Potential Antitumor Agents. 5. Methylated α-(N)-Heterocyclic Carboxaldehyde Thiosemicarbazones†

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Several monomethylated derivatives of α-(N)-heterocyclic carboxaldehyde thiosemicarbazones were prepared to define the molecular dimensions compatible with antineoplastic activity. These were the thiosemicarbazones of 3-, 4-, and 5-methylisoquinoline-1-carboxaldehyde and of 3-, 4-, 5-, and 6-methylpyridine-2-carboxaldehyde. Tests for tumor-inhibitory potency indicate that in general the pyridine derivatives were better inhibitors of the growth of the L1210 lymphoma than were the isoquinolines. Introduction of a 6-Me group in the pyridine ring and of an analogous 3-Me substituent in the isoquinoline structure resulted in compounds with no antineoplastic activity indicating an apparent intolerance to substitution at the α′ position to the heterocyclic N atom for inhibitory action. Me substituents at the other positions of these ring systems did not significantly increase the carcinostatic potency of the parent compounds.

A variety of thiosemicarbazones of α-(N)-heterocyclic carboxaldehydes have been shown to be potent inhibitors of transplanted rodent neoplasms,[1] spontaneous lymphomas of dogs,[2] and DNA viruses of the Herpes family.[3] Investigation of the structure-activity relationships required for antineoplastic activity[4] have indicated that certain of the overall molecular dimensions of 1-formylisoquinoline thiosemicarbazone (IQ-1), one of the most active members of this series,[5] could be modified with the retention of high biological activity. Thus, substituents such as 5-OH and 5-OAc were found to confer therapeutic indices to the resultant derivatives that were greater than for the parent compound.[4a] Substitution of these groups (*i.e.*, OH and OAc) in the 4 position of the isoquinoline ring resulted in compounds which were both less active and less toxic than the parent compound against sarcoma 180 ascites cells in mice; the sodium salt of the 4-OH derivative, however, was considerably more efficacious than the parent compound on the L1210 lymphoma.[4c] Sodium salts of several other OH derivatives of both isoquinoline and pyridine ring systems have also been prepared as a means of solubilizing for parenteral administration these extremely insoluble compounds; in many instances such solubilization conferred greater therapeutic gain.[6] The structural specificity of the formyl thiosemicarbazone side chain was found to be critical, since modifications made at the various positions of the side chain resulted in either a decrease or a complete loss of antitumor activity.[4b]

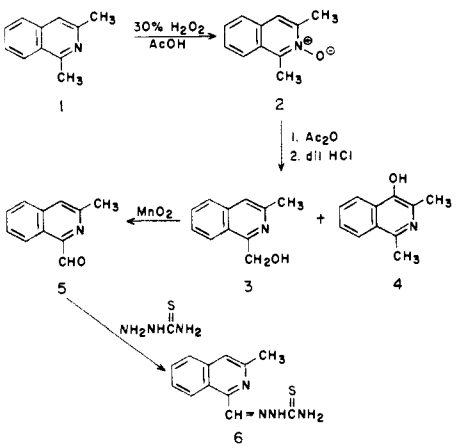
The biochemical basis for the growth-inhibitory activity of IQ-1 has also been studied in our laboratory.[7] The investigations indicate that in mammalian cells the primary site of action of these agents is the biosynthesis of DNA, with the location of the metabolic lesion being the conversion of ribonucleotides to deoxyribonucleotide forms.[8] Blockade of the formation of RNA and protein also occurs, but these pathways are considerably less susceptible to drug-induced inhibition. A similar mechanism of action appears to be operative with both 3-hydroxy-2-formylpyridine thiosemicarbazone and 5-hydroxy-2-formylpyridine thiosemicarbazone,[9] two thiosemicarbazone derivatives of the pyridine ring system with relatively great therapeutic indices as antineoplastic agents.[10]

It was deemed desirable to further define the molecular dimensions compatible with the biological activity of this relatively new class of antineoplastic agents. To this end a number of monosubstituted alkylated derivatives of the two most active heterocyclic ring systems in this series, the isoquinoline and pyridine, have been synthesized and biologically evaluated.

Chemistry. The syntheses of Me-substituted derivatives of isoquinoline- and pyridinecarboxaldehyde thiosemicarbazones were initiated from corresponding dimethyl-substituted heterocyclic ring systems. Lutidines were obtained commercially and dimethylisoquinolines were synthesized utilizing the Bischler-Napieralski reaction. Considerable difficulties were encountered in the dehydrogenation with Pd of 3,4-dihydroisoquinolines when prepared according to a published procedure.[11] Dehydrogenation of 1,3-dimethyl-3,4-dihydroisoquinoline or 1,4-dimethyl-3,4-dihydroisoquinoline with Pd at 200° produced in part unknown compounds which were found to have no aromatic protons in nmr; the identification of these compounds is under investigation. Dehydrogenation was carried out in good yield, however, by heating the dihydroisoquinolines with Ph_2S_2 and removing the formed thiophenol by distillation.

Direct oxidation of 1,3- and 1,4-dimethylisoquinolines (**1** and **7**) with SeO_2 resulted in poor yields of the respective 1-carboxaldehydes. These compounds were therefore synthesized by rearrangement of their *N*-oxides with Ac_2O

Scheme I



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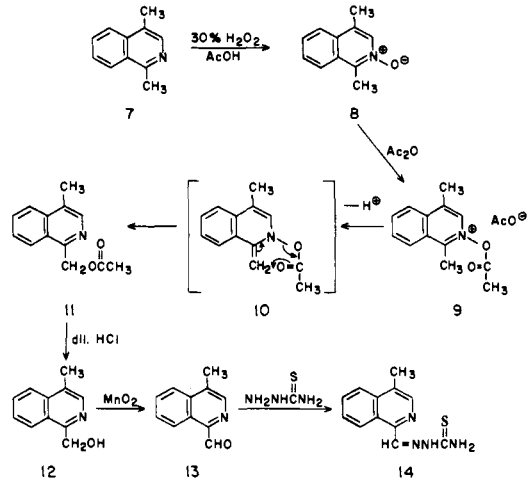
Scheme II

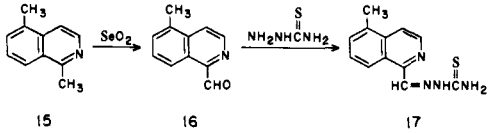


(Schemes I and II). The rearrangement of 1-methylisoquinoline *N*-oxide has been previously reported and a possible mechanism proposed.[4c] A similar mechanism would be expected to be operative in the transformation reactions of **1** (Scheme I). Two compounds, 3-methyl-1-hydroxymethylisoquinoline (**3**) and 1,3-dimethyl-4-hydroxyisoquinoline (**4**), were isolated after acid hydrolysis of the mixture of esters obtained by heating 1,3-dimethylisoquinoline *N*-oxide (**2**) with Ac_2O. Oxidation of **3** with MnO_2 followed by reaction with thiosemicarbazide gave the desired thiosemicarbazone **6**.

Treatment of 1,4-dimethylisoquinoline *N*-oxide (**8**) with Ac_2O (Scheme II) produced mainly 4-methyl-1-acetoxymethylisoquinoline (**11**). A minor isomer, possibly 1-methyl-4-acetoxymethylisoquinoline, was present as a contaminant. Positive identification of this trace material was not accomplished. Compound **11** was hydrolyzed with dil HCl to yield 4-methyl-1-hydroxymethylisoquinoline (**12**) and then purified by alumina column chromatography. The reaction of **8** with Ac_2O contrasts with the attempted rearrangement of 4-methylisoquinoline *N*-oxide by Robison and Robison[12] who could not isolate a discrete chemical material from the polymeric mass which was produced. In the case of **9**, with a Me group in position 1, the rearrangement presumably proceeds by an intramolecular mechanism through intermediates **9** and **10** in a manner analogous to that proposed for 2-picoline *N*-oxide.[13] Nucleophilic attack of the *N*-oxide O on Ac_2O results in a formation of intermediate **9**. Abstraction of a proton from **9** by AcO^- follows to produce the anhydro base **10** which undergoes an intramolecular rearrangement. Compd **12** was then oxidized with MnO_2 to the corresponding carboxaldehyde **13** which on reaction with thiosemicarbazide yielded the desired product **14**.

Selective oxidation of the 1-Me group in 1,5-dimethylisoquinoline (**15**) to the corresponding 1-carboxaldehyde (**16**) was achieved in fair yield with SeO_2 (Scheme III). Compd

Scheme III



16 on reaction with thiosemicarbazide produced the desired derivative (**17**). Methylated pyridine-2-carboxaldehydes, the precursors of the synthesis of thiosemicarbazones, were prepared by previously published procedures[14] utilizing the rearrangement of corresponding lutidine *N*-oxides with Ac_2O.

Nmr Studies. Nmr spectral parameters for the compds prepared were in accord with the structures proposed; differentiation of the various isomers from nmr results has been possible. All peaks assigned to replaceable protons were confirmed by addition of CF_3CO_2H.

From the relatively abundant number of compds investigated in the present study, as well as previously,[4c] some useful empirical results have been obtained which can be used to characterize compds in the isoquinoline series. As an example, unless C-8 or the ring N contains substituents (*e.g.*, N → O), the chemical shift for a 1-Me group occurs between δ 2.81 and 2.92. Similarly, doubly bonded electronegative substituents on the C directly bonded to C-1 cause a paramagnetic shift of the proton resonance at C-8 into the region δ 9.13–9.26. This C-8 proton multiplet lies to lower field than the other ring protons.

An interesting long-range coupling in isomers I and II of 3,4-dihydroisoquinolines (Figure 1) has been used to establish the cyclized nature of the compounds. In I, the 1-Me substituent (δ 2.32) is a triplet, $J_{1\text{-}CH_3,H\text{-}3} = 1.5$ Hz, due to long-range coupling to the two H-3's (dark lines). On the other hand, in II the Me is a doublet showing a 5-bond long-range coupling, $J_{1\text{-}CH_3,H\text{-}3} = 1.9$ Hz, due to coupling with the single proton on C-3. Such long-range coupling must involve the heterocyclic N atom. Double irradiation of the 1-Me resonance lines in I reduced the multiplet structure at δ 3.42 to an octet which is the AB subspectrum of the $ABMX_3$ spin system. In the case of the 5-Me compounds, only in **16** was a splitting resolved between the 5-Me group and the protons at C-4 and C-6. The 5-Me group in this compound was a triplet, $J = 0.7$ Hz.

Biological Results and Discussion. The antitumor activity of methylated α-(N)-heterocyclic carboxaldehyde thiosemicarbazones against the L1210 lymphoma in mice is given in Table I. The findings indicate that, in general, the pyridine derivatives were better inhibitors of the L1210 lymphoma than the isoquinoline series. Although the parent compound, IQ-1, doubled the average life-span of tumor-bearing mice as compared to untreated controls, the Me-substituted derivatives **6**, **14**, and **17** increased the average survival time to a lesser extent. In the pyridine series 2-formylpyridine thiosemicarbazone (PT) was the most active agent causing more than a 2-fold increase in the average survival time. However, this compound was relatively toxic as shown by a 14.4% loss in body weight at the optimal dose level of 5 mg/kg administered twice daily. Introduction of a 3-Me group (**18**) did not result in therapeutic improvement over PT; however, **19**, with a 4-Me substituent, was not only found to be equally active with PT as a tumor inhibitor, but was also much less toxic causing only 4.3% loss in body weight. Compd **20** having a 5-Me substituent was relatively nontoxic as shown by a minimal loss in body weight, but still increased the average survival time to 18.2 days. Intro-

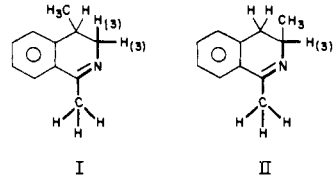


Figure 1.

Table I. Effect of Me-Substituted α-(N)-Heterocyclic Carboxaldehyde Thiosemicarbazones on the Survival Time of Mice Bearing the L1210 Lymphoma

Compound	Maximum effective daily dose, mg/kg[a]	Average Δ weight, %[b]	Average survival, days
None		+ 5.7	8.0
IQ-1	20	− 0.9	16.6
6	20	+ 8.5	10.8
14	20	+11.5	13.6
17	20	+ 8.0	8.4
PT	5	−14.4	21.6
18	15	−10.5	17.4
19	5	− 4.3	20.6
20	15	− 0.5	18.2
21	40	+ 1.6	12.2

[a]Administered twice daily for 4 consecutive days at 12-hr intervals beginning 24 hr after tumor transplantation. [b]Average wt change from onset to termination of drug treatment.

Table II. Methylated α-(N)-Heterocyclic Carboxaldehyde Thiosemicarbazones

Compound	Me Substitution position	Mp, °C dec	Yield, %	Crystn solvent	Formula	Analyses
Isoquinolines						
6	3	207–208	87	THF–cyclohexane	$C_{12}H_{12}N_4S$	C, H, N, S
14	4	214–215	70	EtOH–H_2O	$C_{12}H_{12}N_4S$	C, H, N, S
17	5	233–235	83	*a*	$C_{12}H_{12}N_4S$	C, H, N, S
Pyridines						
18	3	214–216[b]		EtOH	$C_8H_{10}N_4S$	C, H, S
19	4	193–195[c]		EtOH–H_2O	$C_8H_{10}N_4S$	C, H, S
20	5	224–225[d]		EtOH	$C_8H_{10}N_4S$	C, H, S
21	6[e]					

[a]Recrystn was not necessary. [b]Reported mp 209°.[14a] [c]Reported mp 194–196°.[14b] [d]Reported mp 220.5°.[14a] [e]This derivative was generously supplied by Mr. Frederic A. French, Mount Zion Hospital and Medical Center, Chemotherapy Research Laboratory, Palo Alto, Calif.

duction of a 6-Me group in PT (21) resulted in a compound with only minimal biological activity. A similar result was obtained in the isoquinoline series where a 3-Me substituent, which may be considered to be analogous to the 6-Me group of pyridine series, resulted in a compd (6) with much lower antineoplastic activity. Compd 17 which contains a 5-Me group in the isoquinoline ring also showed no antineoplastic activity. This derivative (17) has been reported to be equal in activity to 14 as an inhibitor of the target enzyme ribonucleoside diphosphate reductase *in vitro*.[15] This difference might be explained by the extreme water insolubility of 17 which leads to poor uptake of the compound by neoplastic cells.

Experimental Section

Melting points were determined with a Thomas-Hoover capillary melting point apparatus and are uncor. Elemental analyses§ were performed by the Schwarzkopf Microanalytical Laboratory, Woodside, N. Y., and the Baron Consulting Co., Orange, Conn. Nmr spectra were detd with a Bruker HFX-3 spectrometer operating at 90 MHz and fitted with an H-P frequency counter. Chemical shifts (δ) are given in ppm downfield from TMS. Spectra were obtd in DMSO-d_6 (s, singlet; d, doublet; m, multiplet; dd, doublet of doublets, etc). Only those resonance signals necessary for differentiating the various compds are described.

Antitumor Screening. The thiosemicarbazones were tested for antineoplastic activity in mice bearing the L1210 lymphoma. Complete details of the screening procedure have been described earlier.[6] The ascites tumor was transplanted by inoculating BDF_1 mice ip with approximately 4×10^6 tumor cells. Drugs were administered ip in fine suspension beginning 24 hr after tumor implantation and treatment was continued twice daily at 12-hr intervals for 4 consecutive days. Determination of antineoplastic activity was based upon the prolongation of survival time afforded by the drug treatment.

Dimethyl-3,4-dihydroisoquinolines were synthesized by cyclizing the corresponding amides by published procedures[11] according to the Bischler–Napieralski reaction.

1,3-Dimethylisoquinoline (1). An equimolar mixture of 1,3-dimethyl-3,4-dihydroisoquinoline (9.27 g) and 12.71 g of Ph_2S_2 was heated to 225° in an oil bath permitting the by-product C_6H_5SH to distill off. The residue was acidified with 10% HCl, the impurities were extd with Et_2O, and the aq layer was basified with NaOH soln. The mixt was then extd with Et_2O, dried (Na_2SO_4), filtered, and flash evapd, and the residual oil was distd at 90–93° (0.4 mm) to give 7.95 g (89%) of pure colorless product: nmr δ 2.85 (s, 3 H, 1-CH_3), 2.55 (s, 3 H, 3-CH_3).

1,4-Dimethylisoquinoline (7). The procedure employed was similar to the prepn of 1. The residual oil was distd at 100–105° (0.3 mm) to yield 7.3 g (80%) of pure colorless compd: nmr δ 2.86 (s, 3 H, 1-CH_3), 2.48 (s, 3 H, 4-CH_3), 7.39 (broad s, 1 H, H-3).

1,5-Dimethylisoquinoline (15). This compd was synthesized according to Späth, *et al.*:[11] mp 92–93° (reported 97–98°); nmr δ 2.89 (s, 3 H, 1-CH_3), 2.61 (s, 3 H, 5-CH_3), 8.40 (d, 1 H, J = 7.0 Hz, H-3).

1,3-Dimethylisoquinoline *N*-Oxide (2). A 40% soln of AcO_2H (6.1 ml) was mixed with 0.46 g of NaOAc and added slowly to 4.74 g of 1 preheated at 80°. The mixt was heated for 4 hr with stirring and then allowed to remain at room temp overnight. The AcOH was removed by flash evapn, 2 was then extd with Et_2O, solvent was removed, and the compd was distd at 153–155° (0.2 mm) to yield 4.07 g (78%) of pure material which crystd on standing: mp 50–51°; nmr δ 2.76 (s, 3 H, 1-CH_3), 2.50 (s, 3 H, 3-CH_3). *Anal.* ($C_{11}H_{11}NO$) C, H, N.

1,4-Dimethylisoquinoline *N*-oxide (8) was synthesized according to the procedure of Robison and Robison[12] for the synthesis of isoquinoline *N*-oxide. Recrystn from EtOAc and cyclohexane (Norit A) gave pale, tan-colored crystals: yield 58%; mp 150–151°; nmr δ 2.68 (s, 3 H, 1-CH_3), 2.50 (s, 3 H, 4-CH_3), 8.10 (broad s, 1 H, H-3). *Anal.* ($C_{11}H_{11}NO$) C, H, N.

Rearrangement of 1,3-Dimethylisoquinoline *N*-Oxide. Compd 2 (4.01 g) was heated at 120° in 40 ml of Ac_2O for 2 hr. The mixt was concd to give a dark oil which distd at 130–150° (0.35 mm) yielding 4.4 g (88%) of an oily mixt of esters. To 4.28 g of this mixt of esters 100 ml of 10% HCl was added and heated for 1 hr at 100°. The soln was made alk with NaOH soln (pH 11.0) and 3-methyl-1-hydroxymethylisoquinoline (3) was extd with Et_2O, dried (Na_2SO_4), and concd. The residual solid was recrystd from petr ether to give 1.26 g (37%) as colorless crystals: mp 103–104°; nmr δ 2.60 (s, 3 H, CH_3), 5.05 (s, 2 H, CH_2). *Anal.* ($C_{11}H_{11}NO$) C, H, N.

The alk layer was neutralized to pH 7.0 with 10% HCl, whereupon 4-hydroxy-1,3-dimethylisoquinoline (4) pptd. The ppt was filtered, washed with H_2O and dried. Recrystn from EtOAc contg a small amt of MeOH yielded 0.7 g (20%) of pure material: mp 169–170°; nmr δ 2.84 (s, 3 H, 1-CH_3), 2.77 (s, 3 H, 3-CH_3), 3.86 (broad s, 1 H, OH). *Anal.* ($C_{11}H_{11}NO$) C, H, N.

Rearrangement of 1,4-Dimethylisoquinoline *N*-Oxide. Compd 8 (1.5 g) was heated at 110° in 1.5 ml of Ac_2O for 2 hr. Excess Ac_2O was removed leaving a dark oil which was extd with Et_2O. The Et_2O was removed and then the oil was distd at 167–170° (0.5 mm). The pale yellow oil crystd upon cooling: yield 1.14 g (61%); mp 73–76°. Several recrystns from petr ether raised the mp to 76–77°. The 4-methyl-1-acetoxymethylisoquinoline (11) was possibly contaminated with the 4-acetoxymethyl isomer and was purified after acid hydrolysis.

Compd 11 (0.8 g) was heated at 100° in 20 ml of 10% HCl for 1 hr. The soln was basified with NaOH soln and extd with Et_2O. The Et_2O layer was dried (Na_2SO_4), filtered, and concd to give 4-methyl-1-hydroxymethylisoquinoline (12). The crude 12 was

§Where analyses are indicated only by symbols of the elements, analytical results obtained for those elements are within 0.4% of the theoretical value.

chromatogd on 60 g of silica gel (100–200 mesh) and eluted with EtOAc (500 ml). The solvent was removed and the residue was recrystd from hexane to give colorless crystals: 0.27 g (42%); mp 87–88°; nmr δ 2.56 (s, 3 H, 4-CH_3), 5.05 (d, 2 H, CH_2). *Anal.* ($C_{11}H_{11}NO$) C, H, N.

3-Methylisoquinoline-1-carboxaldehyde (5). Compd **3** (0.38 g, 2.2 mmoles) was dissolved in 20 ml of C_6H_6 and 0.35 g (4.0 mmoles) of MnO_2 was added. The mixt was refluxed for 4 hr and filtered, and the C_6H_6 was removed to yield **5**. Recrystn from petr ether gave colorless needles: 0.27 g (72%); mp 71–72°; nmr δ 10.22 (s, 1 H, CHO), 2.71 (s, 3 H, 3-CH_3). *Anal.* ($C_{11}H_9NO$) N.

4-Methylisoquinoline-1-carboxaldehyde (13) was synthesized by the same procedure as 5, oxidizing 12 with MnO_2. Recrystn from petr ether yielded colorless crystals in 70% yield: mp 59–60°; nmr δ 10.21 (s, 1 H, CHO), 2.68 (s, 3 H, 4-CH_3). *Anal.* ($C_{11}H_9NO$) N.

5-Methylisoquinoline-1-carboxaldehyde (16). Compd **15** (0.32 g, 2 mmoles) was dissolved in 25 ml of dioxane and 0.22 g (2 mmoles) of SeO_2 was added slowly. The mixt was refluxed for 2.5 hr and filtered, dioxane was removed, and the residue was extd with dil HCl. The acid layer was filtered and made alk with $NaHCO_3$, and the resulting ppt was collected, washed (H_2O), and dried. Crystn from hexane (Norit) yielded colorless fibrous material: 0.25 g (72%); mp 108–109°; nmr δ 10.28 (s, 1 H, CHO), 8.81 (d, 1 H, J = 5.7 Hz, H-3), 8.18 (dd, 1 H, J = 5.5 and 0.8 Hz, H-4), 2.69 (t, 3 H, 5-CH_3). *Anal.* ($C_{11}H_9NO$) N.

Thiosemicarbazones. The thiosemicarbazones were prepd by treating alcoholic solns of the corresponding carboxaldehydes with an aq soln of thiosemicarbazide contg a few drops of dil AcOH. Relevant data concerning these compds are listed in Table **II**.

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Specificity of Inhibition of Coenzyme Q-Enzyme Systems by Lipoidal Benzoquinone Derivatives†

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5-Substituted 2,3-dimethoxy-6-phytyl-1,4-benzoquinones were found to inhibit mitochondrial NADH-oxidase and succinoxidase systems from beef heart. The most effective group in the 5 position was OH for the 6-phytyl derivatives. The 5-Cl and 5-Br derivatives were less inhibitory than the 5-OH derivatives, and in diminishing degree. The 5-MeO derivative was essentially noninhibitory. 6-Alkyl- and 6-isoprenyl-2,3-dimethoxy-5-hydroxy-1,4-benzoquinones were similarly evaluated. Inhibition of the NADH-oxidase system was greatest when the hydroxyquinone possessed a side chain of from 16 and 17 C. Inhibition of the succinoxidase system was relatively nonspecific in respect to the side chain. The succinoxidase system was generally more sensitive to most of the benzoquinones tested than was the NADH-oxidase system.

Mitochondrial reconstruction[1,2] and spectrophotometric investigations on the kinetics of coenzyme Q turnover during electron transport[3] are responsible for the view that coenzyme Q participates in the primary electron transport sequence. Coenzyme Q has a widespread distribution in biological systems[4] including the malarial parasite.[5-8] Mammalian succinoxidase and NADH-oxidase systems have been extensively studied and may be considered representative of the coenzyme Q electron transport sequences.

Preliminary structure-activity investigations demonstrated that mitochondrial succinoxidase activity was inhibited by various antimalarial naphthoquinone analogs, and the activity was restored by coenzyme Q and its derivatives.[9] Next, chloroquine and a new napthoquinone antimalarial, 2-ω-cyclohexyloctyl-3-hydroxy-1,4-naphthoquinone, were shown to inhibit beef heart mitochondrial succinoxidase systems.[10] Again, the inhibitory action of antimalarial agents was reversed by coenzyme Q.

These findings link one kind of inhibition of mitochondrial electron transport at the coenzyme Q loci to chemotherapy of malaria. CoQ_8 is the dominant CoQ of *Plasmodium*.[5-8] A series of benzoquinones structurally related to known antimalarial naphthoquinones, which were also succinoxidase inhibitors,[9] have been synthesized.[11] These benzoquinones also have structural resemblance to coenzyme Q. They represent potential antagonists of coenzyme Q function in mitochondrial electron transport; hence, they also represent potential antimalarial activity.

Separate sites for the function of coenzyme Q in the succinoxidase and NADH-oxidase systems in beef heart[12,13] and yeast[13,14] mitochondria have been implicated by studies on the structural specificity of coenzyme Q. These investigations demonstrated that the function of coenzyme Q in the

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†Coenzyme Q. 141.