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Irreversible Enzyme Inhibitors. 191.[†,1] Hydrophobic Bonding to Some Dehydrogenases by 6-, 7-, or 8-Substituted-4-hydroxyquinoline-3-carboxylic Acids

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Twenty-eight derivatives of 4-hydroxyquinoline-3-carboxylic acid bearing aryl, aralkyl, aralkoxy, or aroxyalkoxy groups at the 6, 7, or 8 positions were investigated as inhibitors of glutamate, glyceraldehyde phosphate, lactate, and malate dehydrogenases. The best hydrocarbon interactions were seen with malate dehydrogenase; for example the $6\text{-}C_6H_5O(CH_2)_4O$ group (12) gave a 190-fold increment in binding over the parent quinoline-3-carboxylic acid and a 740-fold increment over the substrate, L-malate. Weaker hydrocarbon interactions (10- to 20-fold increments) were seen with glutamate or lactate dehydrogenase, but none was seen with glyceraldehyde phosphate dehydrogenase.

In the first paper from this laboratory on inhibitors of glutamate, glyceraldehyde phosphate, lactate and malate dehydrogenases, the 4-hydroxyquinoline-3-carboxylic acid (1) (Table I) system was selected for further structural modification.‡ Since hydrophobic bonding can greatly enhance inhibitor binding,[3] a search for such a hydrocarbon interaction has now been made with aryl, aralkyl, and aralkyloxy derivatives substituted on the 6, 7, or 8 positions of 4-hydroxyquinoline-3-carboxylics; the results are the subject of this paper.

Enzyme Results. The best hydrocarbon interactions in Table I were seen with malate dehydrogenase. The 6-$C_6H_5(CH_2)_3O$ (9), $6\text{-}C_6H_5O(CH_2)_4O$ (12), and 6-$C_6H_5O(CH_2)_5O$ (13) groups gave 190-, 130-, and 110-fold increments in binding, respectively, compared to the parent 1. Poorer hydrophobic bonding (16-fold) was seen with the 7-$C_6H_5CH_2$ (15) and 8-$C_6H_5O(CH_2)_3O$ (19) substituents.
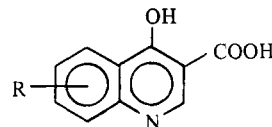
The best hydrocarbon interaction (22-fold) on lactate dehydrogenase was also seen with the $6\text{-}C_6H_5(CH_2)_3O$ (9) substituent; the $6\text{-}C_6H_5O(CH_2)_5O$ (13) was about half as effective.

Hydrocarbon interaction with glutamate dehydrogenase was poor, the 6-$C_6H_5CH_2$ (5), $6\text{-}C_6H_5(CH_2)_2O$ (8), 6-$C_6H_5(CH_2)_3O$ (9), or $6\text{-}C_6H_5O(CH_2)_2O$ (10) substituents showing a 10- to 12-fold increment. No significant hydrocarbon interaction was seen with glyceraldehyde phosphate dehydrogenase; the most potent compound was the 6-$C_6H_5CH_2O$ (7) derivative.

The $6\text{-}C_6H_5(CH_2)_3O$ (9) group stands out as the best across the 4 enzymes, being complexed 37-, 100-, and 740-fold better, respectively, than the substrate, L-glutamate, L-lactate, or L-malate.

Some of the inhibitors were converted to their 1-Me derivatives; this change was previously shown not to be detrimental to binding in the parent series (**1** *vs.* **22**) or 6-MeO series (**2** *vs.* **23**).[1] 1-Methylation (**24–32**) was not detrimental to binding with glutamate and lactate dehydrogenase; in contrast, it was surprising to note that 1-methylation was detrimental to binding, when the inhibitors contained

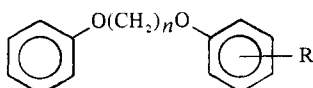Table I. Inhibition[a] of Four Dehydrogenases by

No.	R	Glu-DH	GPDH	LDH	MDH
1[c]	H	600	>1600[d]	440	520
2[c]	6-MeO	430	460	230	>400[d]
3	6-C_4H_9-n	140	1100	160	250
4	6-C_6H_5	94	390	67	290
5	6-$C_6H_5CH_2$	56	1400	160	190
6[g]	6-C_6H_5O	75	530	96	170
7	6-$C_6H_5CH_2O$	97	280	110	110
8	6-$C_6H_5(CH_2)_2O$	55	520	78	73
9	6-$C_6H_5(CH_2)_3O$	55	360	20	2.7
10	6-$C_6H_5O(CH_2)_2O$	50	430	60	43
11	6-$C_6H_5O(CH_2)_3O$	210	400	59	9.5
12	6-$C_6H_5O(CH_2)_4O$	120	330	59	3.9
13	6-$C_6H_5O(CH_2)_5O$	76	>800[d]	37	4.6
14	7-C_6H_5	260[e]	650[e]	170	67
15	7-$C_6H_5CH_2$	470	>4000[d]	110	33
16	7-$C_6H_5O(CH_2)_3O$	>220[d]	1100[e]	100	400
17	8-C_6H_5	570	530[e]	110	260
18	8-$C_6H_5CH_2$	340	1000[e]	770	140
19	8-$C_6H_5O(CH_2)_3O$	>800[d]	>800[d]	>200[d]	33
20[h]	7,8-Benzo	99	670	140	130
21[i]	7,8-(Pyrido-2,3)	410	680	110	520
22[c]	1-Me	500	590	74	520
23[f]	1-Me-6-MeO	270	330	150	200
24	1-Me-6-C_6H_5	>200[d]	>100[d]	>200[d]	>400[d]
25	1-Me-6-$C_6H_5CH_2$	>200[d]	>100[d]	>200[d]	>400[d]
26	1-Me-6-$C_6H_5CH_2O$	140	280	110	>100[d]
27	1-Me-6-$C_6H_5O(CH_2)_3O$	330[e]	>450[d]	84	>120[d]
28	1-Me-7-$C_6H_5CH_2$	460[e]	1100[e]	100	400
29	1-Me-7-$C_6H_5O(CH_2)_3O$	>200[d]	>600[d]	110	>280[d]
30	1-Me-8-C_6H_5	270	>400[d]	57	>400[d]
31	1-Me-8-$C_6H_5CH_2$	330	400	110	320
32	1-Me-8-$C_6H_5O(CH_2)_3O$	>160[d]	300[e]	46	>160[d]

[a]The technical assistance of Nancy Middleton, Pauline Minton and Diane Shea with these assays is acknowledged. [b]I_{50} = concn for 50% inhibition of Glu-DH = glutamate dehydrogenase, GPDH = glyceraldehyde phosphate dehydrogenase, LDH = lactate dehydrogenase, and MDH = malic dehydrogenase when assayed[2] with 2 mM L-glutamate, 0.25 mM glyceraldehyde phosphate, 2 mM pyruvate, and 2 mM L-malate, respectively. [c]Data from ref 2. [d]No inhibition at 0.25 this concn, the max solubility. [e]Estimated from the inhibition at max solubility, which is less than the I_{50}. [f]Data from ref 1. [g]For synthesis see Riegel, *et al.*[4] [h]For synthesis see Foster, *et al.*[5] [i]See ref 6.
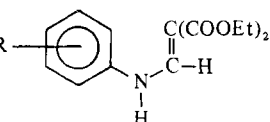
Table II. Properties of

No.	R	n	Method	Mp, °C[a]	Yield, %	Formula[b]
65	4-NHCOCH$_3$	2	A	171–173	36	C$_{16}$H$_{17}$NO$_3$
66	4-NH$_2$	2	B	112–113	86	C$_{14}$H$_{15}$NO$_2$
67	4-NHCOCH$_3$	3	A	125–127	86	C$_{17}$H$_{19}$NO$_3$
68	4-NH$_2$·HCl	3	B	165–167	67	C$_{15}$H$_{17}$NO$_2$·HCl·0.25H$_2$O
69	4-NHCOCH$_3$	4	A	140–141	80	C$_{18}$H$_{21}$NO$_3$
70	4-NH$_2$·HCl	4	B	196–197	56	C$_{16}$H$_{19}$NO$_2$·HCl
71	2-NO$_2$	3	A	Oil[c]	87	

[a]Recrystd from EtOH. [b]*Anal.* C, H, N. [c]Used crude in next step.

Table III. Physical Properties of

No.	R	Time[a]	Mp, °C[b]	Yield, %	Formula[c]
49	4-C$_4$H$_9$-n[d]	30	Oil[e]	100[f]	
50	4-C$_6$H$_5$[d]	25	90–92	72	C$_{20}$H$_{21}$NO$_4$
51	4-C$_6$H$_5$CH$_2$[g]	45	64–65	86	C$_{21}$H$_{23}$NO$_4$
52	4-C$_6$H$_5$CH$_2$O[d]	40	113–116	84	C$_{21}$H$_{23}$NO$_5$
53	4-C$_6$H$_5$(CH$_2$)$_2$O[h]	30	Oil[e]	64[f]	
54	4-C$_6$H$_5$(CH$_2$)$_3$O[h]	30	63–65	86	C$_{23}$H$_{27}$NO$_5$
55	4-C$_6$H$_5$O(CH$_2$)$_2$O	30	92–93	88	C$_{22}$H$_{25}$NO$_6$
56	4-C$_6$H$_5$O(CH$_2$)$_3$O	30	71–73	85	C$_{23}$H$_{27}$NO$_6$
57	4-C$_6$H$_5$O(CH$_2$)$_4$O	30	89–90	85	C$_{24}$H$_{29}$NO$_6$
58	4-C$_6$H$_5$O(CH$_2$)$_5$O[i]	60	71–74	77	C$_{25}$H$_{31}$NO$_6$
59	3-C$_6$H$_5$[j]	30	Oil[e]	100[f]	
60	3-C$_6$H$_5$CH$_2$[g]	30	61–63	78	C$_{21}$H$_{23}$NO$_4$
61	3-C$_6$H$_5$-O(CH$_2$)$_3$O[k]	30	68–70	81	C$_{23}$H$_{27}$NO$_6$
62	2-C$_6$H$_5$[d]	30	64–65	55	C$_{20}$H$_{21}$NO$_4$
63	2-C$_6$H$_5$CH$_2$[g]	30	88–89	45	C$_{21}$H$_{23}$NO$_4$
64	2-C$_6$H$_5$O(CH$_2$)$_3$O[l]	30	60–61	67	C$_{23}$H$_{27}$NO$_6$

[a]Time in min for previously described method.[2] [b]Recrystd from EtOH. [c]*Anal.* C, H, N. [d]Starting arylamine is commercially available. [e]Used crude in next step. [f]Not homogeneous on tlc, but suitable for further transformations. [g]Starting arylamine prepd *via* method given in ref 7. [h]See ref 8 for starting arylamine. [i]See ref 9 for starting arylamine. [j]Nitro compound is commercially available; it was reduced catalytically to the amine. [k]Starting arylamine prepd by method of Lourens.[10] [l]Arylamine from hydrogenation of 71 used crude.

large substituents (**24–33**), to malate and glyceraldehyde phosphate dehydrogenase.
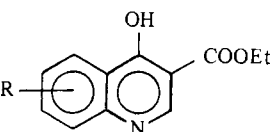
Chemistry. The compounds in Table I were synthesized by the general methods previously described; that is, the appropriate arylamine was condensed with diethyl ethoxymethylenemalonate (Table III), then thermally cyclized (Table IV) and saponified to the desired inhibitors (Tables I, VI). The 1-Me derivatives in Table VI were synthesized by methylation as the appropriate ester in Table IV, then saponified by the methods previously described.

Experimental Section

1-(*p*-Acetamidophenoxy)-4-phenoxybutane (**69**). **Method A.** A mixt of 6.6 g (44 mmoles) of 4-hydroxyacetanilide, 9.1 g (40 mmoles) of 4-phenoxybutyl bromide, and 5.5 g (80 mequiv) of K$_2$CO$_3$ was stirred in 40 ml of dry DMF for about 18 hr. The reaction mixt was poured onto 100 g of ice and dild to 500 ml with H$_2$O. The product was collected and recrystd from EtOH to give 9.6 g (80%): mp 140–141°. *Anal.* (C$_{18}$H$_{21}$NO$_3$) C, H, N. See Table II for other compounds prepared in this manner.

1-(*p*-Aminophenoxy)-4-phenoxybutane Hydrochloride (**70**). **Method B.** A mixt of 5.0 g (17.5 mmoles) of 69, 10 ml of EtOH, and 25 ml of 6 *N* HCl were refluxed for 3 hr. The solid was collected from the cooled reaction mixt and recrystd from EtOH to

Table IV. Physical Properties of

No.	R	Time[a]	Solvent[b]	Mp, °C	Yield, %	Formula[c]
33	6-C$_4$H$_9$-n	60	A	250–252	62[e]	C$_{16}$H$_{19}$NO$_3$
34	6-C$_6$H$_5$	30	B	300–303 dec	70	C$_{18}$H$_{15}$NO$_3$
35	6-C$_6$H$_5$CH$_2$	45	A	265–266	77	C$_{19}$H$_{17}$NO$_3$
36	6-C$_6$H$_5$CH$_2$O	30	C	279–280 dec	71	C$_{19}$H$_{17}$NO$_4$
37	6-C$_6$H$_5$(CH$_2$)$_2$O	30	A	231–235	50[d]	
38	6-C$_6$H$_5$(CH$_2$)$_3$O	30	D	236–238	46[d]	
39	6-C$_6$H$_5$O(CH$_2$)$_2$O	30	D	231–236	68[d]	
40	6-C$_6$H$_5$O(CH$_2$)$_3$O	30	D	214–216	70	C$_{21}$H$_{21}$NO$_5$
41	6-C$_6$H$_5$O(CH$_2$)$_4$O	30	D	218–219	75	C$_{22}$H$_{23}$NO$_5$
42	6-C$_6$H$_5$O(CH$_2$)$_5$O	45	A	182–184	58[d]	
43	7-C$_6$H$_5$	30	E	290–295	45[d,e]	
44	7-C$_6$H$_5$CH$_2$	30	D	268–270	57[d]	
45	7-C$_6$H$_5$O(CH$_2$)$_3$O	30	D	256–259	62[d]	
46	8-C$_6$H$_5$	30	D	254–256	65	C$_{18}$H$_{15}$NO$_3$
47	8-C$_6$H$_5$CH$_2$	30	D	231–232	79	C$_{19}$H$_{17}$NO$_3$
48	8-C$_6$H$_5$O(CH$_2$)$_3$O	60	A	133–134	77	C$_{21}$H$_{21}$NO$_5$

[a]Min for thermal ring closure.[2] [b]Recrystn solvents: A, EtOH; B, HOAc; C, pyridine; D, 2-methoxyethanol; E, EtOH-H$_2$O. [c]*Anal.* C, H, N. [d]Product gels, occludes solvent, is not homogeneous in tlc, but is suitable for further transformation. [e]Overall for 2 steps.

Table V. Physical Properties of
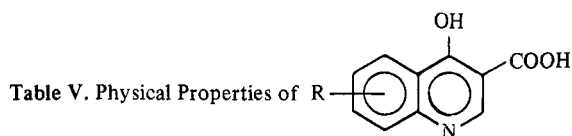
No.	R^a	Solvent^b	Mp, °C	Yield, %	Formula^c
3	6-C_4H_9-n	A	235–236 dec	89	$C_{14}H_{15}NO_3$
4	6-C_6H_5	B	288–289 dec	78	$C_{16}H_{11}NO_3$
5	6-$C_6H_5CH_2$	B	262–263 dec	42	$C_{17}H_{13}NO_3$
7	6-$C_6H_5CH_2O$	C	264–265 dec	66	$C_{17}H_{13}NO_4$
8	6-$C_6H_5(CH_2)_2O$	C	247–249 dec	63	$C_{18}H_{15}NO_4$
9	6-$C_6H_5(CH_2)_3O$	D	247–248 dec	72	$C_{19}H_{17}NO_4$
10	6-$C_6H_5O(CH_2)_2O$	D	235–237 dec	54	$C_{18}H_{15}NO_5$
11	6-$C_6H_5O(CH_2)_3O$	A	202–205	98	$C_{19}H_{17}NO_5$
12	6-$C_6H_5O(CH_2)_4O$	A	237–239	58	$C_{20}H_{19}NO_5$
13	6-$C_6H_5O(CH_2)_6O$	A	215–217	45	$C_{21}H_{21}NO_5$
14	7-C_6H_5	D	279–280 dec	10	$C_{16}H_{11}NO_3 \cdot 0.25H_2O$
15	7-$C_6H_5CH_2$	A	268 dec	37	$C_{17}H_{13}NO_3$
16	7-$C_6H_5O(CH_2)_3O$	A	218–221	63	$C_{19}H_{17}NO_5$
17	8-C_6H_5	A	247–248	58	$C_{16}H_{11}NO_3$
18	8-$C_6H_5CH_2$	C	258–259	33	$C_{17}H_{13}NO_3$
19	8-$C_6H_5O(CH_2)_3O$	C	247–248	56	$C_{19}H_{17}NO_5$

^{a}Compds in Table IV saponified by previously described procedure.[2] ^{b}Recrystn solvents: A, EtOH; B, HOAc; C, 2-methoxyethanol; D, 2-methoxyethanol-H_2O. ^{c}Anal. C, H, N.

Table VI. Physical Properties of
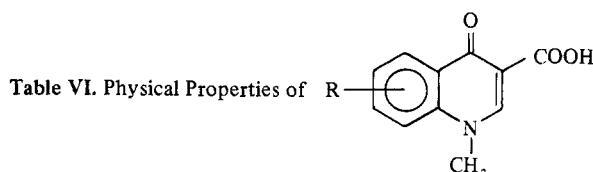
No.	R^a	Solvent^b	Mp, °C	Yield, %	Formula^c
24	6-C_6H_5	A	251–252	82	$C_{17}H_{13}NO_3$
25	6-$C_6H_5CH_2$	B	238–240	40	$C_{18}H_{15}NO_3$
26	6-$C_6H_5CH_2O$	B	253–255	58	$C_{18}H_{15}NO_4$
27	6-$C_6H_5O(CH_2)_3O$	B	194–195	59	$C_{20}H_{19}NO_5$
28	7-$C_6H_5CH_2$	B	235–237	66	$C_{18}H_{15}NO_3$
29	7-$C_6H_5O(CH_2)_3O$	A	177–179	16	$C_{20}H_{19}NO_5$
30	8-C_6H_5	B	278–280	63	$C_{17}H_{13}NO_3$
31	8-$C_6H_5CH_2$	A	191–215^d	40	$C_{18}H_{15}NO_3$
32	8-$C_6H_5O(CH_2)_3O$	A	172–175	46	$C_{20}H_{19}NO_5$

^{a}By alkylation and saponification of compds in Table IV according to the previously described procedure.[2] ^{b}Recrystn solvents: A, EtOH; B, 2-methoxyethanol. ^{c}Anal. C, H, N. ^{d}Retains this mp after addl recrystns.

give 2.75 g (56%), mp 196–197°. Anal. ($C_{16}H_{19}NO \cdot HCl$). See Table II for other compounds prepared in this manner.

The free amine was liberated by partitioning the HCl salt be-

tween 2 N NaOH and CH_2Cl_2. The org phase was dried ($MgSO_4$), spin-evapd and used in the next step.[2]

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Irreversible Enzyme Inhibitors. 192.[†,1] Hydrophobic Bonding to Some Dehydrogenases with 5-Substituted-4-hydroxyquinoline-3-carboxylic Acids

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Fifteen derivatives of 4-hydroxyquinoline-3-carboxylic acid bearing aryl, aralkyl, aralkoxy, or aroxyalkoxy substituents at the 5 position and Cl, Me, or H at the 8 position were synthesized and evaluated as inhibitors of glutamate, lactate, malate, and glyceraldehyde phosphate dehydrogenases; good to excellent hydrophobic interaction was observed with the first three enzymes, the 8-Cl-5-$C_6H_5(CH_2)_6$ substituent (12) giving the best interaction. 12 was complexed to malate dehydrogenase 1100-fold more effectively than the parent 4-hydroxyquinoline-3-carboxylic acid (1) and 4300-fold more effective than L-malate; 12 complexed to glutamate dehydrogenase 250-fold better than the parent 1 and 500-fold better than L-glutamate. Furthermore, 12 was complexed to lactate dehydrogenase 65-fold better than the parent 1 and 300-fold better than the substrate, pyruvate.

The possible utility of inhibitors or glutamate, glyceraldehyde phosphate, lactate, and malate dehydrogenases for

treatment of cancer cells in the resting phase (G_0) was discussed in a previous paper.[2] 4-Hydroxyquinoline-3-carboxylic acid (1) was selected[2] for further study to determine if potency could be increased by hydrophobic bonding with