Me_2CO, $CHCl_3$, or CCl_4. Anal. ($C_{15}H_{14}OS_3$) C, H, S.

TLC (CCl_4) showed that with **16** (50 mg) in refluxing MeOH (10 mL), change from an original single spot for **16** to a total of three spots (16 plus the two symmetrical disulfides) began to occur at ~19 h; **16** itself showed no change in TLC after ~5 months at 5 °C.

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Design, Synthesis, and Correlation Analysis of 7-Substituted 4-Hydroxyquinoline-3-carboxylic Acids as Inhibitors of Cellular Respiration

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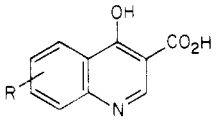
Fifteen 7-substituted 4-hydroxyquinoline-3-carboxylic acids have been designed to minimize covariance between the physicochemical substituent parameters: π, MR, and σ_p. The molecules have been synthesized and evaluated for their ability to inhibit the respiration of Ehrlich ascites cells as a whole cell model and for their ability to inhibit malate dehydrogenase as an intracellular target enzyme model. Correlation analysis indicates that ascites cell inhibition is linearly related to π and that malate dehydrogenase inhibition is linearly related to MR.

Explorations of potential applications of quantitative structure–activity relationships (QSAR, correlation analysis) continue in our attempts to characterize and exploit metabolic and structural differences between normal and malignant tissue for purposes of chemotherapy. Alterations in the neoplastic cell membranes have been reported which result in changes in antigenic and transport properties.[1] Renewed and more detailed examinations of glycolysis and respiration pathways have been conducted.[2] Some cancer cells have exhibited abnormal levels or activities of lactate dehydrogenase,[3,4] malate dehydrogenase,[3-6] and other enzymes.[7,8] Selective inhibition of these enzymes in neoplastic tissue should increase the potential for chemotherapy of solid tumor systems as well as provide new candidates for inclusion in combination therapy. Selectivity, of course, is the key and is extremely critical

in order to minimize cytotoxicity to normal cell populations. This can potentially be achieved through rational, stepwise development of agents which can simultaneously exploit cell membrane transport characteristics as well as enzyme differences in respiratory pathways.

The design by Baker[9-13] of an extensive series of 4-hydroxyquinoline-3-carboxylic acids (1) as inhibitors of glyceraldehyde-3-phosphate dehydrogenase (1.2.1.12), lactate dehydrogenase (1.1.1.27), malate dehydrogenase (1.1.1.37), and glutamate dehydrogenase (1.4.1.2) has been followed by an elegant correlation analysis of the data by Hansch.[14] These reports have led to the selection of this



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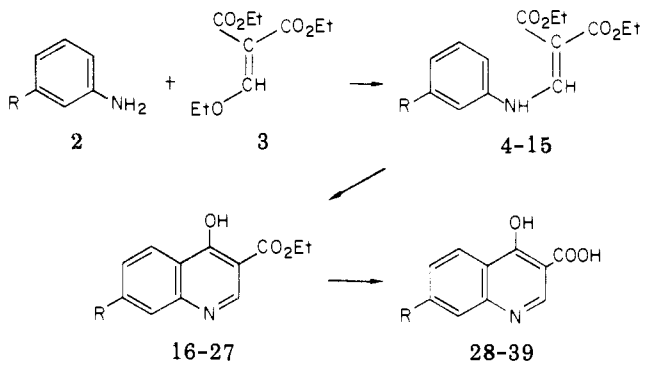
class of compounds for further correlation analysis with the objective of identifying the physicochemical properties of 1 required for transport. The 7 position of 1 has been chosen for structural modification in this study for several reasons. Only a few 7-substituents were prepared and evaluated by Baker consequently precluding any clear-cut definition of physicochemical influences on enzyme binding in this region. Additionally, this substituent position may prove to be a point of enzymic bulk tolerance and thus be suitable for modulation of transport properties. The published correlation analysis strongly suggests that the area of the malate dehydrogenase binding site which comes into contact with the 6,7,8 positions of the quinoline nucleus is polar in nature and correlates with molar refractivity (MR). In contrast, it seemed highly probable that cell membrane transport would follow changes in octanol-water partition coefficient. As a consequence it was most critical that a series of congeners be designed such that enzymic and cellular correlations would allow one to distinguish between MR influences and π influences. With these objectives in mind, a set of 7-substituted 4-hydroxyquinoline-3-carboxylic acids was selected to maximize the range in partition coefficient (over 7 log units) and to minimize, as much as feasible, any covariance with molar refraction or with electronic parameters. The series has been synthesized and evaluated for inhibition of Ehrlich ascites cell respiration as a whole cell model and for inhibition of malate dehydrogenase as a target enzyme model. These results with subsequent correlation analysis are the subject of this report.

Chemistry. Many of the desired congeners were readily available via established procedures as illustrated in Scheme I. Properties and reaction conditions are summarized in Table I. With two exceptions, the requisite meta-substituted anilines (2) were commercially available or could be derived by reduction of analogous nitrobenzenes. The 3,4-dichlorobenzyloxy congener was prepared by treatment of *m*-nitrophenol with 3,4-dichlorobenzyl chloride, followed by reduction to the aniline. Nitration of phenylmethyl sulfone with potassium nitrate and sulfuric acid,[15] followed by reduction using stannous chloride and hydrochloric acid,[16] afforded the *m*-methyl sulfone of aniline. In most instances condensation of the anilines 2 with diethoxymethylenemalonate (3) gave the anilinomethylenemalonates 4-15 which were smoothly converted to the esters 16-27 by thermal cyclization in diphenyl ether. Base-catalyzed hydrolysis then led to the 7-substituted 4-hydroxyquinoline-3-carboxylic acids (28-39). The 7-cyano analogue 24 gave a mixture of nitrile

Scheme I



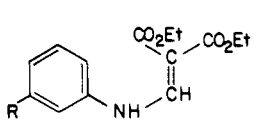
2 3 4-15 16-27 28-39

and carboxamide upon treatment with base. This mixture was completely converted to 37 with concentrated sulfuric acid. All attempts to prepare the 7-hydroxy congener 40 by cleavage of the corresponding 7-methoxy derivative 31, using 48% hydrobromic acid, pyridine hydrochloride, or 47% hydroiodic acid, proved fruitless. Treatment of 34 with hydrobromic acid and glacial acetic acid did result in cleavage of the benzyl moiety to give 40. Efforts to prepare the 7-sulfonic acid 42 by starting with *m*-aminobenzenesulfonic acid or with *m*-aminobenzenesulfonyl fluoride failed upon attempted condensation with diethoxymethylenemalonate. While preparation of *m*-sulfonamidoanilinomethylenemalonate was achieved, subsequent cyclization could not be realized. Consequently, the synthesis of 41 and 42 was approached through an alternative procedure beginning with the 7-nitro-4-hydroxyquinoline-3-carboxylic acid ethyl ester (27). Reduction of 27 to 7-amino-4-hydroxyquinoline-3-carboxylic acid ethyl ester (43), followed by diazotization and treatment with sulfur dioxide in acetic acid–cuprous chloride, afforded the corresponding 7-sulfonyl chloride 45. The 7-chlorosulfonyl-4-hydroxyquinoline-3-carboxylic acid ethyl ester (45) was converted to 42 by base-catalyzed hydrolysis. Synthesis of 41 was realized by treatment of the 7-sulfonyl chloride 45 with concentrated ammonium hydroxide, followed by hydrolysis of the ester 46 with dilute sodium hydroxide (Scheme II).

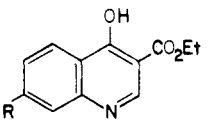
The apparent partition coefficients for all congeners except 35 and 42 were measured by determining the distribution between octanol and pH 7.4 phosphate, Ringer.[21,22] Compound 42, the 7-sulfonate, was apparently too hydrophilic to allow accurate determination. The log *P* (apparent) for 35 was calculated by adding the published π values[20] for two chloro substituents to the measured log *P* (apparent) for 34.

Biological Results. Compounds 28-42 were evaluated for their ability to inhibit the respiration of Ehrlich ascites cell suspensions as a whole cell model and these results compared to the inhibition of malate dehydrogenase as a model intracellular target enzyme. Ehrlich ascites tumor cells have been extensively utilized as a system for study of various biochemical properties of intact cells.[17-19] The respiration rates were followed potentiometrically by oxygen electrode and the percent inhibition of oxygen uptake was correlated with inhibitor concentration by the method of least squares to obtain values for 50% inhibition (I_{50}). Malate dehydrogenase was selected to monitor enzyme inhibition because of its key role in the citric acid cycle and also because this enzyme appeared to be the most susceptible to inhibition in Baker's studies.[9-13] Influences on the dehydrogenase reaction were followed spectrophotometrically in the forward direction by monitoring the increase in absorption at 340 nm due to the production of

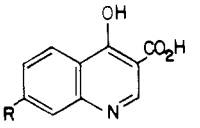
Table I. Physical and Chemical Properties



4-15



16-27



28-42

No.	R	Rxn temp, °C (time)	Mp, °C (lit.)	Yield, %
4	H	100-103 (2 h)	48-49 (50)[a]	92
5	Cl	100 (6 h)	56-57 (55-56)[b]	89
6	F	108-109 (16 h)	47-48 (44-45)[c]	80
7	OCH_3	105 (23 h)	Oil[d]	
8	$COCH_3$	110-112 (23 h)	85-86	80
9	$N(CH_3)_2$	114 (22 h)	49-51	92
10	$OCH_2C_6H_5$	114-116 (20 h)	70-71 (71)[e]	97
11	$OCH_2C_6H_3$-3,4-Cl_2	112-114 (24 h)	81-82	
12	CN	111-112 (24 h)	112-114	86
13	SO_2CH_3	114 (24 h)	112-113	88
14	CO_2Et	110-112 (26 h)	52-53[f]	97
15	NO_2	110-111 (27 h)	83-84 (81-82)[g]	85
16	H	258-259 (1 h)	271-273 (270)[a]	55
17	Cl	258-259 (1 h)	296-299 (295-297)[b]	70
18	F	258-259 (30 min)	308-309 (309-310)[c]	70
19	OCH_3	250-253 (20 min)	275-276 (275)[d]	70
20	$COCH_3$	253-254 (15 min)	302-303	86
21	$N(CH_3)_2$	253-254 (20 min)	289-290	65
22	$OCH_2C_6H_5$	258-259 (30 min)	292-293 (295)[e]	92
23	$OCH_2C_6H_3$-3,4-Cl_2	257-258 (15 min)	297-298	88
24	CN	252-254 (20 min)	310	95
25	SO_2CH_3	256-257 (20 min)	306-307	98
26	CO_2Et	253-254 (20 min)	305[f]	63
27	NO_2	258-259 (45 min)	325-326 (>300)[g]	96

No.	R	Recrystn solvent	Mp, °C (lit.)	Yield, %	Analyses
28	H	HOAc	265 dec (267-268)[h]	94	
29	Cl	HOAc	273-274 dec (273-274)[b]	98	
30	F	HOAc	267-268 dec	100	C, H, N
31	OCH_3	HOAc	266 dec (257-260)[d]	92	
32	$COCH_3$	DMF	281-282 dec	92	C, H, N
33	$N(CH_3)_2$	Nitrobenzene	272-273 dec	96	C, H, N
34	$OCH_2C_6H_5$	DMF	259-260 dec (273)[e]	96	
35	$OCH_2C_6H_3$-3,4-Cl_2	DMF	274-275 dec	89	C, H, N
36	NO_2	HOAc	282 dec (287)[g]	97	
37	$CONH_2$	DMF	307-308 dec	92	C, H, N
38	COOH	DMF	>325	100	C, H, N
39	SO_2CH_3	DMF	283-284 dec	100	C, H, N, S
40	OH	DMF	284-285 dec	100	C, H, N
41	SO_2NH_2	DMF	282-283 dec	80	C, H, N, S
42	SO_3H	Dilute HCl	>350	87	C, H, N, S

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NADH. Enzyme inhibition by compounds **28** (7-H) and **31** (7-OCH_3) could not be measured as no effect on the enzymic reaction could be seen at the limits of their respective solubilities. In general, inhibition of both test systems was observed to increase with increases in group size and lipophilic character.

Analyses and Discussion. The biological data for compounds **28-42** were subjected to computerized multiparameter regression analysis in an effort to delineate structural effects on ascites cell respiratory inhibition and on malate dehydrogenase enzyme inhibition. As usual, the I_{50} values, in moles per liter, have been converted to the negative logarithms (pI_{50}). The biological activities and substituent constants utilized in the regression analysis reported below are presented in Table II. Every potentially meaningful combination of π, π^2, MR, MR^2, σ_p, σ_p^2, σ_p^-, σ_p^+, F, R, $\pi \times MR$, $\pi \times \sigma_p$, and $MR \times \sigma_p$ has been examined with eq 1-4 surfacing as the most significant

$$pI_{50}\ (\text{ascites}) = 0.46\ (\pm 0.11)\ \pi + 3.22\ (\pm 0.16) \quad (1)$$

$$n = 14;\ s = 0.280;\ r = 0.933$$

$$pI_{50}\ (\text{ascites}) = 0.45\ (\pm 0.29)\ MR + 2.68\ (\pm 0.47) \quad (2)$$

$$n = 14;\ s = 0.554;\ r = 0.699$$

$$pI_{50}\ (\text{malate}) = 0.26\ (\pm 0.22)\ \pi + 3.31\ (\pm 0.45) \quad (3)$$

$$n = 13;\ s = 0.716;\ r = 0.604$$

$$pI_{50}\ (\text{malate}) = 0.70\ (\pm 0.17)\ MR + 2.29\ (\pm 0.30) \quad (4)$$

$$n = 13;\ s = 0.315;\ r = 0.939$$

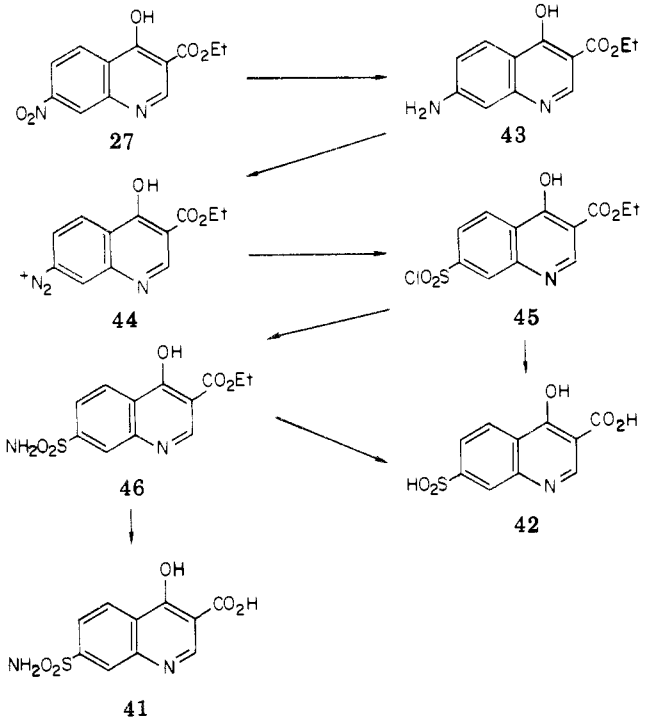
correlations of ascites cell (eq 1 and 2) and of malate dehydrogenase (eq 3 and 4) inhibition.

Table II. Biological and Physicochemical Data for 7-R-4-Hydroxyquinoline-3-carboxylic Acids

				Ascites			MDH		
Compd	R	MR[a]	π[b]	I_{50}[c]	pI_{50} (obsd)	pI_{50} (calcd[d])	I_{50}[c]	pI_{50} (obsd)	pI_{50} (calcd[e])
28	H	0.103	0.0	1.06 (0.17)	2.98	3.22	[f]		2.36
29	Cl	0.603	0.55	0.14 (0.07)	3.84	3.28	3.62 (0.55)	2.44	2.71
30	F	0.092	0.06	0.50 (0.82)	3.30	3.25	10.55 (11.32)	1.98	2.35
31	OCH_3	0.787	0.49	0.52 (0.34)	3.28	3.45	[f]		2.84
32	$COCH_3$	1.118	-0.39	0.79 (0.46)	3.10	3.04	0.91 (0.03)	3.04	3.07
33	$N(CH_3)_2$	1.555	1.10	0.47 (0.11)	3.33	3.74	0.48 (0.03)	3.32	3.38
34	$OCH_2C_6H_5$	3.174	1.81	0.038 (0.014)	4.41	4.07	0.032 (0.006)	4.49	4.51
35	$OCH_2C_6H_3$-3,4-Cl_2	4.174	3.23[g]	0.015 (0.013)	4.82	4.73	0.005 (0.002)	5.32	5.21
36	NO_2	0.736	-0.40	0.58 (0.14)	3.24	3.04	1.89 (0.67)	2.72	2.80
37	$CONH_2$	0.981	-1.18	5.82 (2.50)	2.24	2.67	0.74 (0.58)	3.13	2.98
38	COOH	0.605	-2.80	5.77 (0.76)	2.24	1.92	1.07 (0.77)	2.97	2.71
39	SO_2CH_3	1.349	-1.39	1.79 (0.89)	2.75	2.58	0.66 (0.06)	3.18	3.23
40	OH	0.285	0.06	0.91 (1.24)	3.04	3.25	0.49 (0.15)	3.31	2.49
41	SO_2NH_2	1.228	-1.36	3.40 (0.54)	2.47	2.59	0.95 (0.06)	3.02	3.15
42	SO_3^-	0.971	-4.76[a]	1.30 (0.64)	2.88[f]	1.01	2.14 (1.53)	2.67	2.97

[a] Taken from ref 20. [b] From measured log P (apparent) using redisilled octanol and pH 7.4 phosphate, Ringer; compound **28** has log P = 0.23. [c] I_{50} is in moles per liter $\times 10^3$; for ascites the number in parentheses is the standard deviation for several I_{50} determinations; for MDH the number in parentheses is the 95% confidence interval determined according to ref 23; $pI_{50} = -\log I_{50}$. [d] Calculated via eq 1. [e] Calculated via 4. [f] Omitted from the correlations. [g] π for 34 plus 2 $\times$ 0.71 (Cl).

Scheme II



In these equations, the numbers in parentheses are the 95% confidence intervals associated with the respective coefficients, n is the number of data points used in the correlation, s is the standard deviation, and r is the correlation coefficient. Of the total of 15 compounds evaluated, several were deleted from the correlation analysis for the following reasons. The sulfonate analogue **42** was very poorly fit in the ascites correlations with an observed activity which was considerably higher than predicted. As noted earlier, it was not possible to measure the partition coefficient for this congener, therefore requiring an estimate of its π-substituent constant which was obviously much too negative. Compounds **28** and **31** were necessarily left out of the enzyme correlations due to a lack of an estimate of their biological activities. Interestingly, the 7-sulfonate **42** is fairly well fit by eq 4 where π is not involved. Note that MR, molar refractivity, taken from published listings,[20] has been scaled by 0.1 to allow more direct comparison with π.

It is readily apparent that eq 1 and 4 are the superior relationships accounting for structural influences on ascites and malate dehydrogenase inhibition, respectively. Addition of electronic terms, squared terms, or cross products gave no improvement in either case. Equation 1 is particularly fascinating since one would not expect a linear relationship to hold over such a large π range (6 log units). One can speculate that a nonlinear or parabolic relationship must exist but has been obscured by characteristics influencing enzyme interaction. The effects of changes in target enzyme interaction on changes in whole cell activity are most obvious if eq 2 is compared to eq 4. The higher slope (0.70 vs. 0.45) associated with MR in eq 4 implies that enzyme inhibition increases at a greater rate than ascites inhibition with increasing MR. This more rapid increase in enzyme inhibition is apparently sufficient to overcome the effects of inferior ascites membrane transport. For example, a molecule such as **35**, which is highly lipophilic, would not be expected to pass readily through the cell membrane. However, this is not reflected in an anticipated decrease in ascites respiratory inhibition because of extremely potent enzyme inhibition since compound **35** also has a very high MR value. The equations in MR must be utilized in this comparison since MR is obviously a contributory element to the whole cell activity while π is clearly not a factor in isolated enzyme inhibition. One might expect, under the circumstances, that ascites respiratory inhibition would be influenced by a combination of π and MR; however, the ascites equation in these two parameters affords only a slight and statistically insignificant improvement in correlation coefficient and standard deviation. Finally, the quality of eq 1 supports the conclusion that lipophilic character is the most critical feature in determining the extent of membrane transport and thus ascites cell respiratory inhibition.

Turning to the malate dehydrogenase relationships (eq 3 and 4), it is clear that the correlations published by Hansch[14] have been substantiated by those reported here. The influence of MR at the 7 position has been much more sharply defined by the choice of substituents which minimize covariance between π and MR ($r^2 = 0.36$). Thus, the possibility of hydrophobic interactions in that portion of the enzyme immediately adjacent to the 7 position of

the bound quinoline nucleus has been eliminated, and a tolerance for polar or bulky substituents established. Molar refractivity has been found to correlate well with enzyme binding in a number of investigations.[24-26] It has been interpreted as a steric parameter or as a polar interaction parameter depending upon the specific circumstances of each study. The parameter does parallel molecular weight and molecular volume as pointed out by Hansch.[20,24] However, in most instances where a positive coefficient has been associated with MR, polar interactions seem to be the preferred explanation with the assumption that increased steric bulk will normally exert a negative influence on biological response. For the present case of malate dehydrogenase inhibition, the choice between ionic or hydrogen bond interaction on one hand and bulk-induced conformational perturbation on the other must await further investigation.

Conclusions

Correlation analysis of a carefully designed set of enzyme inhibitors has afforded highly significant relationships between π and ascites cell respiratory inhibition and between MR and malate dehydrogenase inhibition. The process of substituent selection as employed in this study cannot be overemphasized. Covariance between MR and π is all too often a serious obstacle preventing delineation of substituent effects. The result of consciously minimizing this covariance has, in these investigations, been clearly demonstrated.

The purpose of Baker's original studies on dehydrogenase inhibitors was to develop a new class of antineoplastic agents, respiratory inhibitors. We have now shown that the 4-hydroxyquinoline-3-carboxylic acids are inhibitors of intact cell respiration although we have not as yet been able to fully characterize transport properties, apparently because of extremely potent enzyme inhibition. The observation that relatively weak malate dehydrogenase inhibitors (e.g., compounds **28–31**) do inhibit respiration of the ascites cells may be a reflection of membrane transport and of inhibition of alternative intracellular enzymes. In general, there appears to be a qualitative correlation between ascites cell and malate dehydrogenase inhibition which supports the hypothesis that the enzyme that has been monitored is one of the principal intracellular target sites.

Finally, the MR correlation with enzyme inhibition suggests that the 7 position can be utilized in one of two ways in the design of potent respiratory inhibitors. First, it seems that increases in molar refractivity of 7-substituents will afford more potent enzyme inhibitors. One must anticipate that limits in membrane transport as well as enzyme binding properties do exist such that potency cannot be increased indefinitely. Second, the 7 position of molecules possessing highly lipophilic substituents at other points on the quinoline nucleus (e.g., the 5 position) could be utilized to introduce polar, hydrophilic substituents that could improve membrane transport without impairment of enzyme binding. Both of these possibilities are currently under investigation.

Experimental Section

All melting points were taken on a Mel-Temp apparatus and are uncorrected. Elemental analyses were carried out by Midwest Microlab, Ltd., Indianapolis, Ind., and were within 0.4% unless otherwise noted. IR, NMR, and UV data were consistent with the assigned structures. UV spectra were recorded on a Beckman Acta V spectrophotometer. IR spectra were recorded on a Beckman IR-33 spectrophotometer as neat oils, KBr pellets, or Nujol mulls. NMR spectra were determined on a Hitachi Perkin-Elmer R-24 high-resolution NMR spectrometer. Malate dehydrogenase and NAD^+ were obtained from the Sigma Chemical Co.

Synthesis. 4,7-Dihydroxyquinoline-3-carboxylic Acid (40). A suspension of 3.0 g (0.01 mol) of 7-benzyloxy-4-hydroxyquinoline-3-carboxylic acid (**34**) in 20 mL of a 1:1 mixture of 48% HBr and glacial acetic acid was heated at reflux for 4 h. The reaction was allowed to cool to room temperature and the solid product isolated by vacuum filtration, washed with H_2O, and air-dried to give 2.1 g (100%). Recrystallization from aqueous DMF afforded pure **40**: mp 284–285 °C dec; IR (KBr) 3240 (OH), 3040 (CH), 1690 (C=O), 1615, 1440, 1210 cm^{-1}. Anal. ($C_{10}H_7NO_4$) C, H, N.

3-Nitrophenylmethyl Sulfone. Following the procedure of Twist,[15] 6.83 g (0.0675 mol) of KNO_3 was slowly added to a stirred solution of 7.0 g (0.045 mol) of phenylmethyl sulfone in 23 mL of concentrated H_2SO_4 at 10 °C. The reaction mixture was warmed to 90 °C for 1 h and poured over ice water to give product in the form of a yellow solid. The solid was isolated by filtration in vacuo, washed with cold H_2O, and air-dried to give 8.5 g (94%) which was recrystallized from EtOH: mp 147–149 °C (lit.[15] mp 146 °C).

3-Aminophenylmethyl Sulfone. 3-Nitrophenylmethyl sulfone (7.0 g, 0.033 mol) was slowly added to a stirred solution of 35 g of $SnCl_2 \cdot 2H_2O$ in 50 mL of concentrated HCl. The reaction mixture was then stirred for 3.5 h at room temperature; the yellow precipitate was isolated by filtration in vacuo, washed with concentrated HCl, and dissolved in 200 mL of H_2O. The solution was made strongly basic with 15% NaOH and extracted with $CHCl_3$, and the extract was dried (Na_2SO_4). The $CHCl_3$ was evaporated in vacuo to give a pale yellow oil which solidified on standing (5.5 g, 92%). Recrystallization from water afforded pure product: mp 54–55 °C (lit.[16] mp 58 °C).

Diethyl 3-Methylsulfonylanilinomethylenemalonate (13). A mixture of 5.1 g (0.03 mol) of 3-aminophenylmethyl sulfone and 6.5 g (0.03 mol) of diethyl ethoxymethylenemalonate was stirred at 114 °C for 24 h. During the heating, the reaction mixture turned to a viscous orange oil and 1.7 mL of EtOH was collected via a Dean-Stark trap. The reaction mixture solidified upon cooling to room temperature and was recrystallized from EtOH to give **13**: 8.9 g (87%); mp 112–113 °C; IR (KBr) 3025 (CH), 1700, 1650 (C=O), 1305, 1250, 1145 cm^{-1}.

Ethyl 7-Methylsulfonyl-4-hydroxyquinoline-3-carboxylate (25). Diethyl 3-methylsulfonylanilinomethylenemalonate (**13**) (6.0 g, 0.0176 mol) was added with stirring to 125 mL of phenyl ether at 254 °C over a period of 10 min. As the reaction mixture was heated for an additional 20 min at 256–257 °C, a white precipitate separated and 0.7 mL of EtOH was collected via a Dean-Stark trap. Upon cooling to room temperature the precipitate was isolated by filtration in vacuo, washed with phenyl ether and petroleum ether, and air-dried to give **25**: 5.1 g (98%). Recrystallization from DMF afforded pure **25**: mp 306–307 °C dec; IR (KBr) 3100 (CH), 1700 (C=O), 1295, 1195, 1135 cm^{-1}.

7-Methylsulfonyl-4-hydroxyquinoline-3-carboxylic Acid (39). A suspension of 4.0 g (0.0136 mol) of crude **25** in 80 mL of 10% aqueous NaOH was heated at reflux for 4 h. The initially heterogeneous reaction mixture become clear within 30 min. The reaction was allowed to cool to room temperature and acidified with dilute HCl. The precipitate which formed was isolated by filtration in vacuo, washed with H_2O, and air-dried to give **39**: 3.6 g (99%). Recrystallization from aqueous DMF afforded analytically pure **39**: mp 283–284 °C dec; IR (KBr) 3270 (OH), 3100 (CH), 1705 (C=O), 1315, 1145 cm^{-1}. Anal. ($C_{11}H_9NO_5S$) C, H, N, S.

4-Hydroxyquinoline-3-carboxylic Acid-7-Sulfonic Acid (42). A solution of 5.2 g (0.02 mol) of ethyl 7-nitro-4-hydroxyquinoline-3-carboxylate in 40 mL of concentrated HCl was added dropwise to a stirred solution of 20 g of $SnCl_2 \cdot 2H_2O$ in 20 mL of concentrated HCl. The ensuing exothermic reaction afforded a precipitate within a few minutes but was stirred for 6 h at room temperature. The yellow precipitate was isolated by filtration in vacuo, washed with 10 mL of concentrated HCl, suspended in 100 mL of H_2O, and made alkaline with NH_4OH. The resulting white precipitate was isolated, washed with cold H_2O, resuspended in 100 mL of H_2O, and acidified with dilute HCl to give a yellow precipitate. The precipitate was finally isolated by filtration in vacuo, washed with cold dilute HCl, and air-dried to give 4.3 g

(81%) of ethyl-7-amino-4-hydroxyquinoline-3-carboxylic acid hydrochloride (43). This material was purified by dissolving it in hot H_2O, treating with activated charcoal, and reprecipitating with concentrated HCl: mp 226–227 °C dec.

Without further purification, a solution of 6.0 g (0.019 mol) of 43 in 40 mL of concentrated HCl diluted with 8.0 mL of H_2O was treated with a solution of 1.72 g (0.019 mol) of $NaNO_2$ in 10 mL of H_2O at 0 °C. The resulting orange mixture was stirred for 30 min at 0 °C and added slowly to a suspension of 1.1 g of CuCl in 50 mL of glacial HOAc saturated with SO_2 at 0 °C. The mixture was stirred for 45 min at 5–10 °C and diluted with 75 mL of H_2O, and the precipitate was isolated by filtration, washed with cold H_2O, and air-dried to give 45: 6.0 g (85%); mp 282–285 °C.

A solution of 5.1 g (0.016 mol) of crude 45 in 50 mL of 10% aqueous NaOH was heated at reflux for 4.5 h. The reddish brown reaction mixture was cooled to 60 °C, treated with 1.5 g of activated charcoal, and filtered to give a yellow solution which deposited a pale yellow precipitate upon acidification (HCl). The precipitate was isolated by filtration in vacuo, washed with cold H_2O, and air-dried to give 3.8 g (87% from 45) of the desired product, 42. Reprecipitation from aqueous solution upon treatment with concentrated HCl afforded analytically pure 42: mp >350 °C; IR (KBr) 3000, 2700, 1625, 1280, 1215, 1070 cm^{-1}. Anal. ($C_{10}H_7NO_6S$) C, H, N, S.

7-Sulfonamido-4-hydroxyquinoline-3-carboxylic Acid (41). A solution of 6.0 g (0.019 mol) of crude 45 in 250 mL of NH_4OH was treated with 2.0 g of activated charcoal, filtered to give a pale green solution, and allowed to stand overnight (14 h) at 4 °C. Upon evaporation of the solvent at room temperature in vacuo, a gelatinous white residue was obtained. A precipitate, which formed upon acidification (dilute HCl), was isolated by filtration in vacuo, washed with H_2O, and air-dried to give 46: 5.0 g (88%). The solid was purified by dissolution in hot DMF and precipitating with ether: mp 252–255 °C dec.

A solution of 4.0 g (0.0135 mol) of crude 46 in 100 mL of 5% aqueous NaOH was heated at reflux for 4.5 h. The reaction mixture was cooled to 60 °C, treated with 1.0 g of activated charcoal, and filtered to give a bright yellow solution which deposited a white granular solid upon acidification (dilute HCl). The solid was isolated by filtration in vacuo, washed with cold H_2O, and air-dried to give 41: 2.9 g (80% from 46). Recrystallization from aqueous DMF afforded analytically pure products: mp 282–283 °C dec; IR (KBr) 3220, 3080, 1690, 1325, 1150 cm^{-1}. Anal. ($C_{10}H_8N_2O_5S$) C, H, N, S.

Biological Testing. A. Ascites. The Ehrlich ascites tumor cells were maintained by weekly transfer of 0.1–0.2 mL of the cell-rich ascites fluid each from Swiss white mice with 7–10 day tumors. For respiration studies, approximately 4 mL of the ascites fluid was withdrawn from one mouse following cervical dislocation. The cells were washed in isotonic saline before use and were suspended in sufficient saline to give approximately 0.30 g/mL and were maintained in ice until used. In each case 0.3 mL of this suspension was sufficient for individual respiration rate determinations. In measurements of respiratory inhibition the cells were incubated with pure dimethyl sulfoxide (0.1–0.3 mL) as a control or with dimethyl sulfoxide plus inhibitor (0.1–0.3 mL) as treated in sufficient glucose-free phosphate, Ringer, to give a total volume of 3.0 mL. The incubation period was 30 min at 37 °C at which time the respiration rate was recorded using a Yellowsprings Model 53 oxygraph. Dose–response relationships were generated from measurements at a minimum of four concentrations in triplicate.

B. Enzyme Inhibition. Concentrated malate dehydrogenase was diluted with pH 7.4 Tris buffer to a concentration sufficient to give 0.01–0.02 OD unit change per minute at 340 nm under the following conditions. In a cuvette were placed 0.10 mL of 2 mM L-malate neutralized with NaOH, 0.10 mL of 0.80 mM NAD^+ in H_2O, 2.50 mL of 0.05 M Tris buffer (pH 8.4), and 0.20 mL of Me_2SO ± inhibitor; if turbidity appeared the inhibitor concentration was reduced. A zero baseline was obtained while the cuvette temperature was allowed to equilibrate to the sample chamber which was maintained at 25 °C. The reaction was initiated by addition of 10 μL of appropriately diluted malate dehydrogenase. Enzyme inhibition was measured at four inhibitor concentrations in triplicate and the I_{50} calculated from the dose–response line derived by the method of least squares.

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