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Irreversible Enzyme Inhibitors. 190.†,[1] Inhibition of Some Dehydrogenases by 1-Substituted-1,4-dihydro-4-quinolone-3-carboxylic Acids

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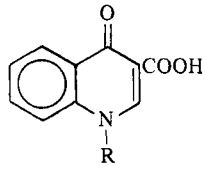
Fifteen 1-alkyl, 1-aralkyl, and 1-aryloxyalkyl derivatives of 1,4-dihydro-6-methoxy-4-quinolone-3-carboxylic acid were synthesized and evaluated as inhibitors of four dehydrogenases, namely, glutamate, glyceraldehyde phosphate, lactate, and malate. No hydrophobic bonding was observed, but good bulk tolerance for large substituents at the 1 position was apparent. Since bulk tolerance is present at the 1 position, several of these 1-substituted 4-quinolone-3-carboxylic acids should be convertible to candidate irreversible inhibitors by attachment to the appropriate derivative of benzenesulfonyl fluoride; furthermore by attachment to Sepharose, affinity columns for purification of the dehydrogenases should arise.

The chemotherapeutic utility of inhibitors of 3 dehydrogenases involved in glucose metabolism, as well as L-glutamate dehydrogenase, was discussed in the previous paper.[1] We observed that 1-methyl-4-quinolone-3-carboxylic acid (1, R = Me) was complexed to these enzymes as good or somewhat better than their respective substrates, glutamate, glyceraldehyde phosphate, pyruvate, and malate. This study



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has now been extended to larger R groups to see if (a) there was bulk tolerance for these larger groups within the enzyme-inhibitor complexes,[2] and (b) could hydrophobic bonding regions on the enzymes be detected. The results are the subject of this paper.

Enzyme Results. The key reaction for synthesis of the compounds in Table I is alkylation of a 3-carbethoxy-4-hydroxyquinoline. Since it is just as easy to synthesize this key intermediate from an arylamine with a substituent as without, four 1-methyl-4-quinoline-3-carboxylic acids were investigated for inhibition; these were 6-MeO (7), 8-MeO (9), 8-Cl (11), and H (3). Little difference in inhibition of the 4 dehydrogenases by the 4 compounds was observed. Therefore the 6-MeO series was arbitrarily chosen for further work.

Fifteen 1-alkyl, aralkyl, or aryloxyalkyl derivatives (7, 12–25) were synthesized and evaluated as inhibitors of glyceraldehyde phosphate dehydrogenase, lactate dehydrogenase, malate dehydrogenase, and glutamate dehydrogenase (Table I). With the exception of 24 and those compounds where solubility hampered measurement, no more than a 5-fold difference in inhibition of a given enzyme by the 15

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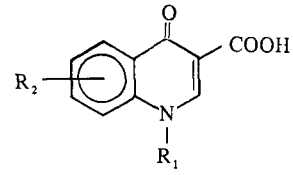
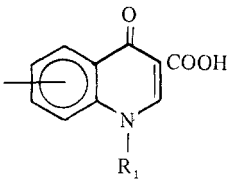
Table I. Inhibition[a] of Four Dehydrogenases by



No.	R_2	R_1	I_{50},[b] μM Glu-DH	GPDH	LDH	MDH
2[c]	H	H	600	>1600[d]	440	520
3[c]	H	CH_3	500	590	74	520[e]
4	H	C_4H_9-*n*	380	530	83	650[e]
5	H	$C_6H_5CH_2$	280	780	63	870[e]
6[c]	6-MeO	H	430	460	230	>400[d]
7	6-MeO	CH_3	270	330	150	200
8[c]	8-MeO	H	540	550	98	650
9	8-MeO	CH_3	320	300	58	340[e]
10[c]	8-Cl	H	330	750	300	410
11	8-Cl	CH_3	290	310	63	300[e]
12	6-MeO	C_4H_9-*n*	250	310	58	220
13	6-MeO	C_6H_{13}-*n*	190	300	62	200
14	6-MeO	$C_6H_5CH_2$	230	300	68	190
15	6-MeO	$C_6H_5(CH_2)_3$	290	310	50	230
16	6-MeO	$C_6H_5O(CH_2)_2$	290	310	72	>200[d]
17	6-MeO	$C_6H_5O(CH_2)_3$	240	320	140	180
18	6-MeO	$C_6H_5O(CH_2)_4$	200	310	87	220
19	6-MeO	α-$C_{10}H_7CH_2$	>250[d]	260	52	>160[d]
20	6-MeO	*m*-$NO_2C_6H_4CH_2$	450[e]	290	66	200[e]
21	6-MeO	*p*-$NO_2C_6H_4CH_2$	750[e]	230	51	>400[d]
22	6-MeO	*m*-$NH_2C_6H_4CH_2$	400[e]	300	69	500[e]
23	6-MeO	*p*-$HOOCC_6H_4CH_2$	250	310	54	300[e]
24	6-MeO	*p*-$NO_2C_6H_4O(CH_2)_4$	98	95	31	90
25	6-MeO	*p*-$NH_2C_6H_4O(CH_2)_4$	450[e]	200	80	900[e]

[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, GDPH = glyceraldehyde phosphate dehydrogenase, LDH = lactate dehydrogenase, and MDH = malate dehydrogenase when assayed[1] with 2 m*M* L-glutamate, 0.25 m*M* glyceraldehyde phosphate, 2 m*M* pyruvate, and 2 m*M* L-malate, respectively. [c] Data from ref 1. [d] No inhibition at 0.25 this concn, the max solubility. [e] Estimated from V_0/V_I observed at max solubility which is less than I_{50}.

Table II. Physical Properties of 

No.	R_2	R_1	Methods[a]	Mp, °C	Solvent[b]	Yield,[c] %	Formula[d]
4	H	C_4H_9-*n*	A, B	164–166	A	12	$C_{14}H_{15}NO_3$
5	H	$C_6H_5CH_2$	A, B	232–234	B	27	$C_{17}H_{13}NO_3$
7	6-MeO	CH_3	A, B	308–309 dec	C	77	$C_{12}H_{11}NO_4$
9	8-MeO	CH_3	A, B	250–252	B	82	$C_{12}H_{11}NO_4$
11	8-Cl	CH_3	A, B	253–256	B	38	$C_{11}H_8ClNO_3$
12	6-MeO	C_4H_9-*n*	A, B	219–221	B	82	$C_{15}H_{17}NO_4$
13	6-MeO	C_6H_{13}-*n*	A, B	136–138	B	72	$C_{17}H_{21}NO_4$
14	6-MeO	$C_6H_5CH_2$	A, B	263–265	C	58	$C_{18}H_{15}NO_4$
15	6-MeO	$C_6H_5(CH_2)_3$	A, B	175–177	C	44	$C_{20}H_{19}NO_4$
16	6-MeO	$C_6H_5O(CH_2)_2$	A, B	230–231	C	60	$C_{19}H_{17}NO_5$
17	6-MeO	$C_6H_5O(CH_2)_3$	A, B	174–175	C	70	$C_{20}H_{19}NO_5$
18	6-MeO	$C_6H_5O(CH_2)_4$	A, B	160–161	C	85	$C_{21}H_{21}NO_5$
19	6-MeO	α-$C_{10}H_7CH_2$	C, B	233–234	B	42	$C_{22}H_{17}NO_4$
20	6-MeO	*m*-$NO_2C_6H_4CH_2$	C, D	277–279	C	74[e]	$C_{18}H_{14}N_2O_6$
21	6-MeO	*p*-$NO_2C_6H_4CH_2$	C, D	269–271	C	79	$C_{18}H_{14}N_2O_6$
22	6-MeO	*m*-$NH_2C_6H_4CH_2$	E	289–291 dec	C	35[e]	$C_{18}H_{16}N_2O_4$
23	6-MeO	*p*-$HOOCC_6H_4CH_2$	C, B	308–309 dec	D	88[e]	$C_{19}H_{15}NO_6$
24	6-MeO	*p*-$NO_2C_6H_4O(CH_2)_4$	C, D[f]	209–210	C	38	$C_{21}H_{20}N_2O_7$
25	6-MeO	*p*-$NH_2C_6H_4O(CH_2)_4$	F	159–162	B	43[e]	$C_{21}H_{22}N_2O_5$

[a]Methods: A, alkylation by method previously described;[1] B, saponification by the previously described method C;[1] C–F, see Experimental Section. [b]Recrystn solvents: A, *i*-PrOH–H_2O; B, EtOH; C, 2-methoxyethanol; D, 2-methoxyethanol–H_2O. [c]Yield overall for alkylation and saponification unless otherwise indicated. [d]Analyzed for C, H, N. [e]Yield in last step. [f]For the starting *p*-nitrophenoxybutyl bromide see Ashley, *et al.*[8]

compounds was observed. The superior effect of the *p*-nitrophenoxy derivative (**24**) can be attributed to an electronic effect when compared to the corresponding *p*-aminophenoxy derivative (**25**).

The following important conclusions can be drawn: (a) no hydrophobic bonding could be detected with 1 substituents; (b) bulk tolerance[2] for groups as large as *m*-aminobenzyl (**22**) or *p*-aminophenoxybutyl (**25**) was apparent; (c) **22**, **23**, and **25** should be useful for conversion to candidate irreversible inhibitors of the 4 dehydrogenases by acylation with appropriate derivatives of $PhSO_2F$;[3] and (d) **22**, **23**, and **25** would make good candidates for attachment to Sepharose to give affinity columns[4–7] suitable for purification of the 4 dehydrogenases.

Chemistry. The compounds in Table I were prepared by alkylation of the appropriate ethyl 4-hydroxyquinoline-3-carboxylate[1] with the appropriate halide and NaH in DMF;[1] in most cases, the intermediate alkylated quinolones were saponified without further purification.[1] Since saponification decomposed the nitro compounds (**20**, **21**, **24**), the ester group was hydrolyzed with 6 *N* HCl. Reduction of **20** and **24** afforded the amines (**22**, **25**).

Experimental Section

Melting points were taken in capillary tubes on a Mel-Temp block and are uncor. Each analytical sample had an ir spectrum compatible with its structure and was homogeneous on tlc on Brinkmann silica gel GF. All analytical samples gave combustion values for C, H, N within 0.4% of theoretical.

Ethyl 6-Methoxy-1-(*p*-nitrobenzyl)-1,4-dihydro-4-quinolone-3-carboxylate (26). Method C. A mixt of 6.0 g (24.2 mmoles) of ethyl 4-hydroxy-6-methoxy-3-quinolinecarboxylate[1] and 1.2 g (29 mequiv, 58% in mineral oil) of NaH in 80 ml of dry DMF was heated at 50–70° until H_2 ceased to evolve. Then 7.34 g (43 mmoles) of *p*-nitrobenzyl chloride was added and the reaction mixt was heated at 70° for an additional 4 hr. The dark reaction mixt was poured into 850 ml of water and stirred for 0.5 hr, then the product was collected on a filter. Two recrystns from 2-methoxyethanol afforded 5.75 g (62%) of yellow crystals, mp 239–242°. A small sample was recrystd from 2-methoxyethanol for analysis, mp 240°. *Anal.* ($C_{20}H_{18}N_2O_6$) C, H, N.

In like manner, **ethyl 6-methoxy-1-(*m*-nitrobenzyl)-1,4-dihydro-4-quinolone-3-carboxylate (27)**, mp 216–219°, and **ethyl 6-methoxy-1-(*p*-cyanobenzyl)-1,4-dihydro-4-quinolone-3-carboxylate (28)**, mp 227–230°, were obtd in 77 and 63% yields, respectively, suitable for further transformations.

6-Methoxy-1-(*m*-nitrobenzyl)-1,4-dihydro-4-quinolone-3-carboxylic Acid (20). Method D. A mixt of 0.50 g (1.31 mmoles) of **27** and 10 ml of 6 *N* HCl was refluxed for 2 hr. After cooling the mixt was filtered and the product recrystd from 2-methoxyethanol: yield, 350 mg (74%) of pale yellow plates; mp 277–279°. *Anal.* ($C_{18}H_{14}N_2O_6$) C, H, N.

1-(*m*-Aminobenzyl)-6-methoxy-1,4-dihydro-4-quinolone-3-carboxylic Acid (22). Method E. To 4.5 g (11.8 mmoles) of **27** and 2.8 g (23.5 mg-atoms) of Sn was added in portions of 60 ml of 12 *N* HCl over 30 min. This was followed by 40 ml of H_2O; the mixt was heated on a steam bath for 2 hr, then left overnight. The solid was collected and dissolved in 600 ml of H_2O, and the soln was partially neutralized with $NaHCO_3$ until no more product pptd. The brown powder was collected on a filter, then extracted with 350 ml of boiling 2-methoxyethanol and filtered. On cooling the 2-methoxyethanol soln gave 0.41 g (11%) of white powder; mp 275–284° slow dec. The residue was extracted again and in like manner another crop of 0.87 g (total 35%), mp 273–280° dec, was obtd. A small portion of the first crop was recrystd from 2-methoxyethanol to give a white powder, mp 289–291° dec. *Anal.* ($C_{18}H_{16}N_2O_4$) C, H, N. The second crop was suitable for further transformations.

1-(*p*-Aminophenoxybutyl)-6-methoxy-1,4-dihydro-4-quinolone-3-carboxylic Acid (25). Method F. A mixt of 1.0 g (2.42 mmoles) of **24**, 100 mg of 10% Pd/C, and 100 ml of 2-methoxyethanol was shaken with H_2 at 2–3 atm overnight. The soln was filtered, then dild with an equal vol of Et_2O and satd with HCl gas to ppt the salt. The HCl salt was dissolved in H_2O by addn of 6 *N* NaOH. The soln was clarified with charcoal, then acidified to about pH 3 with HCl. The free base was recrystd from EtOH to give 0.40 g (43%): mp 159–162°. *Anal.* ($C_{21}H_{22}N_2O_5$) C, H, N.

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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-$C_6H_5O(CH_2)_4O$ 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-$C_6H_5O(CH_2)_4O$ (**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-$C_6H_5(CH_2)_3$ O (**9**) substituent; the 6-$C_6H_5O(CH_2)_5$ O (**13**) was about half as effective.

Hydrocarbon interaction with glutamate dehydrogenase was poor, the 6-$C_6H_5CH_2$ (**5**), 6-$C_6H_5(CH_2)_2O$ (**8**), 6-$C_6H_5(CH_2)_3O$ (**9**), or 6-$C_6H_5O(CH_2)_2O$ (**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.

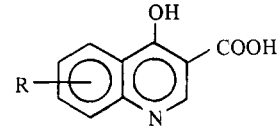
The 6-$C_6H_5(CH_2)_3O$ (**9**) group stands out as the best across the 4 enzymes, being complexed 37-, 100-, and 740-fold better, respectively, than the substrate, L-glutamate, L-lactate, or L-malate.

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

†This work was generously supported by Grant CA-08695 from the National Cancer Institute, U. S. Public Health Service.

‡See ref 2 for a discussion of the possible chemotherapeutic utility of these inhibitors.

Table I. Inhibition[a] of Four Dehydrogenases by



No.	R	I_{50},[b] μM 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, GDPH = glyceraldehyde phosphate dehydrogenase, LDH = lactate dehydrogenase, and MDH = malic dehydrogenase when assayed[2] with 2 m*M* L-glutamate, 0.25 m*M* glyceraldehyde phosphate, 2 m*M* pyruvate, and 2 m*M* 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.