Quantitative Structure–Activity Relationships Involving the Inhibition of Glycolic Acid Oxidase by Derivatives of Glycolic and Glyoxylic Acids

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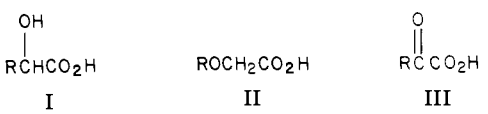
The enzyme glycolic acid oxidase oxidizes glycolate to glyoxylate and glyoxylate to oxalate. Three series of compounds related to the natural substrates, substituted glycolic, oxyacetic, and glyoxylic acids, have been investigated as inhibitors of this enzyme using the techniques of regression analysis and quantitative structure–activity relationships. The best overall correlation with inhibitory potencies was found with the Hansch hydrophobic parameter π. The classical electronic parameters σ_p, σ_m, $\mathcal{F}$, and $\mathcal{R}$ performed poorly. For the substituted glyoxylic acids, a dummy parameter relating to the presence of a nucleophilic group in close proximity to the α-carbonyl of the glyoxylate group was found to be highly significant. The syntheses of six novel glycolic and glyoxylic acids are described.

The enzyme glycolic acid oxidase (glycolate:O_2 oxidoreductase, EC 1.1.3.1), purified from liver, catalyzes the oxidation of glycolic acid to oxalic acid via glyoxylic acid.[1-3] Experimental evidence has been presented which suggests that glycolic acid oxidase (GAO) is one of the enzymes involved in the metabolic production of oxalate.[4,5] Another enzyme implicated in oxalate production is lactate dehydrogenase (LDH),[6] while xanthine oxidase is probably of only minor importance.[7]

Primary hyperoxaluria is a genetic disease in which the overproduction of oxalate leads to systemic deposits of calcium oxalate, generally resulting in death before the end of the third decade.[8,9] No specific treatment for primary hyperoxaluria exists at the present time.[8,9] Reduction of the rate of conversion of glyoxalate to oxalate by inhibition of GAO or LDH might, therefore, be potentially lifesaving in the treatment of this disease. While an inhibitor of LDH in man might prove highly toxic, it is possible that inhibition of GAO could proceed without unacceptable consequences. It has been demonstrated that perfusion of rat livers with DL-β-phenyllactate, an inhibitor of GAO, did reduce the rate of oxalate production.[4] Thus, there is an interest in developing potent inhibitors of GAO for study in the treatment of genetic hyperoxalurias.

Schuman and Massey have determined the inhibition constants for the inhibition of GAO by six straight-chain monocarboxylic acids and three small dicarboxylic acids.[3] They demonstrated that the free energy of inhibition of GAO by the monocarboxylic acids was proportional to the number of carbon atoms in the alkyl chain, indicating a hydrophobic component to the inhibitor binding.[3] They also demonstrated that the increase in free energy of binding observed for the smallest dicarboxylic acid, oxalic acid, compared to that observed for a small monocarboxylic acid, such as acetic acid, was too large to be explained by a mechanism in which both carboxylate groups bound to the same cationic group on the enzyme.[3] Their results were thermodynamically consistent with the electrostatic interaction of the carboxylate groups with two positively charged groups in close proximity on the enzyme.[3]

We have investigated three series of compounds, substituted glycolic acids (I), substituted oxyacetic acids (II),



I II III

and substituted glyoxylic acids (III), all related to the natural substrates of GAO, as possible inhibitors of this enzyme. We report here some quantitative structure–activity relationships for each of these three classes which have been conducted in order to explore more completely the hydrophobic and electronic contributions to the inhibition of this enzyme.

Method. The activity of an inhibitor at a given concentration was expressed as its fractional inhibition, $f_I = R_I/R_C$ where R_I and R_C are the initial rates in the presence and absence of the inhibitor, respectively. In all cases investigated, f_I could be related to the total concentration, $[I]_t$, by the relationship:

$$f_I = (1 + I_{50}/[I]_t)^{-1}$$

where I_{50} is the inhibitor concentration, in molar units, required to produce a fractional inhibition of 0.5. I_{50} values were obtained from graphical interpolation of f_I vs. $[I]_t$ plots or from extrapolation by use of a rearrangement of the above equation:

$$I_{50} = \frac{(1 - f_I)[I]_t}{f_I}$$

This extrapolation was not projected for observed f_I values more than 0.3 unit from 0.5. The I_{50} values were then converted to pI_{50} values ($= -\log_{10}[I_{50}]$) for use as dependent variables in quantitative structure–activity relationships. f_I values in the range 0.3 to 0.7 produced by fixed concentrations of inhibitor have been found by repetitive measurements (data not shown) to be associated with a nearly uniform standard deviation $\delta_{f_I} \leq 0.03$. This leads to standard deviations of the computed pI_{50} values of less than 0.05. The errors in the experimental pI_{50} values are, therefore, conservatively estimated to be ± 0.10. The constants σ_p, σ_m, $\mathcal{F}$, and $\mathcal{R}$ were taken from the literature where available.[10,11] Values for π were taken from these compilations or calculated from the additivity characteristic of this hydrophobic parameter.[12]

Values of the group molar refractivity, MR, were used to estimate the steric bulk contributions of these substituents.[10] While MR is only an approximate measure of substituent steric effects and is known to be related to the electronic polarizability of the substituent,[13] the heterogeneous nature of the substituents employed in this work ruled out the use of more theoretically justified parameters such as Es^c,[14] which has been tabulated only for a limited series of substituents.[15] Also of importance here was the fact that rules have been described for the derivation of MR values for larger and more complicated substituents from smaller fragments.[16,17] The quantitative structure–activity relationships were investigated by means of a stepwise regression analysis technique.[18] The stepwise regression equations were computed using the STEPWISE

0022-2623/79/1822-0608$01.00/0

Table I. Structures, Inhibitory Potencies, and Physical Parameters for a Series of Substituted Glycolic Acids

$$\begin{array}{c} OH \\ | \\ RCHCO_2H \\ (d,l) \end{array}$$

no.	R	pI_{50}	π	π^2	σ_p	$\mathcal{F}$	$\mathcal{R}$	MR
1[a]	C_6H_5-	2.40	1.96	3.84	0.0	0.0	0.0	25.36
2[c]	4-I-C_6H_4-	3.79	3.08	9.49	0.18	0.40	−0.19	38.12
3[c]	4-Cl-C_6H_4-	3.23	2.67	7.13	0.23	0.41	−0.15	30.11
4[c]	4-C_6H_5O-C_6H_4-	3.80	4.04	16.32	−0.03	0.34	−0.35	52.01
5[c]	4-C_6H_5-C_6H_4-	4.40	3.92	15.37	−0.01	0.08	−0.08	49.69
6[c]	4-F-C_6H_4-	2.40	2.10	4.41	0.06	0.43	−0.34	24.95
7[b]	4-(1-c-C_4H_4N)-C_6H_4-	4.16	2.91	8.47	0.37	0.50	−0.09	46.90
8[c]	4-Br-C_6H_4-	3.39	2.82	7.95	0.23	0.44	−0.17	32.96
9[a]	4-(c-C_6H_{11})-C_6H_4-	4.52	4.47	19.98	−0.22			51.02
10[c]	3-NO_2-C_6H_4-	2.75	1.68	2.82		0.67	0.16	31.69
11[c]	2-Cl-C_6H_4-	2.77	2.67	7.13		0.41	−0.15	30.11
12[c]	$CH_3CH_2SCH_2$-	2.72	1.53	2.34				23.07
13[a]	$C_6H_5CH_2$-	3.79	2.01	4.04				30.01
14[c]	$C_6H_5CH{=}CH$-	3.33	2.68	7.18				34.17
15[a]	c-C_6H_{11}-	2.40	2.51	6.30				26.69
16[c]	$(CH_3)_2CHCH_2$-	2.60	2.01	4.04				19.62
17[b]	5-$C_6H_5CH_2$-2-(c-C_4H_3S)-	3.98	3.62	13.10				53.02
18[b]	5-(4-Cl-C_6H_4)-2-(c-C_4H_3S)-	4.29	4.28	18.32				53.37
19[b]	4-C_6H_5-$C_6H_4SCH_2$-	5.00	4.74	22.47				63.27
20[c]	$CH_3SCH_2CH_2$-	2.30	1.53	2.34				23.09
21[a]	3,5,7-$(CH_3)_3$-(c-$C_{10}H_{15}$)-	3.40	4.98	24.80				54.49

[a] Obtainable from commercial sources. [b] Synthesis described under Experimental Section. [c] The syntheses of these compounds have been described in the literature. The reference for each of these compounds is: **2**, 3, 6, **8**, ref 25; 4, ref 26; **5**, ref 27; 10, ref 28; 11, ref 29; **12**, ref 30; **14**, ref 31; 16, ref 32; **20**, ref 33.

procedure of the SAS·76[19] system.

Parameters were considered to be worth including when they entered the relationship at greater than the 0.50 significance level. Additionally, N, the number of compounds included in the analysis, and R^2, the square of the multiple regression coefficient (which is equal to the fraction of the total variance explained by the equation),[18] are listed for each regression equation.

Results and Discussion

Substituted Glycolic Acids (Table I). The entire set of these compounds, **1–21**, can be fitted by stepwise regression to an equation relating pI_{50} to the Hansch hydrophobic parameters π and π^2 and the steric parameter MR. Of these, the most significant is MR, with neither π or π^2 as additional parameters entering the regression equation above the 0.50 significance level (eq 1). It should

$$pI_{50} = (0.054 \pm 0.007)\text{MR} + 1.36 \quad (1)$$

$$N = 21,\ R^2 = 0.771$$

be noted that for the substituents of Table I, π and MR are fairly well correlated with a simple correlation coefficient of 0.93. Thus, π alone can fit the inhibition data nearly as well, as shown in eq 2.

$$pI_{50} = (0.6 \pm 0.1)\pi + 1.66 \quad (2)$$

$$N = 21,\ R^2 = 0.618$$

Table I contains a subgroup of nine para-substituted phenylglycolic acids for which Hammett σ_p values are known, **1–9**, and another subgroup of eight such compounds for which the $\mathcal{F}$ and $\mathcal{R}$ values of Swain and Lupton have also been tabulated, **1–8**. For neither subset were any of these electronic parameters found significant above the 0.50 level. For the nine para-substituted phenylglycolic acids, MR was again found to be the only significant parameter (eq 3). As for the complete set of glycolic acids,

$$pI_{50} = (0.06 \pm 0.01)\text{MR} + 1.03 \quad (3)$$

$$N = 9,\ R^2 = 0.847$$

π and MR are closely correlated, with a correlation coefficient of 0.92.

Substituted Oxyacetic Acids (Table II). The pI_{50} values for the entire set of these compounds, **22–52**, can be fitted to π, π^2, and MR. Both π and π^2, but not MR, are found to enter the regression equation above the 0.50 significance level (eq 4).

$$pI_{50} = (0.8 \pm 0.3)\pi - (0.10 \pm 0.05)\pi^2 + 1.80 \quad (4)$$

$$N = 31,\ R^2 = 0.452$$

While both π and π^2 are significant in the above equation, clearly other unidentified factors must be of major importance, since only 45% of the variance is explained by eq 4.

The compounds in Table II consist mainly of phenoxyacetic acids which are para (**23–32**), ortho (**33–41**), and meta (**42–49**) substituted. Correlations with π, π^2, and MR can be made for each of these subsets and also with the relevant electronic parameters for those members of each subset for which these data are available.

Para-Substituted Phenoxyacetic Acids (22–32). Stepwise regression analysis reveals that for the subgroup of ten para-substituted phenoxyacetic acids for which σ_p, $\mathcal{F}$, and $\mathcal{R}$ values are known, **22–31**, none of these electronic parameters are significant above the 0.50 level. For the complete subgroup of para-substituted compounds, **22–32**, only π and π^2 enter the regression equation above to the 0.50 significance level (eq 5).

$$pI_{50} = (1.5 \pm 0.5)\pi - (0.21 \pm 0.08)\pi^2 + 1.04 \quad (5)$$

$$N = 11,\ R^2 = 0.689$$

Ortho-Substituted Phenoxyacetic Acids (22 and 33–41). The data for these compounds yielded the anomalous result that π^2 was more significnt than π, with π^2 alone entering the equation above the 0.50 significance level (eq 6).

$$pI_{50} = (0.10 \pm 0.04)\pi^2 + 2.41 \quad (6)$$

$$N = 10,\ R^2 = 0.528$$

Table II. Structures, Inhibitory Potencies, and Physical Parameters for Substituted Oxyacetic Acids

$ROCH_2CO_2H$

no.	R	pI_{50}	π	π^2	σ_p	σ_m	$\mathcal{F}$	$\mathcal{R}$	MR
22[a]	C_6H_5-	2.74	1.96	3.84	0.0	0.0	0.0	0.0	25.36
23[a]	4-NO_2-C_6H_4-	2.96	1.68	2.82	0.78		0.67	0.16	29.53
24[a]	4-$(CH_3)_3C$-C_6H_4-	3.64	3.94	15.52	−0.20		−0.07	−0.13	46.01
25[a]	4-HO-C_6H_4-	2.64	1.29	1.66	−0.37		0.29	−0.64	27.18
26[a]	4-Cl-C_6H_4-	3.80	2.67	7.13	0.23		0.41	−0.15	30.11
27[a]	4-CH_3O-C_6H_4-	2.64	1.94	3.76	0.27		0.26	−0.51	31.85
28[a]	4-C_6H_5-C_6H_4-	3.80	3.92	15.37	−0.01		0.08	−0.08	49.69
29[b]	4-(CH_3COCH=CH-)-C_6H_4-	3.85	1.90	3.61	−0.01		0.28	−0.27	45.43
30[a]	4-NH_2-C_6H_4-	2.09	0.73	0.53	−0.66		0.02	−0.68	29.53
31[b]	NO_2CH=CH-C_6H_4-	3.14	2.07	4.28	0.26		0.33	−0.05	40.75
32[b]	4-[$C(CH_3)_2CH_2C(CH_3)_3$]-C_6H_4-	3.27	4.96	24.60					62.52
33[b]	2-NH_2CO-C_6H_4-	2.57	0.47	0.22			0.24	0.14	34.14
34[a]	2-CH_3-C_6H_4-	3.55	2.52	6.35			−0.04	−0.13	30.07
35[a]	2-Cl-C_6H_4-	3.27	2.67	7.13			0.41	−0.15	30.11
36[a]	2-NO_2-C_6H_4-	2.49	1.68	2.82			0.67	−0.16	29.53
37[a]	2-CH_3O-C_6H_4-	2.80	1.94	3.76			0.26	−0.51	31.85
38[b]	2-Br-C_6H_4-	2.31	2.82	7.95			0.44	−0.17	32.69
39[b]	2-HO-C_6H_4-	2.62	1.29	1.66			0.29	−0.64	27.18
40[a]	2-(CH_2=$CHCH_2$)-C_6H_4-	3.80	3.06	9.36					38.82
41[b]	2-(CH_3CH=$CHCH_2$)-C_6H_4-	3.82	3.62	13.10					43.44
42[a]	3-CH_3O-C_6H_4-	3.00	1.94	3.76		0.12	0.26	−0.51	31.85
43[a]	3-Cl-C_6H_4-	3.43	2.67	7.13		0.37	0.41	−0.15	30.11
44[a]	3-CH_3-C_6H_4-	3.57	2.52	6.35		−0.07	−0.04	−0.13	30.07
45[b]	3-I-C_6H_4-	3.35	3.08	9.49		0.35	0.40	−0.19	38.12
46[b]	3-CH_3CONH-C_6H_4-	2.28	0.99	0.98		0.21	0.28	−0.26	39.26
47[a]	3-NO_2-C_6H_4-	3.09	1.68	2.82		−0.71	0.67	0.16	29.53
48[b]	3-CF_3-C_6H_4-	3.46	2.84	8.07		0.43	0.38	0.19	29.35
49[b]	3-C_2H_5O-C_6H_4-	3.09	2.34	5.48		0.10	0.22	−0.44	36.67
50[a]	C_6H_5CH=$CHCH_2$-	2.57	3.06	9.36					38.80
51[a]	2-naphthalyl-	3.09	3.28	10.76					40.77
52[b]	$C_6H_5CH_2$-	3.10	2.01	4.04					30.01

[a] Obtainable from commercial sources. [b] The syntheses of these compounds have been described in the literature. The reference for each of these compounds is: **29**, ref 34; **31**, ref 35; **32**, ref 36; **33**, ref 37; **38**, **39**, and **45**, ref 38; **41**, ref 39; **46**, ref 40; **48**, ref 41; **49**, ref 42; **52**, ref 43.

Table III. Structures, Inhibitory Potencies, and Physical Parameters for a Series of Substituted Glyoxylic Acids

$RCCO_2H$ (O)

no.	R	pI_{50}	π	π^2	MR
53[c]	4-C_6H_5-C_6H_4-	3.21	3.92	15.37	49.69
54[a]	C_6H_5-	2.64	1.96	3.84	25.36
55[c]	2-NO_2-5-$C_6H_5CH_2O$-$C_6H_3CH_2$-	4.19	3.39	11.49	67.50
56[c]	2-NO_2-6-CH_3-$C_6H_3CH_2$-	4.02	2.21	4.88	40.96
57[c]	3,4-$(CH_3O)_2C_6H_3CH_2$-	3.85	1.92	3.69	43.69
58[a]	$C_6H_5CH_2$ -	4.12	2.01	4.04	30.01
59[a]	2-NO_2-$C_6H_4CH_2$-	4.37	1.73	2.99	36.34
60[c]	3-HO-4-CH_3O-$C_6H_3CH_2$-	3.64	1.32	1.74	38.67
61[c]	4-Cl-C_6H_4CH=CH-	3.57	3.39	11.49	39.17
62[c]	3-CH_3O-C_6H_4CH=CH-	3.40	2.66	7.08	41.01
63[c]	3,4-Cl_2-C_6H_3CH=CH-	4.10	4.05	16.40	44.17
64[c]	2,4-$Cl_2C_6H_3CH$=CH-	4.10	4.09	16.73	44.17
65[c]	2-Cl-C_6H_4CH=CH-	3.80	3.39	11.49	39.17
66[c]	3,4-$(CH_3O)_2C_6H_3CH$=CH-	2.92	2.64	6.97	47.85
67[a]	$CH_3(CH_2)_3$-	4.00	4.26	18.15	19.59
68[c]	5-C_6H_5-2-(c-C_4H_3S)-	4.52	3.57	12.74	48.37
69[b]	5-$C_6H_5CH_2$-2-(c-C_4H_3S)-	4.70	3.62	13.10	53.02
70[b]	5-(4-Cl-C_6H_4-)-2-(c-C_4H_3S)-	5.05	4.28	18.32	53.37

[a] Obtainable from commercial sources. [b] Synthesis described under the Experimental Section. [c] The syntheses of these compounds have been described in the literature. The reference for each of these compounds is: **53**, ref 44; **55**, ref 45; **56**, ref 46; **57**, ref 47; **60**, ref 48; **61**, **63**, and **64**, ref 49; **62**, ref 50; **65**, ref 51; **66**, ref 52; **68**, ref 53.

When only π was included as an independent variable (eq 7) the fit was almost as good as for eq 6.

$$pI_{50} = (0.42 \pm 0.16)\pi + 2.08 \quad (7)$$

$$N = 10,\ R^2 = 0.465$$

The near equivalence of eq 6 and 7 is reflected in the large correlation between π and π^2, 0.971.

Meta-Substituted Phenoxyacetic Acids (22 and 42–49). For this subgroup, of the six parameters considered, π, π^2, σ_m, $\mathcal{F}$, $\mathcal{R}$, and MR, only π and σ_m enter the regression equation above the 0.50 significance level to yield eq 8.

$$pI_{50} = (0.65 \pm 0.1)\pi - (0.4 \pm 0.2)\sigma_m + 1.71 \quad (8)$$

$$N = 9,\ R^2 = 0.861$$

Substituted Glyoxylic Acids (Table III). Stepwise

Table IV. Comparison of Observed and Predicted pI_{50} Values

substituted glycolic acids					substituted oxyacetic acids							substituted glyoxylic acids				
		predicted					predicted							predicted		
no.	obsd	eq 1	eq 2	eq 3	no.	obsd	eq 4	eq 5	eq 6	eq 7	eq 8	no.	obsd	eq 9	eq 10	eq 11
1	2.40	2.73	2.81	2.68	**22**	2.74	3.02	3.17	2.81	2.90	2.98	**53**	3.21	4.12	3.82	3.79
2	3.79	3.42	3.47	3.51	**23**	2.96	2.89	2.97				**54**	2.64	3.64	3.40	3.42
3	3.23	2.98	3.23	2.99	**24**	3.64	3.50	3.69				**55**	4.19	3.96	4.51	4.52
4	3.80	4.17	4.03	4.41	**25**	2.64	2.69	2.62				**56**	4.02	3.69	4.27	4.30
5	4.40	4.04	3.96	4.26	**26**	3.80	3.28	3.55				**57**	3.85	3.64	3.39	3.41
6	2.40	2.71	2.90	2.65	**27**	2.64	3.01	3.16				**58**	4.12	3.65	3.41	3.43
7	4.16	3.89	3.37	4.08	**28**	3.80	3.50	3.69				59	4.37	3.61	4.20	4.21
8	3.39	3.14	3.32	3.17	**29**	3.85	2.99	3.13				60	3.64	3.56	3.32	3.30
9	4.52	4.12	4.29	4.34	30	2.09	2.34	2.02				61	3.57	3.96	3.68	3.69
10	2.75	3.07	2.65		31	3.14	3.07	3.24				**62**	3.40	3.78	3.52	3.55
11	2.77	2.98	3.23		**32**	3.27	3.46	3.32				63	4.10	4.16	3.85	3.82
12	2.72	2.60	2.56		33	2.57	2.16		2.43	2.28		64	4.10	4.18	3.87	3.83
13	3.79	2.98	2.84		34	3.55	3.23		3.07	3.13		**65**	3.80	3.96	3.67	3.69
14	3.33	3.20	3.24		**35**	3.27	3.28		3.16	3.19		66	2.92	3.77	3.51	3.55
15	2.40	2.80	3.14		36	2.49	2.89		2.70	2.78		**67**	4.00	4.24	3.92	3.86
16	2.60	2.42	2.84		**37**	2.80	3.01		2.80	2.89		**68**	4.52	4.01	4.55	4.56
17	3.98	4.22	3.79		**38**	2.31	3.32		3.24	3.25		69	4.70	4.03	4.57	4.57
18	4.29	4.24	4.17		39	2.62	2.68		2.58	2.62		70	5.05	4.24	4.76	4.69
19	5.00	4.78	4.44		40	3.80	3.38		3.39	3.35						
20	2.30	2.61	2.55		**41**	3.82	3.48		3.78	3.59						
21	3.40	4.30	4.58		**42**	3.00	3.01				2.92					
					43	3.43	3.28				3.29					
					44	3.57	3.23				3.37					
					45	3.35	3.39				3.56					
					46	2.28	2.51				3.26					
					47	3.09	2.89				3.08					
					48	3.46	3.33				3.37					
					49	3.09	3.17				3.18					
					50	2.57	3.38									
					51	3.09	3.43									
					52	3.10	3.04									

regression reveals that only π^2 enters the regression equation above the 0.50 significance level (eq 9). Only a

$$pI_{50} = (0.03 \pm 0.02)\pi^2 + 3.68 \quad (9)$$

$$N = 23, R^2 = 0.068$$

small fraction of the variance is explained by this equation.

Table III contains a subgroup of six compounds containing heteroatoms in close proximity to the point of attachment of the substituent to the α-keto group of the glyoxylic acid moiety. This subgroup consists of three compounds with aromatic *o*-nitro groups, **55**, **56**, and **59**, and three substituted 2-thienyl compounds, **68–70**. Inspection of residuals from eq 9 revealed that the observed pI_{50} values were greater than calculated for each member of this subgroup. Accordingly, a dummy parameter, D, was created and given a value of 1.0 for each of these six compounds and 0.0 for all the others.[18] The resulting regression equation, eq 10, reveals a decided increase in

$$pI_{50} = (0.04 \pm 0.02)\pi^2 + (0.8 \pm 0.2)D + 3.26 \quad (10)$$

$$N = 18, R^2 = 0.590$$

the goodness of fit. Both π^2 and D enter this equation above the 0.50 significance level.

Once again π^2 was slightly more significant than π. If only π and D are included in the regression equation, the fit is almost as good (eq 11).

$$pI_{50} = (0.2 \pm 0.1)\pi + (0.8 \pm 0.2)D + 3.05 \quad (11)$$

$$N = 18, R^2 = 0.566$$

The pI_{50} values predicted from eq 1–11 may be compared to the observed pI_{50} values in Table IV.

Inspection of eq 1–11 allows certain conclusions to be drawn. In nearly every case, except for the substituted glycolic acids, eq 1 and 3, a hydrophobic parameter was found to be significant. Even in these two cases, the large correlation between π and MR for these substituents implies that MR values contain a substantial hydrophobic component.[20] The data of Tables II and III, for which MR was not found to be significant, showed much smaller correlations between π and MR, 0.68 and 0.31, respectively. In all cases, the predominant hydrophobic term had a positive coefficient, while for two equations, 4 and 5, a π^2 term with a negative coefficient was also observed. This is the form to be expected for a parabolic dependence of a biological response on π which is increasingly unfavorable above some optimal π value, π_0.[21] For a series of inhibitors binding to a purified enzyme preparation, such a parabolic dependence would not be caused by transport barriers, such as intervening membranes, or nonselective binding to extraneous biological material but might be due to a limited steric bulk tolerance at the enzyme active site[22] or to the interaction of inhibitors with a hydrophobic region of limited area near the active site. If bulk steric effects contributed to eq 4 and 5, one would expect to find a significant contribution from the MR term, which is not observed. Furthermore, when the condition for entry of parameters is relaxed (not shown), MR is found to enter regression eq 4 and 5 with a positive coefficient, which is not indicative of steric repulsion. The optimal π values can be calculated for eq 4 and 5 and are found to be 4.0 and 3.6, respectively. The other relationships showed no evidence for an optimal π value. Schuman and Massey[3] had previously shown for a series of straight-chain monocarboxylic acids that their binding affinity to pig liver glycolate oxidase was correlated in a linear manner with the number of carbon atoms (n) in the alkyl residue if the logarithm of the inhibition constant, log K_i, is plotted vs. n. This linear relationship held for the largest alkyl residue investigated, $n = 6$ ($\pi = 3$). Thus, is can be concluded that the glycolate oxidase inhibitor binding site is able to accommodate a wide variety of hydrophobic carboxylic acids

easily with an optimum π value greater than or equal to the average of the above two optimum values, 3.8.

In three cases, the ortho-substituted phenoxyacetic acids (eq 6) and the glyoxylic acids (eq 9 and 10), the π^2 term was found to be more significant than the π term. We feel that this is artifactual, however, for in all these cases the correlation between π and π^2 was greater than 0.97. This large correlation means that the two parameters are nearly equivalent, and in a small set of data the π^2 term might by chance be found to be more significant.

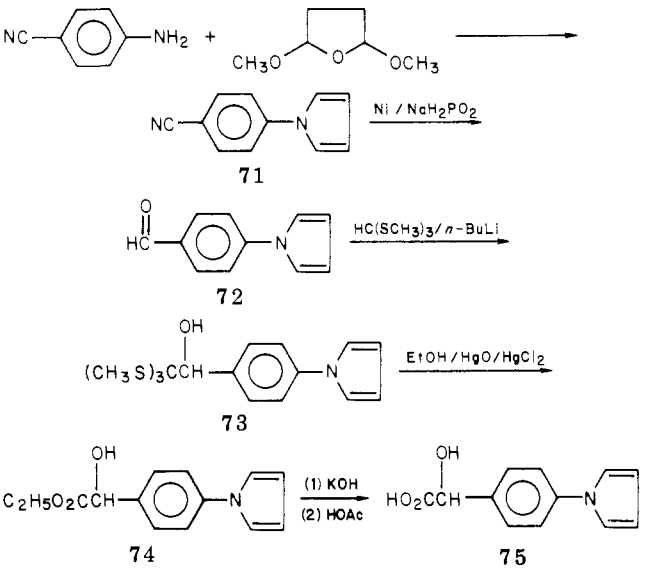
Only for one case, the meta-substituted phenoxyacetic acids, was an electronic parameter found to be significant above the 0.50 level (eq 8). Inhibitors have been postulated to interact with two positively charged groups at the active site of glycolate oxidase,[3] so electron-donating substituents capable of increasing the negative charge at the carboxylate group and the keto, hydroxyl, or ether oxygen next to the carboxylate group would be expected to increase the strength of binding to the active site. Since electron-donating substituents have negative values for σ[23] and $\mathcal{F}$,[24] one would expect to find negative coefficients for these parameters, as is true for σ_m in eq 8. Inductive effects should be much more evident for the ortho and para substituents, however, so it is possible that the significance of σ_m is a coincidence resulting from the small number of compounds included in eq 8. π and σ_m are not significantly correlated for this set of data, having a correlation coefficient of 0.45. The marginal importance of σ_m, together with the lack of significance of σ_p, $\mathcal{F}$ and $\mathcal{R}$, indicates that these standard electronic parameters are at best of only limited importance in describing the inhibition of GAO.

Electronic parameters were not included for the substituted glyoxylic acids of Table III because this set of compounds was too heterogeneous and did not include a large enough subset for which the parameters could apply (such as mono- or disubstituted phenylglyoxylic acids). It is possible, however, that the dummy parameter introduced for the subset of six compounds with heteroatoms near the glyoxylic acid carbonyl could represent an electronic effect. This could be either an inductive effect coupling to these carbonyls or a direct through-space interaction with the enzyme binding site. Introduction of a similar parameter for the substituted glycolic acids of Table I was not possible because of an insufficient number with heteroatoms near the point of attachment of the substituent. The phenoxyacetic acids of Table II were used together with a dummy parameter for all ortho heteroatom-containing phenoxyacetic acids. Thus, compounds **33–39** were assigned a dummy parameter value of 1.0, while all other compounds received a value of 0.0. Multiple regression analysis, however, revealed that the dummy parameter was not significant above the 0.50 level.

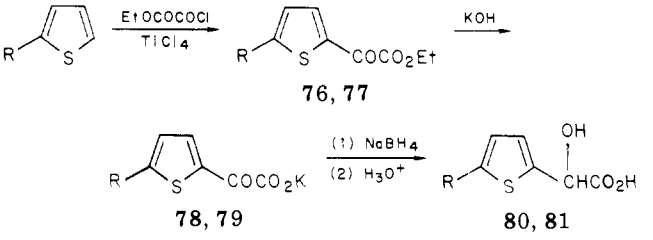
The modest values of the correlation coefficients associated with eq 1–11 indicate that additional factors must still be considered for a complete understanding of the binding of inhibitors to glycolate oxidase. The results of this study, however, emphasize the importance of the hydrophobic parameter for these three related classes of compounds. While specific electronic effects might emerge as additional compounds in these classes are evaluated, the hydrophobic component is likely to remain the most important.

Chemical Synthesis. All of the compounds in Tables I–III were obtained from the Merck Sample Collection. Of these, 28 compounds are available from commercial sources, 36 compounds have been described in the literature (references are indicated in footnotes to the tables), and the remaining 6 compounds are novel and their syntheses are shown in Schemes I–III and described in detail under the Experimental Section.

Scheme I. Synthesis of 71–75

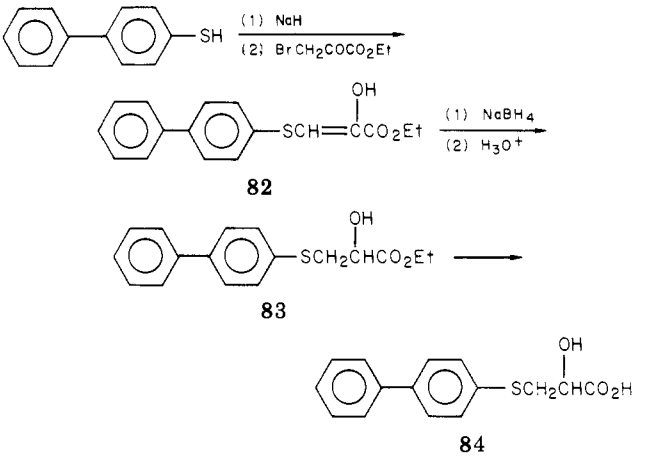


Scheme II. Syntheses of 76–81



76, 78, 80, R = CH_2-Ph; 77, 79, 81, R = *p*-Cl-Ph-

Scheme III. Syntheses of 82–84



Experimental Section

Materials. Sodium glycolate was obtained from J. T. Baker and was used without further purification. Sodium 2,6-dichlorophenol-indophenol (NaDCIP) was obtained from Nutritional Biochemical Corp. and Sigma Chemical Co. Purity was checked by absorbance spectrophotometry. All inorganic salts and buffers were of reagent grade. The pig liver GAO was purified according to the general procedure of Schuman and Massey[2] and had an average specific activity of 5 based on the glycolate–DCIP assay of Schuman and Massey.[2] NMR spectra were obtained on a Varian EM 360 spectrometer.

Assay Procedure. The enzyme activity was measured by following the rate of reduction of NaDCIP by sodium glycolate in the presence of enzyme. This reaction was followed spectrophotometrically at 600 nm. All assays were conducted at 25

°C in a 0.10 M phosphate buffer, pH 7.0, containing 3 mM EDTA. Initial substrate concentrations were 5×10^{-5} M NaDCIP and 2×10^{-4} M sodium glycolate. Reactions were initiated by the addition of enzyme. Initial rates during the period from 1 to 3 min after the addition of enzyme were recorded on a Beckman Acta M-VI spectrophotometer. One control was run simultaneously with three test reactions, and all initial rates were adjusted to a common control rate.

Synthesis. 4-(1-Pyrrolyl)benzonitrile (71). A mixture of 4-aminobenzonitrile (50.0 g, 0.423 mol), 2,5-dimethoxytetrahydrofuran (58.8 g, 0.445 mol), and 4Å molecular sieves (184 g) in toluene (560 mL) was heated under reflux for 32 h. The solvent was evaporated. The residue was washed with petroleum ether and then recrystallized from diisopropyl ether to yield 42.5 g (60%) of **71**, mp 104–106 °C. This material was used without further purification for the synthesis of **72**. Anal. ($C_{11}H_8N_2$) C, H, N.

4-(1-Pyrrolyl)benzaldehyde (72). Moist Raney nickel (5 g) was added to a stirred mixture of **72** (7.5 g, 0.0446 mol) and NaH_2PO_2 (15.0 g, 0.17 mol) in H_2O (54 mL), HOAc (54 mL), and pyridine (108.5 mL).[54] Stirring was continued at 40–45 °C for 1.5 h. The catalyst was separated by filtration and washed with H_2O followed by EtOAc. The filtrate was diluted with H_2O and extracted with EtOAc. The extract was concentrated under reduced pressure and the concentrate diluted with H_2O to obtain 6.5 g (85%) of crystalline **72**, mp 92–95 °C. This material was used without further purification for the synthesis of **73**. Anal. ($C_{11}H_9NO$) C, H, N.

Trimethyl 2-Hydroxy-2-[4-(1-pyrrolyl)phenyl]trithioorthoacetate (73). A solution of *n*-butyllithium in hexane (9.2 mL, 2.3 M) was added dropwise to a stirred solution of trimethyl orthothioformate (3.24 g, 0.021 mol) in dry THF (25 mL) at −78 °C. After stirring at −78 °C for 0.75 h, a solution of **72** (3.42 g, 0.02 mol) in THF (25 mL) was added dropwise. Stirring at −78 °C was continued for 2 h. Acetic acid (1.2 mL) was added, and the reaction mixture was diluted with H_2O (200 mL) and extracted with CH_2Cl_2 (2 × 35 mL). The combined extracts were washed with H_2O, dried over Na_2SO_4, and filtered, and the filtrate was evaporated. The residue was recrystallized from *i*-PrOH to yield 4.5 g (69%) of **73**, mp 101–103 °C. Anal. ($C_{15}H_{19}NOS_3$) C, H, N.

Ethyl 4-(1-Pyrrolyl)phenylglycolate (74). A mixture of **73** (3.25 g, 0.01 mol), HgO (6.50 g, 0.03 mol), and $HgCl_2$ (8.15 g, 0.03 mol) in EtOH (250 mL) was stirred under N_2 and heated under reflux for 24 h. The reaction mixture was filtered with CH_2Cl_2 washing. The filtrate was concentrated under reduced pressure and the residue was dissolved in CH_2Cl_2 (300 mL). This solution was washed with H_2O (2 × 50 mL), 4 M aqueous NH_4Cl (2 × 50 mL), and brine (2 × 20 mL). The solution was dried over $MgSO_4$ and filtered, and the filtrate was evaporated. The residue (2.6 g) was recrystallized from *i*-PrOH to yield 1.64 g (67%) of **74**: mp 126.5–128.5 °C; ^1H NMR ($CDCl_3$) δ 1.26 (t, 3 H, CH_3, J = 7 Hz), 3.55 (s, 1 H, OH, exchanged by D_2O), 4.25 (q, 2 H, OCH_2–, J = 7 Hz), 5.18 (s, 1 H, methine H), 6.30 (q, 2 H, pyrrole, J = 2 Hz), 7.02 (q, 2 H, pyrrole, J = 2 Hz), 7.40 (s, 4 H, aromatic). This material was used directly for the synthesis of **75**. Anal. ($C_{14}H_{15}NO_3$) C, H, N.

4-(1-Pyrrolyl)phenylglycolic Acid (75). A mixture of **74** (0.49 g, 0.002 mol), EtOH (10 mL), dioxane (1.2 mL), and 2 M aqueous KOH (1.5 mL) was stirred at 27 °C for 3 h and then evaporated to dryness. The residue was dissolved in H_2O. The solution was acidified with HOAc and extracted with EtOAc (3 × 15 mL). The combined extracts were washed with H_2O, dried over $MgSO_4$, and filtered, and the filtrate was evaporated. The residue was recrystallized from H_2O to give 0.19 g (44%) of **75**: mp 168–168.5 °C; ^1H NMR (Me_2SO-d_6) δ 5.19 (s, 1 H, methine), 6.35 (t, 1 H, pyrrole, J = 2 Hz), 7.38 (t, 1 H, pyrrole, J = 2 Hz), 7.58 (s, 4 H, aromatic). Anal. ($C_{12}H_{11}NO_3$) C, H, N.

Ethyl 5-Benzylthien-2-ylglyoxylate (76). To a stirred mixture of 2-benzylthiophene[55] (17.4 g, 0.1 mol) and EtOCOCOCl (13.6 g, 0.1 mol) in C_6H_6 (200 mL) cooled in an ice bath was added dropwise $TiCl_4$ (18.9 g, 0.1 mol) in C_6H_6 (50 mL). The mixture was stirred for 3 h at 27 °C and then thoroughly mixed with ice–H_2O (50 mL). The reaction mixture was extracted with CH_2Cl_2. The extract was evaporated and the residue was chromatographed on silica gel with CCl_4–Cl_3CCH_3 (65:35) elution to obtain **76**, which was further purified by distillation [bp 115 °C (0.4 Torr)] to give 4.6 g (16.7%) of **76**. Anal. ($C_{15}H_{14}O_3S$) S.

5-Benzylthien-2-ylglyoxylic Acid (Potassium Salt) (78). A mixture of **76** (1.37 g, 0.005 mol), KOH (0.66 g, 0.01 mol), dioxane (3.3 mL), EtOH (33 mL), and H_2O (6.6 mL) was stirred for 3 h at 27 °C. The reaction mixture was concentrated and chilled to induce crystallization of 0.9 g (64%) of **78**: ^1H NMR (D_2O) δ 4.07 (s, 2 H, CH_2), 6.92 (d, 1 H, thiophene, J = 4 Hz), 7.23 (s, 5 H, aromatic), 7.74 (d, 1 H, thiophene, J = 4 Hz). Anal. ($C_{13}H_9KO_3S$) C, H, S.

5-Benzylthien-2-ylglycolic Acid (80). To a solution of **78** (0.71 g, 0.0025 mol) in CH_3OH (10 mL) and H_2O (2 mL) was added $NaBH_4$ (0.1 g) with stirring. After 3 h, the reaction mixture was diluted with H_2O (20 mL), the pH was adjusted to 1–2 with 6 N aqueous HCl, and the solution was extracted with CH_2Cl_2. The organic solution was dried over Na_2SO_4 and filtered, and the filtrate was evaporated. Recrystallization of the crystalline residue from CCl_4–Cl_3CCH_3 (2:1) gave 0.5 g (82%) of **80**: mp 81–82 °C; ^1H NMR ($CDCl_3$) δ 4.06 (s, 2 H, CH_2), 5.32 (s, 1 H, methine), 6.63 (d, 1 H, thiophene, J = 4 Hz), 6.89 (d, 1 H, thiophene, J = 4 Hz), 7.11 (s, 5 H, aromatic). Anal. ($C_{13}H_{12}O_3S$) C, H, S.

Ethyl 5-(4-Chlorophenyl)thien-2-ylglyoxylate (77). This compound was prepared from 2-(4-chlorophenyl)thiophene (3.89 g, 0.02 mol), EtOCOCOCl (2.73 g, 0.02 mol), and $TiCl_4$ (3.79 g, 0.02 mol) in benzene (30 mL) as described for **76**. The product crystallized from CH_2Cl_2 to give 5.43 g (92%) of **77**: ^1H NMR ($CDCl_3$) δ 1.39 (t, 3 H, CH_3, J = 6.5 Hz), 4.41 (q, 2 H, CH_2, J = 6.5 Hz), 7.30 (d, 1 H, thiophene, J = 4 Hz), 7.1–7.7 (m, 4 H, aromatic), 8.07 (d, 1 H, thiophene, J = 4 Hz). This material was used without further purification for the synthesis of **79**.

5-(5-Chlorophenyl)thien-2-ylglyoxylic Acid (Potassium Salt) (79). A solution of **77** (4.15 g, 0.014 mol) in EtOH (70.5 mL), dioxane (8.5 mL), and 2 M KOH (10.6 mL) was stirred for 18 h. The potassium salt was collected by filtration of the resulting suspension, washed with EtOH, and recrystallized from hot H_2O to give 3.2 g (75%) of **79**: mp 339–343 °C; ^1H NMR (CF_3COOD) δ 7.36 (d, 2 H, aromatic, J = 8.5 Hz), 7.50 (d, 1 H, thiophene, J = 4 Hz), 7.66 (d, 2 H, aromatic, J = 8.5 Hz), 8.45 (d, 1 H, thiophene, J = 4 Hz). Anal. ($C_{12}H_6ClKO_3S$) C, H, S.

5-(4-Chlorophenyl)thien-2-ylglycolic Acid (81). This compound was prepared from **79** (2.71 g, 0.009 mol) using $NaBH_4$ (0.34 g, 0.009 mol) in CH_3OH (45 mL) and H_2O (10 mL) as described for **80**. The product precipitated from the acidified aqueous solution and was collected by filtration. Recrystallization from CH_3CN gave 1.78 g (74%) of **81**: mp 158–160 °C; ^1H NMR (Me_2SO-d_6) δ 5.31 (s, 1 H, methine), 7.05 (d, 1 H, thiophene, J = 4 Hz), 7.34 (d, 1 H, thiophene, J = 4 Hz), 7.38 (d, 2 H, aromatic, J = 9 Hz), 7.60 (d, 2 H, aromatic, J = 9 Hz). Anal. ($C_{12}H_9ClO_3S$) C, H, S.

Ethyl 3-(4-Biphenylylthio)-2-oxopropionate (82). To a solution of 4-biphenylylthiol (2.79 g, 0.015 mol) in dry THF (30 mL) and $HCON(CH_3)_2$ (6 mL) was added portionwise NaH (0.375 g, 0.0156 mol) with stirring under N_2. The resulting solution was added dropwise over 45 min to a solution of ethyl bromopyruvate (3.0 g, 0.0154 mol) in dry THF (30 mL) with stirring under N_2 and cooling to −7 to −10 °C. Stirring was continued for 1 h at 25 °C followed by quenching in ice–H_2O (250 mL). The reaction mixture was extracted with CH_2Cl_2 (3 × 50 mL). The combined extracts were washed with H_2O (3 × 50 mL), dried over Na_2SO_4, and filtered, and the filtrate was evaporated. The residue was recrystallized from CCl_4 to give 1.56 g (35%) of **82**, mp 154.5–156 °C. Anal. ($C_{17}H_{16}O_3S$) C, H, S.

Ethyl 3-(4-Biphenylylthio)lactate (83). To a stirred suspension of **82** (1.44 g, 0.0048 mol) in *i*-PrOH (24 mL) was added $NaBH_4$ (0.183 g, 0.0048 mol). After 2 h, H_2O (75 mL) and 2 N HCl (0.5 mL) were added. The mixture was extracted with CH_2Cl_2 (2 × 150 mL). The combined extracts were dried over Na_2SO_4 and filtered, and the filtrate was evaporated. The residue was chromatographed on silica gel (120 g) using Cl_3CCH_3 as eluent to obtain 0.35 g (24%) of **83**: mp 101.5–102.5 °C (EtOH); ^1H NMR (THF-d_8) δ 1.28 (t, 3 H, CH_3, J = 7 Hz), 3.13 (d, 1 H, 1 H of SCH_2, J = 6 Hz), 3.30 (d, 1 H, 1 H of SCH_2, J = 4 Hz), 4.12 (q, 2 H, OCH_2, J = 7 Hz), ~4.28 (m, 1 H, methine), 4.81 (d, 1 exchangeable H, OH, J = 6 Hz), 7.25–7.7 (m, 4 H, aromatic), 7.55 (s, 5 H, aromatic). Anal. ($C_{17}H_{18}O_3S$) C, H, S.

3-(4-Biphenylylthio)-2-hydroxypropionic Acid (84). A mixture of **83** (0.093 g, 0.31 mmol), 1 N NaOH (0.5 mL), H_2O (2

mL), and THF (1 mL) was stirred overnight at 25 °C. The mixture was acidified with 1 N HCl (0.5 mL) and extracted with CH_2Cl_2 (3 × 5 mL). The combined extracts were dried over Na_2SO_4 and filtered, and the filtrate was evaporated. The residue was recrystallized from CH_3CN to yield 0.065 g (77%) of **84**, mp 175.5–176 °C. Anal. ($C_{15}H_{14}O_3S$) C, H, S.

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Quantitative Structure–Activity Relationship of 5-(X-Benzyl)-2,4-diaminopyrimidines Inhibiting Bovine Liver Dihydrofolate Reductase

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The inhibitory effect for a set of 23 5-(X-benzyl)-2,4-diaminopyrimidines acting on bovine liver dihydrofolate reductase (DHFR) has led to the following quantitative structure–activity relationship (QSAR): $\log 1/C = 0.62\pi_3 + 0.33\sum\sigma + 4.99$, where $r = 0.931$ and $s = 0.146$. C in this expression is the molar concentration of inhibitor producing 50% inhibition, π_3 is the hydrophobic parameter for substituents on the 3 position of the phenyl moiety, and $\sum\sigma$ is the sum of the Hammett σ constants for the 3, 4, and 5 substituents of the phenyl ring.

The selective inhibition of dihydrofolate reductase (DHFR) continues to be one of the most promising leads for the medicinal chemist seeking means for controlling bacterial and parasitic diseases as well as cancer. One of the most interesting features of this enzyme is the great variation in inhibition by nonclassical inhibitors that one finds with enzyme from different sources. Burchall[1] has shown the variability of the inhibitory power of tri-

0022-2623/79/1822-0614$01.00/0