(53) H. H. Jaffé, Chem. Rev., 53, 191 (1953).
(54) R. W. Taft, Jr., in "Steric Effects in Organic Chemistry", M. S. Newman, Ed., Wiley, New York, N.Y., 1956, p 556.
(55) D. H. McDaniel and H. C. Brown, J. Org. Chem., 23, 420 (1958).
(56) E. C. Jorgensen, R. O. Muhlhauser, and R. A. Wiley, J. Med. Chem., 12, 689 (1969).
(57) L. B. Kier and J. R. Hoyland, J. Med. Chem., 13, 1182 (1970).
(58) S. H. Unger and C. Hansch, J. Med. Chem., 16, 745 (1973).

Quantitative Structure–Activity Studies on Monoamine Oxidase Inhibitors

Carl Lynn Johnson

Department of Pharmacology, Mount Sinai School of Medicine, City University of New York, New York, New York 10029.
Received June 5, 1975

Quantitative structure–activity studies were carried out on a series of *N*-isopropylaryl hydrazides which inhibits monoamine oxidase (MAO). The inhibitory potencies of these compounds on MAO were found to correlate with the electron-withdrawing capacity of the aryl ring substituents as estimated by both empirical Hammett σ constants and electronic indices from molecular orbital calculations. Based on these correlations and previously published data on other classes of MAO inhibitors, a general model for the inhibitor pharmacophore is proposed: potent MAO inhibitors should contain an electron-rich functional group in the plane of and approximately 5.3 Å from the center of an aromatic ring; electron-withdrawing groups on the aromatic ring or replacing the phenyl ring with certain types of heterocyclic rings will tend to increase the potency.

The importance of monoamine oxidase (MAO) in controlling the levels of neurotransmitters has prompted interest in learning the mechanism of substrate oxidation and the nature of the active site as well as in understanding the molecular mechanisms of MAO inhibition by a wide variety of chemical compounds, all of which exhibit a pharmacological profile including potent antidepressant activity. Some of the known MAO inhibitors[1,2] include arylalkylhydrazines, aryl hydrazides, arylpropargylamines, arylcyclopropylamines, aryloxycyclopropylamines, *N*-cyclopropylaryloxyethylamines, β-carbolines, and α-methylated arylalkylamines. A common structural feature of both substrates and these various classes of inhibitors is an amino or imino group, which is assumed to play an essential role in complex formation at the active site. The aryl portion of the MAO inhibitors is not an absolute requirement for inhibition of the enzyme, but substituents on the aryl ring or modification of the type of ring markedly affect potency.

One approach to understanding the role of the aryl portion of these compounds in MAO inhibition and to gain insight into the relationships between chemical structure and pharmacological activity is to carry out quantitative structure–activity relationships (QSAR)[3] on a series of related molecules. This approach requires, in addition to the estimate of the biological activity of the molecules, a set of parameters—either empirically or theoretically derived—which describes in numerical terms the structural characteristics of the compounds. Several QSAR studies have dealt with both substrates[4] and inhibitors[5-7] of MAO. The study of Fujita[7] is especially extensive, comparing the QSAR results for many classes of inhibitors.

One group of MAO inhibitors not previously considered in a QSAR study is the aryl hydrazides. In the present communication we report on the QSAR for a series of hydrazides and compare the results with those for other classes of MAO inhibitors in an attempt to arrive at a general picture of the inhibitory pharmacophore. A preliminary report of some of these results has appeared.[8]

Methods. Molecular orbital (MO) calculations were carried out by the CNDO/2 method[9] with the CNINDO computer program.[10] Coordinates were generated with the MBLD program[11] from crystallographic data and/or standard bond angles and bond lengths.[12] Calculations were performed on a CDC 6600 computer. Multiple regression analysis was carried out with a stepwise regression program adapted for the PROPHET[13] time-sharing system. Hammett σ constants were taken from McDaniel and Brown[14] and octanol–H_2O partition coefficients were taken from the compilations of Hansch and co-workers.[15,16]

Results and Discussion

Empirical Structure–Activity Relationships. The in vivo potencies of a series of 23 aryl hydrazides of the general structure $ArCONHNHCH(CH_3)_2$ have been published[17] and are listed in Table I. The potencies are reported as the Marsilid Index (M.I.) taking iproniazid (4-pyridyl derivative) as the standard (M.I. = 100). The Marsilid Index, designed to measure the relative degree of inhibition of brain MAO, is defined as the ratio of the increase in serotonin in rat brain produced by a substance (in amount equimolar to 100 mg of iproniazid/kg) to the increase in serotonin produced by 100 mg of iproniazid/kg.

The electronic substituent constant, σ, and the hydrophobic or lipid solubility constant, log P, are listed in Table I. Since Hammett σ constants are available only for the phenyl derivatives, the empirical QSAR studies were restricted to these ten compounds. Multiple regression analysis led to eq 1–7. The numbers in parentheses in the regression equations represent the standard errors of the regression coefficients; n is the number of compounds; r is the multiple correlation coefficient; s is the standard deviation of the regression; and F is the F test for the significance of the regression. A significant correlation ($p < 0.01$) of the Marsilid Index and σ values was obtained (eq 1). The correlation with log P was much poorer (eq 2). Including a log P term with σ did not improve the correlation (eq 3). The major deviations from the above

$$\text{M.I.} = 88.25\ (\pm 23.09)\ \sigma + 83.37 \quad (1)$$
$$n = 10;\ r = 0.804;\ s = 31.4;\ F = 14.6$$

$$\text{M.I.} = 32.80\ (\pm 13.74)\ \log P + 0.64 \quad (2)$$
$$n = 10;\ r = 0.646;\ s = 30.3;\ F = 5.7$$

$$\text{M.I.} = 70.51\ (\pm 24.41)\ \sigma + 17.13\ (\pm 11.30)\ \log P + 44.33 \quad (3)$$
$$n = 10;\ r = 0.856;\ s = 29.1;\ F = 9.6$$

Table I. Marsilid Index and Structural Parameters for *N*-Isopropyl Hydrazides

Derivative	σ[a]	Log P[b]	Q_0[c] I	Q_0[c] II	Q_1[c] I	Q_1[c] II	M.I. (obsd)[d]
2-Thienyl		1.61	1.4202	1.4028*	1.0505	1.0360	198
3-Thienyl		1.61	1.3975*	1.3962	0.9983	0.9881*	192
2-Pyridyl		0.32	1.4033*	1.3879	0.9927	0.9905*	191
2-Furyl			1.4239	1.3988*	1.0883	1.0745	156
2-Pyrazinyl			1.4049*	1.3908	1.0122	1.0096*	152
4-Chlorophenyl	0.23	2.84	1.4006*		1.0012*		134
3-Nitrophenyl	0.71	1.85	1.4007*	1.3982	1.0140*	1.0144	125
3-Chlorophenyl	0.37	2.84	1.4037*	1.4024	1.0149*	1.0150	123
3-Pyridyl		0.32	1.4072*	1.4080	1.0310	1.0308*	111
4-Pyridyl		0.32	1.3973		0.9779		100
4-Isopropylphenyl	−0.15	3.66	1.4105*	1.4104	1.0375*	1.0374	97
3-Pyrazolyl			1.4058*	1.4000	1.0399*	1.0452	84
3,4-Dimethylphenyl	−0.24	3.25	1.4081*	1.4076	1.0218*	1.0218	76
4-Methoxyphenyl	−0.27	2.11	1.4134*	1.4132	1.0574*	1.0573	63
2-Aminophenyl	−0.66	0.90	1.4106*	1.4161	1.0624	1.0539*	60
5-(4-Methyl)thiazolyl		0.44	1.4442	1.4114*	1.1109	1.0835*	38
4-(1,2,3-Triazolyl)			1.4111*	1.4110	1.0783*	1.0842	38
4-Aminophenyl	−0.66	0.90	1.4115*	1.4115	1.0470*	1.0469	15
4-Hydroxyphenyl	−0.36	1.46	1.4137*	1.4134	1.0591*	1.0590	11
3-Pyridazyl			1.3984	1.3874	0.9868	0.9880	0
2-Hydroxyphenyl	−0.36	1.46	1.4188*	1.4072	1.0728	1.0779*	0
5-(2,4-Dimethyl)pyrimidyl		−0.40	1.4181*	1.4204	1.0851*	1.0763	0
4-(3,5-Dimethyl)isoxazolyl		1.20	1.4469	1.4483	1.2524	1.2611	0

Derivative	M.I. (calcd) Eq 6	Eq 10	Eq 13
2-Thienyl		139	89[e]
3-Thienyl		189	174
2-Pyridyl		134	170
2-Furyl		177	20[e]
2-Pyrazinyl		119	135
4-Chlorophenyl	106	160	150
3-Nitrophenyl	152	159	127
3-Chlorophenyl	120	131	126
3-Pyridyl		97	97
4-Pyridyl		191[e]	193[e]
4-Isopropylphenyl	71	66	85
3-Pyrazolyl		111	81
3,4-Dimethylphenyl	62	89	114
4-Methoxyphenyl	59	38	50
2-Aminophenyl	23[e]	65	56
5-(4-Methyl)thiazolyl		57	3
4-(1,2,3-Triazolyl)		60	12
4-Aminophenyl	23	56	68
4-Hydroxyphenyl	51	35	47
3-Pyridazyl		181[e]	174[e]
2-Hydroxyphenyl	51[e]	−13	13
5-(2,4-Dimethyl)pyrimidyl		−6	0
4-(3,5-Dimethyl)isoxazolyl		−280[e]	−299[e]

[a] Hammett constants from McDaniel and Brown.[14] σ_p values were used for the two ortho derivatives. [b] Octanol-H_2O partition coefficients from the work of Hansch and co-workers.[15,16] Values for isomers (e.g., 2-pyridyl and 3-pyridyl) were estimated from the experimental value of another isomer (e.g., 4-pyridyl). [c] π-Electron densities on the carbonyl (Q_0) and the ring carbon atom to which the hydrazide group is attached (Q_1). As described in the text, two planar conformations were calculated. The columns labeled I give the electron densities for the low-energy conformation; those labeled II give the densities for the higher energy conformation. Both conformations were used in the preliminary regression analysis; the conformations that deviated most from the regression equations were then eliminated and the regression analysis was repeated to generate the equations given in the text. The asterisk (*) indicates which of the conformations was used in deriving eq 10 and 13. [d] Observed potencies expressed as the Marsilid Index from Zeller et al.[17] [e] Not included in the derivation of the regression equation.

equations were the 4-OH and 2-OH derivatives. Elimination of these two compounds yielded eq 4 and 5. Again, including the log P term did not improve the correlation.

$$\text{M.I.} = 73.05\ (\pm 15.72)\ \sigma + 93.11 \quad (4)$$
$$n = 8;\ r = 0.885;\ s = 20.6;\ F = 21.6$$

$$\text{M.I.} = 63.94\ (\pm 16.45)\ \sigma + 10.22\ (\pm 7.86)\ \log P + 68.85 \quad (5)$$
$$n = 9;\ r = 0.915;\ s = 19.5;\ F = 12.9$$

Since the substituent constants for ortho derivatives are not well defined, the analysis was repeated eliminating the 2-OH and 2-NH_2 derivatives (eq 6 and 7). In this case

$$\text{M.I.} = 94.05\ (\pm 21.94)\ \sigma + 84.85 \quad (6)$$
$$n = 8;\ r = 0.868;\ s = 25.9;\ F = 18.4$$

$$\text{M.I.} = 80.64\ (\pm 15.01)\ \sigma + 21.41\ (\pm 7.11)\ \log P + 33.61 \quad (7)$$
$$n = 8;\ r = 0.955;\ s = 16.9;\ F = 26.1$$

including the log P term produced a significant ($p < 0.05$)

improvement in the correlation. Thus, the significance of hydrophobic bonding or partitioning effects in determining potency in this series of compounds is ambiguous on the basis of these empirical correlations with the limited number of compounds that could be considered. It is unambiguous, however, that the major determinant of potency is the electronic effects of the ring substituent; electron-withdrawing groups increase potency.

Molecular Orbital Calculations. The use of empirical parameters precluded analysis of the majority of the hydrazide derivatives because substituent constants that estimate electronic effects are not available for ring systems other than benzene. CNDO–MO calculations were performed to generate a set of electronic parameters for all derivatives. In the present study, the free base form of the hydrazides was used. However, the results on the cation form, previously published,[8] are very similar to those reported here. The geometry chosen for the hydrazide side chain was based on the x-ray crystallographic structure[18] of iproniazid and a theoretical (PCILO) conformational analysis[19] of the same compound, both of which indicated that the hydrazide group was essentially coplanar with the aryl group. Since for most of the derivatives considered here there were two possible planar conformations (involving rotation of the aryl group by 180°), calculations were carried out on both conformations. The decision as to which conformation to retain for the multiple regression analysis is necessarily an arbitrary one. One could, for example, eliminate the higher energy conformation based on the CNDO total energy calculation. Alternatively, one could use averaged electronic parameters, perhaps weighted according to the energies of the two conformations. However, the energy difference between the two conformations was small in most cases: less than 5 kcal/mol for 21 of the 23 compounds and less than 1 kcal/mol for 13 of these compounds. It should also be noted that a complete conformational analysis of iproniazid by the CNDO method showed that the conformation with the hydrazide group perpendicular to the 4-pyridyl ring was a few kilocalories per mole more stable than the coplanar conformation (unpublished data). This result is in conflict with the crystallographic[18] and PCILO[19] studies of iproniazid. In view of the questionable significance of small energy differences obtained by semiempirical MO methods and the possibility that drug–solvent or drug–receptor interactions could determine the true conformer, it was decided to include both conformations in the preliminary regression analysis and then to delete the conformations that did not fit the regression equations.

In our QSAR studies we routinely examine the net atomic charges, frontier densities and virtual frontier densities at each atom, the energies of the highest occupied and lowest empty molecular orbitals, and dipole moments for possible correlations with biological activity. The appearance of, say, a charge at a particular atom in a correlation equation may, but does not necessarily, imply an involvement of this atom in a specific interaction with the receptor. The correlations described in this paper are an attempt to account for the biological data in terms of the general electron-withdrawing or -donating characteristics of different aryl rings.

Examining plots of the Marsilid Index against the various MO parameters showed that most of the data could be correlated with certain MO parameters. The "best" single-parameter correlations that were obtained involved the π-electron densities of the carbonyl oxygen (Q_0) or the ring carbon (Q_1) to which the hydrazide group was attached. When all 23 compounds of Table I were included in the analysis, statistically significant ($p < 0.01$) correlations were obtained between the Marsilid Index and either Q_0 (eq 8) or Q_1 (eq 9). These equations were

$$\text{M.I.} = -3900\ (\pm 1080)\ Q_0 + 5579 \quad (8)$$
$$n = 23;\ r = 0.619;\ s = 53.2;\ F = 13.0$$

$$\text{M.I.} = -618.4\ (\pm 218.7)\ Q_1 + 731.3 \quad (9)$$
$$n = 23;\ r = 0.525;\ s = 57.6;\ F = 8.0$$

obtained by using the particular conformation of each derivative (shown with an asterisk in Table I) which gave the best fit. An examination of the residuals from eq 8 indicated that three compounds, 4-pyridyl (iproniazid), 3-pyridazyl, and 4-(3,5-dimethyl)isoxazolyl, were poorly fit by the equation. Elimination of these three derivatives led to eq 10. The 4-pyridyl and 3-pyridazyl derivatives

$$\text{M.I.} = -9511\ (\pm 1125)\ Q_0 + 13491 \quad (10)$$
$$n = 20;\ r = 0.894;\ s = 30.0;\ F = 71.5$$

would be predicted to be among the most active derivatives (i.e., M.I. $\sim$ 200), whereas their activities were actually 100 and 0, respectively. The inactivity of the 3-pyridazyl derivative is not explained by the MO calculations. It is possible that this compound is exceptionally unstable; there appears to be no information in the literature regarding the stabilities of these hydrazide derivatives. The third derivative would be predicted to have a "negative" M.I. and, in fact, was inactive. Hence, of the original 23 derivatives, only two are badly predicted by eq 10. It should be noted, however, that another possible explanation for the inactivity of the isoxazolyl derivative is the steric effect of the two *o*-methyl groups which may prevent this compound from assuming a conformation in which the hydrazide group is coplanar with the ring. At the same time it must be pointed out that the assumption that the coplanar conformation is involved in binding to the enzyme is based solely on the x-ray study[18] and a theoretical conformational analysis[19] of iproniazid. Such studies do not rule out the possibility that the drug–enzyme interactions affect the conformations of the inhibitor.

In eq 9, three major deviants were observed, 2-thienyl, 2-furyl, and 3-pyridazyl, and elimination of these compounds gave eq 11. Again, the inactive derivative, 4-

$$\text{M.I.} = -748\ (\pm 171)\ Q_1 + 863 \quad (11)$$
$$n = 20;\ r = 0.717;\ s = 43.7;\ F = 19.1$$

(3,5-dimethyl)isoxazolyl, was predicted to have a large negative Marsilid Index and elimination of this compound led to eq 12. The major deviant from eq 12 was the

$$\text{M.I.} = -1531\ (\pm 215)\ Q_1 + 1670 \quad (12)$$
$$n = 19;\ r = 0.865;\ s = 30.8;\ F = 50.6$$

4-pyridyl derivative and elimination of this compound yielded eq 13.

$$\text{M.I.} = -1789\ (\pm 187)\ Q_1 + 1942 \quad (13)$$
$$n = 18;\ r = 0.923;\ s = 24.3;\ F = 92.1$$

In general, one might expect Q_0 and Q_1 to reflect the same electron distribution effects and hence to be internally correlated (eq 14). However, this correlation is

$$Q_0 = 0.171\ (\pm 0.016)\ Q_1 + 1.230 \quad (14)$$
$$n = 23;\ r = 0.916;\ s = 0.004;\ F = 109.4$$

somewhat biased by the outlier data point for the 4-(3,5-dimethyl)isoxazolyl derivative and elimination of this

compound leads to a marked drop in the correlation coefficient (eq 15).

$$Q_0 = 0.145\ (\pm 0.027)\ Q_1 + 1.257 \quad (15)$$
$$n = 22;\ r = 0.765;\ s = 0.004;\ F = 28.2$$

Including a second MO parameter in the correlation equations invariably resulted in a loss in the overall significance of the regression as indicated by F tests. Including a term for lipid solubility or hydrophobic bonding capacity (we were able to estimate log P for only 18 of the 23 compounds) resulted in a marked loss in significance compared to the single parameter equations (eq 16–19).

$$\text{M.I.} = -9662\ (\pm 1257)\ Q_0 + 13697 \quad (16)$$
$$n = 16;\ r = 0.899;\ s = 30.9;\ F = 59.1$$

$$\text{M.I.} = -9791\ (\pm 1358)\ Q_0 - 2.414\ (\pm 7.456)\ \log P + 13881 \quad (17)$$
$$n = 16;\ r = 0.900;\ s = 31.9;\ F = 27.7$$

$$\text{M.I.} = -1829\ (\pm 210)\ Q_1 + 1980 \quad (18)$$
$$n = 15;\ r = 0.924;\ s = 25.4;\ F = 75.8$$

$$\text{M.I.} = -1917\ (\pm 226)\ Q_1 - 6.303\ (\pm 6.098)\ \log P + 2081 \quad (19)$$
$$n = 15;\ r = 0.930;\ s = 25.3;\ F = 38.6$$

The data set used in the above equations was the same as for eq 10 and 13 except for those derivatives for which log P values were unavailable. Thus, the multiple regression analysis using MO parameters and a larger number of compounds suggested that the borderline significance of the hydrophobic term found in the empirical correlation (eq 7) was probably fortuitous.

These correlations both confirm and extend the results obtained using the empirical substituent constant, σ, and suggest that, with few exceptions, substituents or heterocyclic rings that lead to decreased electron density in the region of the hydrazide group will increase the activity of the derivative as an MAO inhibitor. We can predict with considerable confidence that phenyl derivatives with strong electron-withdrawing groups would be good candidates for synthesis and testing. It should be emphasized that for the types of heterocyclic rings included in this study it was by no means obvious a priori which would be electron-withdrawing and which electron-donating. MO calculations therefore provided an indispensable tool for predicting these electron shifts. Clearly, calculations on other heterocyclic ring systems could suggest what derivatives would be worthwhile synthesizing and testing.

A few brief comments on the physical significance of the observed correlations may be warranted. Topliss and Costello[20] have pointed out the possibilities of "chance" correlations which may arise when large numbers of independent variables are examined by multiple regression techniques. However, others have on occasion misinterpreted the results reported by Topliss and Costello. While it is true that with 30 independent variables examined and 20 observations an average correlation coefficient of about 0.9 can be expected by chance alone, this is only true when an average of 6.3 variables is included in the final regression equation.[20] When only a single parameter is included in the regression, as is the case in all the results reported here, the probability of obtaining such high correlation coefficients purely by chance is extremely small. Furthermore, the fact that biological activity correlates with electron-withdrawing capacity estimated either by Hammett σ constants or by MO parameters suggests that the MO correlation is not a chance correlation. Whether the correlation is physically meaningful in terms of drug mechanism is, of course, a completely different question. The correlation with the π-electron density on the carbonyl oxygen could imply that this atom reacts with an active site on the enzyme, but, since most classes of MAO inhibitors do not contain such a group, other explanations should be considered. It is possible that the correlation with the π-electron density on this atom is indirect, reflecting the extent of conjugation of the carbonyl group and the aromatic ring. This latter factor will be an important determinant of the conformation of the molecule: that is, the mutual orientation of the hydrazide side chain and the aromatic ring. Thus, if coplanarity of side chain and ring are important for optimum fit of inhibitor to the enzyme surface, it is clear how activity could be correlated with the π-electron density of the oxygen atom. This possibility suggests that detailed comparisons of the conformational preferences of the various aryl hydrazides using MO theory may be informative. Alternatively, if the aromatic ring of the inhibitors reacts with the enzyme surface through specific interactions (e.g., mutual polarization or charge transfer) it is possible that the electron density on the ring carbon atom C_1 is an indirect reflection of the ability of the ring to engage in such reactions. This suggests that comparisons of the molecular reactivity patterns of the various aryl hydrazides could be a fruitful approach. Methods for generating such reactivity patterns have been developed recently and are being actively pursued in this laboratory.[21]

In contrast to most of the correlation equations obtained by Fujita,[7] we found no evidence for the presence of hydrophobic effects in the hydrazide series. However, for in vivo systems such effects may be expressed in two ways: penetration to the site of action and hydrophobic bonding to the enzyme. High lipid solubility may decrease penetration into the brain and at the same time increase binding to the enzyme.[7] It is possible, therefore, that these two effects balance one another in the aryl hydrazide series. We also found no evidence for the presence of steric effects, with the possible exception of steric inhibition of coplanarity which could be important for the ortho-substituted derivatives (in particular, the pyrimidyl, isoxazolyl, and 2-hydroxyphenyl compounds which were inactive).

Comparison of the QSAR for Various MAO Inhibitor Classes. A study comparing the QSAR results for a large number of MAO inhibitor classes has recently been published.[7] Although there were some differences among the various classes, part of which could be due to differences in enzyme sources, experimental techniques, and choice of derivatives, etc., it is remarkable that many of the correlation equations showed similar regression coefficients. All showed an apparent steric inhibition effect; most showed a hydrophobic bonding effect, although in some cases the coefficient was positive and in others negative; and most showed an electronic effect. It is notable that in all cases where an electronic effect was demonstrable, the coefficient of this term was positive indicating that electron-withdrawing groups increase MAO inhibitory activity. Thus, our results obtained for the aryl hydrazides are quite comparable to the results for other classes of inhibitors, at least in terms of electronic effects.

It seems probable that the primary interaction of MAO inhibitors with the enzyme is through the side-chain amino group. It is not known whether this involves a simple electrostatic interaction of the cationic form of the amino group with an anionic group in the enzyme or a more specific interaction such as nucleophilic attack of the free base form of the amino group on an electrophilic site in

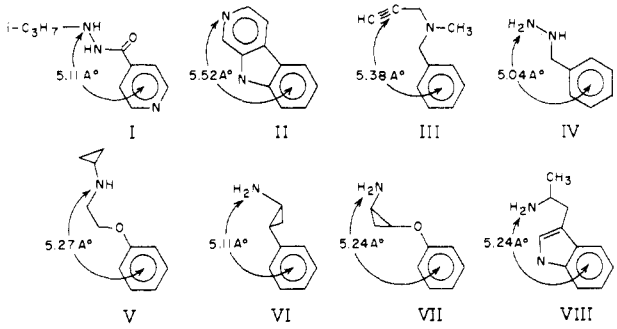


Figure 1. Comparison of the molecular structures of different classes of MAO inhibitors. The classes represented are aryl hydrazides (I), β-carbolines (II), propargylamines (III), benzylhydrazines (IV), *N*-cyclopropylphenoxyethylamines (V), *trans*-phenylcyclopropylamines (VI), *cis*-phenoxycyclopropylamines (VII), and α-methyltryptamines (VIII). In all molecules, the amino (or imino or acetylenic) group is in the plane of the aromatic ring. The distance between this group and the center of the aromatic ring, shown in the figure, was calculated using standard bond angles and bond lengths.[12] No attempt was made to optimize these angles or lengths.

the enzyme. In addition to this primary interaction, MAO inhibitors probably also interact through the aromatic ring. Based on the present study of the aryl hydrazides and the previously published results on other classes of inhibitors,[7] it appears likely that the aromatic rings of most, if not all, MAO inhibitors would react with electron-rich sites in the enzyme. If the interaction of aryl ring and enzyme involves charge-transfer reactions, then enzyme residues such as tyrosine or tryptophan would be good candidates for the electron-donor role.

In his comparative study, Fujita[7] noted that the magnitude of the electronic effects in the different classes of inhibitors, as estimated by the coefficient of the σ term in the regression equations, did not vary much and was not related to the distance between the aromatic moiety and the side-chain nitrogen function. This was interpreted as evidence that the electronic effects were directed to the aromatic ring rather than the side chain. The recent study of Martin et al.[22] suggested a parabolic dependence of inhibitory potency on the side-chain nitrogen pK_a for a series of propynylamine derivatives. The authors interpreted this correlation as implying that the electronic effects were mediated through changes in the reactivity of the side chain. One arm of the parabolic correlation would be consistent with the results of Fujita[7] and those reported here: electron-withdrawing groups increased the potency of the propynylamines as the pK_a was decreased from 9.0 to 6.2. Below 6.2, however, electron withdrawal decreased potency, presumably due to a change in the rate-limiting step of the inhibition mechanism.[22]

The remarkable similarity in the electronic effects observed for the different inhibitor classes encouraged us to look for structural features shared by all. Three-dimensional models of the various inhibitors studied by Fujita[7] as well as the aryl hydrazides were constructed in the PROPHET[13] computer using standard bond angles and bond lengths. Then, using PROPHET's ability to manipulate molecules in the same coordinate space and to rotate single bonds, we examined the structural relationships of the amino groups to the aromatic rings in the various inhibitors. We found that it was possible to orient the amino (or imino) group of all the inhibitors studied in the same relative position with respect to the aromatic ring with the single exception of the propargylamines, in which compounds the acetylenic carbon atom takes the place of the nitrogen atom (Figure 1). The distance between the nitrogen or acetylenic carbon and the center of the aromatic ring for the eight classes of inhibitors ranged from 5.04 to 5.52 Å, with an average of 5.24 ± 0.16 Å. On this basis we suggest that the most active inhibitors should contain an electron-rich functional group (amino/imino or acetylenic) in the plane of an aromatic ring and approximately 5.25 Å from the center of the ring. In accord with this suggestion is the fact that *trans*-phenoxycyclopropylamine, in which this distance is 5.9 Å, is less potent[23] than the cis isomer, in which the distance is 5.2 Å; and the fact that *cis*-phenylcyclopropylamine, in which the distance is 3.3 Å, is less potent[23] than the trans isomer, in which the distance is 5.1 Å.

Conclusions

The quantitative structure–activity studies reported here for a large number of aryl hydrazide inhibitors of monoamine oxidase suggest that the potencies of this series of compounds are largely determined by the relative degree of electron withdrawal in the aryl portion of the molecule. Comparison of these results with previously published structure–activity studies on other classes of inhibitors indicates that these electronic effects are common to most if not all MAO inhibitors. On the basis of these comparisons, we suggest as a minimal requirement for potent MAO inhibitory activity that a molecule should contain an electron-rich functional group (e.g., amino nitrogen or acetylenic carbon) in the plane of and approximately 5.25 Å from the center of an aromatic ring. Superimposed on this gross structural effect are the more subtle effects due to variations in the structure of the aromatic portion of the molecule. In particular, electron-withdrawing groups on the phenyl ring or replacing the phenyl ring with certain types of heterocyclic rings will tend to increase the potency of the inhibitor in a predictable manner.

In the present communication we have demonstrated the utility of molecular orbital calculations in quantitative SAR studies, in particular, the extension of the QSAR approach to compounds that could not be handled with the usual empirical parameters. The results obtained here may be useful in the design of more active aryl hydrazide inhibitors and, in view of the similarities in the electronic effects for many different classes of inhibitors, may have implications for the design of MAO inhibitors in general. The vast majority of structural modifications in the various inhibitor classes has been made by changing phenyl-ring substituents. Our results suggest that modification of the aryl ring itself would also be a useful approach for obtaining more active compounds.

Acknowledgment. This research was supported by Grant MH-17489 from the National Institute of Mental Health. The author gratefully extends his appreciation to Drs. H. Weinstein and J. P. Green for many helpful discussions during the preparation of this manuscript and to Dr. W. F. Raub and the Chemical/Biological Information-Handling Program, Division of Research Resources, National Institutes of Health, for providing access to the PROPHET system time-sharing computer.

References and Notes

(1) J. H. Biel, A. Horita, and A. E. Drukker, *Med. Chem., Ser. Monogr.*, 4 (1), 359 (1964).
(2) C. L. Zirkle and C. Kaiser, *Med. Chem., Ser. Monogr.*, 4 (1), 445 (1964).
(3) C. Hansch, *Int. Encycl. Pharmacol. Ther.*, 1, 75 (1973).
(4) C. Hansch, E. J. Lien, and F. Helmer, *Arch. Biochem. Biophys.*, 128, 319 (1968).
(5) R. W. Fuller, M. M. Marsh, and J. Mills, *J. Med. Chem.*, 11, 397 (1968).

(6) E. Kutter and C. Hansch, *J. Med. Chem.*, **12**, 647 (1969).
(7) T. Fujita, *J. Med. Chem.*, **16**, 923 (1973).
(8) C. L. Johnson, *Proc. Natl. Comput. Conf. Exposition*, **43**, 473 (1974).
(9) J. A. Pople and G. A. Segal, *J. Chem. Phys.*, **44**, 3289 (1966).
(10) P. A. Dobosh, Quantum Chemistry Program Exchange, Program 141, Indiana University.
(11) M. S. Gordon, Quantum Chemistry Program Exchange, Program 135, Indiana University.
(12) Kennard et al., Ed., "Molecular Structures and Dimensions", Vol. A1, International Union of Crystallography, Polycrystal Book Service, Pittsburgh, Pa., 1972.
(13) The PROPHET system is a specialized computer resource developed by the Chemical/Biological Information-Handling Program of the National Institutes of Health and oriented toward the needs of pharmacologists and medicinal chemists studying the relationship between chemical structure and biological activity. A detailed description of the system features appears in *Proc. Natl. Comput. Conf. Exposition*, **43**, 457 (1974).
(14) D. H. McDaniel and H. C. Brown, *J. Org. Chem.*, **23**, 420 (1958).
(15) C. Hansch, A. Leo, S. H. Unger, K. H. Kim, D. Nikaitani, and E. J. Lien, *J. Med. Chem.*, **16**, 1207 (1973).
(16) A. Leo, C. Hansch, and D. Elkins, *Chem. Rev.*, **71**, 525 (1971).
(17) P. Zeller, A. Pletscher, K. F. Gey, H. Gutmann, B. Hegedus, and O. Straub, *Ann. N.Y. Acad. Sci.*, **80**, 555 (1959).
(18) P. C. Chieh and G. J. Palenik, *J. Chem. Soc. A*, 576 (1971).
(19) J.-L. Coubeils, P. Courriere, and B. Pullman, *C. R. Hebd. Seances Acad. Sci., Ser. D*, **273**, 1164 (1971).
(20) J. G. Topliss and R. J. Costello, *J. Med. Chem.*, **15**, 1066 (1972).
(21) H. Weinstein, S. Maayani, S. Srebrenik, S. Cohen, and M. Sokolovsky, *Mol. Pharmacol.*, **9**, 820 (1973); S. Srebrenik, H. Weinstein, and R. Pauncz, *Chem. Phys. Lett.*, **20**, 419 (1973).
(22) Y. C. Martin, W. B. Martin, and J. D. Taylor, *J. Med. Chem.*, 18, 883 (1975).
(23) C. L. Zirkle, C. Kaiser, D. H. Tedeschi, R. F. Tedeschi, and A. J. Berger, *J. Med. Chem.*, **5**, 1265 (1963).

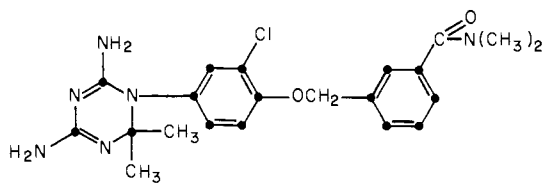
Inhibition of Dihydrofolate Reductase. Structure–Activity Correlations of Quinazolines[1]

James Y. Fukunaga, Corwin Hansch,* and Edward E. Steller

Department of Chemistry, Pomona College, Claremont, California 91711. *Received October 10, 1975*

A quantitative structure–activity relationship (QSAR) has been formulated for quinazolines causing 50% inhibition of liver dihydrofolate reductase. The QSAR for the quinazolines is compared with QSAR for triazine and pyrimidine inhibitors. The three QSAR suggest new possibilities for the design of inhibitors of mammalian dihydrofolate reductase.

Dihydrofolate reductase has become a focal point for research since the discovery that it is a key enzyme in a variety of biochemical processes. The finding that methotrexate, a potent inhibitor of dihydrofolate reductase, is effective in cancer chemotherapy has stimulated great interest in finding differential inhibitors for tumor and normal human enzyme. The pioneering work of Baker and co-workers has resulted in the development of a triazine (I) which now shows promise in clinical trials in cancer chemotherapy.



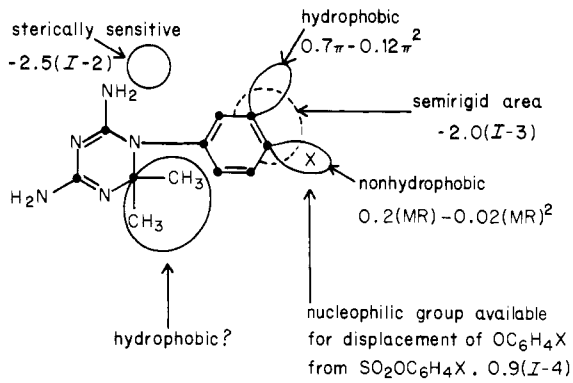
I

We have been interested in formulating QSAR from ligand interactions with dihydrofolate reductase in order to map the region around the active site. From this knowledge we would hope to be able to design more effective inhibitors.

Baker made a series of about 260 derivatives of I in which he varied substituents in the 2, 3, and 4 positions of the *N*-phenyl ring. The concentration of the triazine necessary to produce 50% inhibition of dihydrofolate reductase from tumor tissue was determined. Equation 1 was developed from this study.[2]

In eq 1, n represents the number of data points used in deriving the equation, r is the correlation coefficient, and s the standard deviation from the regression. Of the variables, π-3 refers to the hydrophobic interactions of substituents in the 3 position of the *N*-phenyl moiety,

Chart I



$$\log 1/C = 0.68(\pi\text{-}3) - 0.12(\pi\text{-}3)^2 + 0.23(\text{MR-}4) - 0.024(\text{MR-}4)^2 + 0.24(I\text{-}1) - 2.53(I\text{-}2) - 1.99(I\text{-}3) + 0.88(I\text{-}4) + 0.69(I\text{-}5) + 0.70(I\text{-}6) + 6.49 \quad (1)$$

$$n \quad r \quad s$$
$$244 \quad 0.923 \quad 0.377$$

MR-4 refers to molar refractivity of substituents in the 4 position, and I-1–I-6 are indicator variables. I-1 takes the value of 1 for data points based on enzyme from Walker tumor and zero for those from L1210 leukemia enzyme. I-2 assumes the value of 1 for substituents in the 2 position, I-3 is for rigid structures (C_6H_5 or $CONHC_6H_5$ attached in the 3 or 4 position), I-4 is given the value of 1 for congeners having the active leaving group $-SO_2OAr$, I-5 receives the value of 1 for derivatives with a flexible bridge [$-O(CH_2)_x-$] between the *N*-phenyl group and a second phenyl ring, and I-6 assumes the value of 1 for certain amide functions between rings.