Transformation of Heterocyclic Reversible Monoamine Oxidase-B Inactivators into Irreversible Inactivators by *N*-Methylation

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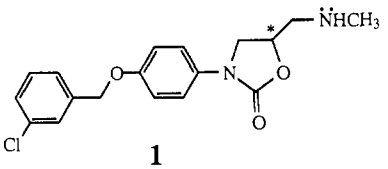
3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (1) is a secondary amine known to be a potent time-dependent irreversible inactivator of monoamine oxidase B (MAO-B). The primary amine analogues of derivatives of 1, as well as of the corresponding dihydrofuranone and pyrrolidinone, had been shown to be time-dependent, but reversible, inhibitors of MAO-B. Here it is shown that the primary amine analogue of 1 is a time-dependent reversible inhibitor of MAO-B and that the secondary and tertiary amine analogues of the corresponding oxazolidinone, dihydrofuranone, and pyrrolidinone are time-dependent irreversible inhibitors of MAO-B. The reaction leading to the irreversible enzyme adduct formation with 1 can be reversed by increasing the temperature. These results are consistent with a stabilizing stereoelectronic effect on the enzyme adduct caused by *N*-methylation which hinders free rotation and prevents the sp^3-orbital containing the nitrogen nonbonded electrons from being *trans* to the active site amino acid leaving group.

Monoamine oxidase (EC 1.4.3.4; MAO), a flavoenzyme involved in the catabolism of a variety of amine neurotransmitters, exists in two isozymic forms known as MAO-A and MAO-B which have different substrate specificities.[1] Inhibitors of MAO-A are used in the treatment of depression,[2] and inhibitors of MAO-B are used in the treatment of Parkinson's disease.[3] 3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(methylamino)methyl]-2-oxazolidinone (1, MD 780236) was reported to be a



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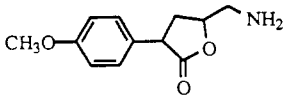
selective irreversible MAO-B inhibitor,[4] and its mechanism of inactivation of MAO-B was investigated.[5] Our results suggested that inactivation resulted from the formation of a covalent adduct to an active site amino acid residue having structure 2 (Scheme I). The stability of 2 was proposed[5] to arise from the electron-withdrawing effect of the heterocycle, which would stabilize the sp^3 hybridization and diminish adduct breakdown by elimination to 3. On the basis of this hypothesis we synthesized two other inactivators of MAO-B, namely, 5-(aminomethyl)-3-(4-methoxyphenyl)dihydrofuran-2(3*H*)-one[6] (4) and



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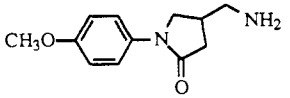

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4-(aminomethyl)-1-(4-methoxyphenyl)-2-pyrrolidinone[7] (5). Unlike 1, which is a secondary amine, 4 and 5 are primary amines. Although 4 and 5 also were time-dependent inhibitors, they exhibited biphasic kinetics, and dialysis of the inhibited enzyme led to complete return of enzyme activity. When MAO-B was inactivated by 1, monophasic kinetics were observed, and no enzyme activity returned upon dialysis.

Previously, we showed that good substrates for MAO-B could be transformed into time-dependent inhibitors by *N*-methylation.[8] This was rationalized in terms of a stereoelectronic effect (Scheme II). A gauche-butane interaction between the *N*-methyl group and the groups on the adjacent carbon atom of the main chain (R') could increase the torsional energy, thereby hindering rotation and preventing the sp^3-orbital containing the nitrogen nonbonded electrons from attaining a *trans* geometry relative to the amino acid residue leaving group. That would result in the stabilization of the adducts (2a) and diminish breakdown to 3. In this paper we utilize this hypothesis to rationalize the cause for the reversibility of inhibition of MAO by 4 and 5 and the irreversibility of inhibition by 1 and other *N*-methylated heterocyclic analogues.

Results

Chemistry. Syntheses of 3-(4-Methoxyphenyl)-5-[(*N*-methylamino)methyl]-2-oxazolidinone (10a) and 5-[(*N*,*N*-Dimethylamino)methyl]-3-(4-methoxyphenyl)-2-oxazolidinone (11a). The syntheses follow the general synthetic route outlined in Scheme III. Condensation of anisidine and glycidol (6) at 60 °C under argon produced aminodiol 7.[9] Reaction of 7 with dimethyl carbonate in toluene at reflux afforded oxazolidinone 8.[5] Mesylation of the hydroxyl group was accomplished by treatment with methanesulfonyl chloride and triethyl amine in dichloromethane at 0 °C under argon to give mesylate 9.[10] Both 10a and 11a were synthesized by reaction of 9 with either aqueous methylamine or dimethylamine, respectively, in sealed tubes at 80 °C for 18 h.

Compound 11b was synthesized by reaction of 1 with formaldehyde and formic acid at 90 °C. Compound 12b was prepared by hydrogenolysis of the corresponding azide.

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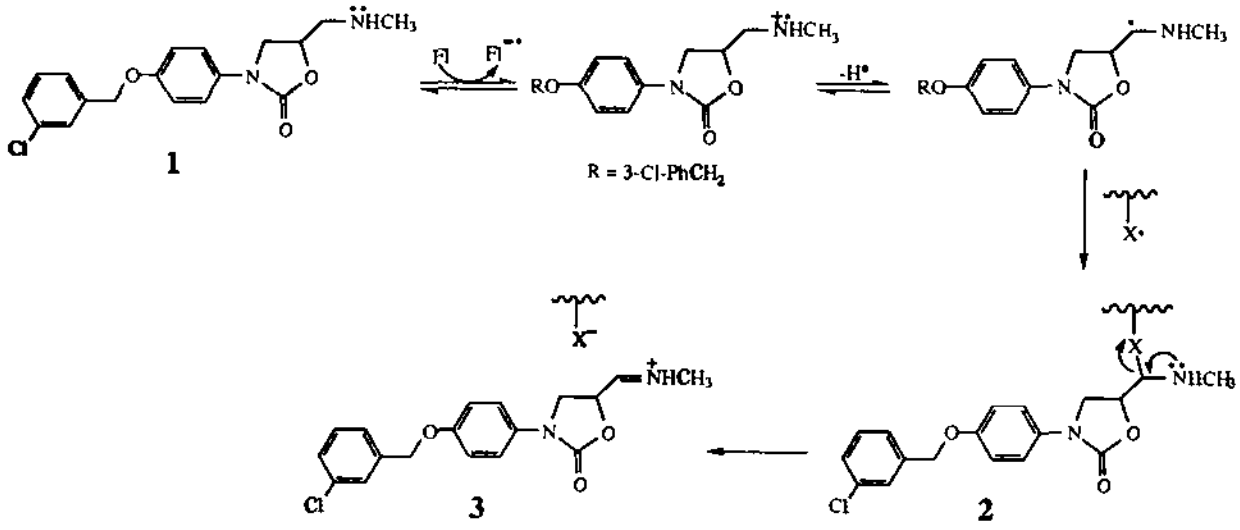
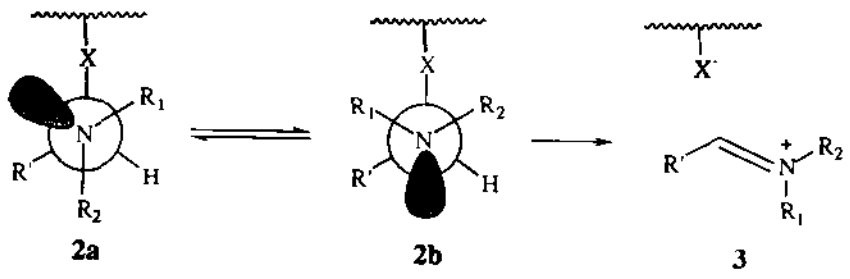
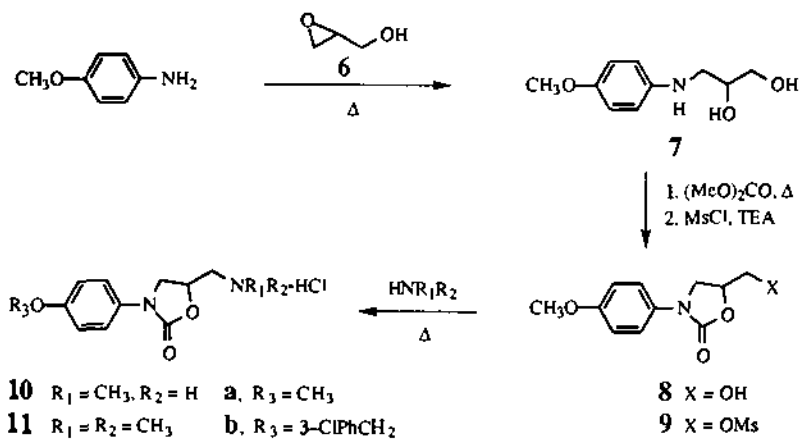
Scheme I



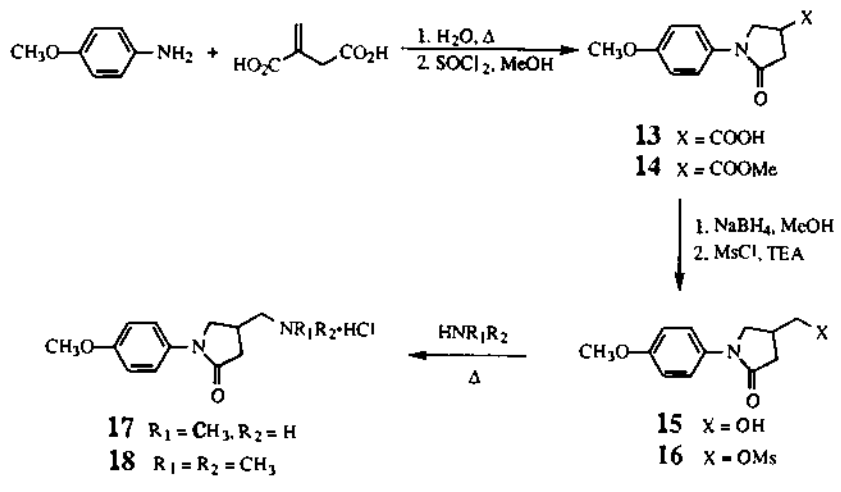
Scheme II



Scheme III



Scheme IV



Syntheses of 1-(4-Methoxyphenyl)-4-[(*N*-methylamino)methyl]-dihydropyrrolin-2-one (17) and 4-[(*N*,*N*-Dimethylamino)methyl]-1-(4-methoxyphenyl)dihydropyrrolin-2-one (18). The syntheses followed the general route shown in Scheme IV. Reaction of anisidine with itaconic acid in refluxing water generated acid **13**,[11] which was esterified by treatment with thionyl chloride in methanol at 0 °C to give methyl ester **14**.[12] Selective reduction of the ester functionality to alcohol **15**,[13] followed by mesylation, produced **16**.[10] Both **17** and **18** were synthesized by reaction of **16** with either aqueous methylamine or aqueous dimethylamine, respectively, in sealed tubes at 80 °C for 18 h.

Scheme V

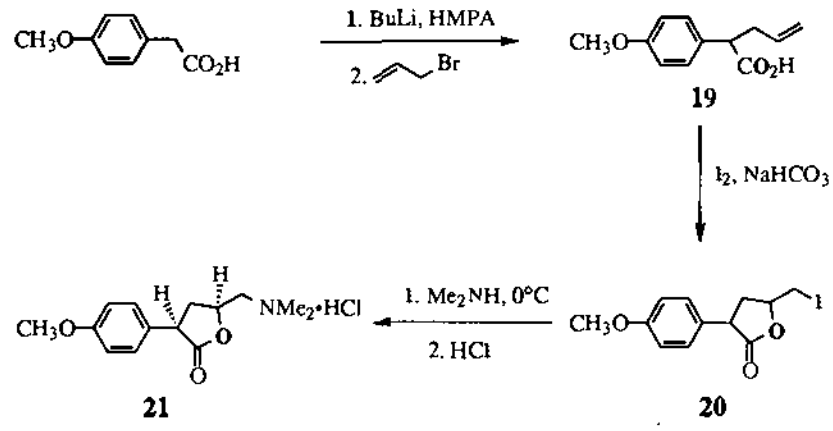


Table 1. Kinetic Constants for Secondary and Tertiary Amine Inactivators

compd	K_I (mM)	$k_{inact} \times 10^{-3}$ (min^{-1})
10a	4.7	1.9
10b	0.2	25
11a	13.3	3.0
11b	0.5	82
12a[a]	12.5	0.8
12b	0.067	140
5[b]	6.7	0.67
17	50	3.5
18	300	3.5
4 (*cis*)[a]	6.7	4.4
21	200	17

[a] Taken from ref 6. [b] Taken from ref 7.

Synthesis of 3-(4-Methoxyphenyl)-5-[(*N*,*N*-dimethylamino)methyl]dihydrofuran-2(3*H*)-one (21). The synthesis of **21** (Scheme V) began with alkylation of *p*-methoxyphenylacetic acid with allyl bromide to give 2-(*p*-methoxyphenyl)-4-pentenoic acid (**19**).[14] Iodolactonization of **19** generated iodo lactone **20**.[15] Reaction of **20** with aqueous dimethylamine at 0 °C afforded **21**.

Enzymology. Incubation of MAO B with compounds **10a**, **10b**, **11a**, **11b**, **12b**, **17**, **18**, and **21** resulted in time-dependent inactivation of the enzyme with pseudo-first-order kinetics. The kinetic constants (Table I) were determined from a replot of the half-lives of inactivation at a given concentration of inactivator.[16] Dialysis of inactivated MAO-B by these compounds at room temperature for 18 h resulted in no return of MAO-B activity. At 37 °C, however, enzyme inactivated by the primary amine **12b** fully regained activity in 18 h, whereas enzyme inactivated by the secondary amine **10b** regained only 4% of activity, and that inactivated by the tertiary amine **11b** did not regain any activity. At 45 °C **12b**-inactivated MAO-B regained complete activity in less than 1 h and **10b**-inactivated MAO-B regained only 23% of its activity after 18 h.

Discussion

The previously reported primary amine heterocycles (4 (*cis* or *trans*)[6] and 5[7]) did not contain the 4-(3-chlorophenyl)methoxyl side chain found in 1. However, the reversibility of inactivation of MAO by 4 and 5 does not stem from the lack of this side chain, because the primary amine oxazolidinone analogue **12b** also is a reversible time-dependent inhibitor, although not as readily reversible as are 4 and 5. The increased stability of **12b** relative to 4 and 5 may stem from additional binding interactions of the 4-(3-chlorophenyl)methoxyl side chain with the active site. Conversion of the primary amines 4 (*cis*), 5, or **12a** to the corresponding secondary (**17** or **10a**) or tertiary amine analogues (**21**, **18**, or **11a**) transforms these formerly reversible MAO-B inactivators into irreversible inactivators. Therefore, *N*-methylation appears to be mostly responsible for irreversible adduct formation. The transformation of reversible time-dependent inhibitors into irreversible time-dependent inhibitors by *N*-methylation can be rationalized from the viewpoint of a stereoelectric effect as was done for conversion of MAO substrates into inactivators by *N*-methylation.[8] Breakdown of the proposed enzyme adduct (2, Scheme I) to give active enzyme and the corresponding iminium ion (3) would be facilitated by rotation of the C–N bond to a point where the sp^3-orbital containing the nitrogen nonbonded electrons becomes *trans* to the leaving group (**2b**, Scheme II), i.e., the active site amino acid residue. Hindrance to C–N bond rotation, therefore, could stabilize 2 as depicted by **2a**. This hindrance could arise from gauche–butane interactions between the *N*-methyl group and either the enzyme amino acid residue to which the inactivator is attached (X) or the heterocyclic ring (R'). If the orbital containing the nonbonded electrons is locked in a position gauche to the leaving group (**2a**), then adduct stability would be enhanced considerably.

If hindered bond rotation is important, then an increase in temperature should be useful to overcome the torsional energy barrier, and should lead to reactivation of the enzyme. This, in fact, was observed. As the temperature of the dialysis buffer was increased, the amounts of reactivated enzyme also increased. Although 1 (i.e., **10b**) is an irreversible inactivator at 25 °C, when the temperature is raised, the adduct begins to break down and give active enzyme (see Results). This temperature dependence could account for the discrepancy between the reported in vitro[4,5] irreversibility and the in vivo[17] reversibility of 1. Similar temperature-dependent enzyme reactivation was observed with **12b** except reactivation was faster.

Conclusion

N-Methylation of various heterocyclic time-dependent reversible inhibitors of MAO transforms these inhibitors into time-dependent irreversible inhibitors. The more recent interest in reversible inhibitors of MAO to avoid hypertensive problems associated with peripheral MAO-A inhibition[18] suggests that primary amine design may be beneficial over design of secondary or tertiary amines. If irreversible inhibition is desired, however, then secondary or tertiary amines should be contemplated.

Experimental Section

General. NMR spectra were recorded either on a Varian EM-390 90 MHz, a Varian Gemini-300 300 MHz, or on a Varian XL-400 400-MHz spectrometer. Chemical shifts are reported as δ values in parts per million downfield from Me_4Si as the internal standard in $CDCl_3$. Thin-layer chromatography was performed on EM/UV silica gel plates with a UV indicator. Melting points were obtained with a Fisher-Johns melting point apparatus and are uncorrected. Elemental combustion analyses were performed by G. D. Searle Analytical Laboratory, Skokie, IL. Mass spectra were recorded on a VG Instruments VG70-250SE high-resolution spectrometer. Column chromatography was performed with Merck silica gel (230–400 mesh). All chemicals were purchased from Aldrich Chemical Co. and were used without further purification. THF and ether were freshly distilled from sodium. Methylene chloride was dried by passage over an alumina column. Triethylamine was distilled from BaO before use. Glassware was dried in an oven overnight when dry conditions were required. All reactions were carried out in an atmosphere of inert gas (nitrogen or argon).

3-[(4-Methoxyphenyl)amino]-1,2-propanediol (7). A mixture of *p*-anisidine (5.0 g, 40.6 mmol) and glycidol (6, 0.7 mL, 11.0 mmol) was heated at 65 °C for 8 h under an atmosphere of argon. The excess *p*-anisidine was removed in vacuo and the product was distilled at 160–170 °C/1.0 mmHg to give a yellowish solid (1.4 g, 64%): mp 70–72 °C (lit.[5] mp 72–73 °C); ^{1}H NMR (DMSO-d_6) δ 6.50–6.75 (m, 4 H), 4.95 (br t, 1 H), 4.71 (d, 1 H), 3.57 (t, 1 H), 3.63 (m, 4 H), 3.40 (t, 2 H), 3.10 (m, 2 H), 2.84 (m, 2 H); HRMS (EI) calcd for $C_{10}H_{15}NO_3$ 197.1052, found 197.1055.

5-(Hydroxymethyl)-3-(4-methoxyphenyl)-2-oxazolidinone (8) and 5-[[(methylsulfonyl)oxy]methyl]-3-(4-methoxyphenyl)-2-oxazolidinone (9) were synthesized by the procedures previously reported.[5]

3-(4-Methoxyphenyl)-5-[(*N*-methylamino)methyl]-2-oxazolidinone Hydrochloride (10a). A mixture of aqueous methylamine (5 mL) and mesylate 9 (100 mg, 0.33 mmol) dissolved in THF (1.0 mL) was heated in a sealed tube at 80 °C for 18 h. The mixture was cooled to room temperature and extracted with methylene chloride. The organic extract was washed with 10% aqueous ammonia and dried over magnesium sulfate, and the solvent was removed. The resultant residue was redissolved in methanol and gaseous HCl was bubbled into the methanol solution to saturation. The methanol was removed *in vacuo*, and the residue was recrystallized from methanol–ethyl acetate, giving the product as white crystals (76 mg, 85%): mp 204–206 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.34 (d, J = 9.0 Hz, 2 H), 6.98 (d, J = 9.0 Hz, 2 H), 5.08 (m, 1 H), 4.23 (t, J = 9.0 Hz, 1 H), 3.80 (s, 3 H), 3.78 (m, 1 H), 3.45 (m, 2 H), 2.80 (s, 3 H); ^{13}C NMR δ 156.95, 156.67, 130.07, 123.01, 114.71, 69.54, 55.66, 51.26, 49.05, 33.28. Anal. ($C_{12}H_{17}ClN_2O_3$) C, H, N.

5-[(*N*,*N*-Dimethylamino)methyl]-3-(4-methoxyphenyl)-2-oxazolidinone Hydrochloride (11a). The same procedure as for the preparation of 9 was employed, except aqueous dimethylamine was used. The title compound was obtained in a 79% yield as a crystalline solid: mp 191–192 °C; ^{1}H NMR (300 MHz, D_2O) δ 7.38 (d, J = 9.0 Hz, 2 H), 7.05 (d, J = 9.0 Hz, 2 H), 5.25 (m, 1 H), 4.30 (t, J = 9.0 Hz, 1 H), 3.81 (s, 3 H), 3.70 (dd, J = 9, 11 Hz, 1 H), 3.50 (d, J = 11 Hz, 1 H). Anal. ($C_{13}H_{19}ClN_2O_3$) C, H, Cl, N.

3-[4-[(3-Chlorophenyl)methoxy]phenyl]-5-[(dimethylamino)methyl]-2-oxazolidinone (11b). Compound 1 (16 mg) was dissolved in 0.4 mL of 88% formic acid and 0.4 mL of aqueous formaldehyde and then was heated in an oil bath at 90 °C for 24 h. After being allowed to cool, 10% aqueous hydrochloric acid (0.2 mL) was added, the volatiles were removed by evaporation, and the residue was evaporated from toluene. The resulting white solid was recrystallized from ethyl acetate–ethanol–cyclohexane: mp 183.5–184.5 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.54 (s, 1 H), 7.41 (m, 5 H), 7.11 (d, 2 H), 5.28 (m, 1 H), 5.18 (s, 2 H), 4.86 (HDO), 4.33 (dd, 1 H), 3.86 (dd, 1 H), 3.73 (dd, 1 H), 3.56 (d, 1 H), 3.04 (s, 6 H); HRMS (EI) calcd for $C_{19}H_{21}ClN_2O_3$ 360.1241, found 360.1236.

5-(Aminomethyl)-3-(4-methoxyphenyl)-2-oxazolidinone hydrochloride (12a) was prepared by the previously reported procedure.[5]

(*S*)-5-(Aminomethyl)-3-[4-[(3-chlorophenyl)methoxy]phenyl]-2-oxazolidinone Hydrochloride (12b). (*S*)-3-[4-[(3-chlorophenyl)methoxy]phenyl]-5-(hydroxymethyl)-2-oxazolidinone[5] (200 mg, 0.6 mmol) was dissolved in pyridine (4 mL) and cooled in an ice bath, and then tosyl chloride (114 mg, 0.6 mmol)

was added. After being stirred for 35 min under nitrogen, the reaction mixture was stored in a refrigerator for 16 h. Ethyl acetate (50 mL) was added and was washed twice with 10% HCl (25 mL) and then with 25 mL each of saturated sodium bicarbonate and brine. After being dried over magnesium sulfate, the solvent was evaporated under vacuum and the resulting orange oil was chromatographed on silica gel (EtOAc). The light orange oily solid that was obtained after evaporation of the solvent was dissolved in DMF (1 mL) and sodium azide (195 mg, 3 mmol) was added. The mixture was stirred for 1 h at 50 °C then at 70 °C overnight. The reaction mixture was diluted with water (20 mL) and extracted with dichloromethane (3 × 15 mL). The organic extracts were combined and dried ($MgSO_4$), and the solvent was evaporated. The product was dissolved in 3:1 methanol–ethyl acetate (8 mL) and Lindlar's catalyst (50 mg) was added. After being evacuated and purged with hydrogen three times, the mixture was stirred at room temperature under an atmosphere of hydrogen for 3 h. The reaction mixture was filtered and the solvent was evaporated. The resulting oil was dissolved in chloroform and HCl gas was bubbled in. The solvent was removed and the resulting solid was recrystallized from ethanol and then from ethanol–ether to give a white solid (60 mg, 27%); mp 242–245 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.56 (s, 1 H), 7.43 (m, 5 H), 7.12 (d, 2 H), 5.20 (s, 2 H), 5.11 (m, 1 H), 4.85 (HDO), 4.33 (dd, 1 H), 3.90 (dd, 1 H), 3.47 (d, 2 H); HRMS (EI) calcd for $C_{17}H_{17}ClN_2O_3$ 332.0928, found, 332.0938.

1-(4-Methoxyphenyl)-2-oxopyrrolidine-4-carboxylic Acid (13). Anisidine (23.5 g, 0.19 mol) was added to a solution of itaconic acid (24.8 g, 0.19 mol) in 190 mL of water. The resulting mixture was heated at reflux for 3 h. The aqueous solution was cooled to room temperature, and the crystalline solid that formed was collected by filtration and washed twice with cold water, giving **10** (43.2 g, 97%): mp 168–170 °C; ^{1}H NMR (90 MHz, $CDCl_3$/DMSO) δ 7.45 (d, 2 H), 6.87 (d, 2 H), 6.05 (bs, 1 H), 4.00 (d, 2 H), 3.78 (s, 3 H), 3.32 (m, 1 H), and 2.80 (dd, 2 H); IR (KBr) 3020 (br, s), 1725 (s), 1646 cm^{-1} (s); HRMS (EI) calcd for $C_{12}H_{13}NO_4$ 235.0844, found 235.0840.

Methyl 1-(4-Methoxyphenyl)-2-oxopyrrolidine-4-carboxylate (14). To a solution of **13** (21.0 g, 89.3 mmol) in 100 mL of methanol at 0 °C was added thionyl chloride (6.5 mL, 89.3 mmol) via a syringe. The resulting mixture was stirred at 0 °C for 1.5 h, and then the temperature was allowed to rise to room temperature. The mixture was stirred for 3 h at room temperature, then the methanol was removed in vacuo, and the residue was bulb-to-bulb distilled at 220–230 °C/2 mmHg. The title product was obtained as a white solid in 93% yield: mp 86–88 °C; ^{1}H NMR (90 MHz, $CDCl_3$) δ 7.46 (d, 2 H), 6.90 (d, 2 H), 4.03 (dd, 2 H), 3.75 (s, 3 H), 3.73 (s, 3 H), 3.35 (m, 1 H), 2.85 (dd, 2 H); IR (KBr) 1733 (s), 1691 (s), 1513 cm^{-1} (s); HRMS (EI) calcd for $C_{13}H_{15}NO_4$ 249.1001, found 249.1001. Anal. ($C_{13}H_{15}NO_4$) C, H, N.

4-(Hydroxymethyl)-1-(4-methoxyphenyl)-2-pyrrolidinone (15). To a stirred solution of **14** (1.1 g, 4.4 mmol) in 40 mL of methanol under N_2 at room temperature was added sodium borohydride (335 mg, 8.8 mmol) portionwise. The resulting cloudy solution was heated at reflux for 2 h, more sodium borohydride (190 mg, 5.0 mmol) was added, and reflux was continued for 3 h. The resulting mixture was chilled to 0 °C, the residual sodium borohydride was destroyed by successive addition of water (5 mL) and 10% HCl (5 mL), and the methanol was removed in vacuo. The aqueous residue was extracted with ethyl acetate (3 × 25 mL), and the combined organic extracts were extracted with saturated sodium bicarbonate and dried over magnesium sulfate. Removal of the ethyl acetate and purification of the resulting crude product by silica gel column chromatography (R_f = 0.25, ethyl acetate) gave a white crystalline solid (0.65 g, 67%): mp 111–113 °C; ^{1}H NMR (300 M Hz, $CDCl_3$) δ 7.50 (d, J = 9 Hz, 2 H), 6.90 (d, J = 9 Hz, 2 H), 3.92 (dd, 1 H), 3.80 (s, 3 H), 3.65 (m, 3 H), 3.10 (br s, 1 H), 2.70 (m, 2 H), 2.36 (m, 1 H); ^{13}C NMR (300 MHz, $CDCl_3$) δ 173.36, 156.75, 132.31, 122.10, 114.07, 64.21, 55.50, 51.83, 35.31, 33.10; IR (KBr) 3361 (br, s), 1647 (s), 1515 cm^{-1} (s); HRMS (EI) calcd for $C_{12}H_{15}NO_3$ 221.1052, found 221.1051. Anal. ($C_{12}H_{15}NO_3$) C, H, N.

4-[[(Methylsulfonyl)oxy]methyl]-1-(4-methoxyphenyl)-2-pyrrolidinone (16). To a stirred solution of **15** (0.55 g, 2.5 mmol) in 20 mL of anhydrous methylene chloride at 0 °C under nitrogen was added triethylamine (0.5 mL, 3.6 mmol) via syringe followed by methanesulfonyl chloride (0.23 mL, 3.0 mmol). The reaction was complete, as evidenced by TLC analysis, in 30 min after stirring at 0 °C. The resulting mixture was extracted successively with 15 mL each of water, 10% HCl, and saturated sodium bicarbonate solution. The organic layer was dried over magnesium sulfate and the solvent was removed in vacuo to give white crystals (0.68 g, 92%): mp 105–107 °C; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.40 (d, 2 H), 6.85 (d, 2 H), 4.27 (dd, 1 H), 4.22 (dd, 1 H), 3.93 (dd, 1 H), 3.73 (s, 3 H), 3.65 (dd, 1 H), 3.00 (s, 3 H), 2.89 (m, 1 H), 2.73 (dd, 1 H), 2.38 (dd, 1 H); IR (KBr) 1694 (s), 1516 (s), 1357 (s), 1350 (s), 1250 (s), 1162 cm^{-1} (s); HRMS (EI) calcd for $C_{13}H_{17}NO_5S$ 299.0827, found 299.0818. Anal. ($C_{13}H_{17}NO_5S$) C, H, N.

1-(4-Methoxyphenyl)-4-[(*N*-methylamino)methyl]-2-pyrrolidinone Hydrochloride (17). The mesylate **16** (1.0 g, 3.37 mmol) was dissolved in 5 mL of THF and was added to a precooled aqueous solution of methylamine (40% aqueous solution, 4.0 mL) in a resealable tube. The tube was sealed and the mixture was heated in an oil bath at 80 °C for 18 h, after which time TLC indicated that all of the starting material was consumed (R_f = 0.35, ethyl acetate). The mixture was cooled to room temperature and concentrated in vacuo, and the residue was extracted with ethyl acetate. The organic layer was extracted with 10 mL of 10% KOH solution and dried over magnesium sulfate. After removal of the organic solvent, the residue was redissolved in methanol, gaseous HCl was bubbled in to saturation, the methanol was removed, and the resulting solid was recrystallized from ethanol, ethyl acetate, and cyclohexane (1:10:2), giving the product as white crystals (0.52 g, 57%): mp 158–160 °C. ^{1}H NMR (400 MHz, D_2O) δ 7.40 (d, J = 9.0 Hz, 2 H), 7.10 (d, J = 9.0 Hz, 2 H), 4.10 (dd, J_1 = 8 Hz, J_2 = 10 Hz, 1 H), 3.90 (s, 3 H), 3.75 (dd, J_1 = 6.5 Hz, J_2 = 10 Hz, 1 H), 3.32 (dd, J_1 = 2.5 Hz, J_2 = 7.0 Hz, 2 H), 3.05 (h, J = 8 Hz, 1 H), 2.93 (dd, J_1 = 9.0 Hz, J_2 = 17.0 Hz, 1 H), 2.80 (s, 3 H), 2.57 (dd, J_1 = 7.5 Hz, J_2 = 17.0 Hz, 1 H); ^{13}C NMR, δ 178.21, 160.52, 151.07, 127.97, 117.58, 58.51, 56.54, 54.65, 38.64, 36.21, 31.80. Anal. ($C_{13}H_{19}ClN_2O_2$) C, H, N.

4-[(*N*,*N*-Dimethylamino)methyl]-1-(4-methoxyphenyl)-2-pyrrolidinone Hydrochloride (18). The same procedure as for the preparation of **17** was employed, except aqueous dimethylamine was used. The title compound was prepared in a 74% yield: mp 198–199 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.40 (d, J = 9.0 Hz, 2 H), 7.10 (d, J = 9.0 Hz, 2 H), 4.13 (t, J = 9.0 Hz, 1 H), 3.90 (s, 3 H), 3.77 (t, J = 9.0 Hz, 1 H), 3.46 (d, J = 7.0 Hz, 2 H), 3.18 (p, J = 7.0 Hz, 1 H), 3.00 (s, 6 H), 2.95 (dd, J_1 = 9.0 Hz, J_2 = 17.0 Hz, 1 H), 2.60 (dd, J_1 = 7.5 Hz, J_2 = 17.0 Hz, 1 H). Anal. ($C_{14}H_{21}ClN_2O_2$) C, H, Cl, N.

2-(4-Methoxyphenyl)-4-pentenoic Acid (19). To a solution of *p*-methoxyphenylacetic acid (15 g, 90 mmol) in 300 mL of anhydrous THF in an ice bath under argon was added a solution of *n*-butyllithium (2.5 M, 72 mL, 180 mmol) dropwise over 45 min. After the addition was complete, the mixture was stirred at 0 °C for 1 h, then at room temperature for 2 h. Upon addition of hexamethylphosphoramide (15 mL), a yellowish homogeneous solution resulted, which was stirred for 30 min and then cooled to 0 °C. Allyl bromide (8 mL, 92.4 mmol) was added via syringe, and then the resulting mixture was stirred at 0 °C for 1 h and at room temperature for 4 h. The reaction was quenched by addition of an aqueous 10% HCl solution (200 mL) at 0 °C. The resulting two-layer mixture was concentrated in vacuo and then was extracted with ethyl acetate (2 × 200 mL). The combined organic extracts were washed with brine and dried over sodium sulfate. After removal of the solvent in vacuo, the residue was distilled at 175–180 °C/5 mmHg. The product was obtained as a colorless oil, which solidified to a semisolid upon standing (17.5 g, 95%); ^{1}H NMR (400 MHz, $CDCl_3$) δ 10.50 (br, 1 H), 7.35 (d, 2 H), 6.90 (d, 2H), 5.80 (m, 1H), 5.10 (dd, 2 H), 3.80 (s, 3 H), 3.62 (t, 1 H), 2.85 (m, 1 H), 2.55 (m, 1H); IR (neat) 3100 (br, s), 1714 (s), 1614 (s).

5-(Iodomethyl)-3-(4-methoxyphenyl)furan-2(3*H*)-one (20). A solution of **19** (7.5 g, 36.4 mmol) in ether (45 mL) was added to saturated sodium bicarbonate (180 mL) at 0 °C under nitrogen. Iodine (27.7 g, 109 mmol) in 75 mL of THF was added at 0 °C over 45 min, then the mixture was stirred at 0 °C for 4 h. The reaction was quenched by addition of saturated sodium thiosulfate at 0 °C, and the resulting clear solution was concentrated in

vacuo. The aqueous layer was extracted with ethyl acetate (3 × 150 mL), and the combined extracts were washed with brine and dried over magnesium sulfate. Removal of the solvent resulted in a yellowish oil (10 g, 83%), which was identified by NMR spectral analysis as a mixture of the *cis*- and *trans*-iodo lactones in a ratio of 2:1. The diastereomeric iodo lactones could be separated by careful silica gel column chromatography eluting with a mixture of hexane and ethyl acetate (2:1) to give two products.

***cis*-20**: A colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.21 (d, 2 H), 6.90 (d, 2 H), 4.48 (m, 1 H), 3.90 (dd, 1 H), 3.80 (s, 3 H), 3.50 (dd, 1 H), 3.37 (dd, 1 H), 2.90 (m, 1 H), and 2.10 (q, 1 H); IR 1771 cm^{-1}.

***trans*-20**: A colorless oil; ^{1}H NMR (400 MHz, $CDCl_3$) δ 7.18 (d, 2 H), 6.90 (d, 2 H), 4.70 (m, 1 H), 3.97 (t, 1 H), 3.80 (s, 3 H), 3.43 (dd, 1 H), 3.35 (dd, 1 H), 2.55 (m, 1H); IR 1772 cm^{-1}.

***cis*-5-[(*N*,*N*-Dimethylamino)methyl]-3-(4-methoxyphenyl)-dihydrofuran-2(3*H*)-one Hydrochloride (21)**. A mixture of two isomeric iodo lactones (1.5 g, 4.5 mmol) was dissolved in 10 mL of THF. This was slowly added to an aqueous solution of dimethylamine (40%, 5.0 mL) at 0 °C in an ice bath. The mixture was stirred for 18 h; during this period the temperature was slowly raised to room temperature. The resultant mixture was poured into 50 mL of ethyl acetate. The organic layer was extracted with 20 mL each of water and 10% KOH solution and dried over magnesium sulfate. After removal of the organic solvent, the residue was redissolved in methanol, and gaseous HCl was introduced to saturation. The methanol was removed, and the resulting solid was recrystallized twice from ethanol, ethyl acetate, and cyclohexane (1:10:2), giving white crystals of pure *cis*-isomer (0.4 g, 31%): mp 183–185 °C; ^{1}H NMR (400 MHz, D_2O) δ 7.34 (d, J = 9.0 Hz, 2 H), 7.08 (d, J = 9.0 Hz, 2 H), 5.20 (m, 1 H), 4.25 (dd, $J1$ = 9.0 Hz, $J2$ = 8.5 Hz, 1 H), 3.90 (s. 3 H), 3.64 (d, J = 6.0 Hz, 2 H), 3.07 (s, 6 H), 2.95 (m, 1 H), 2.25 (q, J = 11 Hz, 1 H). Anal. ($C_{14}H_{20}ClNO_3$) C, H, Cl, N.

Enzyme and Assays. Bovine liver MAO-B was isolated according to the method of Salach[19] having a specific activity 4–6 units/mg. MAO activity was assayed by a modified published procedure[20] in Tris buffer (100 mM, pH 9.0) at 25 °C with cinnamylamine as substrate.

Time-Dependent Inactivation Experiments (General Methods). Solutions (180 μL each) of an inactivator hydrochloride at various concentrations and a control containing no inactivator in potassium phosphate buffer (100 mM, pH 7.40) in the presence of 2.0 mM glutathione were preincubated at 25 °C. To these solutions was added MAO-B (20 μL of 6 mg/mL). After being mixed the samples were incubated at 25 °C, periodically agitated, and assayed for MAO activity by adding 10 μL of the mixture to 490 μL of cinnamylamine solution in Tris buffer (0.50 mM, pH 9.0) as described above. The enzyme activity thus determined was corrected against a control containing no inactivator. Kinetic constants (K_I and k_{inact}) were determined as described by Kitz and Wilson.[16]

Reactivation Studies (General) MAO B (~1.0 mg) was incubated with an inactivator at an appropriate concentration in a total volume of 1200 μL in potassium phosphate buffer (100 mM, pH 7.4) in the presence of 2.0 mM glutathione at 25 °C until about 90% of the enzyme was inactivated. The incubation mixture and a control (1000 μL each) were transferred to dialysis bags (Spectrum, 12 000 MW cutoff) and were dialyzed against 4 L of potassium phosphate buffer (100 mM, pH 7.0) in the presence of 0.1% Triton X-100 at various temperatures. The MAO-B activity was assayed by periodically removing 20 μL of the mixture and adding to 480 μL of a cinnamylamine solution in Tris buffer (0.50 mM, pH 9.0) as described above. The enzyme activity thus determined was corrected against the uninactivated control.

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