4-Hydroxyquinoline-3-carboxylic Acids as Inhibitors of Cell Respiration. 2. Quantitative Structure–Activity Relationship of Dehydrogenase Enzyme and Ehrlich Ascites Tumor Cell Inhibitions[1]

Eugene A. Coats,* Kishorkant J. Shah, Stanley R. Milstein, Clara S. Genther, Dilip M. Nene, Jeffrey Roesener, James Schmidt, Michael Pleiss, Ellen Wagner,

College of Pharmacy, University of Cincinnati, Cincinnati, Ohio 45267

and John K. Baker

Department of Medicinal Chemistry, School of Pharmacy, University of Mississippi, University, Mississippi 38677.
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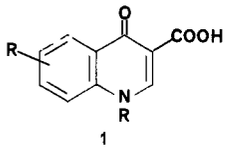
Studies on dehydrogenase enzyme inhibition have been extended with the design, synthesis, and correlation analysis of 7-[(substituted-benzyl)oxy]-, 7-[(substituted-phenethyl)oxy]-, and 7-[(substituted-phenoxy)ethoxy]-4-hydroxyquinoline-3-carboxylic acids. Sixteen new congeners and the fifteen molecules previously synthesized have been tested against cytoplasmic malate dehydrogenase and lactate dehydrogenase, as well as against mitochondrial malate dehydrogenase. The lipophilic congeners show a clear specificity for inhibition of the mitochondrial enzyme. Correlation analysis of the data on the three enzymes allows a comparison of the binding sites in quantitative terms, while examination of the data on inhibition of ascites tumor cell respiration affords an indication of membrane transport. A newly developed high-pressure liquid chromatography based retention index is compared to the octanol–water π constant as a model for hydrophobic interactions.

Biochemical pathways leading to the replication and transcription of DNA continue to be favored targets for the development of enzyme inhibitors useful as antineoplastic agents. However, because such agents are often ineffective in therapy of solid tumors with a small growth fraction, investigators have intensified their efforts to characterize additional metabolic and structural differences between normal and malignant tissues. Thus, alterations in certain enzymes involved in the respiratory pathway were identified and prompted the development of a series of respiratory enzyme inhibitors (1) by B. R. Baker.[2-6]



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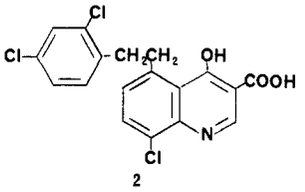
The compounds were evaluated against four isolated enzyme systems: glyceraldehyde-3-phosphate dehydrogenase and lactate dehydrogenase from cytoplasmic glycolysis, and glutamate dehydrogenase and malate dehydrogenase from the mitochondria. Correlation analyses of the data were reported by Hansch and Yoshimoto[7] and supported the qualitative assessment of Baker that hydrophobic groups substituted at the 5 position of the quinoline afford the greatest contribution to enzyme binding. It was also noted that of the four dehydrogenase systems, malate dehydrogenase appeared to be the most susceptible. However, it seemed probable to us that the more potent inhibitors of malate dehydrogenase, such as **2**, were much too lipophilic for effective transport in an



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in vivo system. Consequently, a series of 7-substituted 4-hydroxyquinoline-3-carboxylic acids was designed and synthesized.[8] Since the primary objective of the study was to evaluate transport properties, the congeners were selected to assure a wide range in hydrophobic character. The 7 position was chosen because of the apparent lack of hydrophobic interaction with the enzyme at this point,[7] and thus its potential as a region of bulk tolerance could be evaluated. The ability of the derivatives to enter an intact cell was monitored by evaluating their inhibition of the respiration of Ehrlich ascites cell suspensions as measured by oxygen electrode. The molecules were also tested for their ability to inhibit mitochondrial malic acid dehydrogenase (m-MDH). Correlation analysis of the ascites cell and enzyme data on compounds **10–24** in Tables II and III afforded eq 1 and 2.[8] In the equations, I_{50}

$$\log 1/I_{50}\ (\text{ascites}) = 0.46\ (0.11)\ \pi + 3.22\ (0.16) \quad (1)$$

$$n = 14;\ s = 0.28;\ r = 0.93\ (18\ \text{is omitted})$$

$$\log 1/I_{50}\ (\text{m-MDH}) = 0.70\ (0.17)\ \text{MR} + 2.29\ (0.30) \quad (2)$$

$$n = 13;\ s = 0.32;\ r = 0.94\ (10\ \text{and}\ 12\ \text{were omitted})$$

is the molar concentration of inhibitor providing 50% inhibition, π is the Hansch substituent constant,[9,10] and MR is molar refraction[10,11] as defined by the Lorenz–Lorenz equation. The numbers in parentheses are the 95% confidence intervals associated with the regression coefficients, n is the number of data points, s is the standard

(1) Portions of this work were presented in August, 1979, at the Third Congress of the Hungarian Pharmacological Society, Budapest, Hungary, and in August 1980. See "Abstracts of Papers", Second Chemical Congress of the North American Continent, Las Vegas, NV, 1980; American Chemical Society: Washington, DC, 1980; Abstr MEDI 73.
(2) Baker, B. R.; Bramhall, R. R. *J. Med. Chem.* **1972**, *15*, 230.
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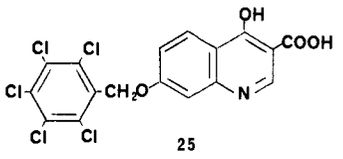
deviation, and r is the correlation coefficient. It was clear from these equations that membrane transport is controlled by lipophilicity while enzyme inhibition is controlled by polar character. The surprising feature was that ascites cell respiratory inhibition gave a linear relationship with π, suggesting that transport across the cell membrane and the mitochondrial membrane was not particularly limited by high lipophilic character. Equation 2 for m-MDH inhibition delineates a strong polar interaction between the 7-substituents and that particular portion of the enzyme binding site. For both test systems, the most active congeners were the benzyloxy and the dichlorobenzyloxy derivatives (**23** and **24** in Tables II and III). As a check on this observation, two additional substituents, the 7-(pentachlorobenzyl)oxy (**25**), and the 7-(α-



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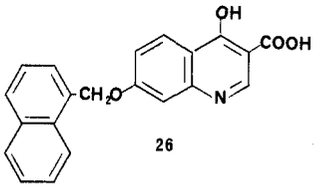

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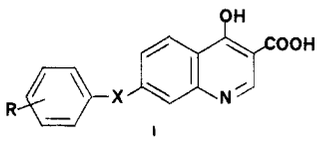
naphthyl)methoxy (**26**) were prepared and evaluated against the tumor cell and the enzyme systems. These particular congeners were chosen so as to increase MR and π without extending the bridge between the aromatic rings and the quinoline nucleus. Both molecules proved to be nearly as active as **23** and **24** and provided additional rationale for further studies which are the subject of this report.

Design. Three sets of congeners have been designed, which differ only in the distance between the aromatic ring and the 7 position of the quinoline nucleus (see structure i). The substituents on the aromatic ring were selected



i

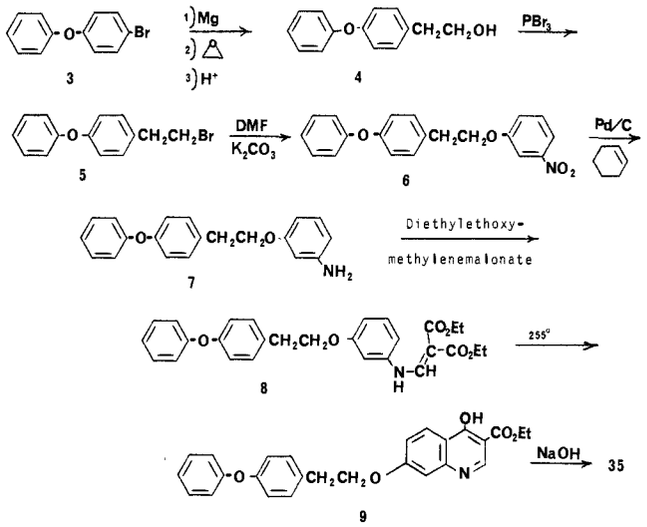
X = $-CH_2O-$; $-CH_2CH_2O-$; $-OCH_2CH_2O-$

R = 4-H; 4-F; 4-Br; 4-OC_6H_5; 3,4-CH=CH-CH=CH-

from published cluster analysis[12] based upon the parameters π, MR, F, and R. The three sets of molecules have been intentionally designed to incorporate well-defined, parallel substituent variations to allow characterization of changes in binding to mitochondrial malate dehydrogenase at three distances from the point of attachment to the parent quinoline nucleus. It was anticipated that this design would provide indications of bulk tolerance, as well as changes in intensity of physicochemical interaction with distance.

Chemistry. Preparation of the desired 7-substituted 4-hydroxyquinoline-3-carboxylic acids is dependent upon the generation of meta-substituted anilines, which can be subjected to the Gould–Jacobs quinoline synthesis. Treatment of *m*-nitrophenol with the appropriate benzyl halide, phenethyl halide, or phenoxyethyl halide in sodium/ethanol (method A) or potassium carbonate/dimethylformamide (method B) provided the *m*-nitrophenyl ethers. Reduction of the nitro group was accomplished by stannous chloride in ethanolic HCl (method C), iron powder in ethanolic HCl (method D), or palladium–carbon/cyclohexene (method E). Where the starting benzyl or phenethyl halides were not available, the corresponding alcohols were treated with phosphorus tribromide or with thionyl chloride. Phenoxybenzyl chloride was prepared by Blanc chloromethylation of phenyl ether.[13,14] Reaction of the appropriate phenols with 1,2-dibromoethane in base gave the desired phenoxyethyl bromides. The synthesis of 7-[(4-phenoxyphenethyl)oxy]-4-hydroxyquinoline-3-carboxylic acid (**35**) is illustrated in Scheme I as an example of the complete sequence. Formation of the Grignard reagent of 4-phenoxyphenyl bromide (**3**) was followed by treatment in situ with ethylene oxide to give 4-phenoxy-2-phenylethanol (**4**),[15-17] which was purified by vacuum distillation. Following conversion to the bromide (**5**) with phosphorus tribromide,[17] reaction with *m*-nitrophenol in potassium carbonate/dimethylformamide gave the *m*-nitrophenyl ether **6** in about 25% yield (from the bromide) with 4-phenoxystyrene as the major byproduct. Reduction with palladium–carbon/cyclohexene[18] proceeded in 95% yield to give **7**. Condensation of the aniline with diethyl ethoxymethylenemalonate afforded **8**, which was cyclized without purification in refluxing diphenyl ether to the quinoline ester **9**. Hydrolysis using 20% NaOH gave **35**. Physical and chemical data for those compounds prepared in this study are summarized in Table I.

Scheme I



The physicochemical properties associated with all congeners are listed in Table II. In addition to the routinely employed substituent constants, the retention index (RI) has been measured for each compound. This retention index scale is derived from reverse-phase high-pressure LC determination of capacity factors, k', for compounds **10–40**, as compared to a standard series of 2-ketoalkanes (C_3–C_{23}).[19,20] It was envisioned that the retention indexes

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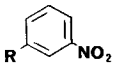
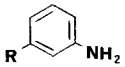
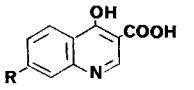
Table I. Physical and Chemical Properties of 7-Substituted 4-Hydroxyquinoline-3-carboxylic Acids and Precursors

no.	R	method[a]	mp, °C	yield, %	method[e]	mp, °C	yield, %	mp, °C	recrystn solvent	mol formula[g]
										
25	$OCH_2C_6Cl_5$	B[b]	191-192	79	C	139-140	84	305-306	DMF	$C_{17}H_8Cl_5NO_4$
26	OCH_2-α-$C_{10}H_7$	A	103-104	69	C	88-91	84[d]	272-273	aq DMF	$C_{21}H_{15}NO_4$
27	$OCH_2C_6H_4$-4-F	A	91-92	77	C	91-92	63	269-270	aq DMF	$C_{17}H_{12}FNO_4$
28	$OCH_2C_6H_4$-4-Br	A	84-86	99[d]	C	92-93	99[d]	276-278	aq DMF	$C_{17}H_{12}BrNO_4$
29	OCH_2-β-$C_{10}H_7$	B	119-120	96[d]	D	118-119	57	264-265	methoxyethanol	$C_{21}H_{15}NO_4$
30	$OCH_2C_6H_4$-4-OC_6H_5	B	79-80	99[d]	D	67-68	71	235-238	EtOH	$C_{23}H_{17}NO_5$
31	$OCH_2CH_2C_6H_5$	A	51-52	33	C	oil	90[d]	239-240	aq DMF	$C_{18}H_{15}NO_4$
32	$OCH_2CH_2C_6H_4$-4-F	A	76-77	20	C	oil	99[d]	242-243	aq DMF	$C_{18}H_{14}FNO_4$
33	$OCH_2CH_2C_6H_4$-4-Br	B	89-90	90[d]	C	oil	92[d]	259-260	2-propanol	$C_{18}H_{14}BrNO_4$
34	OCH_2CH_2-β-$C_{10}H_7$	A/B	oil	65/55[d]	C	oil	95[d]	247-248	2-propanol	$C_{22}H_{17}NO_4$
35	$OCH_2CH_2C_6H_4$-4-OC_6H_5	B	73-73.5	28	E	oil	95[d]	261-262	methoxyethanol	$C_{24}H_{19}NO_5$
36	$OCH_2CH_2OC_6H_5$	A	95-97[c]	53	C	92-95	99[d]	246-247	aq DMF	$C_{18}H_{15}NO_5$
37	$OCH_2CH_2OC_6H_4$-4-F	A	76-78	61	C	153-155	71	247-248	aq DMF	$C_{18}H_{14}FNO_5$
38	$OCH_2CH_2OC_6H_4$-4-Br	A	75-77	49	C	91-93	45	243-245	aq DMF	$C_{18}H_{14}BrNO_5$
39	OCH_2CH_2O-β-$C_{10}H_7$	A	102-105	44	C[f]	118-121	81	258-259	aq DMF	$C_{22}H_{17}NO_5$
40	$OCH_2CH_2OC_6H_4$-4-OC_6H_5	A	95-98	42	D	106-109	82	232-235	aq DMF	$C_{24}H_{19}NO_6$

[a] Method A = Na-EtOH; method B = K_2CO_3-DMF. [b] Acetone-K_2CO_3. [c] Literature[28] mp 94-95 °C. [d] Crude yields. [e] Method C = $SnCl_2$-HCl-EtOH; method D = Fe-HCl-EtOH; method E = Pd-cyclohexene. [f] Also prepared by hydrolysis of corresponding acetamido derivative. [g] Elemental analyses for C, H, and N were within 0.4% of theoretical values with the exception of compound 34 (N: calcd, 3.89; found, 3.03).

might prove to be a valuable alternative to octanol-buffer derived π values in the current study, since congeners at the extremes of the log P scale (e.g., 18 and 25) could not be accurately measured by shake-flask methods.

Biological Results

The newly synthesized congeners were evaluated for their ability to inhibit mitochondrial malate dehydrogenase (m-MDH). The entire set was also tested against cytoplasmic malate dehydrogenase (s-MDH) and skeletal muscle lactate dehydrogenase (LDH-M_4). The malate dehydrogenase enzyme reactions were followed spectrophotometrically in the forward direction by monitoring the increase in absorption at 340 nm due to production of NADH, while lactate dehydrogenase was followed in the reverse direction. In addition to those previously reported,[8] several of the new lipophilic congeners were examined as inhibitors of Ehrlich ascites cell respiration. The I_{50} values in all instances were estimated from least-squares fitting of dose-response plots and have been converted to log $1/I_{50}$ (pI_{50}) for correlation analyses (Tables II and III).

Analysis and Discussion

Physicochemical parameters and biological data in Tables II and III have been subjected to multiparameter regression analysis using a computer program provided by Professor Corwin Hansch, Pomona College, Claremont, CA. The analyses are divided into three sections according to the objective of each set of correlations.

Mitochondrial malate dehydrogenase (m-MDH) binding correlations were developed initially for each of the three sets of parallel congeners to give eq 3–11 (Table IV). The parameters π, σ, RI, and MR were examined for each set of congeners, with σ affording poor correlations in each instance. With only five data points in each set, multiparameter relationships were not considered. Since π, RI, and MR are highly covariant, it was not surprising to find that each gave a relatively good correlation; however, lipophilicity, as accounted for by the HPLC retention index (RI), was clearly superior (eq 4, 7, and 10).

The effect of increasing the bridge between the 7-position of the quinoline nucleus and the substituted aromatic ring is minimal, since the slopes associated with RI for the benzyloxy and phenethyloxy congeners (eq 4 and 7) are identical within confidence limits to the slope associated with RI for the phenoxyethyloxy congeners (eq 10). Combination of the three sets of congeners afforded eq 12 as the best relationship. No improvement was seen upon addition of electronic parameters or upon generation of parabolic relationships in π, MR, or RI. Three benzyloxy congeners, **24–26**, were not included in the study of identically substituted sets because of the desire to maintain strict analogy between each subset as defined by bridge type. These three molecules, which were prepared to assess the effect of high lipophilicity immediately adjacent to the quinoline nucleus, are less active than would be predicted by eq 12. Their inclusion gives eq 13, which exhibits a decrease in slope as well as an expected decrease in the quality of the correlation. Addition of a squared term in RI afforded a highly significant equation (eq 13 vs. 14: $F_{1,15}$ = 45.6450) and allowed the delineation of an optimal lipophilic character in terms of RI. Equation 14 is not as solid as one might like because only one compound, the pentachlorobenzyloxy (**25**), exceeds the apparent optimal RI. These results may be visualized schematically as in

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Table II. Physicochemical and Enzyme Inhibition Data for Substituted 4-Hydroxyquinoline-3-carboxylic Acids

					m-MDH: pI_{50}		s-MDH: pI_{50}		LDH-M_4: pI_{50}	
no.	R	π[a,b]	RI[b,c]	MR[b,d]	obsd[e,f]	calcd[g]	obsd[f,h]	calcd[i]	obsd[f,i]	calcd[k]
10	H	0.0	4.80	0.103	I	2.52	I	2.33	I	2.54
11	SO_2CH_3	−1.39	3.25	1.349	3.18	3.24	3.36 (2.96-3.78)	3.14	3.39 (3.05-3.71)	3.40
12	OCH_3	0.49	5.87	0.787	I	2.91	I	2.80	I	3.04
13	OH	0.06	3.89	0.285	3.31	2.62	3.02 (2.37-3.77)	2.46	2.95 (2.76-3.14)	2.68
14	Cl	0.55	6.18	0.603	2.44	2.81	2.33 (2.25-2.35)	2.68	2.74 (2.53-2.91)	2.91
15	F	0.06	5.67	0.092	1.98	2.51	1.96 (1.57-2.29)	2.32	I	2.53
16	$CONH_2$	−1.18	2.84	0.981	3.13	3.02	3.22 (2.71-3.73)	2.93	I	3.17
17	COOH	−2.80	1.81	0.605	2.97	2.81	2.89 (1.50-3.76)	2.68	2.91 (2.53-3.29)	2.91
18	SO_3	−4.76	1.21	0.971	2.67	3.02	2.51 (2.30-2.72)	2.92	3.06 (2.91-3.20)	3.16
19	SO_2NH_2	−1.36	3.08	1.228	3.02	3.17	3.16 (3.01-3.30)	3.07	3.24 (3.07-3.41)	3.32
20	$COCH_3$	−0.39	4.48	1.118	3.04	3.10	3.04 (2.93-3.25)	3.01	3.25 (2.94-3.53)	3.25
21	NO_2	−0.40	5.32	0.736	2.72	2.88	2.92 (2.91-2.93)	2.77	3.02 (2.91-3.12)	3.00
22	$N(CH_3)_2$	1.10	6.79	1.555	3.32	3.35	3.43 (2.76-4.07)	3.25	3.50 (3.39-3.62)	3.52
23	$OCH_2C_6H_5$	1.81	8.51	3.174	4.49	4.29	I	3.93	I	4.25
24	$OCH_2C_6H_3$-3,4-Cl_2	3.06	10.79	4.174	5.32	4.86	I	4.16	I	4.52
25	$OCH_2C_6Cl_5$	5.34	13.70	5.664	5.32 (5.28-5.65)	5.72	I	4.24	I	4.66
26	OCH_2-α-$C_{10}H_7$	3.13	9.26	4.715	4.88 (4.31-5.22)	5.17	I	4.23	I	4.61
27	$OCH_2C_6H_4$-4-F	1.95	8.70	3.163	4.40 (4.30-4.49)	4.28	I	3.93	I	4.25
28	$OCH_2C_6H_4$-4-Br	2.67	9.86	3.959	5.17 (4.99-5.34)	4.74	I	4.13	I	4.48
29	OCH_2-β-$C_{10}H_7$	3.13	10.51	4.715	5.39 (5.28-5.49)	5.17	I	4.23	I	4.61
30	$OCH_2C_6H_4$-4-OC_6H_5	3.89	11.23	5.839	5.83 (4.85-7.19)	5.82	4.51 (3.47-5.58)	4.23	4.56 (3.80-5.38)	4.65
31	$OCH_2CH_2C_6H_5$	2.30	9.19	3.639	4.42 (4.29-4.57)	4.55	3.81 (3.33-4.19)	4.06	I	4.40
32	$OCH_2CH_2C_6H_4$-4-F	2.44	9.32	3.628	4.83 (4.26-5.39)	4.55	I	4.05	I	4.40
33	$OCH_2CH_2C_6H_4$-4-Br	3.16	10.49	4.424	5.60 (5.18-6.13)	5.01	4.04 (3.66-4.42)	4.20	4.75 (4.50-4.86)	4.57
34	OCH_2CH_2-β-$C_{10}H_7$	3.62	11.24	5.180	5.66 (5.48-5.84)	5.44	4.14 (3.92-4.37)	4.25	I	4.65
35	$OCH_2CH_2C_6H_4$-4-OC_6H_5	4.38	11.54	6.304	5.74 (5.38-6.06)	6.09	4.21 (3.93-4.49)	4.18	I	4.62
36	$OCH_2CH_2OC_6H_5$	1.83	8.54	3.858	4.22 (3.75-4.64)	4.68	I	4.11	I	4.45
37	$OCH_2CH_2OC_6H_4$-4-F	1.97	8.81	3.847	4.74 (4.56-4.91)	4.67	I	4.10	I	4.45
38	$OCH_2CH_2OC_6H_4$-4-Br	2.69	10.17	4.643	5.29 (5.04-5.55)	5.13	I	4.22	I	4.60
39	OCH_2H_2O-β-$C_{10}H_7$	3.15	10.29	5.399	5.80 (5.38-6.34)	5.57	I	4.25	I	4.66
40	$OCH_2CH_2OC_6H_4$-4-OC_6H_5	3.91	10.77	6.523	5.61 (5.45-5.78)	6.21	4.09 (3.99-4.15)	4.15	I	4.60

[a] Values for 10-17 and 19-23 are from measured log P (apparent).[8] Remaining π values were calculated using additivity principles.[10] [b] Parameter covariances, r^2, are as follows: π vs. RI = 0.95; π vs. MR = 0.76; MR vs. RI = 0.82 (10 and 12 excluded). [c] HPLC retention index, scaled by 0.01, see ref 19 and 20. [d] Molar refractivity, scaled by 0.1, values calculated using additivity principles or taken from ref 10. [e] Log $1/I_{50}$ for mitochondrial malate dehydrogenase: values for 10-24 were previously reported (ref 8). [f] I_{50} values are in mole per liter. Values in parentheses are 95% confidence intervals. Compounds which were inactive at the limits of solubility are denoted by I. [g] Calculated via eq 21. [h] Log $1/I_{50}$ for cytoplasmic malate dehydrogenase. [i] Calculated via eq 19. [j] Log $1/I_{50}$ for skeletal-muscle lactate dehydrogenase. [k] Calculated via eq 16.

Figure 1. Equations 4, 7, 10, and 12–14 support the existence of a hydrophobic pocket near the 7 position of the quinoline nucleus in the mitochondrial malate dehydrogenase inhibitor binding site. Binding of the smaller, nonaromatic congeners, previously studied,[8] seems to occur at a polar site (eq 2) such that there may be a progression from a polar interaction right at the 7 position to a lipophilic interaction as one moves out from the point of the quinoline ring attachment (Figure 1).

In addition to the characterization of binding to mitochondrial malate dehydrogenase, a major objective of these investigations has been the development of selective in-

Table III. Inhibition of Ehrlich Ascites Cell Respiration

no.	pI_{50}[a] obsd[b]	pI_{50}[a] calcd[d]	no.	pI_{50}[a] obsd[b]	pI_{50}[a] calcd[d]
10	2.98	3.13	26	4.07 (0.06)	4.13
11	2.75	2.63	27	3.78 (0.21)	4.04
12	3.28	3.43	28	4.27 (0.12)	4.21
13	3.04	2.85	29		4.28
14	3.84	3.51	30	4.00 (0.06)	4.35
15	3.30	3.38	31		4.12
16	2.24	2.48	32		4.14
17	2.24	2.08	33		4.28
18	2.88[c]	1.83	34		4.35
19	2.47	2.57	35		4.37
20	3.10	3.03	36		4.01
21	3.24	3.28	37		4.06
22	3.33	3.66	38		4.25
23	4.41	4.01	39		4.26
24	4.82	4.31	40		4.31
25	4.37 (0.15)	4.44			

[a] Log $1/I_{50}$ is in moles per liter. [b] Compounds 10-24 were previously reported.[8] Compounds 25-28 and 30 were determined in this study. Numbers in parentheses are standard deviations. Compounds 29 and 31-40 have not been evaluated. [c] Omitted from the correlations. [d] Calculated via eq 26.

Table IV

eq no.	equation	n	s	r	F
	Benzyloxy Congeners (23 and 27-30)				
3	pI_{50} = 0.69 (0.22) π + 3.18 (0.62)	5	0.12	0.99	100.11
4	pI_{50} = 0.52 (0.13) RI + 0.01 (1.23)	5	0.09	0.99	173.23
5	pI_{50} = 0.52 (0.23) MR + 2.88 (0.99)	5	0.16	0.97	51.40
	Phenethyloxy Congeners (31-35)				
6	pI_{50} = 0.61 (0.58) π + 3.31 (1.91)	5	0.31	0.89	11.11
7	pI_{50} = 0.52 (0.34) RI − 0.10 (3.56)	5	0.23	0.94	23.03
8	pI_{50} = 0.43 (0.54) MR + 3.25 (2.55)	5	0.38	0.83	6.55
	Phenoxyethyloxy Congeners (36-40)				
9	pI_{50} = 0.66 (0.67) π + 3.34 (1.88)	5	0.36	0.88	9.98
10	pI_{50} = 0.62 (0.42) RI − 0.89 (4.15)	5	0.26	0.94	21.57
11	pI_{50} = 0.47 (0.59) MR + 2.83 (2.94)	5	0.42	0.83	6.48
	Combined Sets (23-40)				
12	pI_{50} = 0.52 (0.11) RI − 0.02 (1.14)	15	0.20	0.94	97.81
13	pI_{50} = 0.30 (0.14) RI + 2.11 (1.46)	18	0.37	0.74	19.89
14	pI_{50} = 3.00 (0.85) RI − 0.13 (0.04) RI^2 − 12.10 (4.55)	18	0.19	0.94	60.52
	RI_0 = 11.91 (11.53-12.56)				

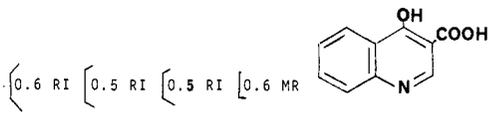

Figure 1. m-MDH inhibitor binding analysis.

hibitors of this enzyme. Consequently, all compounds were evaluated as inhibitors of cytoplasmic malate dehydrogenase (s-MDH) and of skeletal muscle lactate dehydrogenase (LDH-M_4). The more lipophilic congeners were not sufficiently active against these two enzymes to allow establishment of I_{50} values within the limits of solubility, although 11 molecules did exhibit measurable inhibition of LDH-M_4 while 17 molecules exhibited measurable inhibition of s-MDH. The lower pH of the LDH test system was presumably responsible for the decrase in measurable activities as compared to the s-MDH system. Since the least number of molecules were active against LDH, this set was utilized as the basis for comparison with s-MDH and m-MDH. Equations 15 and 16 (Table V) were developed from the LDH data. Equation 17 was derived from the s-MDH data using exactly the same congeners as those for the LDH equations, while eq 18 and 19 correlate all available data on s-MDH. Equation 20 is the comparable relationship for m-MDH, while eq 21 includes all active compounds against m-MDH. Finally, eq 22–24 provide an indication of the correlations between the three enzyme systems. In all cases, the correlations involving MR were superior to those involving RI, π, or Hammett σ. One notes immediately that the enzymes are similar with respect to the correlation with molar refraction (MR); however, the character of this correlation differs markedly. Equation 15 for LDH is virtually identical with eq 17 for s-MDH, but the coefficients for MR in these two equations are only half that seen for m-MDH (eq 20). Although the parabolic equation (eq 16) for LDH is statistically significant ($F_{1,8}$ vs. eq 15 = 10.4975), the optimum molar refraction is ill-defined (note large confidence interval). Nonetheless, this may explain why the larger, more lipophilic quinoline substituents were not active. Addition of an MR^2 term to eq 17 correlating s-MDH inhibition did not provide similar improvement; however, if all 17 active s-MDH inhibitors are employed, eq 18 and 19 emerge (eq 18 vs. 19: $F_{1,14}$ = 6.7112), which are virtually identical with the LDH eq 15 and 16, thus supporting the similarity between these enzymes. Correlation of the 11 molecule base set with m-MDH activity afforded eq 20. As mentioned above, the coefficient associated with MR in this equation is twice that seen for LDH and s-MDH (eq 15,

Table V

eq no.	equation	n	s	r	F
15	pI_{50} (LDH-M_4) = 0.36 (0.08) MR + 2.79 (0.20)	11	0.20	0.96	95.60
16	pI_{50} (LDH-M_4) = 0.79 (0.31) MR − 0.07 (0.05) MR^2 + 2.46 (0.27)	11	0.14	0.98	103.50
	MR_0 = 5.56 (4.47-11.82)				
17	pI_{50} (s-MDH) = 0.32 (0.12) MR + 2.65 (0.28)	11	0.28	0.90	40.15
18	pI_{50} (s-MDH) = 0.29 (0.09) MR + 2.61 (0.29)	17	0.38	0.87	47.96
19	pI_{50} (s-MDH) = 0.75 (0.39) MR − 0.07 (0.06) MR^2 + 2.25 (0.39)	17	0.33	0.92	34.46
	MR_0 = 5.29 (4.30-15.07)				
20	pI_{50} (m-MDH) = 0.62 (0.14) MR + 2.40 (0.34)	11	0.35	0.96	99.68
21	pI_{50} (m-MDH) = 0.58 (0.06) MR + 2.46 (0.24)	29	0.34	0.96	329.86
22	pI_{50} (m-MDH) = 1.69 (0.50) s-MDH − 1.95 (1.62)	11	0.44	0.93	58.86
23	pI_{50} (m-MDH) = 1.67 (0.35) LDH-M_4 − 2.21 (1.22)	11	0.33	0.96	114.34
24	pI_{50} (LDH-M_4) = 0.97 (0.30) s-MDH + 0.30 (0.97)	11	0.26	0.93	53.83

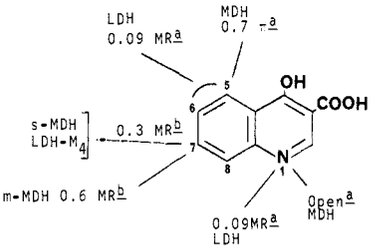

Figure 2. Comparative analysis of dehydrogenase inhibitor binding. [a]Reference 7. [b]This work.

17, and 18), indicating a much more dramatic increase in activity with changes in MR. In this case, when all 29 m-MDH inhibitors were included to give eq 21, the coefficient for MR was unchanged and the relationship remained linear, demonstrating unequivocally that the mitochondrial enzyme is quite different from the cytoplasmic enzymes. An alternative means of comparing the three enzymes is to derive correlations between the respective biological activities. Using once again the 11 congeners which were active in all three systems, eq 22–24 were developed. Although eq 22 and 23 are identical, the lower standard deviation and higher correlation coefficient for eq 23 indicate a somewhat tighter fit between m-MDH and LDH. The greater sensitivity of m-MDH to structural changes is reflected in the coefficients associated with s-MDH and LDH activity in these equations. A shortcoming of regression analysis must be recognized here, since only the active molecules can be included in a direct comparative analysis as provided by eq 22–24. While there may be similar binding mechanisms for the congeners with smaller polar substituents, the enzymes are obviously not similar with respect to binding of the larger, more lipophilic, aromatic substituents. The direct correspondence between LDH and s-MDH is again born out by the one to one relationship obtained with eq 24. This is especially intriguing since the LDH reaction was inhibited in the reductive direction (pyruvate → lactate), while the s-MDH reaction was inhibited in the oxidative direction (L-malate → oxaloacetate). The respective reverse reactions were examined for all compounds and were not significantly influenced. These results are illustrated diagramatically in Figure 2, which also includes the data developed by Hansch[7] on the quinoline congeners studied by Baker.[2-6] The comparative analyses are in full accord with the numerous studies on the structural and kinetic characteristics of LDH-M_4, m-MDH, and s-MDH. X-ray crystallographic analyses of LDH-M_4 and s-MDH have established that these two enzymes are quite similar in the cofactor binding region.[21] A degree of homology has also been found in the active sites of these two enzymes, as well as in the overall backbone conformations.[21] Fluorescence and thermodynamic properties exhibited by s-MDH and LDH-M_4 upon substrate binding were found to be very similar.[22] While m-MDH and s-MDH both contain two subunits and have molecular weights in the range of 68 000–72 000,[23] the results of amino acid analysis[24] and of several kinetic investigations[25-27] indicate that differences are to be expected in the primary, secondary, and tertiary structures of these two enzymes. These differences are supported by the detailed QSAR of aromatic congener inhibition of m-MDH and the comparative analyses which suggest that the binding site or some portion of it may be more flexible and more lipophilic than that of the two cytoplasmic dehydrogenases.

While the major thrust of these investigations has been to develop potent and selective dehydrogenase inhibitors, the ability of the compounds to inhibit the respiration of Ehrlich ascites cell suspensions has been monitored to provide an indication of membrane-transport properties. It was surprising to find that respiratory inhibition was a linear function of lipophilicity as indicated by eq 1. Therefore, in the course of the current study, five additional congeners have been evaluated in this whole cell test system in an effort to test the limits of the linear relationship. Inclusion of these additional molecules and reformulation of the correlations afforded eq 25 and 26.

$$pI_{50}\ (\text{ascites}) = 0.21\ (0.05)\ \text{RI} + 2.05\ (0.35) \quad (25)$$

$$n = 19;\ s = 0.31;\ r = 0.92;\ F = 87.53$$

$$pI_{50}\ (\text{ascites}) = 0.46\ (0.18)\ \text{RI} - 0.02\ (0.01)\ \text{RI}^2 + 1.29\ (0.59) \quad (26)$$

$$n = 19;\ s = 0.25;\ r = 0.95;\ F = 70.85;\ \text{RI}_0 = 13.51\ (10.88\text{–}26.78)$$

With the use of the HPLC retention index (RI) as a measure of lipophilicity, a nonlinear relationship has begun to emerge, eq 26. Corresponding equations using π or MR are statistically poorer and do not allow definition of optimal values in either parameter. Although eq 26 is a statistically significant improvement over eq 25 ($F_{1,16}$ = 9.6461), the confidence limits associated with RI_0 are large, and only one compound, the pentachlorobenzyloxy (**25**), exceeds this optimum value. It is apparent that membrane transport is not a severely limiting factor over a wide range in lipophilicity, at least for the conditions and cell system employed here.

Experimental Section

Melting points were taken on a Mel-Temp apparatus and are uncorrected. Elemental analyses were performed by Midwest Microlab, Ltd., Indianapolis, IN, and are within 0.4% of the theoretical values unless otherwise noted. Infrared and proton magnetic resonance spectra were in accord with assigned structures for all compounds.

3-[(4-Bromobenzyl)oxy]-1-nitrobenzene. Method A. A solution of 3.0 g (0.021 mol) of 3-nitrophenol in 30 mL of anhydrous EtOH was added to a solution of 0.49 g (0.021 mol) of sodium metal in 30 mL of anhydrous EtOH under N_2 at room temperature. After the solution was stirred for 0.5 h, a solution of 4-bromobenzyl bromide (5.24 g, 0.021 mol) in 30 mL was added, and the resulting mixture was stirred and heated at reflux under N_2 for 21 h. After the mixture was refrigerated overnight, the deposited crystalline mass was isolated by filtration in vacuo and washed with successive 200-mL portions of ice–water, 5% NaOH, and ice–water to give 6.5 g (100%) of crude product. Recrystallization from absolute ethanol gave white needles, mp 84–86 °C. Anal. ($C_{13}H_{10}BrNO_3$) C, H, N.

3-(2-Naphthylmethoxy)-1-nitrobenzene. Method B. A solution of 6.5 g (29.4 mmol) of 2-(bromomethyl)naphthalene in 10 mL of anhydrous DMF was added dropwise to a refluxing mixture of 4.0 g (28.8 mmol) of 3-nitrophenol and 7.96 g (57.6 mmol) of K_2CO_3 in 40 mL of anhydrous DMF. After the addition, heating was discontinued, and the tan reaction mixture allowed

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to stir at room temperature overnight. The mixture was combined with 600 mL of ice–water and stirred for 2 h, and the tan solid was isolated by filtration in vacuo. Recrystallization from 95% EtOH gave 7.1 g (88%) of product, mp 119–120 °C. Anal. ($C_{17}H_{13}NO_3$) C, H, N.

3-[(4-Bromobenzyl)oxy]aniline. Method C. A mixture of finely ground $SnCl_2 2H_2O$ (2.2 g, 9.73 mmol), 4 mL of concentrated HCl, and 10 mL of absolute EtOH was heated on a steam bath until a solution formed. Solid 3-[(4-bromobenzyl)oxy]nitrobenzene (1.0 g, 3.24 mmol) was added and heating was continued until solution was again complete. Cooling in ice afforded the crystalline stannic chloride salt of the product, which was isolated by filtration in vacuo and treated with 20% NaOH (50 mL). This alkaline mixture was then extracted with Et_2O (3 × 30 mL) and the extracts were dried ($MgSO_4$) and evaporated to give 0.58 g (64%) of crude product. Recrystallization from aqueous EtOH gave white crystals, mp 92–93 °C. Anal. ($C_{13}H_{12}BrNO$) C, H, N.

3-(2-Naphthylmethoxy)aniline. Method D. A solution of 10 g (35.8 mmol) of 3-(2-naphthylmethoxy)nitrobenzene in 300 mL of hot absolute EtOH was added to a mechanically stirred, refluxing, mixture of 20 g (358 mmol) of Fe powder, 5 mL of concentrated HCl, and 10 mL of H_2O in 100 mL of absolute EtOH. The resulting mixture was heated at reflux for 6 h and treated, hot, with 20 mL of 20% NaOH. The hot mixture was filtered with light suction, and the filtrate deposited crystalline product upon cooling: yield 5.40 g (60%); mp 118–119 °C (from 95% EtOH). Anal. ($C_{17}H_{15}NO$) C, H, N.

4-Phenoxyphenethyl Alcohol (4). A modification of previously reported procedures[15-17] was employed. In a three-neck, round-bottom flask (500 mL) were placed 5 g of finely ground Mg turnings and 3 g of iodine crystals. The flask was fitted with an overhead stirrer, a condenser with a drying tube, and a dropping funnel. Through the funnel was run 20 mL of dry Et_2O into the flask, and a reaction was initiated. Meanwhile the dropping funnel was filled with 25 g (0.1 mol) of 4-phenoxybromobenzene in 25 mL of dry Et_2O. As the iodine color began to disappear, the 4-phenoxybromobenzene solution was slowly run into the flask. A vigorous reaction started and was maintained throughout the addition. After the addition was complete, the reaction was stirred and refluxed on a water bath for 3 h, then cooled in an ice bath, and treated with a mixture of 15 mL of ethylene oxide and 10 mL of dry Et_2O (under N_2 atmosphere within 10 min). The reaction mixture was stirred for 0.5 h at ice-bath temperature, for 0.5 h at room temperature, and for 0.5 h at reflux. On completion, the mixture was poured into a beaker containing 250 mL of cold 50% H_2SO_4 solution to decompose the Grignard reagent. The mixture was taken up in a separatory funnel, and the ether layer was separated. The aqueous layer was extracted 3 times with 65 mL of ether, and the ether layers were combined, dried over $CaCl_2$, and evaporated to yield a tan oil. Vacuum distillation afforded 12 g (56%) of the desired product, bp 130–150 °C (0.06–0.05 mmHg).

4-Phenoxyphenethyl Bromide (5). In a 50-mL, round-bottom flask fitted with a dropping funnel were placed 2.2 g (0.01 mol) of 4-phenoxyphenethyl alcohol (4) and 10 mL of dry Et_2O. This mixture was cooled in an ice bath and into it, dropwise, was added a mixture of 2.72 g of PBr_3 and 10 mL of dry Et_2O. During the addition the reaction mixture was well stirred. The stirring was continued for 5 h. At the end of this time, excess PBr_3 and Et_2O were removed under vacuum using a water aspirator. The resulting tan oil was vacuum distilled at 110–130 °C (0.075 mmHg) to give the desired product, 2.3 g (83%).

3-[(4-Phenoxyphenethyl)oxy]-1-nitrobenzene (6). In a round-bottom flask fitted with a dropping funnel were placed 4.1 g (0.03 mol) of *m*-nitrophenol, 8.2 g (0.06 mol) of K_2CO_3, and 20 mL of dry DMF. This mixture was stirred for 0.5 h. At the end of this time, 8.3 g of 4-phenoxyphenethyl bromide in 10 mL of dry DMF was slowly added through the dropping funnel. The reaction mixture was stirred for 10 h and then poured into a beaker containing 150 mL of ice–water and stirred for 0.5 h. This mixture was taken up in a separatory funnel and extracted 4 times with 75 mL of CH_2Cl_2. The extracts were collected, dried over $CaCl_2$, and evaporated to dryness, and the resulting tan oil was chromatographed on 30 g of silica gel. Elution with petroleum ether (30–60 °C) afforded 4-phenoxystyrene. The second fraction, eluted with CCl_4/petroleum ether (50:50), gave the desired product. The oil thus obtained was solidified by trituration with an Et_2O/petroleum ether mixture: yield 2.9 g (28%); mp 73–73.5 °C (from EtOH). Anal. ($C_{20}H_{17}NO_4$) H, N; C: calcd, 71.63; found, 72.11.

3-[(4-Phenoxyphenethyl)oxy]aniline (7). Method E. In a three-neck, round-bottom flask were placed 6.7 g (0.02 mol) of the 3-[(4-phenoxyphenethyl)oxy]-1-nitrobenzene, 3 g of Pd/C, 10 mL of absolute EtOH, and 50 mL of cyclohexene. The mixture was refluxed for 6 h and monitored by TLC. The disappearance of starting material as indicated by TLC was considered the end point. At this time, the mixture was filtered and washed with absolute EtOH. The filtrate was evaporated to dryness, yielding 7 as a light yellow oil, 5.8 g (95%), which was used in the subsequent reaction without further purification.

7-[(4-Phenoxyphenethyl)oxy]-4-hydroxyquinoline-3-carboxylic Acid (35). In a round-bottom flask were placed 5.4 g (0.018 mol) of the aniline (7) obtained from the previous step, 3.9 g (0.018 mol) of diethyl ethoxymethylenemalonate, and 30 mL of dry toluene. This mixture was refluxed for 6 h and monitored by TLC. The reaction was stopped when TLC indicated disappearance of the aniline and was then evaporated under vacuum to dryness, resulting in a brown oil, 8.

This oil was mixed with 10–15 mL of diphenyl ether and slowly dropped into a round-bottom flask containing 30–40 mL of refluxing diphenyl ether. Care was taken that the temperature remained at 255–256 °C. After the addition was complete, the mixture was further refluxed for 7–8 min, then cooled to room temperature, poured into a beaker containing 100 mL of petroleum ether, and stirred for 0.5 h. This solution was filtered to afford tan crystalline ethyl 7-[(4-phenoxyphenethyl)oxy]-4-hydroxyquinoline-3-carboxylate (9).

The ester 9 was treated with 30 mL of 20% NaOH at reflux for 30 min. The solution was then cooled to room temperature, neutralized with 10% HCl, and filtered to yield a tan crystalline product, which was recrystallized from methoxyethanol to give 7-[(4-phenoxyphenethyl)oxy]-4-hydroxyquinoline-3-carboxylic acid (35) yield: 1.2 g (16% from 7); mp 261–262 °C. Anal. ($C_{24}H_{19}NO_5$) C, H, N.

Measurement of Retention Indexes. The conditions employed in the reversed-phase, high-pressure LC measurement of capacity factors and the methods of conversion to retention indexes were identical with those reported previously.[20]

Biological Testing. (A) Ascites. Procedures for maintenance and testing of Ehrlich ascites tumor cells have been reported.[8]

(B) Enzyme Inhibition. Mitochondrial malate dehydrogenase (pig heart; catalog no. 410-13), cytoplasmic malate dehydrogenase (pig heart; catalog no. M-7383), lactate dehydrogenase (rabbit muscle; type II; catalog no. L-2500), NAD^+ (catalog no. 260-150), and NADH (catalog no. 340-1100) were obtained from Sigma Chemical Co. and were used without further purification.

Concentrated malate dehydrogenases were diluted with 0.05 M Tris buffer (pH 7.4) containing 1% bovine serum albumin to give 0.022–0.027 OD unit change per minute at 340 nm under the following conditions. In a cuvette were placed 2.4 mL of 0.05 M Tris buffer (pH 8.4), 0.1 mL of 0.06 M L-malic acid (neutralized to pH 7.5), 0.1 mL of 0.03 M NAD^+ (in deionized H_2O), and 0.20 mL of Me_2SO ± inhibitor.

Lactate dehydrogenase was diluted with 0.05 M Tris buffer (pH 7.4) to give 0.022–0.027 OD unit change per minute at 340 nm under the following conditions. In a cuvette were placed 2.0 mL of 0.05 M Tris buffer (pH 7.4), 0.5 mL of 0.006 M pyruvic acid (in pH 7.4 Tris), 0.1 mL of 0.003 M NADH (in pH 7.4 Tris), and 0.20 mL of Me_2SO ± inhibitor.

If turbidity appeared, the inhibitor concentration was reduced. A zero base line was obtained while the cuvette temperature was allowed to equilibrate to the sample chamber, which was maintained at 25 °C. Reactions were initiated by the addition of 0.1 mL of appropriately diluted enzyme. Enzyme inhibition was measured at five inhibitor concentrations in triplicate, and the I_{50} concentration was determined from a least-squares fitting of the dose–response line. The 95% confidence intervals for the I_{50} values were then estimated from the 95% confidence limits associated with the slopes and intercepts of the dose–response regression lines.

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