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Structure-Activity Relationships of Monoamine Oxidase Inhibitors†

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The effect of aromatic substituents on the MAO inhibitory activity of various sets of inhibitors such as aralkylhydrazine derivatives, pargylines, phenylcyclopropylamines, β-carbolines, and α-methyltryptamines is analyzed by means of free-energy-related substituent parameters and regression analysis. In each set of substituted derivatives, the variation in the *in vitro* and/or *in vivo* activity in terms of pI_{50} and $-\log ED_{50}$ can be correlated by a linear combination of hydrophobic, electronic, and steric substituent constants, π, σ, and E_S. By comparison of correlations expressed in the form of an equation, it is indicated that the substituent effects on MAO inhibition are essentially the same in various classes of inhibitors against enzyme from the same origin. Similar physicochemical mechanisms of inhibition against various test systems are also suggested. The role of the aromatic moiety required for potent inhibitory activity seems to be one of interaction as an electron acceptor with the electron-rich site of the enzyme surface.

Since the discovery that the antidepressant activity of iproniazide is due to its high *in vivo* monoamine oxidase (MAO) inhibition, compounds having similar activity have been extensively studied and various classes of inhibitors have been discovered. Among these are compounds (I–X which are classified as (a) hyrazine derivatives or (b) nonhydrazine amino compounds on a structural basis or (a) irreversible long-lasting or (b) reversible short-acting inhibitors on a basis of action pattern[1] (Chart I).

There have been considerable efforts to understand the mechanism of MAO inhibition based upon structure–activity studies. A common structural feature of various classes of inhibitors and substrates is that they have an amino group. Thus, the amino group is considered to play an essential role in orientation and complex formation at the catalytic site of the enzyme.[2] The aryl moieties in various classes of inhibitors are necessary for potent MAO inhibition.[3] Zirkle and associates examined the anti-MAO activity of phenylcyclopropylamine derivatives and β-carbolines. While they proposed specific steric and electronic roles for the cyclopropane ring on the enzyme surface, their conclusion concerning a possible role for the aromatic moiety of inhibitors is that it binds to a complementary flat surface(s) of the enzyme through van der Waals or hydrophobic forces so that the complex formation becomes facilitated.[4] Belleau and Moran proposed that nucleophilicity of the amino nitrogen and polarizability of the α–β carbon–carbon bonds play a dominant role in the inhibitor–enzyme interaction unless stereochemistry of the inhibitor molecule is prohibitive. They thought that the aromatic moiety of various classes of inhibitors interacts not with catalytic but with accessory nonspecific sites on the enzyme surface so that rigid structural requirements do not apply.[2] Despite the fact that ultimate mechanisms may be different in various series of inhibitors, the factors involved in binding with the enzyme do not seem to differ greatly from each other.[3] However, there has been no successful attempt to define common structural requirements for activity of many types of compounds. In this work, we analyze the effect of aromatic substituents on *in vitro* as well as *in vivo* activities in order to rationalize the roles of the aromatic moiety of various classes of inhibitors and to gain insight into general structure–activity relationships of MAO inhibitors.

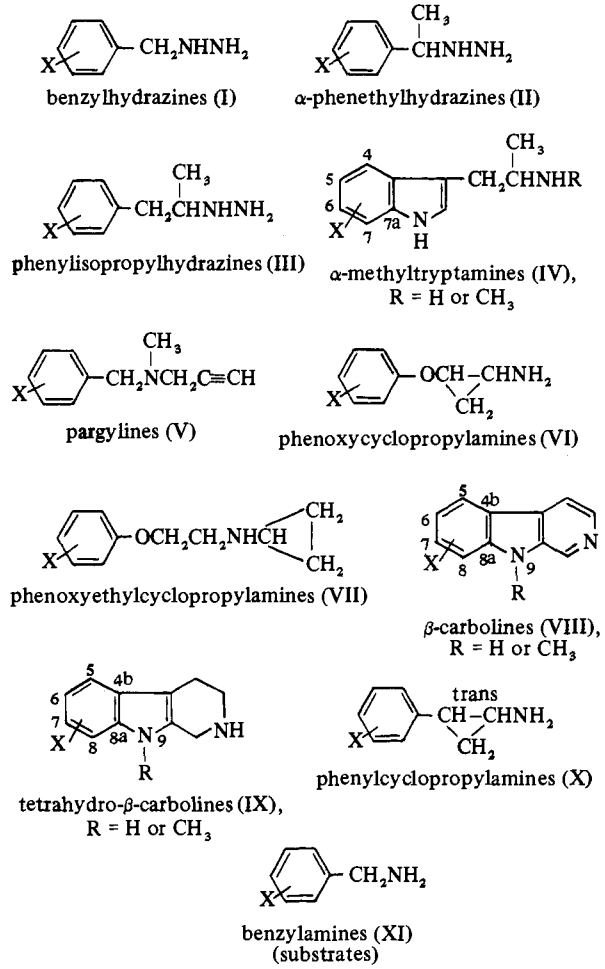
Method

The working hypothesis which we use for strucutre–activity studies is that the variation in a certain biological response of a series of substituted analogs can be analyzed in terms of the variation in hydrophobic, electronic, and steric effects of substituents. With the use of free-energy-related parameters, this situation is formulated as eq 1.[5,6] In this equation, C is the molar concentration required to exhibit

$$\log 1/C = a\pi + \rho\sigma + \delta E_S + c \qquad (1)$$

a standard biological response such as I_{50} concentration for *in vitro* activity and ED_{50} for *in vivo* activity. It is assumed that the *in vivo* concentration required for 50% effective pharmacological response is proportional to ED_{50}. π, σ, and E_S are substituent constants for hydrophobic, electronic, and steric effects, respectively, a, ρ, and δ are susceptibility

Chart I



†Studies on Structure-Activity Relationships. 6.

constants, and c is the intercept. From simultaneous equations, the number of which is equal to that of compounds in a set, the "best" values of a, ρ, δ, and c are determined by means of the method of least squares. In this way, the effects of substituent on the activity can be separated and analyzed quantitatively.

In this work, the π values are those of substituted phenoxyacetic acid series unless otherwise noted.[7,8] For condensed ring compounds, IV, VIII, and IX, π_{ortho}, π_{meta}, and π_{para} values are used for substituents at positions with respect to -NH- or $-NCH_3-$. For the electronic effect of a substituent relative to its ortho position, σ_{para} values are used as an approximation. The σ values are those from a recent tabulation by Tute.[9] E_s values for heteroatom substituents are derived from their van der Waals radius, r_v, by eq 2 according to Kutter and Hansch.[10] The calculations

$$E_s = -1.839r_v + 3.484 \quad (2)$$

were carried out by a FACOM 230/60 computer of the Data Processing Center of this University.

Table I. *In Vitro* Activity of Benzylhydrazines against Guinea Pig Mitochondrial MAO

Substituents	π	σ_2	E_s[3]	pI_{50} Obsd[a]	pI_{50} Calcd[b]	$\lvert\Delta\rvert$
H	0.00	0.00	1.24	6.5	6.47	0.03
4-OMe	−0.04	0.12	1.24	6.7	6.69	0.01
4-Cl	0.70	0.37	1.24	6.6	6.70	0.10
2-Cl	0.76	0.37	1.24	6.7	6.66	0.04
3,4-Cl_2	1.46	0.60	0.27	6.2	6.16	0.04
3,4-$(OMe)_2$	0.08	−0.15	0.69	5.9	5.90	0.00
2,3-$(CH)_4$	1.24[c]	0.04	0.23[d]	5.3	5.34	0.04
4-*i*-Pr	1.40	−0.08	1.24	5.6	5.58	0.02

[a]Taken from A. L. Green, *Biochem. J.*, 84, 217 (1962). From the original set of data, the 4-Me derivative is omitted. [b]Calculated by eq 3. [c]π value for 3,4-$(CH)_4$. [d]The value for half-thickness of phenyl.[10]

Table II. *In Vitro* Activity of α-Methyltryptamines against Guinea Pig Mitochondrial MAO

Substituents	$\pi_{4,6}$	σ_{7a}	E_s[5]	N-Me	pI_{50} Obsd[a]	pI_{50} Calcd[b]	$\lvert\Delta\rvert$
H	0.00	0.00	1.24	0	4.52	4.48	0.04
6-OMe	0.12	0.12	1.24	0	4.12	4.50	0.38
6-Me	0.51	−0.07	1.24	0	4.00	3.84	0.16
4-Me	0.51	−0.07	1.24	0	4.00	3.84	0.16
5-OMe	0.00	−0.27	0.69	0	3.42	3.55	0.13
5-Cl	0.00	0.23	0.27	0	3.67	3.73	0.06
7-Me	0.00	−0.17	1.24	0	4.60	4.27	0.33
5,7-Cl_2	0.00	0.45	0.27	0	4.30	4.00	0.30
N-Me	0.00	0.00	1.24	1	4.63	4.48	0.15
N-Me,6-OMe	0.12	0.12	1.24	1	4.30	4.50	0.20
N-Me,6-Cl	0.76	0.37	1.24	1	4.00	4.12	0.12
N,6-Me_2	0.51	−0.07	1.24	1	3.67	3.84	0.17
N,4-Me_2	0.51	−0.07	1.24	1	4.00	3.84	0.16
N-Me,5-OMe	0.00	−0.27	0.69	1	3.37	3.55	0.18
N-Me,5-Cl	0.00	0.23	0.27	1	3.67	3.73	0.06

[a]Taken from A. W. Lessin, R. F. Long, and M. W. Parks, *Brit. J. Pharmacol. Chemother.*, 29, 70 (1967). The I_{50} values are in terms of moles per liter calculated from the original activity data relative to that of harmaline. The 6-Cl derivative is omitted from the original set of data for the correlation. [b]Calculated by eq 4.

Results

Among numerous sets of activity data in the literature those given in Tables I-IX for compounds I-VI and VIII-X are selected, where the variety of substituents seems to be rich enough for analysis. For these activity values, equations shown in Table X are derived. n is the number of compounds, s is the standard deviation, and r is the correlation coefficient. The values in parentheses are the 95% confidence intervals. Equations 7 and 11 which were derived for phenoxyethylcyclopropylamines[10] and benzylamines (substrates)[11] by Hansch and coworkers are also included for comparison.

The parameter σ_i refers to the electronic effect of substituents relative to the ith position and E_s^i to the steric effect of a substituent at the ith position of the ring system. E_s^{ij} is the sum of E_s values for substituents at ith and jth positions and π is the sum of values for substituents on the aromatic ring except for eq 4, 8, and 9 where π_{ij} means that the substituents at positions except for the ith and jth are not considered. The N-Me is a dummy variable which takes a value of 0 or 1 according to the absence or presence of an N-methyl group and the value, b, describes the effect of N-methylation on the activity.

The level of significance of equations in Table X is examined by F tests. The correlation as a whole and any one of the terms in each equation, except for eq 14a, are justified at better than the 0.90 level of significance in terms of $F_{k-1,n-k}$ and $F_{1,n-k}$ values, respectively, where k is the number of terms in the equation. The addition of a π^2 term does not improve the correlation of these equations.

Table III. Anti-MAO Activity of Pargyline Derivatives *in Vitro* and *in Vivo*

Substituents	π	σ_1	σ_2	E_s[3]	E_s[4]	pI_{50} Obsd[b]	pI_{50} Calcd[c]	$\lvert\Delta\rvert$	−Log min D[a] Obsd[d]	−Log min D[a] Calcd[e]	$\lvert\Delta\rvert$
H	0.00	0.00	0.00	1.24	1.24	6.05	6.49	0.44	4.20	4.07	0.13
2-Cl	0.76	0.23	0.37	1.24	1.24	7.30	7.23	0.07	4.58	4.38	0.20
2-Br	0.84	0.23	0.39	1.24	1.24	7.19	7.29	0.10	4.19	4.40	0.21
2,4-Cl_2	1.46	0.46	0.74	1.24	0.27	7.12	7.20	0.08	4.18	4.19	0.01
2-Me	0.84	−0.17	−0.07	1.24	1.24	6.77	6.74	0.03	3.84	4.01	0.17
4-*i*-Pr	1.40	−0.15	−0.07	1.24	−0.47	5.52	5.65	0.13	3.30	3.13	0.17
4-Ph	1.89	−0.01	0.06	1.24	0.23	6.70	6.53	0.17	3.37	3.60	0.23
3,4,5-$(OMe)_3$	0.20	−0.03	−0.42	0.69	0.69	6.77	6.51	0.26	3.39	3.44	0.05
4-NMe_2	0.18[f]	−0.44[g]	−0.15[g]	1.24	0.63	5.46	5.65	0.19	3.60	3.63	0.03
2,3-$(CH)_4$	1.24[h]	0.04	0.04	0.23[i]	1.24	6.30	5.92	0.38	4.32	4.11	0.21
2-OMe	−0.33	−0.27	0.12	1.24	1.24	7.05	7.02	0.03			

[a]Minimum dose to produce maximal response upon administration of DOPA. [b]From L. R. Swett, W. B. Martin, J. D. Taylor, G. H. Everett, A. A. Wykes, and Y. C. Glasish, *Ann. N. Y. Acad. Sci.*, 107, 891 (1963). The 2-OH derivative is deleted for the analysis of *in vitro* activity from the original set of data. [c]Calculated by eq 5. [d]In terms of dose in mol/kg, mouse, ip, calcd from the original data; see reference in footnote *b*. The 2-OH, 2-OMe, and 3-NO_2 derivatives are not included for correlation. [e]Calculated by eq 13. [f]π value for substituted benzene. [g]σ° (σ normal) value from R. W. Taft, Jr., *J. Phys. Chem.*, 64, 1805 (1960). [h]The value for 3,4-$(CH)_4$. [i]From the half-thickness of phenyl.[10]

As an example, the procedure is illustrated for the set of *in vitro* activity of pargyline derivatives. From the data in Table III we have derived the following equations.

	n	s	r	
$pI_{50} = 0.230\pi + 6.389$	11	0.664	0.246	(16)
$= 1.260\sigma_1 + 6.579$	11	0.598	0.489	(17)
$= 1.584\sigma_2 + 6.421$	11	0.446	0.760	(18)
$= 0.109E_s^3 + 6.447$	11	0.684	0.056	(19)
$= 0.552E_s^4 + 6.125$	11	0.595	0.497	(20)
$= -0.024\pi + 1.603\sigma_2 + 6.438$	11	0.472	0.760	(21)
$= 0.235\pi + 0.143E_s^3 + 6.228$	11	0.702	0.257	(22)
$= 0.680\pi + 0.989E_s^4 + 5.251$	11	0.447	0.788	(23)
$= 1.726\sigma_2 - 0.414E_s^3 + 6.863$	11	0.450	0.786	(24)
$= 1.527\sigma_2 + 0.503E_s^4 + 6.024$	11	0.340	0.884	(25)
$= 0.389\pi + 1.192\sigma_2 + 0.764E_s^4 + 5.547$	11	0.271	0.937	(5)
$= 0.566\pi + 0.463\sigma_1 + 0.908E_s^4 + 5.409$	11	0.464	0.802	(26)

Comparison of single parameter eq 16–20 shows that the most meaningful substituent parameter is σ_2, the electronic effect relative to an ortho position with respect to the side chain. Neither the steric effect nor the hydrophobic effect of 3 and 4 substituents is well related alone to the activity. The correlations obtained for two-parameter eq 21-25 indicate that the steric effect of the 4 substituents is more likely to participate in the activity than that of the 3 substituents. With the use of three parameters, π, σ_2, and E_s^4 together, eq 5 is derived, the correlation of which is justified at better than 0.995 level of significance ($F_{3,7} = 16.80$, $F_{3,7,0.005} = 10.88$). Additions of π, σ_2, and E_s^4 terms over eq 25, 23, and 21, respectively, are also significant at better than 0.90 level (for σ_2 term, $F_{1,7} = 14.74$; for π term, $F_{1,7} = 5.54$; for E_s^4 term, $F_{1,7} = 17.23$; $F_{1,7,0.10} = 3.59$, $F_{1,7,0.05} = 5.59$, $F_{1,7,0.01} = 12.24$). If we use σ_1 instead of σ_2 values with π and E_s^4, the correlation is much poorer as shown in eq 26. Thus, eq 5 is included in Table X.

Unfortunately, the pK_a values are not available for most of the inhibitors. However, we could assume that, being strong to moderate bases,[12-14] they have the value at least higher than the physiological pH values so as to exist mostly as the cationic form under the physiological conditions. Thus, the correlations in Table X can be regarded as being those for activities in terms of concentration of conjugate cations as the first approximation.

Discussions

Equations in Table X are arranged so that the various series of inhibitors are classified according to the enzyme sources and test systems against which the activities are determined. It is easily recognized that the aromatic substituent effects are surprisingly similar to each other when compared within *in vitro* activities against enzymes prepared from the same source. Equations 3 and 4 indicate that the aromatic substituents of benzylhydrazines and α-methyltryptamines exert similar effects hydrophobically, electronically, and sterically on guinea pig liver mitochondrial enzyme. For activities against rat liver mitochondrial enzyme, the hydrophobic effect of substituents is not as significant as electronic and steric effects in pargylines and phenoxycyclopropylamines as shown in eq 5 and 6. These equations are very similar to the result obtained by Kutter and Hansch

Table IV. Anti-MAO Activity of Phenoxycyclopropylamines *in Vitro* and *in Vivo*

Substituents	π	σ_1	E_s^4	pI_{50} Obsd[a]	pI_{50} Calcd	$\lvert\Delta\rvert$	Log $1/ED_{50}$ Obsd[a,b]	Log $1/ED_{50}$ Calcd	$\lvert\Delta\rvert$
H	0.00	0.00	1.24	6.73[c]	6.89	0.16	4.82[c]	4.77	0.05
4-NMe_2	0.18[d]	−0.44[e]	0.63	5.11	5.34	0.23	4.12	4.18	0.06
4-OMe	−0.04	−0.27	0.69	6.00	5.72	0.28	4.25	4.24	0.01
4-F	0.15	0.06	0.78	6.40	6.43	0.03	4.74	4.32	0.42
4-Cl	0.70	0.23	0.27	6.10	6.10	0.00	3.66	3.83	0.17
2-Me	0.84	−0.17	1.24	6.73	6.59	0.14	4.51	4.77	0.26

[a]From J. Finkelstein, E. Chiang, and J. Lee, *J. Med. Chem.*, 8, 432 (1965). The compounds are mixtures of trans and cis isomers in a ratio of *ca.* 3:1. The 4-Me derivative is omitted from the correlations. [b]ED_{50} values are in terms of mol/kg, mouse ip. [c]Calculated from the activity data of pure trans and cis isomers assuming that the activity is additive. [d]π value for substituted benzene. [e]σ° value from R. W. Taft, Jr., *J. Phys. Chem.*, 64, 1805 (1960).

Table V. *In Vitro* Activity of β-Carbolines against Beef Liver Mitochondrial MAO

Substituents	$\pi_{6,8}$	σ_{4b}	$E_s^{6,8}$	N-CH_3	pI_{50} Obsd[a]	pI_{50} Calcd[b]	$\lvert\Delta\rvert$
H	0.00	0.00	2.48	0	4.54	4.59	0.05
6-OMe	−0.04	0.12	1.93	0	4.37	4.25	0.12
6-Me	0.60	−0.07	1.24	0	3.92	3.99	0.07
6-Cl	0.70	0.37	1.51	0	4.62	4.56	0.06
6-NH_2	−1.44[c]	−0.16	1.87	0	3.20	3.18	0.02
8-OMe	−0.33	0.12	1.93	0	3.92	4.08	0.16
8-Me	0.84	−0.07	1.24	0	4.14	4.13	0.01
8-NH_2	−1.44[c]	−0.16	1.87	0	3.24	3.18	0.06
N-CH_3	0.00	0.00	2.48	1	5.00	4.95	0.05
N-CH_3,6-OMe	−0.04	0.12	1.93	1	4.55	4.61	0.06
N,6-Me_2	0.60	−0.07	1.24	1	4.15	4.35	0.20
N,8-Me_2	0.84	−0.07	1.24	1	4.70	4.49	0.21

[a]Taken from B. T. Ho, Ko-Chin Li, K. E. Walker, L. W. Tansey, P. M. Kralik, and W. M. McIsaac, *J. Pharm. Sci.*, 59, 1445 (1970). [b]Calculated by eq 8. [c]The value for substituted phenol.

Table VI. *In Vitro* Activity of H_4-β-Carbolines against Beef Liver Mitochondrial MAO

Substituents	$\pi_{6,8}$	σ_4b	E_S[6]	N-CH_3	pI_{50} Obsd[a]	pI_{50} Calcd[b]	|Δ|
H	0.00	0.00	1.24	0	3.47	3.49	0.02
6-OMe	−0.04	0.12	0.69	0	2.88	3.07	0.19
6-Me	0.60	−0.07	0.00	0	3.17	2.90	0.27
6-F	0.15	0.34	0.78	0	3.28	3.23	0.05
6-Cl	0.70	0.37	0.27	0	3.38	3.15	0.23
6-Br	1.19	0.39	0.08	0	3.34	3.27	0.07
8-Me	0.84	−0.07	1.24	0	3.42	3.93	0.51
8-Cl	0.76	0.37	1.24	0	4.00	3.89	0.11
N-Me	0.00	0.00	1.24	1	5.00	4.52	0.48
N-Me,6-OMe	−0.04	0.12	0.69	1	4.00	4.10	0.10
N,6-Me_2	0.60	−0.07	0.00	1	4.00	3.93	0.07
N-Me,6-F	0.15	0.34	0.78	1	3.92	4.26	0.34
N-Me,6-Cl	0.70	0.37	0.27	1	3.74	4.18	0.44
N,8-Me_2	0.84	−0.07	1.24	1	4.80	4.96	0.16
N-Me,8-Cl	0.76	0.37	1.24	1	5.42	4.92	0.50

[a]Taken from B. T. Ho, W. M. McIsaac, and L. W. Tansey, *J. Pharm. Sci.*, 58, 998 (1969). [b]Calculated by eq 9.

Table VII. *In Vivo* Activity of α-Phenethylhydrazines against Mouse

Substituents	π	σ_2	E_S[4]	Log 1/ED_{50} Obsd[a]	Log 1/ED_{50} Calcd[b]	|Δ|
H	0.00	0.00	1.24	4.43	4.50	0.07
4-F	0.15	0.34	0.78	4.31	4.16	0.15
4-Cl	0.70	0.37	0.27	4.05	4.02	0.03
4-OEt	0.46	0.10	0.69	3.95	4.27	0.31
4-Ph	1.89	0.06	0.23	4.62	4.70	0.08
4-OPh	1.25	0.25	0.69	4.95	4.74	0.21
4-OMe	0.04	0.12	0.69	4.04	3.96	0.08

[a]The ED_{50} values are in terms of mol/kg, mouse, ip, calculated from F. E. Anderson, D. Kaminsky, B. Dubnik, S. R. Klutchko, W. A. Cetenko, J. Gylys, and J. A. Hart, *J. Med. Pharm. Chem.*, 5, 221 (1962), where the activity data are expressed in mg/kg. [b]Calculated by eq 11.

Table VIII. *In Vivo* Activity of Phenylisopropylhydrazines against Mouse

Substituents	π	σ_2	E_S[4]	Log A[a] Obsd[b]	Log A[a] Calcd[c]	|Δ|
H	0.00	0.00	1.24	1.60	1.80	0.20
4-OMe	−0.04	0.12	0.69	0.98	0.91	0.07
3,4-$(OMe)_2$	0.08	−0.15	0.69	0.75	0.83	0.08
3,4,5-$(OMe)_3$	0.20	−0.42	0.69	0.51	0.75	0.24
3,4-(CH_2O_2)	−0.42	−0.16	0.69	1.42	1.17	0.25
3-Cl	0.76	0.23	1.24	1.39	1.29	0.10
2-Me	0.84	−0.07	1.24	1.34	1.24	0.10

[a]A is the activity relative to that of iproniazide = 1 from J. H. Biel, A. E. Drukker, T. F. Mitchell, E. P. Sprengeler, P. A. Nuhfer, A. C. Conway, and A. Horita, *J. Amer. Chem. Soc.*, 81, 2805 (1959). The values are corrected by the relative molecular weight. [b]The 4-*i*-Pr derivative is omitted in the analysis. [c]Calculated by eq 12.

for phenoxyethylcyclopropylamines shown in eq 7.[10] For activities against beef liver mitochondrial enzyme, eq 8 and 9 show that the substituents exhibit similar hydrophobic and steric effects on β-carbolines and tetrahydro-β-carbolines but less significant electronic effect, in accord with eq 10 obtained by Hansch and associates for substrate specificity of benzylamines with beef liver mitochondria.[11] The above results strongly suggest that the role of the aromatic moiety in enzyme–inhibitor complex formation is essentially the same even though the side chain structure varies considerably and regardless of whether the action pattern is reversible or irreversible and competitive or noncompetitive as far as the inhibiton of enzyme from the same source is concerned.

Table IX. *In Vivo* Activity of *trans*-Phenylcyclopropylamines against Rat

Substituents	π	σ_2	E_S[3]	Log 1/ED_{50} Obsd[a]	Log 1/ED_{50} Calcd[b]	|Δ|
H	0.00	0.00	1.24	5.96	5.80	0.16
4-Cl	0.70	0.37	1.24	5.77	5.97	0.20
4-CF_3	1.07[c]	0.43	1.24	5.96	5.80	0.16
4-Me	0.60	0.07	1.24	5.52	5.48	0.04
4-OMe	−0.04	0.12	1.24	5.92	6.05	0.13
3,4-Cl_2	1.46	0.60	0.27	5.36	5.34	0.02
3-Cl	0.76	0.23	0.27	5.19	5.18	0.01
3-CF_3	1.07	0.54	−1.16	4.80	4.80	0.00
3,4-$(OMe)_2$	0.08	−0.15	0.69	4.96	5.19	0.23
3,4-(CH_2O_2)	−0.42	−0.16	0.69	5.72	5.54	0.18

[a]Taken from ref 4. The ortho-substituted derivatives are not included in the analysis. [b]Calculated by eq 15. [c]The value of the meta substituent.

The fact that some "a" values in Table X have a negative sign means that the increase in hydrophobicity of substituents does not necessarily enhance the MAO inhibitory action. It has been generally observed that, for nonspecific binding with protein of various sets of congeners, the slope, a, with increasing log P or π (1-octanol–H_2O) value is around 0.6 ± 0.1.[6] Thus, eq 8–10 would indicate a nonspecific hydrophobic interaction of the aromatic moiety of inhibitors such as β-carbolines and tetrahydro-β-carbolines as well as of substrates such as benzylamines with a complementary fraction on the beef liver mitochondrial enzyme. Since the substrate activity of benzylamines was determined with the intact beef liver mitochondria, the movement of drugs across the mitochondrial membrane seems insensitive to the variation of drug structure.

Equations 3 and 4 mean that the increase in hydrophobicity diminishes the activity against the guinea pig liver mitochondrial enzyme. In eq 4 for α-methyltryptamines, the hydrophobicity parameter is the $\pi_{4,6}$ value which takes care of only 4 and 6 substituents. When the hydrophobicity is considered at the other positions, no acceptable correlation is obtained. Thus, the hydrophobic nature of 5 and 7 substituents does not play a role in the inhibition. Such a hydrophobic effect, specific to the substituent positions, would suggest a specific mode of interaction between inhibitors and enzyme. The complementary surface of the guinea pig liver enzyme would be rather hydrophilic and the hydrophilic region may not spread uniformly. The activity was determined against intact guinea pig liver mi-

Table X. Structure-Activity Correlations of MAO Inhibitors

$$\text{Log } 1/C = a\pi + \rho\sigma + \delta E_S + b(\text{N-Me}) + c$$

Compd ser no.	Test system[a]	Log 1/C	a	ρ	δ	b	c	n	s	r	Eq
				In Vitro Activity							
I	Guinea pig liver mitoch., tyramine	pI_{50}	−0.545 (±0.125)	$1.638\sigma_2$ (±0.271)	0.516 E_S^3 (±0.161)		5.832 (±0.209)	8	0.062	0.996	3
IV	Guinea pig liver mitoch., 5-OH-tryptamine	pI_{50}	$-1.085\pi_{4,6}$ (±0.620)	$1.251\sigma_{7a}$ (±0.714)	1.071 E_S^5 (±0.438)		3.152 (±0.400)	15	0.231	0.862	4
V	Rat liver mitoch. MAO, serotonine	pI_{50}	0.389 (±0.391)	$1.192\sigma_2$ (±0.734)	0.764 E_S^4 (±0.435)		5.547 (±0.588)	11	0.271	0.937	5
VI	Rat liver mitoch. MAO, tyramine	pI_{50}		$1.803\sigma_1$ (±1.461)	1.246 E_S^4 (±0.941)		5.349 (±0.811)	6	0.244	0.950	6
VII	Rat liver mitoch. MAO, kynuramine	pI_{50}	0.180 (±0.180)	$1.752\sigma_1$ (±0.400)	0.766 $E_S^{3,5}$ (±0.150)		3.996 (±0.300)	15	0.203	0.976	7
VIII	Beef liver mitoch. MAO, tryptamine	pI_{50}	$0.590\pi_{6,8}$ (±0.191)	$0.720\sigma_{4b}$ (±0.814)	0.731 $E_S^{6,8}$ (±0.290)	0.361 (±0.230)	2.777 (±0.503)	12	0.144	0.979	8
IX	Beef liver mitoch. MAO, tryptamine	pI_{50}	$0.525\pi_{6,8}$ (±0.515)		0.730 E_S^6 (±0.414)	1.030 (±0.392)	2.586 (±0.511)	15	0.341	0.909	9
XI	Beef liver mitoch., rate of oxidation	Log k[b]	0.623 log P		0.683 E_S^4		0.554	13	0.293	0.872	10
				In Vivo Activity							
II	Mouse, ip, reserpine challenge	$-\text{Log ED}_{50}$	0.606 (±0.464)		0.933 E_S^4 (±0.980)		3.343 (±0.898)	7	0.214	0.876	11
III	Mouse, ip, reserpine challenge	Log A[c]	−0.675 (±0.761)		1.671 E_S^4 (±1.168)		−0.268 (±1.008)	7	0.220	0.894	12
V	Mouse, ip, DOPA potentiation	−Log min D		$0.840\sigma_2$ (±0.464)	0.517 E_S^4 (±0.255)		3.432 (±0.242)	10	0.193	0.928	13
VI	Rat, ip, DOPA potentiation	$-\text{Log ED}_{50}$			0.966 E_S^4 (±0.866)		3.569 (±0.761)	6	0.262	0.840	14
			−0.489 (±1.306)	$0.411\sigma_1$ (±2.041)	0.986 E_S^4 (±1.253)		3.743 (±1.176)	6	0.241	0.936	14[a]
X	Rat, po, tryptamine potentiation	$-\text{Log ED}_{50}$	−0.746 (±0.614)	$1.858\sigma_2$ (±1.370)	0.502 E_S^3 (±0.211)		5.180 (±0.276)	10	0.179	0.939	15

[a]For *in vitro* activity, enzyme preparation and substrate. For *in vivo* activity, test object, route of administration, and test method. [b]k is the rate of oxidation in terms of μmol/(hr g) of mitochondria. [c]A is the activity relative to iproniazide as 1.0.

tochondria. However, the mitochondrial membrane does not seem to be a barrier to drug transport. If an accumulation in the mitochondrial membrane is the cause of negative response to increasing hydrophobicity, it should be nonspecific for substituent positions. Similar substituent effects could be expected for benzylhydrazines if additional members of variously substituted analogs are included in eq 3. Somewhat related stereospecific hydrophobic substituent effects have been found in the case of emulsin-catalyzed hydrolysis of substituted phenyl glucosides[15] and antibacterial activity of kojic acid analogs.[16]

It should be noted that, for *in vivo* activity, the increase in hydrophobicity of inhibitors beyond optimum exhibits a twofold effect on the activity. One is to decrease the effective concentration around the site of action. The more hydrophobic, the more inhibitor molecules would be trapped by lipid phases and sometimes would be metabolized to inactive compounds on the way from the site of administration to the target enzyme. Thus, the activity should be lowered. This effect is much more pronounced in whole animals than in the above cases, which only involve crossing of mitochondrial membranes. Secondly, there is a direct effect on the enzyme. The increase in hydrophobicity would generally favor the complex formation. The negative signs of slope, a, of equations for *in vivo* activities, except for eq 11, would mean that the first effect outweighs the second effect.

Comparing eq 13 for *in vivo* activity with eq 5 for *in vitro* activity of pargylines, we see that the substituent effects are very similar to each other, with the exception of the hydrophobic effect. Equation 13 would indicate that contributions from the two above-mentioned effects compensate each other for *in vivo* activity. Thus, it is anticipated that very similar mechanisms on the enzyme–inhibitor complex formation exist between rat liver enzyme inhibition and *in vivo* inhibition with the use of mouse. For phenoxycyclopropylamine derivatives, a similar situation might exist between *in vitro* and *in vivo* activities as described by eq 6 and 14a. However, the correlation in eq 14a is not justified at better than 0.90 level of significance ($F_{3,2} = 4.72$; $F_{3,2,0.25} = 3.15$; $F_{3,2,0.10} = 9.16$). If variously substituted analogs are added to this series, this situation would be improved substantially.

The value, b, represents the effect of the N-methyl group on the activity. It should be taken as the sum of contributions from hydrophobic, electronic, and steric effects of the group. Comparison of the b value in eq 8 with that in eq 9 shows that the effect of introduction of N-methyl into tetrahydro-β-carbolines is about five ($\approx \log^{-1} 1.030/\log^{-1} 0.361$) times larger than introduction into β-carbolines. Since the ring system of tetrahydro-β-carboline is nonplanar, the interaction with the complementary fraction of the enzyme would be weaker than the totally coplanar β-carbolines. The introduction of a methyl group into the 9 position of tetrahydro compounds, then, would exert a stronger effect on the complex formation than the β-carbolines. Equation 4 for α-methyltryptamines does not indicate a significant contribution of the side chain N-methyl to the activity. The addition of a dummy variable into eq 4 does not improve the correlation. The activity variation in this series is only dependent on the aromatic ring substitution. The hydrophobic, steric, and electronic effects of N-methylation on the activity may act so as to compensate one another.

In equations where the electronic effect plays a significant role in Table X, the ρ values are always positive. Their magnitude does not relate to the distance between aromatic moiety and side chain nitrogen function and also does not vary greatly, the average for eight series of inhibitors being 1.4 ± 0.3. In some series, the variation in the electronic effect of substituents does not seem to contribute significantly to the variation in the inhibitory action. However, even in these series, a positive ρ value could be expected if additional compounds having substituents of varying degrees of electronic effect are included in the correlation as suggested by eq 14a. The same trends were also observed for *in vivo* activity of α-phenethyl- and phenylisopropylhydrazines and *in vitro* activity of tetrahydro-β-carbolines. Thus, it could be considered that the electronic effect of substituents is common in various series of inhibitors and not directed to the side chain nitrogen function; the aromatic ring itself interacts with the enzyme by means of a similar mechanism.

The above discussion on the electronic effect has been based upon equations where activities are correlated in terms of conjugate cations. Recently, a possibility that the conjugate cation is responsible for the inhibition has been suggested by Ho.[17] However, if the binding of the free amino group to an electrophilic center of the enzyme is essential for substrates and inhibitors as suggested by Belleau and Moran,[2] the activity in terms of neutral bases should be analyzed and compared with each other. Although we are unable to estimate accurate activity values for neutral bases because of the lack of available K_A values, they should be expressable by eq 27 where $[H^+]$ is the physiological hydrogen ion concentration, ρ_A is the reaction constant for dissociation equilibrium, σ_A is relative to the position of attachment to the side chain, c' is a constant being equal to $\log [H^+] - \log K_A^0$, and K_A^0 is the value for the unsubstituted standard compound in each series. We have assumed as $pK_A \gg pH$, so that the second term of right side of eq 27 can be ignored to give eq 28.

$$\log \frac{1}{C} + \log \frac{K_A + [H^+]}{K_A} = \log \frac{1}{C} + \log \frac{K_A + [H^+]}{[H^+]} - \rho_A\sigma_A + c' \quad (27)$$

$$\approx \log \frac{1}{C} - \rho_A\sigma_A + c' \quad (28)$$

The magnitude of ρ_A values varies according to the distance between aromatic system and site of dissocation.[18] If a common electronic mechanism is also considered in terms of neutral bases, the effect of substituents may be separated into $\rho\sigma_i$ and $\rho_A\sigma_A$ terms. The $\rho\sigma_i$ term would indicate the common effect on the aromatic ring and $\rho_A\sigma_A$ term would be a measure of nucleophilicity of the neutral nitrogen atom. Hence, as far as the effect on the aromatic ring itself is concerned, the discussion remains the same as the case of cationic species, since the $\rho\sigma_i$ term is common in both cases. The fact that the sign of $\rho_A\sigma_A$ term is negative might mean that the electron-donating aromatic substituent effect favors a nucleophilic attack of neutral nitrogen on an electrophilic center of catalytic site.

The positive sign of ρ values means that the greater the electron withdrawal of the aromatic substituents, the stronger the interaction with the enzyme. In this interaction, the aromatic moiety of inhibitors would act as an electron acceptor. The possibility of a charge-transfer type interaction with MAO surface has been suggested for substrates and inhibitors. MAO is a flavoprotein and the catalytic site of the enzyme is considered to act as an electron acceptor.[2] Thus, the complementary site with which aro-

matic moiety interacts might be located outside the catalytic region of the enzyme as postulated by Belleau and Moran.[2]

The most striking feature common to the activity of various sets of inhibitors is the high dependence on the steric effect. The E_s term is highly significant in equations for all sets of compounds in Table X. By definition, the positive sign of δ values means that the smaller the van der Waals radius of the substituent, the stronger the activity. The E_s constants used for unsymmetrical groups such as nitro and phenyl are derived from their half thickness instead of their width and those for OMe, OPh, and $N(Me)_2$ are from the van der Waals radius of just the oxygen and nitrogen.[10] Thus, the steric effect would be due to a kind of fit to a surface rather than engulfment of the substituent by enzyme, as Kutter and Hansch have proposed for phenoxyethylcyclopropylamines.[10] The effect seems important at meta positions (with respect to the side chain) in some sets and at para positions in other sets. There could be site(s) which inhibit(s) the approach of the aromatic moiety by interfering the complementary substituents. The location of inhibitors on the enzyme would be mainly determined by interactions between side chain structure bearing the nitrogen function and catalytic site of enzyme so that some substituents on the aromatic moiety would not be able to escape from the sterically unfavorable complementary surface. The situation is quite similar to Zeller's models for eutopic and dystopic complexes between benzylamines as substrates and beef liver mitochondrial MAO.[19]

For pargyline derivatives and some others, the activity of ortho-substituted analogs is well correlated; however, this is not true of phenylcyclopropylamine derivatives. Since the ortho substituents generally enhance the inhibitory activity of pargyline while the reverse seems to be the case for phenylcyclopropylamine, the ortho-substituent effect may be dependent on the side chain structure.

The activity data of a few compounds in some sets of inhibitors are not included in the analyses, since preliminary calculations show only very poor correlations for these data points. These compounds are footnoted in corresponding tables. Other factors might be operative for some of these. For instance, in the 2-hydroxyl derivative of pargyline, the hydroxyl group can be hydrogen bonded with the lone pair electrons at the tertiary amino nitrogen so that the interaction of the amino group with the enzyme should be modified. For phenoxycyclopropylamines, the activity of the 4-methyl derivative is considerably higher than that predicted by eq 6. It has been demonstrated recently that the steric effect of the methyl group appears considerably lower than the one expected from its conventionally used group van der Waals radius.[20] Thus, for some poorly correlated derivatives, the substituent parameters may not represent the true situation.

At this point, it is interesting to note that quite similar structure-activity relationships are observed for other enzymatic reactions. With the data in Table XI, eq 29 is de-

		n	s	r	
$\log 1/K_m$ =	$0.300\pi_3(\pm0.236) + 0.593\sigma_1(\pm0.321) + 0.212E_s^3(\pm0.181) + 2.339(\pm0.164)$	14	0.161	0.860	(29)
$\log k$ =	$0.722\pi(\pm0.337) - 1.842\sigma^-(\pm0.484) + 0.557E_s^4(\pm0.404) - 1.892(\pm0.379)$	12	0.288	0.959	(30)

Table XI. Complex Formation of D-Phenylglycines with D-Amino Acid Oxidase

Substituents	π_3	σ_1	E_s^3	Log $1/K_m$[a] Obsd[b]	Calcd[c]	$\lvert\Delta\rvert$
4-NMe_2	0.00	−0.83	1.24	2.07	2.11	0.04
4-NH_2	0.00	−0.66	1.24	2.19	2.21	0.02
4-OH	0.00	−0.37	1.24	2.32	2.38	0.06
4-OMe	0.00	−0.27	1.24	2.22	2.44	0.22
4-Me	0.00	−0.17	1.24	2.52	2.50	0.02
3-NH_2	−1.15[d]	−0.16	0.63	2.22	2.03	0.19
3-Me	0.51	−0.07	0.00	2.64	2.45	0.19
H	0.00	0.00	1.24	2.83	2.60	0.23
4-F	0.00	0.06	1.24	2.65	2.64	0.01
3-OH	−0.49	0.12	0.69	2.18	2.41	0.23
4-Cl	0.00	0.23	1.24	2.67	2.74	0.07
3-F	0.13	0.34	0.78	2.85	2.74	0.11
3-Cl	0.76	0.37	0.27	2.85	2.84	0.01
3-NO_2	0.11	0.71	−1.28[e]	2.41	2.52	0.11

[a] Michaelis constant with hog kidney enzyme at pH 8.5. [b] K_m values are recalculated in terms of the zwitterion form of substrates. The value for the 3-OMe derivative is not included. [c] Calculated by eq 29. [d] From substituted benzyl alcohol. [e] The value for the half-width of the group.[10]

Table XII. Rate of Reaction between Thiamine and Anilines with Thiaminase

Substituents	π	σ^-	E_s^4	Log k[a] Obsd	Calcd[b]	$\lvert\Delta\rvert$
4-CN	0.14	0.90	0.73	−3.43	−3.04	0.39
4-$COCH_3$	−0.11	0.87	0.23	−3.34	−3.45	0.11
3-NO_2	0.47	0.71	1.24	−1.86	−2.17	0.31
3-Br	1.17	0.39	1.24	−1.26	−1.07	0.19
3-Cl	1.04	0.37	1.24	−1.15	−1.13	0.02
4-Br	1.13	0.23	0.08	−1.49	−1.45	0.04
4-Cl	0.93	0.23	0.27	−1.33	−1.49	0.16
H	0.00	0.00	1.24	−0.74	−1.20	0.46
3-Me	0.50	−0.07	1.24	−0.92	−0.71	0.21
4-Me	0.49	−0.17	0.00	−1.10	−1.22	0.12
4-OMe	−0.12	−0.27	0.69	−1.36	−1.10	0.26
4-OH	−0.86	−0.36	0.69	−1.53	−1.47	0.06

[a] k is the rate constant of the base transfer reaction in min^{-1} with 200 units of carp vicera thiaminase, at pH 6.4, where the substrates exist exclusively as neutral base. [b] Calculated by eq 30.

rived for the Michaelis constants, K_m, of substituted D-phenylglycines with D-phenylglycine oxidase.[21] Equation 30, from the data in Table XII, describes substituent effects on the rate of transfer of substituted anilines catalyzed by thiaminase.[22] The substrate specificities for these enzyme reactions have been analyzed only on the basis of the electronic effects of the substituents; the chemical mechanism of action depending upon the electron-withdrawing ability of substituents has been postulated to be biphasic by the original authors.[21,22] The present analyses clearly indicate the importance not only of electronic but also of steric and hydrophobic effects.

It is quite unfortunate that, for most of the MAO inhibitors, physicochemical parameters such as dissociation constants and partition coefficients are not available. For dissociable molecules such as the compounds discussed here, information on the dissociation equilibrium is particularly important in order to separate the effect of dissociation from the total electronic effect of substituents and also to estimate the intrinsic activity of the standard compound in each series. To determine the molecular form responsible for the inhibitory activity is also required before a definite conclusion as to the structure-activity relationships of MAO inhibitors can be made. Nevertheless, the present work re-

veal that the aromatic substituent effects on MAO inhibition are essentially the same in various sets of inhibitors against enzyme from the same origin. It is also suggested that the inhibition against enzymes from various origins involves similar physicochemical mechanism. Although the electronic effect of substituents does not seem to contribute significantly in some series of inhibitors, the most probable role of the aromatic moiety would be to interact as an electron acceptor with the noncatalytic electron-rich site of the enzyme surface. These findings would not have been uncovered unless the structure-activity relationships were described in the form of equations so that the various features among them could be compared quantitatively. The present work also supports the use of E_S parameters in explaining intermolecular steric interactions in biomedical systems developed by Hansch and Kutter.[10,23] It is hoped that the role of side chain structure in the mechanism of MAO inhibitors could be delineated in physicochemical as well as quantitative terms so that a comprehensive structure-activity picture for MAO inhibitors can be drawn.

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A New Nonsteroidal Antiinflammatory Agent. 2-Substituted 5- or 6-Benzothiazoleacetic Acids and Their Derivatives

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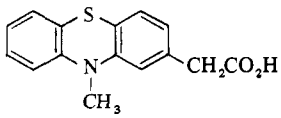
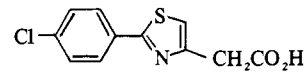
Synthesis of 34 2-substituted benzothiazole compounds with an acetic acid function at the 5 or 6 position was carried out and their antiinflammatory activity was investigated. It was found that the presence of an acetic acid function was important for antiinflammatory activity and also that 2-substituted 5-benzothiazoleacetic acids (6) were better than 2-substituted 6-benzothiazoleacetic acids (7) in antiinflammatory activity.

Many aromatic and heteroaromatic acetic acids have been reported[1-3] as nonsteroidal antiinflammatory agents. Among them, Messer, *et al.*,[4] recently reported on 10-methyl-2-phenothiazinylacetic acid (metiazinic acid, I) and Hepworth, *et al.*,[5] reported on 2-(4-chlorophenyl)thiazol-4-ylacetic acid (fenclozic acid, II), both of which contain nitrogen and sulfur atoms in their skeleton.

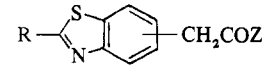


methiazinic acid (I)



fenclozic acid (II)

Little is known about the antiinflammatory activity of the benzothiazole ring system[6] and, moreover, a compound which has an acetic acid function in such a system has not yet been reported at all. Therefore, novel 2-substituted 5- or 6-benzothiazoleacetic acids (6 and 7) and their derivatives were synthesized and their antiinflammatory activities and LD_{50} values were examined.



R = phenyl, mono- or disubstituted phenyl, pyridyl, naphthyl, furyl, benzyl, phenetyl, styryl, and phenoxymethyl; Z = -OH, -OEt, $-NH_2$, or -NHOH

Chemistry. 3-Amino-4-mercaptoacetophenone (1), which was obtained by the reaction of 4-chloro-3-nitroacetophenone with sodium sulfide nonahydrate in water, was condensed with arylcarboxylic acid chlorides or aldehydes to yield the 2-substituted 5-acetylbenzothiazoles (3). 5-Acetylbenzothiazole derivatives 3 were allowed to react with sulfur and morpholine in a Willgerodt-Kindler reaction and the morpholides 5 were isolated as intermediates. These morpholides 5 were hydrolyzed with concentrated hydrochloric acid or 10% aqueous sodium hydroxide solution to yield the 2-substituted 5-benzothiazoleacetic acids (6). In the ring closure with aryl aldehydes, benzothiazoline derivatives 2 were often obtained, but these (2) were easily oxidized to a benzothiazole 3 by refluxing in the presence of