$: C, H, Cl.

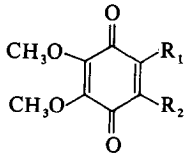
RESULTS OF *IN VIVO* and *IN VITRO* BIOASSAYS

The Walker survival system is a sensitive test system for percentage T/C values, but substantial long-term survivors or cures are not very commonly observed, so that this parameter is of great importance in the search for effective antitumor agent. Our 10 best antitumor analogs of coenzyme Q (Table 1) all showed long-term cures. 5-Phytylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 276371) was the most potent quinone with % T/C = 789 and three out of six cures at 0.78 mg/kg. 6-*n*-Octylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 252188) and 6-phytyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone (NSC 277818) gave six out of six cures at 3.13 mg/kg (% T/C = 584) and four out of four cures at 50 mg/kg (% T/C = 923), respectively. The dodecylquinone (NSC 220334) exhibited five out of six cures (% T/C = 714 at 32 mg/kg). Table 3 lists all 38 analogs of coenzyme Q which have been tested against Walker carcinosarcoma 256 in rats by NCI. All of these quinones except two were active (T/C $\geqslant$125%).

Substitution of a chlorogroup in place of hydrogen on the quinone ring in position 5 almost invariably resulted in markedly increased antitumor activity, as evidenced by 6-*n*-dodecylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 220334), 6-*n*-octadecylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 259277), 6-ω-cyclohexylhexylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 238136), and 6-β-naphthylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 247511) versus 5-*n*-dodecylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 186889), 5-*n*-octadecylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 238135), 5-ω-cyclohexylhexylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 237675), and 5-β-naphthylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 234211), respectively. A methyl group in position 5 instead of hydrogen gave little, if any, increase in percentage T/C, as seen for 6-*n*-hexadecylmercapto-5-methyl-2,3-dimethoxy-1,4-benzoquinone (NSC 247512), 6-β-naphthylmercapto-5-methyl-2,3-dimethoxy-1,4-benzoquinone (NSC 249318), and 6-ω-cyclohexylhexylmercapto-5-methyl-2,3-dimethoxy-1,4-benzoquinone (NSC 249319)

TABLE 3

ANTITUMOR ACTIVITY OF SUBSTITUTED 1,4-BENZOQUINONES AGAINST WALKER CARCINOSARCOMA IN RATS



NSC No.	Structure	Dose (mg/kg)	Toxicity day survivors	Cures	Percentage T/C
265469	$R_1 = Cl$	50	5/6	0/6	175
	$R_2 = SCH_2CH_2CHCH_3$ (CH$_3$)	25	6/6	0/6	195
		12.5	6/6	0/6	205
		6.25	6/6	2/6	547
		3.13	6/6	4/6	820
252188	$R_1 = Cl$	50	0/6	0/6	—
	$R_2 = S(CH_2)_7CH_3$	25	3/6	2/6	—
		12.5	6/6	2/6	363
		6.25	6/6	3/6	580
		3.13	6/6	6/6	584
264705	$R_1 = Cl$	12.5	6/6	1/6	178
	$R_2 = S(CH_2)_9CH_3$	6.25	6/6	1/6	164
		3.13	6/6	0/6	161
		1.56	6/6	0/6	123
		0.78	6/6	0/6	105
220334[20]	$R_1 = Cl$	32	6/6	5/6	714
	$R_2 = S(CH_2)_{11}CH_3$	16	6/6	4/6	713
		8	6/6	3/6	710
		4	6/6	1/6	146
		2	6/6	0/6	103
		1	6/6	0/6	110
254673	$R_1 = Cl$	50	6/6	0/6	142
	$R_2 = S(CH_2)_{13}CH_3$	25	6/6	0/6	168
		12.5	6/6	0/6	137
		6.25	6/6	0/6	132
		3.13	6/6	0/6	108
259277[19]	$R_1 = Cl$	50	6/6	2/6	166
	$R_2 = S(CH_2)_{17}CH_3$	25	6/6	1/6	162
		12.5	6/6	2/6	141
		6.25	6/6	0/6	125
		3.13	6/6	0/6	103
238136[20]	$R_1 = Cl$	50	6/6	0/6	150
	$R_2 = S(CH_2)_6$–C$_6$H$_{11}$	25	6/6	0/6	148
		12.5	6/6	3/6	597
		6.25	6/6	0/6	140
		3.13	6/6	0/6	128

TABLE 3—*continued*

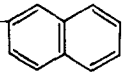
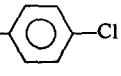
NSC No.	Structure	Dose (mg/kg)	Toxicity day survivors	Cures	Percentage T/C
247511[20]	$R_1 = Cl$ $R_2 = S$- 	50	6/6	0/6	205
		25	6/6	0/6	296
		12.5	6/6	0/6	183
		6.25	6/6	0/6	116
		3.13	6/6	0/6	121
254675[20]	$R_1 = H$ $R_2 = S(CH_2)_{13}CH_3$	50	6/6	0/6	160
		25	6/6	0/6	204
		12.5	5/6	0/6	120
		6.25	6/6	0/6	108
		3.13	6/6	0/6	118
237676[20]	$R_1 = H$ $R_2 = S(CH_2)_{15}CH_3$	32	3/3	0/3	172
		16	3/3	0/3	145
		8	3/3	0/3	160
		4	3/3	0/3	130
		2	3/3	0/3	101
255102[20]	$R_1 = H$ $R_2 = S(CH_2)_{16}CH_3$	50	6/6	0/6	216
		25	6/6	0/6	144
		12.5	6/6	0/6	160
		6.25	6/6	0/6	156
		3.13	6/6	0/6	106
238135[19]	$R_1 = H$ $R_2 = S(CH_2)_{17}CH_3$	50	6/6	0/6	149
		25	6/6	1/6	151
		12.5	6/6	0/6	105
		6.25	6/6	0/6	113
		3.13	6/6	0/6	106
237675[20]	$R_1 = H$ $R_2 = S(CH_2)_6$- 	32	3/3	0/6	160
		16	3/3	0/6	172
		8	3/3	0/6	125
		4	3/3	0/6	101
		2	3/3	0/6	107
265479	$R_1 = Cl$ $R_2 = S(CH_2)_3$- 	12.5	6/6	0/6	298
		6.25	6/6	1/6	326
		3.13	6/6	0/6	236
		1.56	6/6	2/6	195
		0.78	6/6	2/6	154
266761	$R_1 = Cl$ $R_2 = SCH_2$- 	50	5/6	0/6	216
		25	6/6	0/6	195
		12.5	6/6	1/6	205
		6.25	6/6	1/6	246
		3.13	6/6	0/6	127
237677[20]	$R_1 = H$ $R_2 = S(CH_2)_7CH_3$	32	0/3	0/3	—
		16	2/3	0/3	101
		8	3/3	0/3	166
		4	3/3	0/3	172
		2	3/3	0/3	172

TABLE 3—*continued*

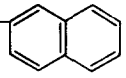
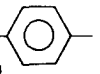
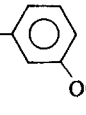
NSC No.	Structure	Dose (mg/kg)	Toxicity day survivors	Cures	Percentage T/C
254674	$R_1 = H$ $R_2 = S(CH_2)_9CH_3$	50	2/6	0/6	—
		25	3/6	0/6	—
		12.5	6/6	0/6	153
		6.25	6/6	0/6	180
		3.13	6/6	0/6	132
186889[19]	$R_1 = H$ $R_2 = S(CH_2)_{11}CH_3$	32	2/6	0/6	—
		16	6/6	0/6	205
		8	6/6	2/6	250
		4	6/6	0/6	192
		2	6/6	1/6	250
		1	6/6	0/6	140
258835	$R_1 = H$ $R_2 = S(CH_2)_3$– 	25	1/6	3/6	789
		12.5	6/6	1/6	605
		6.25	6/6	3/6	789
		3.13	6/6	0/6	135
234214[20]	$R_1 = H$ $R_2 = S$– 	40	6/6	4/6	789
		20	6/6	1/6	223
		10	6/6	1/6	157
		5	6/6	0/6	148
		2.5	6/6	0/6	128
		1.25	6/6	0/6	118
260616	$R_1 = H$ $R_2 = SCH_2$–  $-(CH_2)_{11}CH_3$	50	2/6	0/6	—
		25	5/6	0/6	137
		12.5	6/6	0/6	216
		6.25	6/6	2/6	212
		3.13	6/6	2/6	337
276371[19]	$R_1 = H$ R_2 = S-phytyl	25	6/6	2/6	236
		12.5	6/6	3/6	789
		6.25	6/6	3/6	789
		3.13	6/6	3/6	789
		1.56	6/6	1/6	539
		0.78	6/6	3/6	789
290503	$R_1 = H$ $R_2 = S$– 	50	4/6	0/6	88
		25	6/6	1/6	211
		12.5	6/6	0/6	229
		6.25	6/6	3/6	845
		3.13	6/6	0/6	197
290814	$R_1 = H$ $R_2 = S$– 	50	6/6	1/6	225
		25	6/6	0/6	239
		12.5	6/6	0/6	239
		6.25	6/6	2/6	633
		3.13	6/6	1/6	183

TABLE 3—*continued*

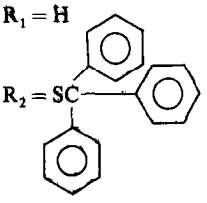
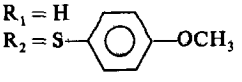
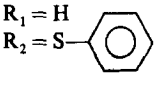
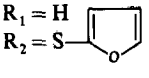
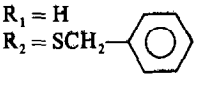
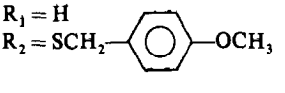
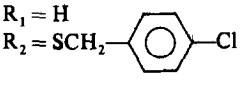
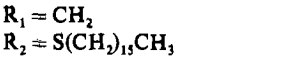
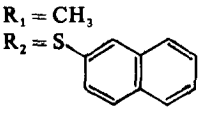
NSC No.	Structure	Dose (mg/kg)	Toxicity day survivors	Cures	Percentage T/C
290819	$R_1 = H$  $R_2 = SC$	50	6/6	2/6	239
		25	6/6	2/6	261
		12.5	6/6	0/6	126
		6.25	6/6	0/6	116
		3.13	6/6	0/6	126
277807	$R_1 = H$  $R_2 = S$ —OCH_3	50	4/6	2/6	96
		25	5/6	1/6	273
		12.5	6/6	*	918
		6.25	6/6	1/6	230
		3.13	6/6	2/6	292
270037	$R_1 = H$  $R_2 = S$	50	6/6	0/6	168
		25	4/6	0/6	174
		12.5	6/6	0/6	188
		6.25	6/6	1/6	156
		3.13	6/6	1/6	132
270038	$R_1 = H$  $R_2 = S$	50	4/6	0/6	152
		25	4/6	0/6	164
		12.5	6/6	2/6	188
		6.25	6/6	0/6	188
		3.13	6/6	0/6	176
274233	$R_1 = H$  $R_2 = SCH_2$	50	1/6	0/6	—
		25	2/6	0/6	—
		12.5	6/6	0/6	193
		6.25	6/6	0/6	193
		3.13	6/6	0/6	189
276022	$R_1 = H$  $R_2 = SCH_2$ —OCH_3	200	0/3	0/3	—
		100	0/3	0/3	—
		50	0/3	0/3	—
		25	3/3	0/3	87
		12.5	3/3	0/3	91
		6.25	3/3	0/3	102
265478	$R_1 = H$  $R_2 = SCH_2$ —Cl	12.5	6/6	0/6	187
		6.25	6/6	1/6	246
		3.13	6/6	2/6	315
		1.56	6/6	0/6	178
		0.78	6/6	0/6	154
247512	$R_1 = CH_2$  $R_2 = S(CH_2)_{15}CH_3$	50	6/6	2/6	166
		25	6/6	0/6	116
		12.5	6/6	0/6	116
		6.25	6/6	0/6	121
		3.13	6/6	0/6	106
249318	$R_1 = CH_3$  $R_2 = S$	50	5/6	0/6	200
		25	6/6	0/6	233
		12.5	6/6	0/6	155
		6.25	6/6	0/6	111
		3.13	6/6	0/6	103

TABLE 3—*continued*

NSC No.	Structure	Dose (mg/kg)	Toxicity day survivors	Cures	Percentage T/C
249319	$R_1 = CH_3$ $R_2 = S(CH_2)_6$—⬡	50	5/6	1/6	283
		25	6/6	0/6	150
		12.5	6/6	0/6	111
		6.25	5/6	0/6	101
		3.13	6/6	0/6	103
274531	$R_1 = CH_3$ $R_2 = S(CH_2)_3$—⬡	50	4/6	0/6	189
		25	6/6	0/6	172
		12.5	6/6	0/6	229
		6.25	6/6	2/6	216
		3.13	6/6	0/6	121
180805[16]	$R_1 = OH$ $R_2 = (CH_2)_5$—⬡	32	6/6	0/6	107
		16	6/6	0/6	106
		8	6/6	0/6	106
		4	6/6	0/6	119
		2	6/6	0/6	106
		1	6/6	0/6	103
277818[23]	$R_1 = OH$ R_2 = phytyl	50	4/4	4/4	923
		25	4/4	*	815
		12.5	4/4	0/4	143
		6.25	4/4	0/4	123
		3.13	4/4	0/4	109
268481[16]	$R_1 = OH$ $R_2 = (CH_2)_9CH_3$	50	6/6	0/6	125
		25	6/6	0/6	109
		12.5	6/6	0/6	118
		6.25	6/6	0/6	120
		3.13	6/6	0/6	117

TABLE 4

INHIBITORY EFFECT OF BENZOQUINONE ANALOGS OF COENZYME Q ON MITOCHONDRIAL SUCCINOXIDASE ACTIVITY

Compounds (NSC No.)	Concentrations[a]	Relative enzyme activity[b]	Nanomoles for 50% inhibition	A.I.[c]
None	—	100	—	—
Standard inhibitor[d]	4	61		
	6	50	3	1
	8	45		
2,3-Dimethoxy-1,4-benzoquinone:				
5-*n*-Octylmercapto- (237677)	500	80	—	>425
	1000	81		
5-*n*-Decylmercapto- (254674)	200	129	—	>425
	1000	116		
5-*n*-Dodecylmercapto- (186889)	500	136	—	>425
	1000	139		
5-*n*-Tetradecylmercapto- (254675)	200	117	—	>425
	1000	106		

TABLE 4—*continued*

Compounds (NSC No.)	Concentrations[a]	Relative enzyme activity[b]	Nanomoles for 50% inhibition	A.I.[c]
5-*n*-Hexadecylmercapto-	500	102	—	>425
(237676)	1000	106		
5-*n*-Heptadecylmercapto-	200	106	—	>425
(255102)	1000	96		
5-*n*-Octadecylmercapto-	500	96	—	>425
(238135)	1000	91		
5-ω-Cyclohexylhexylmercapto-	500	125	—	>425
(237675)	1000	122		
5-ω-Phenylpropylmercapto-	40	60	72	31
(258835)	100	42		
5-β-Naphthylmercapto-	16	68		
(234214)	20	59	25	11
	28	46		
	40	32		
2,3-Dimethoxy-5-chloro-1,4-benzoquinone:				
6-*n*-Octylmercapto-	10	82		
(252188)	20	53	22	9
	40	23		
6-*n*-Dodecylmercapto-	25	79		
(220334)	50	52	50	21
	100	42		
	200	37		
6-*n*-Tetradecylmercapto-	100	59		
(254677)	200	51	210	89
	400	37		
6-*n*-hexadecylmercapto-	200	65		
(237678)	600	48	540	230
	1000	35		
6-ω-Cyclohexylhexylmercapto-	6	70		
(238136)	8	59	106	45
	12	47		
6-β-Naphthylmercapto-	8	70		
(247511)	12	57	15	65
	20	33		
2,3-Dimethoxy-5-methyl-1,4-benzoquinone:				
6-*n*-Hexadecylmercapto-	500	105	—	>425
(247512)	1000	102		
6-ω-Cyclohexylhexylmercapto-	500	142	—	>425
(249319)	1000	148		
6-β-Naphthylmercapto-	20	76		
(249318)	40	57	47	20
	52	45		

[a] Nanomoles in a flask. Content of the mitochondrial protein was 0.588 mg per flask.

[b] Percentage of specific activity in the presence of inhibitor to that of the control. The specific activity of the control was 0.554 ± 0.070 atoms of O_2/mg/min.

[c] Antimetabolite CoQ index is defined as the nanomoles of inhibitor per nanomole of mitochondrial CoQ for approximately 50% inhibition. Mitochondria contained 4.0 nmol of CoQ/mg of protein.

[d] 6-ω-Cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone.

TABLE 5

INHIBITORY EFFECTS OF BENZOQUINONE ANALOGS OF COENZYME Q ON MITOCHONDRIAL NADH-OXIDASE ACTIVITY

Compounds (NSC No.)	Concentration[a]	Relative enzyme activity[b]	Nanomoles for 50% inhibition	A.I.[c]
None	—	100	—	—
Standard inhibitor[d]	4	56		
	5	50	5	3
	6	47		
2,3-Dimethoxy-1,4-benzoquinone	100	52		
5-*n*-Octylmercapto-	200	22	105	60
(237677)	300	16		
5-*n*-Decylmercapto-	40	75		
(254674)	100	56	120	68
	200	27		
5-*n*-Dodecylmercapto-	100	87		
(186889)	200	69	280	159
	300	46		
5-*n*-Tetradecylmercapto-	200	67		
(254675)	300	51	310	176
	400	38		
5-*n*-Hexadecylmercapto-	500	94		
(237676)	1000	50	1000	567
5-*n*-Heptadecylmercapto-	1000	81	—	>567
(255102)				
5-*n*-Octadecylmercapto-	500	99		
(238135)	1000	98	—	>567
5-ω-Cyclohexylhexylmercapto-	60	79		
(237675)	80	68		
	100	55	108	61
	140	32		
5-ω-Phenylpropylmercapto-	10	87		
(258835)	20	71	28	16
	40	44		
5-β-naphthylmercapto-	8	84		
(234214)	16	50	16	9
	24	33		
2,3-Dimethoxy-5-chloro-1,4-benzoquinone	20	86		
6-*n*-Octylmercapto-	30	43	28	16
(252188)	40	28		
6-*n*-Dodecylmercapto-	40	63		
(220334)	60	52	65	37
	120	26		
6-*n*-Tetradecylmercapto-	40	73		
(254677)	100	44	85	48
	200	26		
6-*n*-Hexadecylmercapto-	100	64		
	200	53	230	130
	400	38		

TABLE 5—*continued*

Compounds (NSC No.)	Concentration[a]	Relative enzyme activity[b]	Nanomoles for 50% inhibition	A.I.[c]
6-ω-Cyclohexylhexylmercapto-	20	83		
(238136)	40	54	43	24
	80	24		
6-β-naphthylmercapto-	10	67		
(247511)	16	34	12	7
	20	27		
2,3-Dimethoxy-5-methyl-1,4-benzoquinone:				
6-*n*-Hexadecylmercapto-	500	83		
(247512)	1000	86	—	>567
6-ω-Cyclohexylhexylmercapto-	60	59		
(249319)	100	43	83	47
	160	21		
6-β-Naphthylmercapto-	20	73		
(249318)	30	54	32	18
	40	35		

[a] Nanomoles in a flask. Content of the mitochondrial protein was 0.441 mg per flask.

[b] Percentage of specific activity in the presence of inhibitor to that of the control. The specific activity of the control was 0.948 ±0.079 μatom of O_2/mg/min.

[c] Antimetabolite CoQ index is defined as the nanomoles of inhibitor per nanomole of mitochondrial CoQ for approximately 50% inhibition. Mitochondria contained 4.0 nmol of CoQ/mg of protein.

[d] 6-ω-Cyclohexylpentyl-5-hydroxy-2,3-dimethoxy-1,4-benzoquinone.

versus 5-*n*-hexadecylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 237676), 5-β-naphthylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 234214), and 5-ω-cyclohexylhexylmercapto-2,3-dimethoxy-1,4-benzoquinone (NSC 237675), respectively. 6-*n*-octylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 252188) was toxic at 25 mg/kg (three out of six survivors). Apparently shorter sidechain lengths increase toxicity to rats in this assay (compare NSC 252188 and NSC 220334).

Nineteen of these 38 quinones were tested for their inhibitory effects on mitochondrial succinoxidase and NADH-oxidase activities (Tables 4 and 5, respectively). In general, the quinones were stronger inhibitors of NADH-oxidase than of succinoxidase. The 5-chloro-1,4-benzoquinones were far stronger inhibitors in both enzyme systems than the corresponding "deschloro"-quinones. Although a perfect correlation was not expected, many of the most potent antitumor compounds [6-*n*-octylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 252188) and 6-*n*-dodecylmercapto-5-chloro-2,3-dimethoxy-1,4-benzoquinone (NSC 220334)] were also among the best *in vitro* enzyme inhibitors.

Figures 1 and 2 show the effects of 6-*n*-hexadecylmercapto-5-methyl-2,3-dimethoxy-1,4-benzoquinone (NSC 247512) and 6-ω-cyclohexylhexylmercapto-5-methyl-2,3-dimethoxy-1,4-benzoquinone (NSC 249319) on restoration of CoQ_{10}-depleted succinoxidase and NADH-oxidase systems. NSC 247512 and NSC 249319 are analogs which contain hexadecylmercapto- and cyclohexylhexylmercapto side chains, respectively, instead of the decaprenyl side chain of CoQ_{10}, but each has the 5-methyl group of

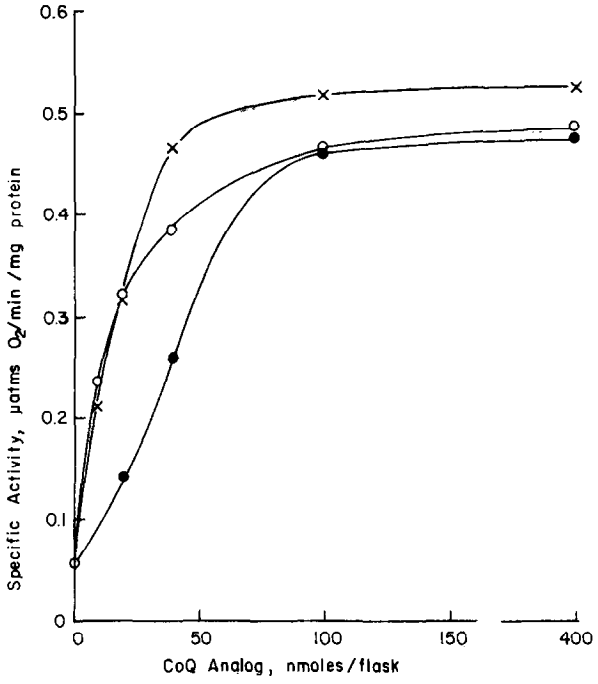


FIG. 1. The effects of 6-alkylmercapto analogs of CoQ on restoration of CoQ-depleted succinoxidase system. (○) CoQ_{10}; (●) 6-hexadecylmercapto analog (NSC 247512); and (×) 6-cyclohexylhexylmercapto analog (NSC 249319).

	Nanomoles					
Concentration	0	10	20	40	100	400
CoQ_{10}	0.052	0.235	0.322	0.387	0.467	0.487
6-Hexadecylmercapto	—	—	0.142	0.259	0.462	0.477
6-Cyclohexylhexylmercapto	—	0.209	0.319	0.466	0.519	0.529

Mitochondrial proteins: 0.71 mg/flask.

CoQ_{10}. From Fig. 1 it can be seen that the NSC 247512 and particularly NSC 249319 are effective in restoring enzymatic activity. NSC 249319 seemed to be more effective than coenzyme Q_{10} in restoring the activity of succinoxidase. However, in the NADH oxidase system NSC 247512 and NSC 249319 were only about 30% as effective in restoration of enzymatic activity as CoQ_{10}. NSC 249319 was active only over a narrow concentration range. These data (Figs. 1 and 2) indicate that replacement of the decaprenyl chain of CoQ_{10} by other moieties and retention of the 5-methyl group can give analogs having agonist activity.

DISCUSSION

A rationale based upon coenzyme Q_{10} for the synthesis of potential antitumor agents constitutes a new approach in the research on chemotherapy of cancer. There are

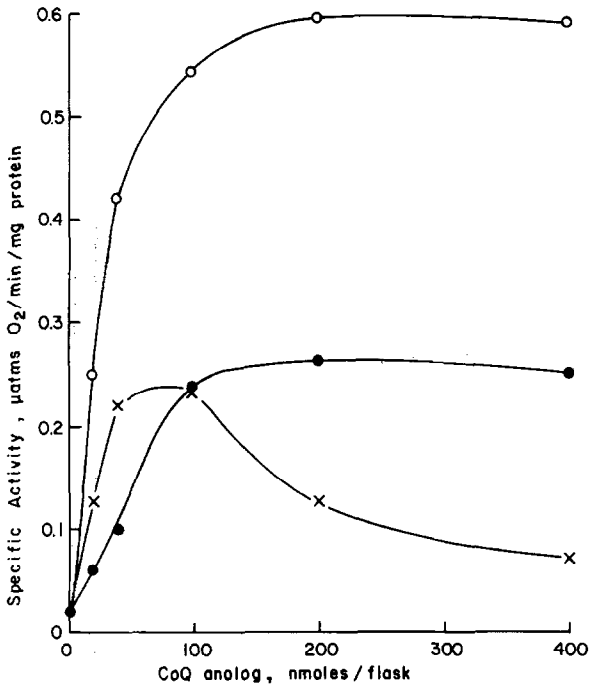


FIG. 2. The effects of 6-alkylmercapto analogs of CoQ on restoration of CoQ-depleted NADH-oxidase system. (O) CoQ_{10}; (●) 6-hexadecylmercapto analog (NSC 247512); and (×) 6-cyclohexylhexylmercapto analog (NSC 249319).

	Nanomoles					
Concentration	0	20	40	100	200	400
CoQ_{10}	0.023	0.251	0.418	0.542	0.595	0.587
6-Hexadecylmercapto	—	0.062	0.097	0.241	0.263	0.250
6-Cyclohexylhexylmercapto	—	0.129	0.220	0.234	0.124	0.071

Mitochondrial protein: 0.54 mg/flask.

multiple sites for potential inhibition of coenzyme Q_{10} enzymes by effective analogs of CoQ_{10}. The three quinone precursors of CoQ imply three enzyme sites in biosynthesis for inhibition, and inhibition may occur at sites of functionality of CoQ_{10} in mitochondria and in the Golgi apparatus.

The inhibitory activities of the analogs of coenzyme Q_{10} may be considered as follows. The three substituents on the benzoquinone nucleus, such as the 2,3-dimethoxy-5-chloro groups in place of 2,3-dimethoxy-5-methyl-moieties of CoQ_{10}, probably contribute to an adverse redox potential of narrow range as based upon the structural specificity of CoQ_{10} for electron transfer. The chloroquinones closely simulate CoQ_{10} in that only the 5-methyl group of these three substituents is changed, and this change is now known to be useful according to the documentation of the best antitumor agents in Table 1.

The 6-decaprenyl side chain of CoQ_{10} is also essential in its own specific way (length and steric nature) for the coenzyme activity of CoQ_{10}, particularly for NADH-oxidase. For example, reduction to a perhydro side chain has been shown to be detrimental to coenzymatic activity. Step-wise shortening by single isoprenoid units of the length of the isoprenoid side chain from seven units to one or two units decreased the coenzymatic activity for NADH-oxidase (*1*). Likewise, the introduction of an oxide group (*21*) or increasing the length beyond 10 units with the inclusion of *cis* double bonds in the isoprenoid side chain is detrimental to the coenzymatic activity of coenzyme Q_{10}, and may provide guidelines for new design of inhibitors and potential antitumor drugs.

For the most part, the analogs (Table 3) which contain sulfur in the side chain adjacent to the quinone ring have little or no side-chain unsaturation. The sulfur atom adjacent to the nucleus could be expected to influence the oxidation–reduction potentials of the quinone analogs. The shorter, saturated, and unbranched side chains, which were incorporated extensively in to these analogs, would significantly differ from the nature and stereochemistry of the 6-decaprenyl side chain and would influence the positioning of the analog in the "lipid milieu" of the mitochondria. It is known that CoQ_{10} is in a "lipid milieu" of the mitochondria, and cardiolipin of the mitochondria has often been thought to be associated with CoQ_{10}.

It may be considered that the altered side chain is situated differently from CoQ_{10} on the "receptor" of the apoenzyme in the lipid complex. Then, of structural necessity, the benzoquinone nucleus is different from the nucleus of CoQ_{10} in its relationship to the isoalloxazine nucleus of flavoprotein and the porphyrin nucleus of cytochrome b_1. It seems reasonable that precise molecular orbital relationships of the planar nuclei of CoQ_{10}, of the isoalloxalloxazine of riboflavin in flavoprotein, and of the porphyrin of cytochrome are essential for the electron-transfer mechanisms existing in the respiratory chain.

The structural differences between the octylmercapto (NSC 252188) and dodecylmercapto (NSC 220334) and octadecylmercapto (NSC 259277) analogs (Table 1) are seemingly of deceptively little consequence in organic chemistry. However, it is evident that the analogs with the octylmercapto side chain gave six out of six cures (% T/C = 584) and the analog with the octadecylmercapto side chain was inactive at about the same dose level. It appears that the structure of the side chain of these analogs is highly important in the design of such antitumor agents.

Our analogs of coenzyme Q_{10} which have been synthesized recently are substantially more potent than those which were synthesized in our early exploration of this approach. Folkers *et al.* (*22*) reported that one of four such analogs was found to be a particularly potent inhibitor of two human cell lines of leukemia. It is believed that the continuing synthesis of such analogs of coenzyme Q_{10} will lead not only to more potent compounds in the present antitumor test system *in vivo* but also to compounds showing effective antitumor activity in other tumor systems.

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