Irreversible Enzyme Inhibitors. 189.†,[1] Inhibition of Some Dehydrogenases by Derivatives of 4-Hydroxyquinoline-2- and -3-carboxylic Acids

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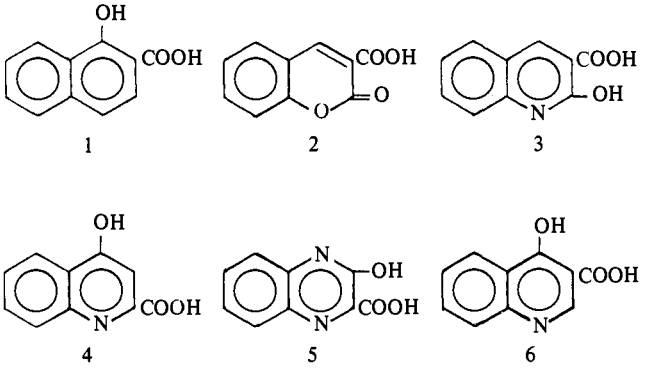
Seventeen derivatives of 4-hydroxyquinoline-3-carboxylic acid and 8 derivatives of 4-hydroxyquinoline-2-carboxylic acid with small substituents were synthesized and investigated as inhibitors of glutamate, glyceraldehyde phosphate, lactate and malate dehydrogenases. The most potent compound against the 4 dehydrogenases was 8-chloro-4-hydroxy-5-methylquinoline-3-carboxylic acid.

The design of antimetabolites for treatment of cancer has usually focused on inhibition of enzymes involved in synthesis of nucleic acid precursors; well-known examples are amethopterin, 6-mercaptopurine, and fluorouracil. These antimetabolites can obviously only function when the precursors and DNA are being synthesized by a tumor just prior to cell division; such drugs are most effective against the growth phase of leukemic cells *in vivo* where division time is 12-24 hr and DNA must be synthesized during this time. Many animal and human solid tumors are slow dividing and do not respond to treatment with inhibitors of DNA synthesis since most of the tumor cells are in a resting phase called stationary phase (G_0) and are not making DNA.[2]

Although resting cells are not synthesizing DNA in order to divide, they are still alive and must be using an energy source, presumably glucose. Therefore, we selected 3 dehydrogenase enzymes involved in glucose metabolism for study of inhibitors: (1) glyceraldehyde phosphate dehydrogenase, the junction point of glucose metabolism *via* fructose 1,6-diphosphate and the pentose shunt *via* ribulose 5-phosphate, (2) lactate dehydrogenase used by cells with a poor O_2 supply, and (3) malate dehydrogenase in the Krebs cycle. A fourth enzyme was selected that connects the glucose energy pathway with amino acid metabolism, namely glutamate dehydrogenase.

In an early paper of this series[3] we reported that 1-hydroxy-2-naphthoic acid (**1**) and several bicyclic heterocyclic carboxylic acids containing an appropriately positioned OH or oxo group (**2-4**) were good inhibitors of lactate and glutamate dehydrogenases; soon after we observed[4] that **5** and **6** also were inhibitors of these 2 enzymes.

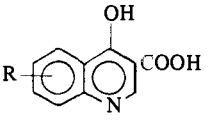


For the current program we wished to limit the 6 structural types to 1 or at the most 2. Such a decision would be based on (a) whether the parent compound or a simple derivative could inhibit all 4 enzymes and (b) ease of synthesis of many derivatives on the benzo moiety where enhanced by small substituents such as Cl, Me, and MeO or hydrophobic bonding by larger substituents can be sought. From the synthetic point of view derivatives of **4** and **6** would have the most flexibility since they can be made in 3 steps from an arylamine. In this paper is reported the inhibiton of the 4 enzymes by simple derivatives of **4** and **6** in the papers that follow, the detection of hydrophobic bonding by aryl, arylalkyl, and aryloxyalkyl substituents on **6** is reported.

Enzyme Results. Inhibition of glutamate (Glu-DH), lactate (LDH) and malate (MDH) dehydrogenases was seen with the parent 4-hydroxyquinoline-3-carboxylic acid (**6**) with I_{50} of about 500 μM, about 0.5 the concn of substrate being dehydrogenated or hydrogenated; although the I_{50} of **6** on glyceraldehyde phosphate dehydrogenase (GPDH) could not be detected due to lack of solubility, some of the derivatives of **6** such as **7**, **14**, and **21** showed good activity (Table I).

Effects by small substituents on Glu-DH were 19-fold be-

Table I. Inhibition[a] of Four Dehydrogenases by



No.	R	I_{50},[b] μM Glu-DH	GPDH	LDH	MDH
6[c]	H	600	1600[d]	440	520
7[e]	6-Cl	550[f]	1100	110	110
8[e]	7-Cl	1700	870	270	330
9[g]	8-Cl	330	750	300	410
10	8-Br	330	860	100	400
11[h]	6-Me	800	3000	210	700
12[h]	8-Me	2100[f]	4700	340	2100[f]
13[e]	8-CF_3	510	1200	170	1400[f]
14[i]	6-MeO	430	460	230	>400[d]
15[j]	8-MeO	540	550	98	650[f]
16[k]	6-AcNH	290[f]	470	86	~500[f]
17[e]	6-NO_2	200	550	110	400[f]
18[k]	8-NO_2	1300[f]	1100	300	>2000[d]
19	5,8-Cl_2	310	330	200	170
20	6,8-Cl_2	300	500	130	63
21	5-Me-8-Cl	110	150	140	120
22[l]	1-Me	500	590	74	520

[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, MDH = malate dehydrogenase as determined in the Experimental Section. [c] See ref 5 and 6 for synthesis. [d] No inhibition at 0.25 this concentration, the maximum solubility. [e] Commercially available. [f] Estimated from V_0/V_I observed at max solubility, which is less than the I_{50}. [g] See ref 7 for synthesis. [h] See ref 5 for synthesis. [i] See ref 8 for synthesis. [j] See ref 9 for synthesis. [k] See ref 6 for synthesis. [l] See ref 10 for synthesis.

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tween **21** and **12**, 31-fold between **21** and **12** on GDPH, only 5-fold between **16** and **6** on LDH, and 33-fold between **20** and **12** on MDH. There was no apparent correlations in these effects when considered from the steric, Hansch π, or Hammett σ viewpoints.

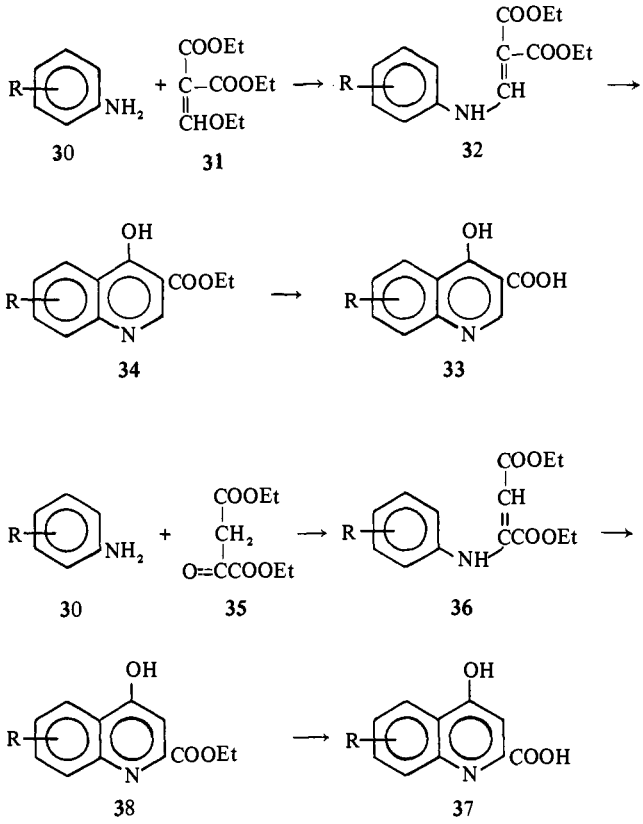
It is noteworthy that the 1-Me derivatives (**22**) was complexed to the enzymes somewhat more effectively than the parent compound (**6**). The most effective compounds when all 4 enzymes are considered were the 5-Me-8-Cl (**21**) and 5,8-Cl_2 (**19**) derivatives. Specificity differences between the enzymes were relatively small; for example, **15** was 5- to 36-fold more effective on LDH than the other 3 enzymes.

In Table II, the parent 4-hydroxyquinoline-2-carboxylic acid (**4**) showed inhibition of all 4 enzymes with I_{50}'s ranging from 68 to 380 μM. Substituent effects were even smaller than in 3-carboxylic acid series, being about 2-fold (excluding **27**). 1-Methylation (**27**) was detrimental to binding to 3 of the 4 enzymes, MDH being the exception.

Either derivatives of the 3-carboxylic acid (**6**) (Table I) or the 2-carboxylic acid (**4**) (Table II) could be used for the search for hydrophobic bonding with larger substituents. The future studies were limited to derivatives of the 3-carboxylic acid (**6**) for 3 reasons: (a) activity was not lost by 1-methylation (compare **22** *vs.* **27**), (b) the effect of substituents was greater, and (c) synthesis proceeded more smoothly. The successful search for hydrophobic bonding with derivatives of **6** is reported in the accompanying papers.

Chemistry. The thermal ring closure of arylaminomethylene malonic esters (**32**) to 4-hydroxyquinoline-3-carboxylic esters (**34**) is known as the Gould-Jacobs reaction;[7-9,14] the intermediates (**32**) are readily prepared by reaction of ethoxymethylenemalonic ester (**31**) with arylamines (**30**). It has been reported[5,6] that meta-substituted arylamino intermediates (**32**) ring close only to the lesser hindered position; in order to synthesize 5-substituted derivatives of **34**, it is therefore necessary to block the more reactive ortho position. Saponification of **34** gave the desired substituted 4-hydroxyquinoline-3-carboxylic acids (**33**) in Table I.

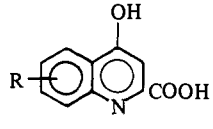
4-Hydroxyquinoline-2-carboxylic esters (**38**) are readily synthesized by thermal ring closure of the appropriate arylaminofumaric esters (**36**),[11,12] the intermediate fumarates are obtained by condensation of an arylamine (**30**) with ethyl oxosuccinate (**35**) in AcOH. Meta-substituted arylaminofumarates ring close to give both possible 5 and



7 isomers of **38**.[9,13] When the intermediate fumarates (**36**) were crystalline and could be purified, ring closure to **38** in diphenyl ether at 260° proceeded smoothly; in contrast, if the intermediate fumarate (**36**) was an oil that could not be purified, highly colored crude products (**38**) were obtained which were purified with considerably more difficulty. Saponification of the esters (**38**) gave the required substituted 4-hydroxyquinoline-2-carboxylic acids (**37**). The 1-methyl derivatives of 4-oxoquinoline-2- and -3-carboxylic acids were synthesized by alkylation of **34** or **38** in DMF with NaH and MeI.[10,15]

Although it is generally accepted from physical evidence that 2- and 4-hydroxyquinolines exist in the oxo form, the 4-hydroxyquinoline-3-carboxylic acids (**33**) and esters (**34**) exist in the OH form,[16,17] presumably due to H bonding between the 4-OH and 3-C=O groups.

Table II. Inhibition[a] of Four Dehydrogenases by



No.	R	I_{50},[b] μM Glu-DH	GPDH	LDH	MDH
4	H	380[c]	290	68[d]	280
23[e]	5-Cl	210	290	57	200
24	6-Cl	200	310	64	300[f]
25[e]	7-Cl	280	340	73	200
26[g]	8-Cl	170	300	55	170
27	1-Me	>6000[h]	>1200[h]	1300	510
28[i]	6-Me	170	320	61	210
29	8-Me	170	310	52	360[f]

[a,b] See Table I. [c] From ref 3. [d] I_{50} = 190 reported in ref 3. [e] See ref 11 and 12 for synthesis. [f] Estimated from V_0/V_I observed at max solubility, which is less than I_{50}. [g] See ref 12 for synthesis. [h] No inhibition at 0.25 this concn (max solubility). [i] See ref 13 for synthesis.

Experimental Section

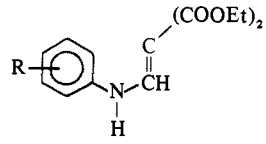
Melting points were taken in capillary tubes on a Mel-Temp block and are uncor. Each analytical sample had an ir spectrum compatible with its structure and was homogeneous on tlc on Brinkmann silica gel GF. All analytical samples gave combustion values for C, H, N within 0.4% of theoretical.

Enzyme Assays. Glutamate, glyceraldehyde phosphate, lactate, and malate dehydrogenases were purchased from Sigma Chemical Co. They were dild with the appropriate buffer to a concn sufficient to give 0.015–0.02 OD unit change per min at 340 mμ under the following conditions.

In a glass cuvette were placed 100 μl of 60 m*M* L-glutamate in 60 m*M* NaOH, 100 μl of 24 m*M* DPN in H_2O, 2.45 ml of 0.05 *M* Tris buffer (pH 8.4), and 0.30 ml of DMSO ± inhibitor; if turbidity obviously appeared or if an increasing OD was observed, the inhibitor concn was halved. When a zero base line was obtd, the enzymatic reaction was initiated with 50 μl of appropriately dild glutamate dehydrogenase. The cuvette concns of L-glutamate and DPN were 2 m*M* and 0.80 m*M*, resp.

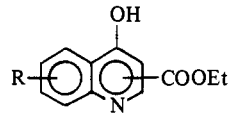
Similarly, malate dehydrogenase was run with cuvette concns of 2 m*M* L-malate and 0.80 m*M* DPN, and lactate dehydrogenase was run with 2 m*M* pyruvate and 0.60 m*M* DPNH in pH 7.4 Tris buffer.

Glyceraldehyde phosphate dehydrogenase was run with 2.25 ml

Table III. Physical Properties of 

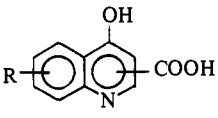
No.	R	Time[a]	Mp,[b] °C	Yield, %	Formula[c]
36	2-Br	90	81-82	28	$C_{14}H_{16}BrNO_4$
37	2,5-Cl_2	120	119-120	86	$C_{14}H_{15}Cl_2NO_4$
38	2,4-Cl_2	120	112-113	86	$C_{14}H_{15}Cl_2NO_4$
39	2-Cl-5-CH_3	30	91-92	32	$C_{15}H_{18}ClNO_4$

[a]Min for method A. [b]Recrystd from EtOH. [c]*Anal.* C, H, N.

Table IV. Physical Properties of 

No.	R	Position of COOEt	Time[a]	Solvent[b]	Mp, °C	Yield, %	Formula[c]
30	8-Br	3	5	A	248-251 dec	27	$C_{12}H_{10}BrNO_3$
31	5,8-Cl_2	3	6	A	203-205 dec	26	$C_{12}H_9Cl_2NO_3$
32	6,8-Cl_2	3	6	B	277-278 dec	50	$C_{12}H_9Cl_2NO_3$
33	5-CH_3-8-Cl	3	0.75	C	196-198	51	$C_{13}H_{12}ClNO_3$
34	6-Cl	2	0.5	B	243	84	$C_{12}H_{10}ClNO_3$
35	8-CH_3	2	0.5	C	141-143	41[d]	$C_{13}H_{13}NO_3$

[a]Hours for method B. [b]Recrystn solvents: A, EtOH; B, pyridine; C, EtOH-H_2O. [c]*Anal.* C, H, N. [d]Intermediate not purified and yield is overall.

Table V. Physical Properties of 

No.	R	Position of COOH	Solvent[a]	Mp, °C dec	Yield,[b] %	Formula[c]
10	8-Br	3	A	270-271	40	$C_{10}H_6BrNO_3$
19	5,8-Cl_2	3	B	279-280	60	$C_{10}H_5Cl_2NO_3$
20	6,8-Cl_2	3	B	310	55	$C_{10}H_5Cl_2NO_3$
21	5,CH_3-8-Cl	3	B	285-286	22	$C_{11}H_8ClNO_3$
24	6-Cl	2	C	283	94	$C_{10}H_6ClNO_3$
29	8-CH_3	2	D	256-259	43	$C_{11}H_9NO_3$

[a]Recrystn solvents: A, pyridine-EtOH; B, 2-methoxyethanol; C, pyridine; D, EtOH-H_2O. [b]Prepd by method C. [c]*Anal.* C, H, N.

of 0.05 *M* Tris buffer (pH 9.0) contg 10 m*M* mercaptoethanol and 1 m*M* Versene, 0.10 ml of 0.4 *M* Na_2HAsO_4, 0.15 ml of 9 m*M* DPN, 0.30 ml of DMSO ± inhibitor, 100 μl of enzyme, and 100 μl of 7.5 m*M* glyceraldehyde phosphate in H_2O; the cuvette concs of glyceraldehyde phosphate and DPN were 0.25 m*M* and 0.45 m*M*, resp.

Diethyl 2-Chloro-5-methylanilinomethylenemalonate (39). Method A. A mixt of 4.0 g (28.3 mmoles) of crude 3-amino-4-chlorotoluene (obtd in 97% yield by catalytic reduction of 4-chloro-3-nitrotoluene in EtOH with a PtO_2 catalyst) and 6.1 g (28.3 mmoles) of diethyl ethoxymethylenemalonate was heated on a steam bath for 30 min, then cooled and twice recrystd from EtOH: yield, 2.81 g (32%); mp 91-93° (Table III). A second crop of usable material was obtd from the combined filtrates.

Diethyl 4-Chloroanilinofumarate (40). To 8.0 g (62 mmoles) of 4-chloroaniline was added 8.3 g (40 mmoles) of the sodium enolate salt of diethyl oxalacetate and 29 ml of glacial AcOH. The reaction mixt was stirred with heating at 40-50° for 4 hr, then left overnight at room temp. The reaction mixt was poured onto ice, made basic with 50% NaOH, and extd with CH_2Cl_2. The org phase was washed with 0.5 *N* HCl followed by 0.5 *N* NaOH and dried (Na_2SO_4); the solvent was removed leaving 8.8 g of the crude yellow product. This material was recrystd from EtOH to give 5.7 g (48%) of product: mp 56-60°. A small sample was recrystd from EtOH for analysis: mp 58-61°. *Anal.* ($C_{14}H_{16}ClNO_4$) C, H, N.

The 2-methylanilinofumarate (41) was prepd in like manner; however, since it was noncryst it was used crude in the cyclization step.

Ethyl 8-Chloro-4-hydroxy-5-methyl-3-quinolinecarboxylate (33). Method B. A mixt of 3.9 g (12.5 mmoles) of 39 and 40 ml of diphenyl ether was heated at 260° for 45 min. After cooling, the mixt was dild with an equal vol of petroleum hexanes; the product was collected and washed with petroleum hexanes. The crude product was dissolved in 70% EtOH and clarified with charcoal. The soln was induced to deposit crystals by concn under a gentle air stream: yield 1.70 g (51%); mp 196-198° (see Table IV).

8-Chloro-4-hydroxy-5-methyl-3-quinolinecarboxylic Acid (21). Method C. To 10 ml of 10% NaOH was added 0.50 g (1.9 mmoles) of 33 and 1 ml of EtOH. The mixt was refluxed for 2 hr, filtered, then acidified with HCl to ppt the free acid. This material was recrystd twice from 2-methoxyethanol: yield, 100 mg (22%); mp 285-286° dec (Table V).

1-Methyl-4-oxo-1,4-dihydro-2-quinolinecarboxylic Acid (27). A mixt of 0.50 g (2.64 mmoles) of 4, 330 mg (8 mmoles, 58% in mineral oil) of NaH, and 15 ml of dry DMF protected from moisture was stirred at 80° for 30 min when H_2 evoln was complete. The soln was cooled to room temp and 2.5 ml of MeI was added. After being stirred for 2 hr, an addl 2.5 ml of MeI was added. After 2 hr more, the solvent was removed *in vacuo*, and the residue was recrystd from H_2O to give 170 mg (32%) of methyl 1-methyl-4-oxo-1,4-dihydro-2-quinolinecarboxylate: mp 145-146°. This was saponified by method C to give 100 mg (63% from ester): mp 186-187° dec. *Anal.* ($C_{11}H_9NO_3 \cdot 0.25H_2O$) C, H, N.

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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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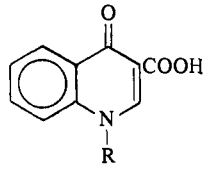
Fifteen 1-alkyl, 1-aralkyl, and 1-aryloxyalkyl derivatives of 1,4-dihydro-6-methoxy-4-quinolone-3-carboxylic acid were synthesized and evaluated as inhibitors of four dehydrogenases, namely, glutamate, glyceraldehyde phosphate, lactate, and malate. No hydrophobic bonding was observed, but good bulk tolerance 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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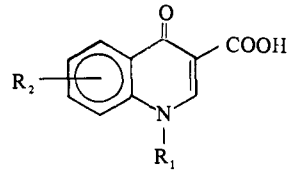
has now been extended to larger R groups to see if (a) there was bulk tolerance for these larger groups within the enzyme-inhibitor complexes,[2] and (b) could hydrophobic bonding regions on the enzymes be detected. The results are the subject of this paper.

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

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

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}.