SAR STUDIES OF FLUORINE-SUBSTITUTED BENZYLAMINES AND SUBSTITUTED 2-PHENYLETHYLAMINES AS SUBSTRATES AND INACTIVATORS OF MONOAMINE OXIDASE B

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To test whether addition of electron-withdrawing substituents to good substrates of monoamine oxidase (MAO) will transform them into inactivators, a series of fluorine-substituted benzylamines and a series of substituted 2-phenylethylamines were synthesized and tested as substrates and inactivators of monoamine oxidase B. All of the compounds were substrates. None of the benzylamines was an inactivator, but several of the phenethylamines were. These results suggest that either stronger electron withdrawing character or additional stabilizing factors are needed to stabilize the hypothesized enzyme adduct.

KEY WORDS: Structure-activity relationships, fluorinated benzylamines, substituted 2-phenylethyl-amines, monoamine oxidase B

INTRODUCTION

Monoamine oxidase (MAO, EC 1.4.3.4), a flavoenzyme that catalyzes the degradation of a variety of amine neurotransmitters, exists in two isozymic forms, MAO A and MAO B,[1] which differ in primary structure[2] and in substrate specificity;[1] MAO A oxidizes norepinephrine and serotonin and MAO B oxidizes benzylamine and 2-phenylethylamine. Compounds that selectively inactivate MAO A exhibit antidepressant activity[3] and those that selectively inactivate MAO B are used in the treatment of Parkinson's disease.[4]

A number of structure-activity relationship studies have been performed on MAO utilizing either substrates or inactivators, but most of these studies were carried out on mixtures of MAO isozymes making interpretations of the results difficult. Zeller[5,6] compared the kinetics of oxidation of 15 substituted benzylamines by beef liver mitochondria. He used ortho-, meta-, and para-substituted benzylamines with the following groups: chloro, iodo, methoxy, methyl, and nitro. No Hammett plot[7] was made, but he concluded that meta-substituted benzylamines are better substrates than the corresponding ortho- or para-substituted ones.

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Hellerman and coworkers[8,9] compared the turnover kinetics of 11 benzylamine derivatives and generated a linear free energy relationship by a comparison of the logarithm of the maximal velocity to σ. There was an inflection point in the plot at $\sigma = 0$; the value of ρ equalled 1.34 for substituents with negative σ values (electron donating) but ρ equalled 0 for substituents with positive σ values (electron withdrawing). No explanation for this change in the substituent effect was given. In general, it was concluded that as the benzyl ring becomes more electron poor, the maximal velocity increases.

Kutter and Hansch[10] analyzed previously reported data concerning MAO inhibitors,[11] focusing on various steric parameters. They concluded that substitution at the meta position was quite important for the binding of the phenoxyethylcyclopropylamine MAO inhibitors, and that the binding was characteristic of binding to a surface rather than to binding in a pocket engulfed in the enzyme. Fujita[12] also analyzed previously published MAO inhibition data from a number of authors using a linear combination of hydrophobic, electronic, and steric substituent constants. He determined that the substituent effects were similar in the various classes of MAO inhibitors that were studied and concluded from this analysis that the probable role of the aromatic moiety was to interact as an electron acceptor with a noncatalytic electron-rich site on the enzyme surface.

Mahmoundian[13] reported a Hansch type analysis for MAO A inhibition by a variety of ring substituted phenylalkylamines both *in vitro* and *in vivo*. He concluded that the presence of a hydrogen acceptor group in the para position and an S configuration of the side chain was favorable for inhibition. The MAO-A inhibitory activity correlated with the sum of the field parameters and the sum of the hydrophobicity of substituents on the phenyl ring with a positive slope, but correlated with the sum of the molar refractivity with a negative slope.

Nishimura *et al.*[14] reported SAR studies on MAO B using analogues of the inactivator 2-(n-pentylamino)acetamide and found that the partition ratio decreased with increasing electron withdrawing ability (as measured by σ_I) of the analogues.

Most recently, Walker and Edmondson[15] reported structure-activity relationship studies on the MAO B-catalyzed oxidation of *para*- and *meta*-substituted benzylamines. They concluded that there was a linear correlation between the K_d values of the *para*-substituted analogues and the hydrophobicity parameter (π); the binding affinity increased with increasing hydrophobicity of the substituent. There also was a small negative contribution to binding by the van der Waals volume (V_w). Flavin reduction rates by the *para*-substituted benzylamines were retarded by increased values of the Taft steric parameter E_s. The *meta*-substituted benzylamine analogues showed a decreased binding affinity with the V_w and no correlation with π. The rate of flavin reduction by the *meta*-substituted analogues was independent of the nature of the substituent.

We have suggested that various heterocycle-containing inactivators appear to function by virtue of their ability to stabilize the purported enzyme adduct by an electron-withdrawing effect.[16-18] If electron-withdrawing character is all that is required for inactivation, we thought that attachment of electron-withdrawing groups to good substrates for the MAO isozymes may provide a simple approach to the design

of selective inactivators of this enzyme. Two series of compounds were prepared to test this electron-withdrawal hypothesis with MAO B, namely, fluorine-substituted benzylamines and substituted 2-phenylethylamines. Almost all of the analogues were excellent substrates for MAO B. None of the benzylamine analogues was an inactivator, but several of the phenylethylamines were weak inactivators.

MATERIALS AND METHODS

Instrumentation

MAO assays were recorded on a Perkin-Elmer Lambda 1 UV/vis spectrophotometer. Wavelength spectra of MAO were recorded on a Beckman DU-40 UV/vis spectrophotometer. NMR spectra were recorded on a Varian Gemini 300 VXR 300-MHz, a Varian XL-400 400-MHz or a JEOL FX-270 270-MHz spectrometer. Proton chemical shifts are reported as δ values in parts per million downfield from tetramethylsilane as an internal standard. Fluorine chemical shifts are reported as δ values in parts per million downfield from trichlorofluoromethane as an internal standard. Mass spectra were obtained on a VG Instruments VG70–250E high resolution spectrometer. Melting points were taken on a Fisher-Johns melting point apparatus and are uncorrected. Flash column chromatography utilized silica gel 60 (230–400 mesh ASTM) (Merck). Elemental analyses (C, H, N) were performed by Oneida Research Services, Inc. (Whitesboro, NY).

Reagents

All chemical were obtained from either Aldrich Chemical Company or Sigma Chemical Company and were used as received except as noted below. Protein assays utilized the Pierce Coomassie protein assay reagent (No. 23200). Dialysis tubing was Spectra/Por 2 and was obtained from Fisher. Prior to use the dialysis tubing was boiled in 1 mM ethylenediaminetetraacetate for 60 min and then washed extensively with doubly distilled deionized water. 4-Nitrobenzylamine and 4-methylbenzylamine were recrystallized from ethanol prior to use. Benzylamine (NMR (D_2O) δ 7.3 (m, 5 H), 4.0 (s, 1 H)), 2-methoxybenzylamine (NMR (DMSO) δ 8.5 (s, 3 H), 7.4 (m, 2 H), 7.0 (m, 2 H), 4.0 (s, 2 H)), 4-methoxybenzylamine (NMR (DMSO) δ 8.5 (s, 3 H), 7.4 (d, 2 H), 7.0 (d, 2 H), 4.0 (s, 2 H)), 2-chlorobenzylamine (NMR (DMSO) δ 8.5 (s, 3 H), 7.5 (m, 4 H), 4.1 (s, 2 H)), 4-chlorobenzylamine (NMR (DMSO) δ 8.5 (s, 3 H), 7.5 (d, 2 H), 7.1 (d, 2 H), 4.1 (s, 2 H)), 2-fluorobenzylamine (NMR (CD_3OD) δ 7.5 (m, 2 H), 7.2 (m, 2 H), 4.2 (s, 2 H)), 3-fluorobenzylamine (NMR (DMSO) δ 8.5 (s, 3 H), 7.4 (m , 2 H), 7.0 (m, 2 H), 4.0 (s, 2 H)), 4-fluorobenzylamine (NMR (CD_3OD) δ 7.5 (m, 2 H), 7.2 (m, 2 H), 4.1 (s, 2 H), 2.3 (s, 3 H)), 2-methylbenzylamine (NMR (D_2O) δ 7.4 (m, 2 H), 7.2 (m, 2 H), 3.9 (s, 2 H)), 2,6-difluorobenzylamine (NMR (CD_3OD) δ 7.4 (m, 1 H), 7.0 (t, 2 H), 4.1 (s, 2 H)), 2,4-difluorobenzylamine (NMR (CD_3OD) δ 7.3 (m, 1 H), 6.9 (m, 2 H), 4.0 (s, 2 H)), β-hydroxyphenethylamine (NMR (DMSO) δ 8.1 (s, 3 H), 7.3 (m, 5 H), 6.1 (d, 1 H), 4.8 (m, 1 H), 2.9 (m, 2 H)), and β-methylphenethylamine (NMR (DMSO) δ 8.1 (s, 3 H), 7.3 (m, 5 H), 3.1 (m, 1 H), 2.9 (d, 2 H), 1.2 (d, 3 H)), were separately dissolved in dry diethyl ether, treated with HCl gas to form the salt,

isolated by filtration and recrystallized from ethanol. β-Methoxyphenethylamine•HCl,[24] β-chloro-phenethylamine•HCl[25] and β-fluorophenethylamine•HCl,[26] were prepared according to literature procedures.

2,4,5-Trifluorobenzylamine•HCl. Tetrahydrofuran (THF, 66 mL) and water (33 mL) were placed in a round bottom flask equipped with a magnetic stirrer. Cobalt (III) chloride hexahydrate (0.45 g, 1.87 mmol) was added to the flask yielding a pink solution, and 2,4,5 trifluorobenzonitrile (2.92 g, 18.6 mmol) was added. The mixture was intermittently cooled in an ice water bath and sodium borohydride (1.8 g, 47 mmol) was added over 8 min. The exothermic reaction produced a black precipitate. After being stirred for 2 h the mixture was treated with ammonium hydroxide (2 mL) and centrifuged to remove the precipitate. The pellet was washed with a small amount of the same solvent mixture and centrifuged. The combined supernatants were rotary evaporated to remove the bulk of the THF and then were extracted with dichloromethane (4×40 mL), dried over magnesium sulfate, and rotary evaporated to yield a yellow oil. The oil was dissolved in dry diethyl ether and hydrogen chloride gas was bubbled through the solution to yield a white solid, which was isolated by filtration, and recrystallized (2×) from methanol/hexane (0.8 g, 22% yield). NMR (DMSO) δ 8.5 (s, 3 H), 7.6 (m, 1 H), 4.0 (s, 2 H); mp 155–158°C; EI HRMS (70 eV) Calcd for $C_7H_6F_3N$ 161.0452. Found 161.0459.

(±)-β-Methoxy-β-trifluoromethylphenethylamine•HCl and pentafluorobenzylamine• HCl. These were prepared following the identical procedure as for 2,4,5-trifluorobenzylamine•HCl from the appropriate nitriles (2.5 g and 3 g, respectively, starting materials).

(±)-β-Methoxy-β-trifluoromethylphenethylamine•HCl: yield, 0.71 g (25%); mp 185–186°C; NMR (DMSO) δ 8.8 (s, 3 H), 7.6 (m, 5 H), 3.7 (s, 2 H), 3.3 (s, 3 H); EI HRMS (70 eV) Calcd for $C_{10}H_{12}F_3NO$ 219.0871. Found 219.0862.

Pentafluorobenzylamine•HCl: yield, 2.33 g (70%); mp 269–270°C; NMR (DMSO) δ 8.5 (s, 3 H), 4.1 (s, 2 H); EI HRMS (70 eV) Calcd for $C_7H_4F_5N$ 197.0264. Found 197.0252.

β, β-Difluorophenylacetamide. Ethyl benzoylformate (3.34 g, 18.9 mmol) was placed in a dry round bottom flask equipped with a reflux condenser under nitrogen. Diethylaminosulfur trifluoride (6.1 g, 37.8 mmol) was added to the flask, and the mixture was stirred at room temperature for 2.5 h. The mixture was poured over ice and extracted with dichloromethane, dried over magnesium sulfate, and rotary evaporated to yield a red oil. The red oil was dissolved in absolute ethanol (25 mL), and anhydrous ammonia gas was bubbled through the solution for 30 min. The reaction vessel was then sealed and stirred overnight. The mixture was rotary evaporated to yield a red-yellow solid, which was recrystallized from carbon tetrachloride/chloroform (2.6 g, 80% yield); mp 133–134°C; NMR (DMSO) δ 8.2 (d, 2 H), 7.5 (m, 5 H);[19] F NMR (DMSO) δ –101 (s). EI HRMS (70 eV) Calcd for $C_8H_7F_2NO$ 171.0495. Found 171.0490.

β, β-Difluorophenethylamine•HCl. Lithium aluminum hydride (3 g, 80 mmol) was placed in a dry 3-neck flask equipped with a reflux condenser and a dropping funnel under nitrogen. Dry diethyl ether (25 mL) was added and the mixture was stirred.

β,β-Difluorophenylacetamide (1.5 g, 9.6 mmol) was dissolved in dry diethyl ether and was added dropwise to the stirred solution. After completion of the addition, the mixture was allowed to reflux for 45 min. The mixture was cooled, and water was carefully added to decompose the excess reductant. The mixture was treated with saturated sodium sulfate and the aluminum salts were removed by filtration. The resulting liquid was rotary evaporated to yield a yellow oil. The oil was purified by silica gel flash chromatography with a 5:1 mixture of ethyl acetate:hexane, and the HCl salt was made by bubbling HCl gas through the purified oil in dry diethyl ether. The salt was washed with acetone to remove colored impurities and was recrystallized from ethanol (yield 1.17 g, 63%); mp 169–170°C, NMR (DMSO) δ 8.9 (s, 3 H), 7.6 (m, 5 H); 3.7 (t, 3 H);[19] F NMR (DMSO) δ –98 (t). EIMS (70 eV) m/z 157.1 (M$^+$, 35), 136 (13), 127 (100), 118 (21), 109 (22), 77 (46). Anal. Calcd for $C_8H_{10}ClF_2N$ C,49.62%; H, 5.25%; N, 7.28%. Found C, 49.52%; H, 5.21%; N, 7.23%.

(R)-β-Methoxy-β-trifluoromethylphenylacetamide. (R)-β-Methoxy-β-(trifluoromethyl) phenylacetic acid (1 g, 4.3 mmol) was placed in a round bottom flask equipped with a magnetic stirrer under nitrogen and diluted with dichloromethane (2 mL). Freshly distilled thionyl chloride (5 mL, 68 mmol) was added to the mixture. The solution was allowed to reflux for 24 h. The excess thionyl chloride was removed by rotary evaporation. The residue was placed in a pressure tube that was cooled in Dry ice. Liquid ammonia (~5 mL) was added to the tube, which was sealed and allowed to stir for 20 min at room temperature, yielding a red solution. The tube was opened, and after evolution of the excess ammonia, a dirty yellow solid appeared. The residue was dissolved in aqueous sodium hydroxide (5%) and was extracted with dichloromethane (3 times). The combined organic layers were dried over magnesium sulfate, and the solvent was removed by rotary evaporation to yield a yellow solid (0.58 g, 58%); mp 125–128°C; NMR (DMSO) δ 8.4 (s, 2 H), 7.6 (m, 5 H), 3.3 (s, 3 H).

(R)-β-Methoxy-β-trifluoromethylphenethylamine•HCl. A borane solution (1 M in THF, 6 mL, 6 mmol) was placed in a three necked flask equipped with a dropping funnel and reflux condenser under nitrogen. (R)-β-Methoxy-β-trifluoromethylphenylacetamide (0.4 g, 1.72 mmol) was dissolved in freshly distilled THF (15 mL), and was added dropwise to the borane solution. The mixture was allowed to reflux for 6 h. The mixture was cooled in an ice bath, and excess reductant was quenched by the careful addition of 6 N HCl (10 mL). The THF was removed by distillation. The solution was made basic with solid NaOH, and was extracted with dichloromethane (3 times). The combined organic layers were extracted with 0.5 M HCl (3 times), and the combined aqueous extracts were rotary evaporated to yield a white solid that was recrystallized from dichloromethane/hexane (0.275 g, 63%); mp 185–186°C; NMR (DMSO) δ 8.8 (s, 3 H), 7.6 (m, 5 H), 3.7 (s, 2 H), 3.3 (s, 3 H); $[\alpha]_D^{25}$ (H$_2$O, 14.86 mg/mL): –10.3°.

(S)-β-Methoxy-β-trifluoromethylphenethylamine•HCl. This compound was prepared by the same procedure as the (R)-isomer starting from (S)-β-methoxy-β-(trifluoro-methyl)phenylacetic acid (1 g, 4.3 mmol); overall yield (0.35 g, 35%); mp 185–186°C; NMR (DMSO) δ 8.8 (s, 3 H), 7.6 (m, 5 H), 3.7 (s, 2 H), 3.3 (s, 3 H); $[\alpha]_D^{25}$ (H$_2$O, 11.55 mg/mL): +10.9°.

Enzyme

Beef liver MAO B was isolated as described previously.[27] The enzyme was stored as a concentrated solution (15–25 mg/mL) in potassium phosphate buffer (50 mM, pH 7.2) at 4°C. The specific activity varied among preparations, ranging from 3.5–7 units per mg, where a unit of activity is the conversion of 1 mol of benzylamine to benzaldehyde per minute at pH 9.0 and 30°C. Enzyme purity was determined by the use of sodium dodecylsulfate polyacrylamide gel electrophoresis. All buffers and enzyme solutions were prepared with doubly distilled deionized water.

Determination of Substrate Kinetics

A modification of the general method of Szutowicz *et al.*[28] for the assay of hydrogen peroxide was used. Solutions of substrate (benzylamine•HCl) were prepared (51.6, 86, 172, 344 and 516 μM, in 50 mM CHES buffer, pH 9.0 with 10 M sodium azide). An assay solution was prepared containing horseradish peroxidase (0.05 mg/mL) and 2,2′-azinobis(3-ethylbenzothiaxoline-6-sulfonic acid) diammonium salt (ABTS, 18 mM) in phosphate-citrate buffer (0.5 M, pH 4.0). Dilute MAO B was prepared by mixing stock MAO B (10 μL) with buffer (190 μL, 50 mM CHES, pH 9.0, 10 μM sodium azide). All solutions and the spectrometer were kept at 25°C with the use of a temperature controlled water bath/recirculator and a block heater. The substrate solutions (495 μL) were incubated with diluted MAO (5 μL) for 2, 3 or 4 min, then they were mixed with the assay solution (500 μL) and the absorbance was measured at 414 nm versus a blank of assay solution (500 μL), dilute MAO (5 μL), and buffer (50 mM CHES, pH 9.0, 10 μM sodium azide). The reported extinction coefficient of 24,600 mol^{-1}cm^{-1} for the ABTS dye was used to convert the absorbance measurements into concentration units. The data were then plotted in a double reciprocal plot (Lineweaver-Burke) for approximate k_{cat} and K_m determination and visual assessment. The kinetic constants were obtained using non-linear regression analysis.[29]

Determination of Inactivation Kinetics

All solutions and the spectrometer cuvette holder were kept at 25°C with the use of a temperature controlled water bath/recirculator and a block heater. The inactivator at various concentrations was prepared in buffer (380 μL, 50 mM CHES, pH 9.0). An assay solution of substrate (2 mM benzylamine•HCl or 2 mM cinnamylamine•HCl) in buffer (50 mM CHES, pH 9.0) was prepared. MAO (20 μL of a 15 μM solution) was added to each of the inactivator solutions. Periodically, aliquots (10 μL) were removed from each inactivator solution, mixed with the assay solution (490 μL), and the change in absorbance with time was observed at 250 nm (benzylamine assay solution) or 290 nm (cinnamylamine assay solution). A plot of the natural logarithm of the activity versus time for each inactivator concentration generated the inactivation rate for each concentration, which was then plotted versus the concentration on a double reciprocal plot[19] for data assessment. Kinetic constants were determined using published non-linear regression programs.[29]

*Determination of Inhibition Kinetics for (±)-β-Methoxy-
β-trifluoromethylphenethylamine•HCl*

A series of solutions (0, 0.513, 1.03, 2.05 mM) was prepared with different concentrations of (±)-β-methoxy-β-trifluoromethylphenethylamine•HCl and benzylamine (0.0675, 0.149, 0.257, 0.743, 1.28 mM) in buffer (50 mM CHES, pH 9.0). A dilute solution of MAO B was prepared (8 μM in 50 mM CHES, pH 9.0 buffer). Each of the amine solutions (495 μL) was mixed with dilute enzyme (5 μL) in a cuvette, and the change in absorbance was measured at 250 nm. A plot of the inverse of activity versus the inverse of the concentration of benzylamine was made for each of the phenethylamine concentrations. The slope of that line was plotted versus the phenethylamine concentration in order to determine inhibition constants.

RESULTS

Substrate Activity of Fluorine-Substituted Benzylamines

The kineties for the oxidation of a series of fluorine-substituted benzylamines is summarized in Table 1. Nearly all of the benzylamine derivatives are good substrates of MAO B.

Linear free energy relationships were developed. A plot of ln k_{cat} versus σ showed a very good correlation ($r^2 = 0.95$, Figure 1). The ρ value for these analogues was −3.8. This ρ value indicates that the turnover number is strongly affected by the electronic character of the aromatic moiety (increasing electron-withdrawing character decreases the rate). A plot of ln (k_{cat}/K_m) versus σ showed similar results (Figure 2; $r^2 = 0.96$ with a ρ value of −2.0). A plot of ln (k_{cat}/K_m) versus the hydrophobicity (log P, Figure 3) did not show a good correlation ($r^2 = 0.72$), but as the hydrophobicity increased, the catalytic efficiency tended to decrease.

TABLE 1
Kinetic Constants of Fluorine-Substituted Benzylamine Derivatives
for Oxidation by MAO B at pH 9.0, 25°C.

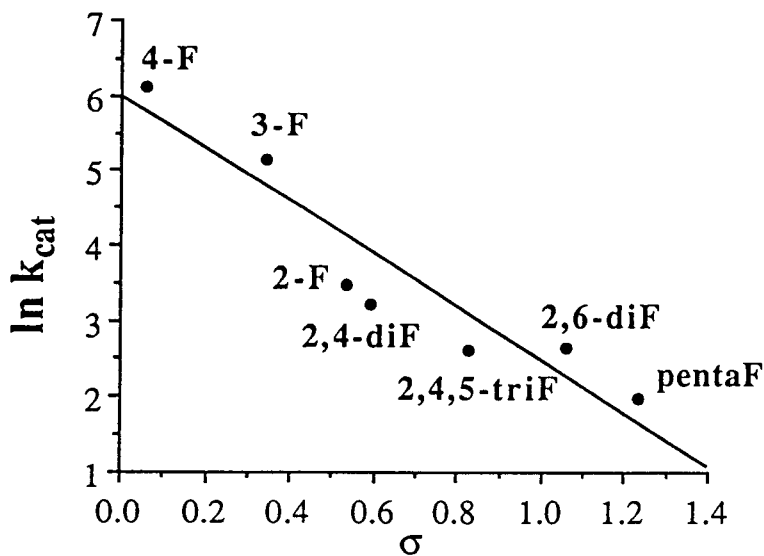| Number	Compound	k_{cat} (min^{-1})	K_m (μM)
1	2-fluorobenzylamine	31	19
2	3-fluorobenzylamine	168	51
3	4-fluorobenzylamine	448	147
4	2,4-difluorobenzylamine	25	15
5	2,6-difluorobenzylamine	14	20
6	2,4,5-trifluorobenzylamine	14	7
7	pentafluorobenzylamine	7	22
8	benzylamine	1024	253

FIGURE 1   Plot of ln k_{cat} versus σ for Fluorinated Benzylamines. The substitution pattern is noted next to each data point. See Table 1 for the kinetics of each data point. See Materials and Methods for procedure.
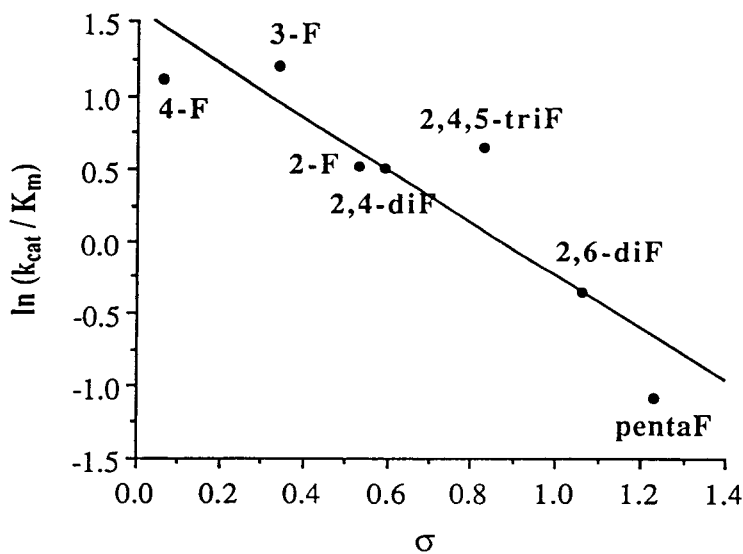

FIGURE 2   Plot of ln (k_{cat}/K_m) versus σ for Fluorinated Benzylamines. The substitution pattern is noted next to each data point. See Table 1 for the kinetics of each data point. See Materials and Methods for procedure.
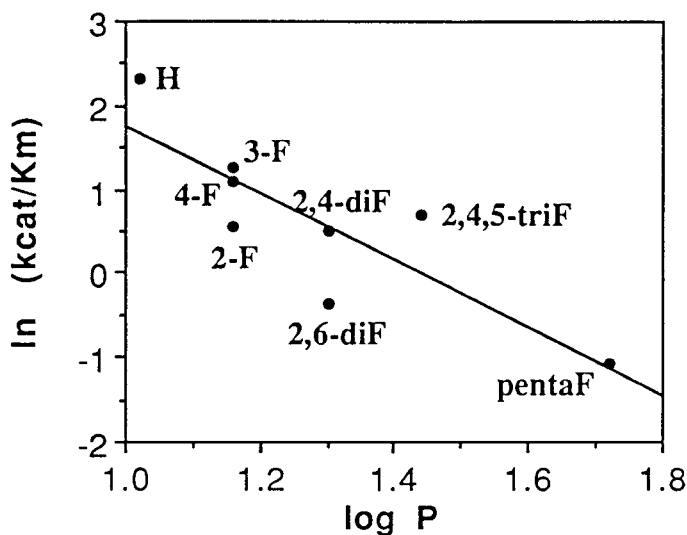
FIGURE 3 Plot of ln (k_{cat}/K_m) versus log P for Fluorinated Benzylamines. The substitution pattern is noted next to each data point. See Table 1 for the kinetics of each data point. See Materials and Methods for procedure.

Substrate Activity of the Substituted 2-Phenylethylamines

The phenethylamine derivatives also were well behaved substrates (Table 2) with one exception, β-methoxy-β-trifluoromethylphenethylamine (15). Compound 15, however, does binds to the enzyme, and shows both inhibition and inactivation characteristics, as is discussed below. The phenethylamine derivatives did not show a correlation for ln (k_{cat}/K_m) versus σ, log P, or the Taft steric constant (E_s).

Inactivation Properties of the Substituted 2-Phenylethylamines

Inactivation kinetics[19] of the 2-phenylethylamine series are summarized in Table 3. Little or no enzyme activity returned upon exhaustive dialysis. Only five of these derivatives were pseudo first-order inactivators. Phenethylamine and β-methylphene-thylamine did not inactivate the enzyme appreciably, and the inactivation solutions soon became cloudy, presumably the result of product precipitation. This made kinetic measurements of the inactivation rates for these two compounds irreproducible.

One of the more potent inactivators was β-methoxy-β-trifluoromethylphenethyl-amine (15); however, its kinetics were poorly behaved. The values listed in Table 3 for this compound (and its enantiomers) are approximate values because the inactivation data deviated from linearity significantly (at higher concentrations of inactivator larger than expected inactivation rates were observed), and, therefore, the data were fitted by hand.

TABLE 2
Kinetic Constants of 2-Phenylethylamine Derivatives for Oxidation by MAO B at pH 9.0, 25°C.
The dashes indicate that the compound did not have a detectable level of substrate activity.

Number	Compound	k_{cat} (min^{-1})	K_m (μM)
9	2-fluorophenethylamine	95	33
10	2-chlorophenethylamine	8	100
11	2-methylphenethylamine	314	11
12	2-hydroxyphenethylamine	169	12
13	2-methoxyphenethylamine	200	879
14	2,2-difluorophenethylamine	152	48
15	2-methoxy-2-trifluoromethylphenethylamine	–	–
16	phenethylamine	189	10

TABLE 3
Kinetic Constants of 2-Phenethylamine Derivatives for Inactivation of MAO B at pH 9.0, 25°C.
The values in the parenthesis are only approximate values, and the dashed lines indicate
that inhibition constants were unattainable.

Number	Compound	k_{inact} (min^{-1})	K_I (mM)
9	2-fluorophenethylamine	0.026	18.6
10	2-chlorophenethylamine	0.012	54.3
11	2-methylphenethylamine	–	–
12	2-hydroxyphenethylamine	0.0063	64
13	2-methoxy phenethylamine	0.31	417
14	2,2-difluorophenethylamine	0.017	6.6
15	(±)-2-methoxy-2-trifluoromethylphenethylamine	(0.57)	(25)
(R)-15	(R)-2-methoxy-2-trifluoromethylphenethylamine	(0.55)	(28)
(S)-15	(S)-2-methoxy-2-trifluoromethylphenethylamine	(0.62)	(24)
16	phenethylamine	–	–

Hammett plots of the natural log of the partition ratio (ln $[k_{cat}/k_{inact}]$) versus σ_R, σ_I, and the Taft steric constant (E_s) were constructed using only the well behaved phenethylamine inactivators; none of these plots shows a correlation.

However, a plot of the ln of the partition ratio versus the hydrophobicity (log P) showed a reasonable correlation (Figure 4) with the exception of one outlying point (**13**). The correlation for the data set, except **13**, is excellent ($r^2 = 0.98$). A plot of ln k_{inact}/K_I versus the hydrophobicity did not show a correlation, but a plot of k_{inact}/K_I versus the E_s (Figure 5) did show a good correlation ($r^2 = 0.93$).
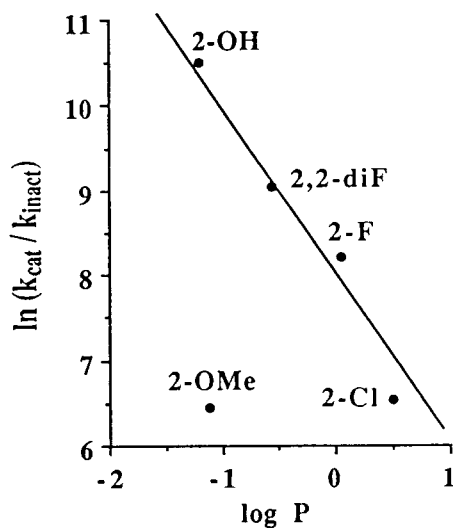
FIGURE 4 Plot of ln (k_{cat}/k_{inact}) versus log P for Substituted Phenylethylamines. The substitution pattern is noted next to each data point. See Tables 2 and 3 for the kinetics of each data point. See Materials and Methods for procedure.
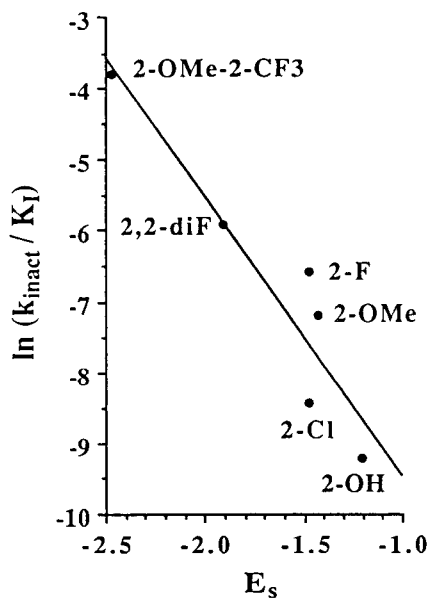

FIGURE 5 Plot of ln (k_{inact}/K_I) versus E_s for Substituted Phenylethylamines. The substitution pattern is noted next to each data point. See Table 3 for the kinetics of each data point. See Materials and Methods for procedure.

DISCUSSION

It is apparent from the results shown in Tables 1–3 that small increases in the electron-withdrawing effect by various substituents is not sufficient to transform substrates into inactivators. The heterocyclic analogues previously studied[16–18] either exhibit a higher degree of electron withdrawing character[20] or have additional binding features that stabilize the enzyme adduct.[21,22]

The fluorine-substituted benzylamine analogues are very good substrates. As the electron-withdrawing character of the benzene ring increases, however, the turnover number decreases. This is consistent with a mechanism involving electron transfer from the amine to the flavin.[23] For electron-donating substituents, Hellerman and coworkers[8,9] found the opposite correlation; it is not known why. The trend of decreasing catalytic efficiency (k_{cat}/K_m) with increased hydrophobicity (Figure 3) is consistent with the "stickiness" associated with binding of hydrophobic substituents to a hydrophobic active site.

The lack of correlation between the catalytic efficiency of 2-phenylethylamines and electronic, hydrophobic, or steric factors indicates that there are many parameters involved in the binding and turnover of these more flexible molecules than in the case of the benzylamines.

The deviation from linear inactivation kinetics for **15** was investigated in an attempt to determine the source of the uncharacteristic behavior. One possibility was that because this compound is chiral, its two enantiomers were reacting differently with the enzyme and causing the unusual kinetics. The two pure enantiomers were synthesized and tested as inactivators. The inactivation kinetics for the racemic compound and for both of the pure enantiomers were the same; therefore, different binding characteristics cannot be the source of the deviation. Reversible inhibition studies also were conducted on racemic β-methoxy-β-trifluoromethylphenethylamine to determine if higher concentrations of inactivator resulted in noncompetitive enhancement of the inactivation; however, these studies indicated that this compound is a competitive inhibitor with a K_i of 3.3 mM.

Electronic and steric factors do not appear to be important to the partition ratio for the 2-phenylethylamines, but there is a good correlation of partition ratio with hydrophobicity. This may indicate that the increased hydrophobicity assists in stabilizing the enzyme adduct.

In conclusion, small increases in the electron-withdrawing properties of substrates for MAO do not appear to be sufficient to transform substrates into inactivators.

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