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Regression Analysis of the Relationship between Physical Properties and the in Vitro Inhibition of Monoamine Oxidase by Propynylamines

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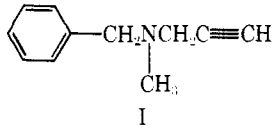
Regression analysis of the potency of inhibition of monoamine oxidase by 47 propynylamines revealed that there are three determinants of inhibitory potency: (1) the smallest substituent on the nitrogen must be methyl or hydrogen in order for any activity to be observed; (2) potency is parabolically related to pK_a—the optimum pK_a is 6.2; and (3) ortho-substituted benzylamine analogs are ten times more potent than predicted on the basis of pK_a values. The optimum pK_a cannot be explained by differences in fraction ionized but rather in terms of the multistep sequence whereby these compounds inhibit MAO. A very slight positive effect of hydrophobicity on potency was found. The potency of several analogs not included in the original analysis was predicted.

The monoamine oxidase, MAO [amine:oxygen oxidoreductase (deaminating), E.C. 1.4.3.4], inhibitor pargyline (I) is by now a well-known drug.[1] At the time of its discovery a number of analogs were prepared and tested. A preliminary discussion of the structure and activity of some of these compounds has also been reported.[2] At the time of the initial evaluation of the analogs, we also measured their pK_a's. Variations in pK_a do not in themselves explain differences in potency, and at the time of the original studies in 1960 we were not able to discern any relationship between physical properties and potency. The introduction of multiple regression analysis to the study of quantitative structure–activity relationships of drugs provided the necessary tool for an examination of our data.[3]



I

There is considerable evidence that pargyline produces its irreversible inhibition of MAO in a several-step sequence. Since pargyline is a competitive inhibitor of MAO when it is added to the enzyme simultaneously with the substrate, the first step in the inhibition is postulated to be a reversible substrate-like binding.[4] In the second step, the MAO oxidizes pargyline by abstracting a proton from one of the methylene groups.[5] Finally, the modified form of the pargyline reacts to form a covalent bond to the enzyme with the result that the enzymatic activity is lost.[4]

One of the objectives of our quantitative structure–activity analysis was to explore the possibility that the relative potency of various analogs which react in such a complex scheme would be correlated with physical properties. If such a correlation were found, would it be consistent with the proposed mode of inhibition of MAO by pargyline? Could the relative potency of the various inhibitors have been predicted by the relative binding constants and maximal velocities of substrates?

Methods. pI_{50}. The approximate pI_{50} value (negative log of the molar concentration which inhibits MAO 50%) was determined as previously described.[2] The reactions were conducted in 10-ml beakers in a Dubnoff shaker. In brief, graded concentrations of the inhibitor were preincubated 30 min at 38° in 0.1 *M* phosphate buffer, pH 7.2, with rat liver mitochondria. The mitochondria has been isolated by differential centrifugation and stored at −5°. After the preincubation, serotonin creatinine phosphate was added to produce a final concentration of 0.01 *M*. The reaction of MAO with the serotonin proceeded for 2.5 hr. By the end of this incubation period the contents of the control beakers had turned dark brown due to reactions of the aldehyde formed in the deamination of serotonin. In contrast, the contents of vessels in which the MAO was completely inhibited were the same off-white color as before the addition of substrate. For evaluation, the contents of the beakers were poured into small test tubes. The color

Table I. Values of Substituent Constants of Aromatic Substituents

Substituent	MR	S	P	π
H	1.03	0.000	0.000	0.00
2-OC_2H_5	12.47	0.844	−1.415	0.18
2-Cl	6.03	0.916	−0.125	0.76
3,4-C_4H_4	8.73[a]	0.000	0.000	0.66[a]
4-Cl	6.03	0.916	−0.125	0.70
2,3-C_4H_4	8.73[a]	−0.013[a]	0.017[a]	0.66[a]
2-OCH_3	7.87	0.634	−1.125	−0.33
3-OCH_3	7.87	0.634	−1.125	0.12
2-CH_3	5.65	0.017	−0.376	0.84
3,4-OCH_2O	4.18[a]	0.000	0.000	−0.26[a]
4-C_6H_5	25.36	0.359	−0.352	1.77
2-F	0.92	0.911	−0.406	0.01
3-Cl	6.03	0.916	−0.125	0.76
4-OCH_3	7.87	0.634	−1.125	−0.04
3-CH_3	5.65	0.017	−0.376	0.51
4-$N(CH_3)_2$	15.60	0.000	0.000	0.18
4-CH_3	5.65	0.017	−0.376	0.60
3-OH	2.85	0.482	−1.448	−0.49
4-OH	2.85	0.482	−1.448	−0.61
4-OC_6H_5	27.68	1.009	−0.995	2.10
4-$CH(CH_3)_2$	14.98	0.000	0.000	1.40

[a] At each position of substitution on the parent benzene ring.

of each tube was compared to a series of standards of pargyline (which produced known inhibition by the Warburg method), and the approximate extent of inhibition of MAO was estimated. The pI_{50} was determined from the plot of log concentration of inhibitor vs. percent inhibition. At least three concentrations which produced between 20 and 80% inhibition were run. The advantages of this method are that it is simple and it uses a physiologically relevant substrate. Since it provides only a crude estimate of potency, a wide range in pI_{50} values is necessary for quantitative structure–activity calculations, and one must be careful not to overfit the data.

pK_a Measurements. The pK_a's were measured by titration of 200 ml of a 0.0025 M solution of the hydrochloride salt with 0.10 N NaOH at 25°. The pK_a values were determined from the graph of pH vs. milliliters of NaOH as the pH at half-neutralization of the compound. Appropriate corrections for hydrolysis were applied to pH values above 9.0. The values are considered to be accurate within 0.1 pH unit.

If the compound or its free base was insoluble at the above concentration, the pK_a was determined at several concentrations of ethanol in water. The "aqueous" pK_a was then estimated by extrapolation of the plot of apparent pK_a vs. ethanol concentration to zero concentration of ethanol. The lowest concentration of ethanol was 10% (v/v) if the compound was soluble in it. The errors involved in this type of determination are much larger than aqueous titration; our estimate of the precision of the pK_a's so determined is 0.25 pH unit.

The reliability of the pK_a measurements was also assessed by considering the correlation of the observed pK values with the Hammett σ constant. Thus, the measured pK_a values of the 18 pargyline analogs substituted in the meta or para position and for which σ values were available correlated with these σ values.[6] The following equation describes the relationship.

$$pK_a = 6.66 - 0.82\,\sigma$$
$$R = 0.82,\ s = 0.18$$

Thus it is concluded that the pK_a measurements are sufficiently accurate to describe differences in fundamental properties of these compounds.

π, MR, S, and P. The values of these physical properties were taken from the references cited below and are listed in Table I. π is a measure of the relative hydrophobic effect of a substituent.[7] It is measured from the influence of that substituent on the logarithm of the octanol–water partition coefficient of a suitable parent molecule, benzene in this case. MR, the molar polarizability, is a rough measure of the size of a substituent.[6] It is calculated by additivity. S and P are constants which reflect the electronic effect of a substituent; S represents the inductive or field effect and P the resonance effect.[8] Hammett σ values are a linear combination of S and P values, which can be regarded as more fundamental properties. For the compounds for which R_2 = $CH_2C{\equiv}CH$ and R_3 = CH_3, Table II, pK_a is a function only of S and P of the ortho position.

$$pK_a = 6.70 - 0.65\ (\pm 0.46)\ S_2 - 1.06\ (\pm 0.40)\ P_2$$
$$n = 20,\ R = 0.81,\ s = 0.28$$

Regression Analyses. These calculations were performed using the Pomona College Medicinal Chemistry Project Program, HANSCH. Every possible combination of predictor variables was considered in the selection of the "best" equation. No equation is presented unless all terms are significant at the 5% level and each term represents a statistically significant (5% level) improvement over the corresponding equation minus that variable.

Initial Design of Analogs. The "lead" in this series was unsubstituted propynylamine (Table III, **53**). It was the 334th compound tested at Abbott Laboratories in the in vitro assay for a nonreversible MAO inhibitor. Propynylamine at 1×10^{-4} M inhibited serotonin oxidation by MAO approximately 30%.

It was reasoned that a propynylamine derivative which also included structural features of a substrate might increase potency. Four molecules were selected for initial synthesis (Table II): N-methyl-N-2-propynylbenzylamine (**26**, pargyline), 2-propynylbenzylamine (**35**), α-methyl-N-2-propynylbenzylamine (**41**), and N,N-di-2-propynylbenzylamine (**45**). Their pI_{50}'s were 6.0, 5.1, 4.6, and 3.8. The best inhibitor was thus more than 100 times more potent than propynylamine. Since amphetamine is a weak MAO inhibitor the next compounds prepared were the propynyl analogs of l- and d-N-methylamphetamine (Desoxyn, **28** and **36**). Their pI_{50}'s are 5.6 and 5.0, respectively.

At this point the N-benzyl-N-methyl analog (pargyline) was most potent; therefore, further synthesis concentrated on derivatives of it (Table II). The 2-ethoxy analog **1** is 30-fold more potent than **26**. Thus the incorporation of a substrate analog portion into propynylamine increased potency 100 times and further molecular modification increased potency another 30 times. In the 60 analogs prepared, an overall increase of 3000 times vs. propynylamine was accomplished.

Regression Analysis of the Relationships between Physical Properties and Potency. The structure, physical properties, and potency of the molecules under consideration are listed in Table II.

For the first regression analyses, subsets of the data were considered. The first subset consisted of analogs which either lack an aromatic ring or in which two or more carbon atoms separate the aromatic ring from the nitrogen atom. Equation 1 (Table IV) is the only statistically significant relationship found. A very negative dependence of potency of pK_a was observed. The range of pK_a values for this data set is 7.4–9.0.

Table II. Inhibition of MAO by Amines, $R_1R_2R_3N$

No.	R_1	R_2	R_3	pK_a	π	pI_{50} (obsd)	pI_{50} (calcd, eq 7)	Deviation
1	$CH_2C_6H_4$-2-OC_2H_5	$CH_2C{\equiv}CH$	CH_3	7.4	0.18	7.5	6.60	0.90
2	$CH_2C_6H_4$-2-Cl	$CH_2C{\equiv}CH$	CH_3	6.3[a]	0.76	7.3	7.27	0.03
3	CH_2-2-$C_{10}H_7$	$CH_2C{\equiv}CH$	CH_3	6.6[a]	1.33	7.3	6.33	0.97
4	$CH_2C_6H_3$-2,4-Cl_2	$CH_2C{\equiv}CH$	CH_3	6.3[a]	1.46	7.1	7.44	−0.34
5	$CH_2C_6H_3$-2,6-Cl_2	$CH_2C{\equiv}CH$	CH_3	6.1[a]	1.52	7.1	7.46	−0.36
6	CH_2-1-$C_{10}H_7$	$CH_2C{\equiv}CH$	CH_3	6.3[a]	1.33	7.0	7.41	−0.41
7	$CH_2C_6H_4$-2-OCH_3	$CH_2C{\equiv}CH$	CH_3	7.9[a]	−0.33	6.8	5.96	0.84
8	$CH_2C_6H_4$-3-OCH_3	$CH_2C{\equiv}CH$	CH_3	6.5	0.12	6.8	6.06	0.74
9	$CH_2C_6H_4$-2-CH_3	$CH_2C{\equiv}CH$	CH_3	6.7	0.84	6.8	7.20	−0.40
10	$CH_2C_6H_3$-3,4-OCH_2O	$CH_2C{\equiv}CH$	CH_3	6.8	−0.53	6.8	5.80	1.0
11	$CH_2C_6H_4$-4-C_6H_5	$CH_2C{\equiv}CH$	CH_3	6.8[a]	1.77	6.7	6.37	0.33
12	$CH_2C_6H_4$-2-F	$CH_2C{\equiv}CH$	CH_3	6.3	0.01	6.7	7.08	−0.38
13	$CH_2C_6H_3$-3,4-Cl_2	$CH_2C{\equiv}CH$	CH_3	7.0[a]	1.46	6.7	6.19	0.51
14	$CH_2C_6H_4$-4-Cl	$CH_2C{\equiv}CH$	CH_3	6.4[a]	0.70	6.6	6.22	0.38
15	$CH_2C_6H_4$-3-Cl	$CH_2C{\equiv}CH$	CH_3	6.2[a]	0.76	6.4	6.25	0.15
16	$CH_2C_6H_3$-2,4-$(OCH_3)_2$	$CH_2C{\equiv}CH$	CH_3	7.6	−0.37	6.4	6.28	0.12
17	$CH_2C_6H_4$-3-CH_3	$CH_2C{\equiv}CH$	CH_3	6.8	0.51	6.4	6.06	0.34
18	$CH_2C_6H_4$-4-OCH_3	$CH_2C{\equiv}CH$	CH_3	6.9	0.04	6.3	5.89	0.41
19	$CH_2C_6H_4$-4-$N(CH_3)_2$	$CH_2C{\equiv}CH$	CH_3	7.1[b]	0.18	6.3	5.81	0.49
20	$CH_2C_6H_4$-4-CH_3	$CH_2C{\equiv}CH$	CH_3	6.9	0.60	6.3	6.03	0.27
21	$CH(CH_3)C_6H_5$	$CH_2C{\equiv}CH$	CH_3	7.3[a]	0.50	6.2	5.75	0.45
22	$CH_2C_6H_4$-3-OH	$CH_2C{\equiv}CH$	CH_3	6.8[a]	−0.49	6.2	5.81	0.39
23	$CH_2C_6H_4$-4-OH	$CH_2C{\equiv}CH$	CH_3	7.2[a]	−0.61	6.1	5.55	0.55
24	$CH_2C_6H_4$-4-O-C_6H_5	$CH_2C{\equiv}CH$	CH_3	6.5[a]	2.10	6.0	6.55	−0.55
25	$CH_2C_6H_4$-4-Cl	$CH_2C{\equiv}CH$	H	7.1	0.19	6.0	5.81	0.19
26	$CH_2C_6H_5$	$CH_2C{\equiv}CH$	CH_3	6.6	0.00	6.0	6.00	0.00
27	$CH_2C_6H_3$-3,4-$(OCH_3)_2$	$CH_2C{\equiv}CH$	CH_3	6.8	0.08	5.8	5.95	−0.15
28	*l*-$CH(CH_3)CH_2C_6H_5$	$CH_2C{\equiv}CH$	H	7.4	0.82	5.6	5.74	−0.14
29	CH_2-2-C_5H_4N	$CH_2C{\equiv}CH$	CH_3	6.0[b]	−1.58	5.5	5.66	−0.16
30	$CH_2C_6H_4$-4-$CH(CH_3)_2$	$CH_2C{\equiv}CH$	CH_3	7.0[a]	1.40	5.5	6.17	−0.67
31	CH_2-c-C_6H_{11}	$CH_2C{\equiv}CH$	CH_3	8.0[a]	0.77	5.4	5.08	0.32
32	$CH_2C_6H_2$-3,4,5-$(OCH_3)_3$	$CH_2C{\equiv}CH$	CH_3	6.2	−0.60	5.3[c]	5.92	−0.62
33	$(CH_2)_5C_6H_5$	$CH_2C{\equiv}CH$	H	8.5[a]	1.70	5.3	4.58	0.72
34	$C(CH_3)_2C_6H_5$	$CH_2C{\equiv}CH$	CH_3	7.7[a]	1.00	5.2	5.50	−0.30
35	$CH_2C_6H_5$	$CH_2C{\equiv}CH$	H	7.3	−0.51	5.1	5.49	−0.40
36	*d*-$CH(CH_3)CH_2C_6H_5$	$CH_2C{\equiv}CH$	H	7.4	0.82	5.0	5.74	−0.74
37	$CH_2C_6H_5$	$CH_2C{\equiv}CCH_3$	CH_3	7.4[a]	0.51	5.0	5.67	−0.67
38	$(CH_2)_3C_6H_5$	$CH_2C{\equiv}CH$	H	8.1	0.51	5.0	4.89	0.11
39	CH_2-3-C_5H_4N	$CH_2C{\equiv}CH$	CH_3	5.8[b]	−1.48	4.8	5.65	−0.85
40	CH_2-4-C_5H_4N	$CH_2C{\equiv}CH$	CH_3	5.6[b]	−1.48	4.7	5.58	−0.88
41	$CH(CH_3)C_6H_5$	$CH_2C{\equiv}CH$	H	7.1	0.20	4.6	5.82	−1.22
42	CH_2-c-C_6H_{11}	$CH_2C{\equiv}CH$	H	8.4	0.27	4.3	4.39	−0.09
43	$(CH_2)_4C_6H_5$	$CH_2C{\equiv}CH$	H	8.2[a]	0.70	4.3	4.80	−0.50
44	CH_3	$CH_2C{\equiv}CH$	H	8.4	−2.69	4.0	3.66	0.34
45	$CH_2C_6H_5$	$CH_2C{\equiv}CH$	$CH_2C{\equiv}CH$	4.6[a]	0.48	3.8[c]	5.30	−1.50
46	$CH_2C_6H_5$	$CH_2C{\equiv}CH$	C_3H_7	7.0[a]	1.02	3.7[c]	6.08	−2.38
47	$(CH_2)_5CH_3$	$CH_2C{\equiv}CH$	H	8.4	−0.14	3.7	4.29	−0.59
48	$CH_2C_6H_5$	$CH_2C{\equiv}N$	CH_3	3.5	−1.32	3.6	3.19	0.41
49	$C(CH_3)_2C_5H_{11}$	$CH_2C{\equiv}CH$	H	9.0[a]	0.47	3.3	3.37	−0.07
50	$CH_2C_6H_5$	$CH_2C{\equiv}CH$	C_2H_5	6.8	0.51	3.1[c]	6.06	−2.96
51	c-C_6H_{11}	$CH_2C{\equiv}CH$	H	8.5	−0.23	3.0	4.10	−1.10

[a] The pK_a was measured in several alcohol-water solutions and extrapolated to 0% alcohol. [b] pK_2. pK_1 is for an ionizable group elsewhere in the molecule. [c] Omitted from the regression equation.

The second subset of compounds was *N*-methyl-*N*-benzyl analogs substituted in the aromatic ring. Equation 2 describes the relationships for this set. A special enhancement of potency by ortho substitution is indicated by the positive coefficient of the molar polarizability of the ortho substituent. The coefficient of pK_a, while still negative, is less than one-half of the previous equation. Since the range in pK_a's was 6.2–7.9 for these compounds, it was suspected that the difference in coefficients of the pK_a term in eq 1 and 2 might be due to an optimum pK_a value between 6.2 and 7.4.

The third subset of molecules considered consisted of all derivatives for which R_3 is CH_3 or H and in which the ring is not substituted. This group of analogs has the largest

Table III. Related Molecules Not Included in Regression Equations

No.	R_1	R_2	R_3	pK_a	π	pI_{50} (obsd)	pI_{50} (calcd eq 7)
52	CH_2-2-C_4H_3S	$CH_2C{\equiv}CH$	CH_3	6.2	−0.34	5.5	6.1
53	H	$CH_2C{\equiv}CH$	H	8.2	−3.20	4.0	4.1
54	$(CH_2)_4CH_3$	$CH_2C{\equiv}CH$	H	8.4[a]	−0.70	3.7	4.4
55	CH_3	$CH_2C{\equiv}CH$	CH_3	8.7[a]	−2.20	3.7	3.6
56	$CH_2C_6H_5$	C_3H_7	CH_3	9.6[a]	0.52	3.0	2.4
57	C_6H_5	$CH_2C{\equiv}CH$	H	3.3	−1.02	<3.0	2.9
58	$CH_2C_6H_4$-2-OC_6H_{13}	$CH_2C{\equiv}CH$	CH_3	7.4	2.67	5.0	7.4
59	$CH_2C_6H_5$	$(CH_2)_2C{\equiv}CH$	CH_3	7.7	0.51	3.1	5.6
60	$CH_2C_6H_5$	$CH_2CH{=}CH_2$	CH_3	7.4	0.3	<3.0	5.6
61	$CH_2C_6H_5$	$CH_2CCl{=}CH_2$	CH_3	6.8	0.7	<3.0	6.1
62	$CH_2C_6H_5$	$CH_2CH{=}CHCl$	CH_3	7.1	1.0	<3.0	6.0
63	$CH_2C_6H_5$	$CH(CH_3)C{\equiv}CH$	CH_3	6.9	0.3	<3.0	6.2
64	$CH_2C_6H_5$	$C(CH_3)_2C{\equiv}CH$	H	7.6	0.6	<3.0	5.8
65		$-(CH_2)_4-$	$CH_2C{\equiv}CH$	8.7[a]	−1.6	<3.0	5.7
66	$CHOHC_6H_5$	$CH_2C{\equiv}CH$	CH_3	6.9	−1.8	<3.0	5.6

[a] Estimated.

Table IV. Regression Equations (±95% Confidence Limits)

Eq no.	Compds included	Intercept	pK_a	pK_a^2	π	Other	Optimum pK_a (95% confidence interval)	n	s	R
1	28, 31, 33, 36, 38, 42–44, 47, 49, 51	15.21 (8.11)	−1.31 (0.99)					11	0.66	0.71
2	1–20, 22–24, 26, 27, 30, 32	9.05 (2.34)	−0.415 (0.350)			0.103 (0.045) MR_2		27	0.37	0.70
3	21, 25, 28, 29, 31, 33–44, 47–49, 51	−3.96 (4.81)	3.17 (1.47)	−0.26 (0.11)	0.32 (0.27)		6.05 (5.40–6.48)	21	0.54	0.82
4	All except 32, 45, 46, 50	−7.10 (4.48)	4.22 (1.33)	−0.34 (0.10)	0.26 (0.18)	0.16 (0.06) MR_2	6.18 (5.81–6.45)	47	0.56	0.88
5	All except 32, 45, 46, 50	−7.93 (5.07)	4.55 (1.50)	−0.37 (0.11)	0.25 (0.20)	0.86 (0.52) S_2	6.19 (5.80–6.48)	47	0.63	0.84
6	All except 32, 45, 46, 50	−6.79 (4.43)	4.31 (1.31)	−0.36 (0.10)	0.26 (0.18)	−1.41 (0.54) P_2	6.02 (5.64–6.30)	47	0.55	0.89
7	All except 32, 45, 46, 50	−7.48 (4.66)	4.38 (1.38)	−0.35 (0.10)	0.25 (0.19)	1.02 (0.45) D_2	6.18 (5.81–6.46)	47	0.58	0.87
8	All[a] except 32, 45, 46, 50	−6.57 (4.88)	3.87 (1.45)	−0.30 (0.11)	0.21 (0.20)	1.05 (0.47) D_2	6.56 (6.17–6.89)	47	0.61	0.81
9	All[b] except 32, 45, 46, 50	3.77 (4.61)	1.84 (1.36)	−0.21 (0.10)	0.24 (0.19)	1.06 (0.45) D_2	4.34 (2.11–5.19)	47	0.58	0.91

[a] Concentration of un-ionized drug which produces 50% inhibition was used as the dependent variable. [b] Concentration of ionized drug which produces 50% inhibition was used as the dependent variable.

range of pK_a values, from 3.5 to 9.0. Equation 3 is the relationship for this series. An optimum pK_a of 6.0 and a small positive hydrophobic effect are observed.

Finally all molecules were treated as one set. Equation 4 expresses the relationships between the variables and all compounds (except **32**) included in eq 1–3. **32** was omitted because it fit the previous equations badly and it was felt that this might be due to the steric crowding of the methoxy groups.

Influence of Ortho Substitution on Potency. Because ortho substitution seemed to enhance potency, other physical properties related to the position of aromatic substitution were investigated as predictor variables. The physical properties (π, MR, S, and P) of the substituent at each position (ortho, meta, para) were considered as separate terms in the regression equation. Equations 5 and 6 are the result. Thus, there are three regression equations (4–6) of approximately equal statistical significance. Each indicated that ortho-substituted analogs are more potent.

In order to determine why three equations rather than one was found, compounds of eq 2 for which we had reliable values of the various parameters were selected and the interrelationships between the physical properties were studied by regression analysis. Equations 9–13 (Table V) are the result. We conclude that within this series of compounds there is so much interrelationship between the physical properties of the 2-substituent that one cannot establish which is the true determinant of potency. Therefore, we summarized the effect of ortho substitution by using a dummy variable.

This variable was set equal to 1.0 if the compound is ortho-substituted, 0.0 if it is not. Equation 7 thus adequately summarizes the structure–activity relationships within the total series.

Table V. Interrelationships between the Ortho Substituent Constants Equations (±95% Confidence Limits)

Eq no.		n	s	R
9	$MR_2 = 1.91\ (1.45) + 5.73\ (3.39)\ S_2$	22	2.97	0.62
10	$MR_2 = 1.85\ (1.12) - 6.27\ (2.38)\ P_2$	22	2.24	0.78
11	$MR_2 = 1.05\ (0.73) + 5.49\ (1.92)\ PI_2 - 7.03\ (1.47)\ P_2$	22	1.35	0.93
12	$S_2 = 0.10\ (0.14) - 0.565\ (0.309)\ P_2$	22	0.293	0.65
13	$S_2 = 0.042\ (0.144) - 0.621\ (0.288)\ P_2 + 0.403\ (0.379)\ PI_2$	22	0.267	0.74

Influence of pK_a on Potency. Since it has been proposed that it is the un-ionized form of benzylamine which binds to (human) MAO,[9] it seemed possible that part of the dependence of potency of pK_a values was due to a change in proportion of drug molecules which are ionized at the pH of the test. Therefore, the pI_{50} value of the *uncharged* form was calculated by addition of the logarithm of the proportion of that particular analog present in the uncharged form to the observed pI_{50}. Equation 8 was calculated from these calculated pI_{50}'s. It can be seen that the dependence of potency on π and D-2 does not change but that the optimum pK_a is somewhat higher although not significantly different.

We have previously presented evidence that it is the protonated species of the β-carbolines which inhibits monoamine oxidase.[10] Thus, we repeated the structure–activity calculations, but this time we used as the activity the pI_{50} corrected to the concentration of the *protonated* species. Equation 9 is the result. Again, all terms are statistically significant. The dependence of potency on π and ortho substitution is identical to the previous equations. An optimum pK_a is still seen; however, in this case the optimum is statistically significantly lower than those from the previous equations.

If the only influence of pK_a on potency were to change the fraction of the various compounds which is in the more active form, then the quantitative structure–activity relationships with either the pI_{50} (un-ionized) or pI_{50} (ionized) would show no dependence on pK_a. Since this is not the case we conclude that the basicity of the nitrogen atom influences potency by mechanisms in addition to changing the fraction of drug ionized.

Prediction of Potency of Other Analogs. Compound **32** was omitted from the calculations, but its activity is as accurately predicted as molecules included in the equation. Three molecules (**45**, **46**, and **50**) were not included in the calculations since these are the only analogs in which R_3 is larger or more lipophilic than methyl. They are much less potent than expected. Thus a lack of "bulk tolerance" for R_3 is indicated.

Finally, we attempted to further verify that scope and predictive ability of equation 8. Our records were searched for data on related analogs; compounds **52–66** (Table III) were found. The potencies of **52–57** were adequately predicted by eq 7. The unexpectedly low potency of molecule **58** suggests that further examination of the effect of 2-substitution might result in the observation of an optimum value of some physical property. The low observed activity of compounds **60–64** emphasizes the key role of the propynyl portion of the molecule. **65** is inactive because all three substituents on the nitrogen are larger or more lipophilic than methyl. The inactivity of molecule **66** cannot be explained by any of the above arguments.

Discussion

Correlation of These Results with the Proposed Mechanism of Action of Pargyline. The upward concave shape of the pK_a vs. potency curve means that at the optimum there is a change in rate-limiting step from one of positive dependence on pK_a to one of negative, or vice versa. As stated in the introduction, pargyline first interacts with MAO in the manner of a typical substrate to form a reactive intermediate.[4,5] The necessity for an unsubstituted propargyl group suggests that this reaction is of the methylene adjacent to the triple bond. Additionally, it has been shown that there is a positive correlation of σ with the V_{max} of benzylamine substrates of bovine kidney MAO.[5] This means that the reaction is facilitated by withdrawal of electrons from the reaction site: removal of a proton.

The implications of the preceeding arguments with respect to analogs of paragyline are that it is expected that compounds which are more efficiently metabolized by the enzyme would more quickly produce a significant concentration of this intermediate. The positive dependence of the reaction on σ explains why inhibitory potency increases for pargyline analogs as the pK_a is decreased from 9.0 to 6.2. (The pI_{50} of pargyline is not constant after 30 min of preincubation.)

The third step in the reaction of pargyline with MAO has been postulated to be a nucleophilic attack by the modified inhibitor on an electron-deficient region of the MAO.[5,11] The rate of this reaction would show a negative correlation with σ since electron-withdrawing groups decrease the electron density (and nucleophilicity) of whichever atom acts as the nucleophile. This explains the decrease in potency of analogs whose pK_a is below the optimum.

Thus it is concluded that the observation of an optimum pK_a value for pargyline analogs is fully consistent with the proposed mechansim of action of pargyline and with structure–activity studies of substrates. The optimum pK_a represents the best compromise between the competing influences of basicity on steps 1 and 2 and on step 3 of the reaction of the inhibitors with MAO.

Electronic Effects of Substitution. An electronic effect on the potency of analogs is seen in molecules **59–62** in which the propynyl group has been modified. These compounds are at least 1000 times less potent than calculated from eq 7. Unpublished observations in our laboratory suggest that these molecules cannot react with MAO in the time-dependent, irreversible sense.

Hydrophobic Effects of Substitution. The above equations suggest a slight, general hydrophobic effect on potency. There is no structure–activity evidence for specific hydrophobic bonding between certain ring positions and the enzyme. This is consistent with the postulate that the substrate-like reaction occurs with the methylene of the propargyl (and not the benzyl) group.

Steric Effects of Substitution. The lack of fit to the general equation of molecules in which R_3 is larger than methyl is best rationalized as a steric effect. These compounds are at least 190 times less active than calculated from eq 7.

Atoms Involved in the Irreversible Bonding of Pargyline to MAO. Substitution at the terminal carbon atom of the propynyl group (**37**) or on the benzylic carbon atom (**21**, **34**, and **41**) produces molecules which fit the equations satisfactorily. On the other hand, mono- or disubstitution of methyl groups for the α-hydrogen atoms of the propynyl group abolishes all inhibitory activity. Thus we conclude

that it is this α-carbon atom at which the first reaction with the enzyme occurs. Further evidence in support of this conclusion is the fact that not only benzyl- but phenethyl- and alkylamines are inhibitors with potency described by eq 7.

Comparison with Other Quantitative Structure–Activity Studies on MAO. Another quantitative structure–activity analysis of pargyline analogs has been reported recently.[12] Unfortunately, the potencies used in the calculations were not those reported in the cited source of data. The SAR analysis of other series included in that report is seriously compromised by the fact that too many variables were examined as possible predictors and compounds were arbitrarily omitted to get a better fit. Thus several of the equations might have been expected by chance.[13]

Studies on the inhibition of MAO by *N*-(phenoxyethyl)-cyclopropylamines also demonstrated electronic, hydrophobic, and steric effects on potency.[14] The dependence of lipophilicity was identical with that calculated above. The electronic and steric effects cannot be directly related between the two series.

The rate of oxidative metabolism of primary amines by rabbit liver MAO is characterized by an optimum log *P* of 2.50 and a positive dependence of pK_a.[15] Since the inhibitors in Table I have log *P*'s (log $P = 1.75 + \pi$) on both sides of the optimum for these substrates but show no optimum, the hydrophobic effects of the two series/activities/enzymes are different. The positive dependence on pK_a is also opposite to the inhibitors and the benzylamine substrate data quoted above.

In the structure–activity analysis of the relative potency of β-carbolines as monoamine oxidase inhibitor, a large influence of hydrophobicity was seen.[10,16] This is in contrast to the results presented for propynylamines. It is possible that there are hydrophobic effects on each of the steps of the enzyme–inhibitor reaction but that these effects are of similar magnitude and of opposite direction.

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Potential Inhibitors of L-Asparagine Biosynthesis. 3.[1] Aromatic Sulfonyl Fluoride Analogs of L-Asparagine and L-Glutamine†

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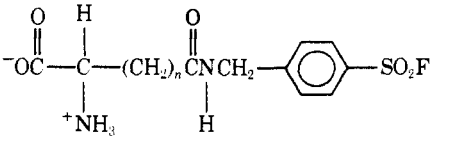
The *N*-[*p*-(fluorosulfonyl)benzyl] derivatives of L-asparagine and L-glutamine (**1a**,**b**) were synthesized as potential inhibitors of L-asparagine synthetase (ASase). Condensation of *p*-(fluorosulfonyl)benzylamine (**2**) with the suitably protected amino acid in the presence of dicyclohexylcarbodiimide, followed by deblocking, afforded **1a** and **1b**. Derivatives **1a** and **1b** at 10 m*M* inhibit ASase isolated from Novikoff hepatoma (rats) by 60 and 46%, respectively. Preliminary results on inhibition of Jensen sarcoma (L-asparaginase sensitive) and JA-1 sarcoma (L-asparaginase resistant) tissue cultures by 0.3 m*M* **1a** (139, 90%) and **1b** (101, 103%), respectively, are discussed.

The finding that L-asparaginase (ASNase) resistant tumors and tumors once sensitive to the deamidase exhibit an increased biosynthesis of L-asparagine via a heightened activity of L-asparagine synthetase[2-5] (ASase) has prompted the search for agents capable of inhibiting the synthetase. Such compounds could be beneficial from two standpoints. First, they could be used against the resistant line, and, second, they could be used in combination with ASNase against sensitive tumors in an attempt to prevent the development of the resistant cells.

Mammalian ASase synthesizes L-asparagine from L-aspartic acid, utilizing L-glutamine as the primary source of nitrogen.[6-8] In addition, it has been observed that the synthetase undergoes product inhibition by L-asparagine.[5,6,8-10] It was felt, therefore, that close analogs of L-asparagine and L-glutamine could possibly inhibit the biosynthesis or interfere with the utilization of L-asparagine in both L-asparaginase sensitive and resistant tumors.



1a, $n = 1$
b, $n = 2$

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