Molecular Size and Flexibility as Determinants of Selectivity in the Oxidation of *N*-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine Analogs by Monoamine Oxidase A and B

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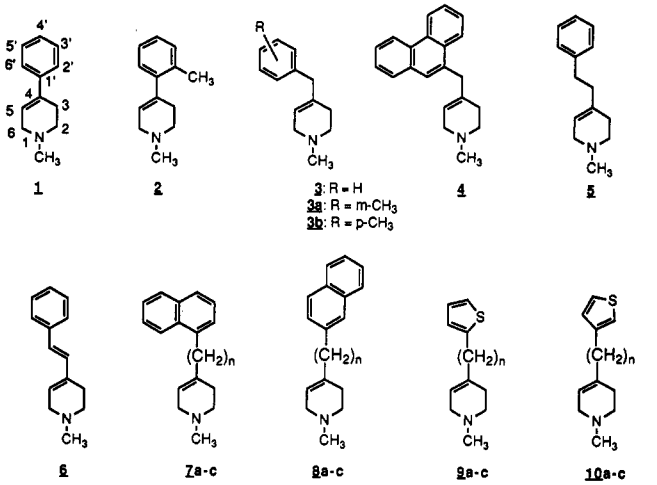
The introduction of a methylene bridge between the phenyl and tetrahydropyridyl moieties of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) results in increased selectivity for monoamine oxidase B (MAO B) over monoamine oxidase A (MAO A). However, lengthening of this bridge results in a total loss of selectivity. In the present study, a number of isomeric 4-naphthyl-, 4-(naphthylalkyl)-, 4-thienyl-, and 4-(thienylalkyl)tetrahydropyridines, conformationally restrained and flexible analogs of MPTP, were synthesized and evaluated as potential selective substrates of MAO A and B. In terms of the parameter (turnover number)/K_m, the bulky naphthyl analogs were invariably better substrates of MAO A than kynuramine, the reference substrate for this enzyme. In addition, all naphthyl analogs, regardless of conformational mobility, were more effective substrates of MAO A than MAO B. Similarly, all thienyl analogs were found to be more effective substrates of MAO B. In contrast to the naphthalenes, the conformationally restrained thiophenes **9a** and **10a** were found to be poor substrates of MAO B, relative to benzylamine, the reference substrate. These results suggest that the selectivity of these compounds for either MAO A or B is determined by the complex interplay of molecular size and flexibility. In this interplay, either one of these two factors may predominate.

Introduction

Monoamine oxidase-catalyzed bioactivation of *N*-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP, **1**; see Chart I) is a key step in the sequence of events culminating in MPTP-induced neurotoxicity.[1-4] This discovery has stimulated great interest in the role of this enzyme in the biotransformation of small organic molecules. Although MPTP is oxidized principally by MAO B,[1,2] one of the two distinct forms of mammalian MAO, seemingly minor structural modifications of MPTP have been shown to alter the selectivity in favor of either MAO A or B. Case in point, 2'-alkylation (exemplified by **2**) has been found to increase preference for MAO A over MAO B.[5,6] On the other hand, the insertion of a methylene bridge between the phenyl and tetrahydropyridyl moieties of MPTP, to yield 4-homo-MPTP, **3**, results in increased selectivity for MAO B.[6]

In an earlier study[7] of flexible MPTP analogs, molecular size and flexibility were identified as important determinants of selectivity for MAO A and B. Specifically, **3** (Chart I) was shown to be twice as selective for MAO B as MPTP. However, the ethylene-bridged analog **5** was oxidized by both enzymes with comparable efficiency. In contrast to the latter, the rigid *trans*-stilbazole **6** was found to be a poor substrate for both MAO A and B.[7,8] In general, bulky MPTP analogs such as **4**, **7b**, and **8b** were shown to be preferentially oxidized by MAO A, while smaller analogs

Chart I



such as **9b** showed a marked preference for MAO B. The substituted thiophene, **9b**, was identified as the most selective substrate for MAO B, while **7b** and **8b** showed the highest level of selectivity for MAO A. On the other hand, MPTP analogs of intermediate size (e.g., **3a**, **3b**, **5**) were oxidized with comparable facility by both enzymes, further supporting the concept of a selectivity continuum. These initial findings prompted continued investigation of the effects of substrate size and flexibility on selectivity for MAO A and B.

A particularly interesting observation that emerged from these studies relates to the selectivity profiles of MPTP, **3**, and **5**. From these three analogs, it is clear that a one-carbon bridge (exemplified by **3**) increases selectivity for

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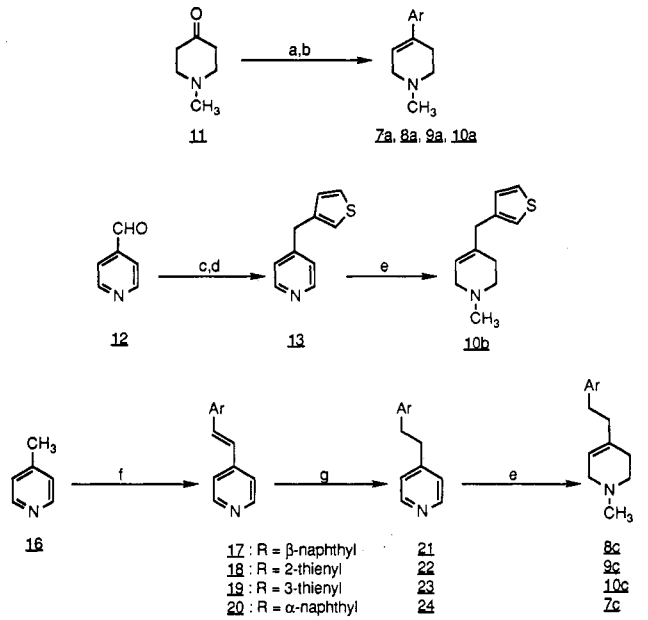
Scheme I[a]



[a] (a) Grignard reagent, THF, reflux; (b) 6 N HCl, reflux; (c) aryl bromide, *n*-BuLi, −78 °C; (d) Zn, HCO_2H, reflux; (e) MeI, $NaBH_4$, MeOH; (f) aryl carboxaldehyde, Ac_2O, reflux; (g) H_2, Pd/C, EtOH.

MAO B. However, selectivity is totally abolished by the two-carbon bridge (compare **3** and **5**), clearly underscoring the role of flexibility in determining selectivity for MAO A and B. In earlier studies,[6,8] increased flexibility was also associated with greater reactivity. As part of our continuing studies on MPTP-like compounds as substrates for MAO A and B, we have further investigated the effect of molecular flexibility on selectivity with compounds **7a–c**, **8a–c**, **9a–c**, and **10a–c**.

Chemistry

The synthesis of the target compounds is outlined in Scheme I. Briefly, the rigid analogs **7a**, **8a**, **9a**, and **10a** were prepared from the reaction of the ketone **11** with the appropriate Grignard reagent or aryllithium intermediate. The resulting alcohols were subsequently dehydrated to yield the desired tetrahydropyridines. The methylene-bridged analog **10b** was synthesized by a procedure reported earlier[8] for the preparation of **7b**, **8b**, and **9b**. Similarly, the *trans*-arylethenylpyridines **17–20** were converted to the corresponding ethylene-bridged tetrahydropyridines **7c**, **8c**, **9c**, and **10c** via a procedure described earlier.[8]

Results and Discussion

The kinetic parameters for the oxidation of various tetrahydropyridines by MAO A and B are given in Table I. Kinetic parameters for known substrates of the two enzymes, benzylamine and kynuramine, are also provided for comparison. In our analysis of the data, we have used the function (turnover number)/K_m (TN/K_m) to provide a useful comparison of the effectiveness of various analogs as substrates of MAO A and B. The selectivity of a given substrate is given by a selectivity index (SI) which is defined as $(TN/K_m)_{MAO\ A}/(TN/K_m)_{MAO\ B}$. By definition, a nonselective substrate would have a selectivity index of unity. Values of the SI are not shown in Table I but have been used to determine the selectivity of the substrates examined. In the ensuing discussion, we make reference to five series of analogs. For the purpose of this discussion, each series contains three analogs (n = 0–2) where n signifies the number of carbon atoms in the alkyl bridge (see Chart I). The reference series for this study consists of MPTP, **3**, and **5**.

Table I. Kinetic Constants for the Oxidation of MPTP Analogs by Highly Purified MOA A and B

	MAO A			MAO B		
compound	TN[a]	K_m[b]	TN/K_m	TN[a]	K_m[b]	TN/K_m
benzylamine	10	0.78	13	283	0.29	963[c]
kynuyramine	146	0.17	860[c]	165	0.084	1964
MPTP[d]	20	0.14	143	204	0.39	523
3[c]	9.2	0.066	139	193	0.154	1250
5[c]	137	0.12	1120	61	0.054	1130
7a	140	0.074	1893	55	0.110	500
7b[c]	443	0.167	2592	89	0.85	105
7c	244	0.076	3187	80	1.28	62
8a	10.2	0.0046	2216	73.5	0.73	101
8b[c]	121	0.036	3344	274	0.84	326
8c	95.9	0.043	2225	79	1.03	76
8a	5.21	0.188	28	12	0.168	71
9b[c]	8.4	0.048	175	337	0.102	3270
9c	42	0.108	389	51.9	0.049	1059
10a	8.14	0.102	80	4.17	0.026	160
10b	13.3	0.136	98	153	0.195	785
10c	29.7	0.13	228	50.7	0.057	890

[a] TN, turnover number (μmol of substrate/min per μmol of enzyme). [b] K_m in millimolar. [c] From ref 7. [d] From ref 6.

An earlier examination of MPTP analogs in mouse brain and liver mitochondrial preparations identified **9a** and **10a** as substrates of "monoamine oxidase".[14–16] However, the rates of oxidation of both compounds were significantly lower than those of MPTP. Additionally, relative to MPTP, the oxidation of **9a** and **10a** was significantly less sensitive to inhibition by deprenyl, a potent and selective inhibitor of MAO B.[14] These results suggested a role for MAO A, known to coexist with MAO B in these mitochondrial preparations, in the oxidation of these two thiophene analogs. The present study utilizes highly purified MAO A and B and thereby permits a clearer definition of the relative involvement of these two enzymes in the oxidation of these and other MPTP analogs.

Monoamine Oxidase A. As evident on Table I, replacement of the phenyl group of MPTP by the 2-thienyl substituent results in a 4-fold decrease in the TN accompanied by a slight increase in the K_m. The combined effect of these changes is a 5-fold decrease in the effectiveness of **9a** as a substrate for MAO A. Changing the substituent from 2-thienyl to 3-thienyl results in a noticeable increase in TN with a concomitant decrease in K_m (**9a** vs **10a**). As a result, **10a** is 3 times more effective than its positional isomer, **9a**. In contrast to benzene, thiophene is characterized by a net dipole moment. Since **9a** and **10a** would be expected to have different net dipole moments, the result of different substitution patterns on the thiophene nucleus, the disparity in their performance may be attributed to the effects of the dipoles on the interaction between these substrates and MAO A.

In contrast to the thiophenes **9a** and **10a**, the naphthyl analogs **7a** and **8a** are more effective substrates than MPTP. Specifically, the α-naphthyl analog **7a** shows a 7-fold increase in TN over MPTP. This increase is accompanied by a 2-fold decrease in the K_m (**7a** vs MPTP). On the other hand, the TN and K_m of **8a** are 2- and 30-fold lower than corresponding values for MPTP. Since **7a** and **8a** differ only in the site of substitution, these differences in TN and K_m may be a reflection of the topography of the substrate binding site of MAO A. The greater

effectiveness of **7a** and **8a** over MPTP and the thiophenes **9a** and **10a** also supports the view that MAO A prefers bulky lipophilic substituents.

The insertion of a methylene bridge between the phenyl and tetrahydropyridyl moieties of MPTP results in a 2-fold reduction in both TN and K_m. The net result is that **3** is as effective a substrate for MAO A as its lower homolog, MPTP. A similar structural modification of **9a** (to yield **9b**) results in a 60% increase in TN accompanied by a 4-fold reduction in K_m. Compound **9b** is thus 6 times more effective than its lower homolog, **9a**. However, the 3-thienyl isomer **10b** shows no increase in effectiveness over the corresponding **10a**, the result of simultaneous increases in both the TN and K_m (compare **10a** to **10b**). In contrast to the thiophenes, both methylene-bridged naphthalenes **7b** and **8b** show greater effectiveness relative to the corresponding lower homologs **7a** and **8a**. For **7b** and **8b** this increase in effectiveness is attributed to the 3- and 12-fold increases, which more than offset corresponding increases in K_m.

As reported earlier,[7] the ethylene-bridged analog **5** is a more effective substrate for MAO A than the corresponding lower homologs **3** and MPTP. This increase is largely the result of a higher TN (see Table I). Similarly, the thienyl analogs **9c** and **10c** also show increased effectiveness, attributable to higher TN, over their corresponding lower homologs. Such increases clearly reflect the role of substrate flexibility on the catalytic efficiency of the enzyme. In spite of the relative flexibility of both ethylene-bridged naphthalenes, only **7c** shows increased effectiveness over its corresponding lower homologs. However, this increase is not due to a higher TN, as is the case with the thiophenes **9c** and **10c**, but to a lower K_m value. In contrast, the β-substituted naphthalene, **8c**, is equally effective as its relatively rigid lower homolog **8a**. Additionally, **8c** is even less effective than the methylene-bridged homolog **8b**. A comparison of the α- and β-substituted naphthalenes (**7a** vs **8a**, **7b** vs **8b**, **7c** vs **8c**) reveals that the TN of the former are consistently higher than those of the latter. Conversely, the K_m values of the β-substituted naphthalenes are consistently lower than those of the corresponding α-substituted compounds. Clearly, substitution at the α and β positions of naphthalene gives rise to molecules of different lengths and shapes. Specifically, the β-substituted naphthalenes are longer and narrower than the corresponding α-naphthyl compounds. These differences in the shape appear to be reflected in the individual parameters, TN and K_m. However, since an increase in TN can be offset by a decrease in K_m, these shape differences become obscured in the parameter TN/K_m.

MAO B. Previous studies have shown that MPTP is oxidized efficiently by MAO B.[3,4] In addition, the analogs **3** and **5** were found to be more effective substrates for this enzyme than the parent, MPTP.[5,8] As shown in Table I, this increase in effectiveness is due to lower K_m values. In this and the 2-thienyl series, a progressive decrease in the K_m becomes apparent as the length of the bridge increases (from $n = 0$ to $n = 1$ and $n = 2$). No such trend is discernible in the K_m values of the naphthyl and 3-thienyl series. However, values for the TN of both the 2-thienyl and β-naphthyl series are characterized by peak values for the methylene-bridged analog of each group. Replacement of the phenyl group with the 2-thienyl fragment results in a less effective substrate, the result of a drastic reduction in TN (compare **9a** to MPTP). Although the 3-thienyl analog **10a** is twice as effective as **9a**, the former is still less effective than MPTP. Similarly, both rigid naphthyl analogs **7a** and **8a** are less effective substrates than MPTP.

Introduction of the methylene bridge in the 2-thienyl series results in a dramatic increase in effectiveness. Compound **9b** is 46 times more effective as a substrate for MAO B than its lower homolog **9a**. Unlike **3** which attributes its superior performance over MPTP solely to a lower K_m, the methylene bridge in **9b** alters both the TN and K_m relative to **9a**. Thus, the effect of the alkyl bridge on individual parameters cannot always be predicted with reliability. While the 3-substituted thiophene **10b** is also a better substrate than the corresponding lower homolog **10a**, the improvement is of lower magnitude than that observed with the corresponding 2-substituted thiophenes. The disparity between the performance of these isomeric methylene-bridged thiophenes further implicates the molecular dipole in their interaction with the enzyme. In this connection, a previous study[6] of simple MPTP analogs concluded that both MAO A and MAO B were sensitive to the electronic character of substituents on the phenyl ring. Among the thiophenes, substitution at C3 appears to be favored when $n = 0$. However, when $n = 1$, the 2-substituted thiophene is clearly superior (compare **9a** to **10a** and **9b** to **10b**). In contrast, the ethylene-bridged analogs **9c** and **10c** are equally effective substrates of MAO B.

Consistent with published reports,[14-16] both rigid thiophenes **9a** and **10a** are significantly less effective substrates of MAO B than MPTP. However, we find, in contrast to these earlier reports, that **10a** is a more effective substrate than **9a**. Surprisingly, the bulky naphthalene **7a** was found to be as effective a substrate for MAO B as MPTP. This observation is attributed to reductions in both V_{max} and K_m, relative to MPTP. In contrast to **7a**, the β-substituted naphthalene **8a** is a very poor substrate for this enzyme. The disparity in performance between **7a** and **8a** is attributed mainly to the 7-fold difference in the their K_m values. As observed earlier with MAO A, both methylene-bridged thiophenes **9b** and **10b** are more effective than MPTP and the corresponding lower homologs **9a** and **10a** (vide supra). However, both methylene-bridged naphthalenes are less effective than MPTP. Owing to its higher K_m value, the methylene-bridged naphthalene **7b** is a less effective substrate than its corresponding lower homolog **7a**. On the other hand, the isomeric **8b** is 3-fold more effective than **8a**, showing once again that the effects of the alkyl bridge are not predictable.

To a first approximation, the effectiveness of **7a**, relative to MPTP, runs counter to our earlier observations that MAO B preferentially oxidizes smaller MPTP analogs. However, the large discrepancy which characterizes the effectiveness of the isomeric naphthalenes as substrates of MAO B points to the importance of molecular shape in determining the nature of MAO–substrate interactions. Since flexible analogs can more easily accommodate the demands of the substrate binding site, the effects of molecular shape should become more apparent in the interaction of MAO B with bulky conformationally restricted substrates as the substituted naphthalenes **7a**, **7b**, **8a**, and **8b**.

Finally, both ethylene-bridged analogs **7c** and **8c** are poor substrates for MAO B relative to their corresponding lower homologs and MPTP. For the α-naphthalenes, one

notes a progressive reduction in effectiveness with increasing alkyl bridge length. In contrast, among the β-substituted naphthalenes, the methylene-bridged analog is clearly the best substrate.

Selectivity. In spite of differences in their relative effectiveness, all the thiophene analogs are more selective for MAO B than MAO A. Since the one- and two-carbon bridges increases the flexibility but have only minimal effects on molecular size, it is not unreasonable to conclude that flexibility alone is insufficient to cause large variations in selectivity.

In both of the thiophene series, selectivity for MAO B is highest for the methylene-bridged compounds, **9b** and **10b**. In addition, both compounds exhibit greater selectivity for this enzyme than MPTP. Similarly, all the naphthalenes show greater selectivity for MAO A than MAO B. In contrast to the thiophenes, the ethylene-bridged analogs **7c** and **8c** are the most selective compounds in each of the naphthalene series. This observation would appear to run counter to the notion that flexibility is generally detrimental to selectivity. However, it is important to note that **7c** and **8c** are also the bulkiest analogs in their respective series. Given the sensitivity of MAO B to substrate size, it may be that in this instance molecular size is the predominant determinant of selectivity.

Above the β-substituted naphthalenes, there is a 3-fold difference in range of selectivity (**8b** vs **8c**). In contrast, the α-substituted naphthalenes show a 14-fold difference in selectivity. These results suggest that the latter group of compounds is better suited to exploring differences between MAO A and MAO B.

Conclusion

In a previous study of MPTP analogs,[7] we observed that bulky lipophilic analogs were oxidized preferentially by MAO A. On the other hand, the smaller analogs were oxidized preferentially by MAO B. Consequently, we concluded that molecular size was an important factor in determining selectivity for MAO A or B. Additionally, we observed that the insertion of an alkyl bridge between the aryl and tetrahyropyridylyl moieties of MPTP caused significant variations in the selectivity of the resulting analogs. In the present study, we have extended these observations to include both semirigid and flexible tetrahydropyridines containing the thienyl and naphthyl moieties. In general, our earlier observations have been sustained. We still find that increasing the length of the alkyl bridge causes variations in relative effectiveness and selectivity. However, no obvious trends relating to the direction of the change can be identified. Thus, the effects of increasing molecular flexibility are unpredictable. To a first approximation, large bulky tetrahydropyridines are oxidized preferentially by MAO A, while those analogs which contain smaller pendant aryl groups are preferentially oxidized by MAO B. However, a more complex picture is suggested by the appearance of **7a**, a bulky molecule which is as effective a substrate for MAO B as MPTP. Although **7a** is 4 times more selective for MAO A than MAO B in these isolated systems, the fractional contribution of the two enzymes to the oxidation of this molecule in vivo is a function of their relative concentrations. Consequently, MAO B, the more abundant form in the primate brain,[17-20] would be expected to play a significant role in the bioactivation of this compound in primates. Alternatively, in the rodent brain where both enzymes exist in essentially similar concentrations,[21,22] MAO A would be more important. The contrasting profiles of the two isomeric naphthalenes **7a** and **8a** for MAO B also suggest that molecular shape may be as important as molecular size in determining effectiveness and thus selectivity for MAO A and B. The latter observation is consistent with an earlier model[23] which delineates differences between the topographies of MAO A and B. According to this model, both enzymes contain two lipophilic pockets, P2′ and P3′, which can accommodate substituents at the C2′ and C3′ positions of the MPTP skeleton. It is postulated that differences in the sizes of these lipophilic pockets largely constitute the basis for selectivity in the oxidation of MPTP-like substrates.[23] That **7c** is significantly more effective for this enzyme than the two thiophenes **9a** and **10** further supports the view that our earlier conclusions regarding the molecular size requirements for this enzyme need to be modified. These results also suggest that until further investigation sheds more light on the topography of MAO B, any claims regarding the molecular size limitation of this enzyme should be viewed with caution.

Experimental Section

General. Synthetic intermediates were purchased from Aldrich, Inc. (Milwaukee, WI) and were used as received. Tetrahydrofuran (THF) was distilled from CaH_2 immediately prior to use. All other reagents and solvents employed were of reagent grade.

All air-sensitive reactions were carried out under nitrogen. Standard handling techniques for air-sensitive materials were employed throughout this study.

Melting points were determined on a Mel-Temp melting point apparatus and are uncorrected. ^1H spectra were recorded on an IBM-Brucker spectrometer at 200 MHz. Both types of spectra are referenced to the deuterium lock frequency of the spectrometer. With this condition, the chemical shifts (in ppm) of the residual protons in the deuterated solvent were as follows: $CHCl_3$, 7.26; DMSO, 2.52; HOD, 4.81. The following abbreviations are used to describe peak patterns when appropriate: b = broad, s = singlet, d = doublet, t = triplet, q = quartet, m = multiplet. Both low- and high-resolution electron-impact MS were performed on an AEI MS-30 instrument. CIMS was performed on a Finnegan 4000 spectrometer.

Preparative chromatography was performed on either a Harrison Research Chromatotron using Merck 60 PF_{254} silica gel (no. 7749) or a Rainin Instruments preparative HPLC using a 41.1-mm-i.d. Dynamax silica column at a solvent delivery rate of 80 mL/min. Analytical TLC was performed on Analtech glass TLC plates coated with silica gel GHLF and were visualized with UV light and/or methanolic iodine.

4-(3-Thienylmethyl)pyridine (13). A solution of 2.5 M *n*-BuLi in hexane (13.2 mL, 33 mmol) was added to a solution of 3-bromothiophene (3.09 mL, 33 mmol) in THF (100 mL) already cooled to −78 °C, and the resulting mixture was stirred for 2 h. A solution of 4-pyridinecarboxaldehyde, **12** (2.8 mL, 30 mmol), in THF (5 mL) was added dropwise over 15 min. The mixture was allowed to come to room temperature and stirred for 16 h. The mixture was then treated with water (100 mL), adjusted to pH 11 with 10% NaOH, and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure to yield 6.45 g (quant) of α-(3-thienyl)-4-pyridinemethanol as a yellow solid. The latter was used in the next reaction without further purification.

Via an earlier procedure,[8] α-(3-thienyl)-4-pyridinemethanol (5.88 g, 31 mmol) was reacted with zinc (10.0 g, 154 mmol) in formic acid (75 mL). The crude product was purified by HPLC (hexane–*i*-PrOH–Et_3N, 95:5:1) to yield 1.47 g (28.0%) of **13** as a yellow oil: ^1H NMR ($CDCl_3$) δ 3.93 (s, 2, CH_2), 6.85 (dd, 1, thienyl C4-H, J = 1.4 Hz, J = 4.9 Hz), 6.93 (dd, 1, thienyl C2-H,

J = 0.7 Hz, J = 2.7 Hz), 7.08 (d, 2, pyridyl β-H, J = 6.0 Hz), 7.25 (dd, 1, thienyl C5-H, J = 3.0 Hz, J = 4.9 Hz), 8.48 (dd, 2, pyridyl α-H, J = 1.6 Hz, J = 4.4 Hz); CIMS (NH_3) m/e (intensity) 175.9 ($M^+ + 1$, 100.0).

Procedure A. 1-Methyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridine (9a). A solution of 2-bromothiophene (10.6 mL, 110 mmol) in THF (25 mL) was added in a slow stream to a stirring suspension of magnesium (2.67 g, 110 mmol) in THF (200 mL), and the mixture was refluxed for 2 h. The solution was cooled in an ice bath, and a solution of 1-methyl-4-piperidone (12.3 mL, 100 mmol) in THF (25 mL) was added dropwise over 15 min. The reaction mixture was refluxed for 8 h, cooled to room temperature, and treated with saturated aqueous NH_4Cl (100 mL). The resulting solution was adjusted to pH 11 with 10% NaOH and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were concentrated under reduced pressure to yield a dark residue. The latter was treated with 6 N HCl (100 mL) and refluxed for 3 h. The solution was cooled to room temperature, neutralized with 10% NaOH, and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4 and concentrated under reduced pressure to give 6.33 g (35.3%) of the free base as a yellow oil. The corresponding hydrochloride was made by bubbling dry HCl(g) through a solution of the free base in i-PrOH–Et_2O: mp 261–63 °C; ^{1}H NMR (CD_3OD) δ 2.91 (b-s, 2, C3-H), 2.98 (s, 3, NCH_3), 3.52 (b-s, 2, C2-H), 3.89 (b-s, 2, C6-H), 6.09 (b-s, 1, C5-H), 7.02 (dd, 1, thienyl C4-H, J = 3.6 Hz, J = 5.1 Hz), 7.16 (d, 1, thienyl C3-H, J = 3.4 Hz), 7.35 (d, 1, thienyl C5-H, J = 5.1 Hz). Anal. ($C_{10}H_{13}$NS·HCl) C, H. N.

1-Methyl-4-(1-naphthyl)-1,2,3,6-tetrahydropyridine (7a). The reaction of 1-bromonaphthalene (4.6 mL, 33 mmol) with magnesium (0.8 g, 33 mmol) followed by 1-methyl-4-piperidone (3.7 mL, 30 mmol) (procedure A) yielded 2.43 g (36.3%) of **7a** as a yellow oil. The hydrochloride was crystallized from i-PrOH–Et_2O: mp 225–227 °C; ^{1}H NMR (CD_3OD) δ 2.66 (b-d, 1, C3-H, J = 18.5 Hz), 3.05 (b-s, 4, NCH_3 and C3-H), 3.50 (dt, 1, C2-H, J = 4.9 Hz, J = 11.0 Hz), 3.68 (dd, 1, C2-H, J = 5.9 Hz, J = 12.1 Hz), 3.85 (b-d, 1, C6-H, J = 16.5 Hz), 4.08 (b-d, 1, C6-H, J = 16.6 Hz), 5.74 (b-s, 1, C5-H), 7.39–8.00 (m, 7, naphthyl). Anal. ($C_{16}H_{17}$N·HCl) C, H, N.

1-Methyl-4-(2-naphthyl)-1,2,3,6-tetrahydropyridine (8a). Via procedure A, 2-bromonaphthalene (6.83 g, 33 mmol) was reacted with magnesium (0.80 g, 33 mmol) and 1-methyl-4-piperidone (3.7 mL, 30 mmol) to yield 1.32 g (19.7%) of **8a** as a light tan solid. The latter was converted to the corresponding hydrochloride and crystallized from EtOH–Et_2O: mp 255–257 °C; ^{1}H NMR (CD_3OD) δ 3.01 (b-s, 5, C3-H and NCH_3), 3.30 (m, 1, C2-H), 3.77 (m, 2, C2-H and C6-H), 4.04 (m, 1, C6-H), 6.26 (b-s, 1, C5-H), 7.47–7.85 (m, 7, naphthyl). Anal. ($C_{16}H_{17}$N·HCl): C, H, N.

1-Methyl-4-(3-thienyl)-1,2,3,6-tetrahydropyridine (10a). A solution of 2.5 M n-BuLi in hexane (13.2 mL, 33 mmol) was added, under N_2, to a solution of 3-bromothiophene (3.09 mL, 33 mmol) in THF (100 mL) already cooled to −78 °C, and the resulting solution was stirred for 2 h. At this time, a solution of 1-methyl-4-piperidone (3.7 mL, 30 mmol) in THF (5 mL) was added dropwise over 15 min. The reaction mixture was allowed to come to room temperature and stirred for 16 h. The mixture was treated with water (100 mL), and the pH of resulting solution was adjusted to 11 with 10% aqueous NaOH. Following extraction of the mixture with CH_2Cl_2 (3 × 100 mL), the combined organic layers were concentrated under reduced pressure to yield a dark brown syrup. The latter was redissolved in 6 N HCl (100 mL) and refluxed for 3 h. The solution was neutralized with 10% NaOH and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic layers were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The crude product was purified by HPLC on silica gel (hexane–i-PrOH–Et_3N, 90:10:1) to yield 1.39 g (25.9%) of a yellow solid which was converted to the hydrochloride in i-PrOH–Et_2O: mp 230–32 °C; ^{1}H NMR (CD_3OD) δ 2.85–2.97 (m, 5, C3-H and NCH_3), 3.30 (m, 1, C2-H), 3.62–3.82 (m, 2, C2-H and C6-H), 4.01 (dd, 1, C6-H, J = 2.6 Hz, J = 14.4 Hz), 6.14 (b-s, 1, C5-H), 7.33 (dd, 1, thienyl C4-H, J = 2.0 Hz, J = 4.6 Hz), 7.42 (m, 2, thienyl C2-H and C5-H). Anal. ($C_{10}H_{13}$NS·HCl) C, H, N.

Procedure B. 1-Methyl-4-(3-thienylmethyl)-1,2,3,6-tetrahydropyridine (10b). Methyl iodide (2.0 mL, 32 mmol) was added to a stirring solution of 4-(3-thienylmethyl)pyridine (1.47 g, 8.4 mmol) in Et_2O (50 mL). After 16 h, the mixture was concentrated under reduced pressure and the residue was dissolved in MeOH (50 mL). $NaBH_4$ (1.15 g, 30 mmol) was added slowly with cooling in an ice bath. The solution was stirred for 3 h and concentrated under reduced pressure. The residue was diluted with water (50 mL), and the solution was acidified to pH 1 with 6 N HCl. After being stirred for 15 min, the solution was adjusted to pH 11 with 10% NaOH and extracted with CH_2Cl_2 (3 × 100 mL). The combined organic extracts were dried over Na_2SO_4, filtered, and concentrated under reduced pressure. The residue was chromatographed on silica gel (hexane–acetone–Et_3N, 50:50:1). The desired fractions were collected and concentrated to give 0.28 g (17.3%) of a yellow oil. The corresponding oxalate, obtained by adding an equimolar amount of oxalic acid to a solution of **10b** in MeOH, was recrystallized from i-PrOH–Et_2O: mp 90–92 °C; ^{1}H NMR (CD_3OD) δ 2.29 (m, 2, C3-H), 2.87 (s, 3, NCH_3), 3.25–3.90 (m, 6, C2-H, C6-H, and CH_2), 5.48 (s, 1, C5-H), 6.92 (dd, 1, thienyl C4-H, J = 1.1 Hz, J = 4.9 Hz), 7.09 (d, 1, thienyl C2-H, J = 2.8 Hz), 7.33 (dd, 1, thienyl C6-H, J = 2.9 Hz, J = 4.9 Hz). Anal. ($C_{11}H_{15}NS·C_2H_2O_4$) C, H, N.

1-Methyl-4-[2-(1-naphthyl)ethyl]-1,2,3,6-tetrahydropyridine (7c). The conversion of **21** (1.17 g, 5.0 mmol) to **7c** (procedure B) yielded 0.53 g (24%) of the free base as a pale yellow oil. The corresponding hydrochloride was recrystallized from i-PrOH–Et_2O: mp 193–195 °C; ^{1}H NMR (CD_3OD) δ 2.43 (m, 4, C3-H and naphthyl-CH_2CH_2), 2.89 (s, 3, NCH_3), 3.21 (m, 3, C2-H and naphthyl-CH_2CH_2), 3.52 (m, 2, C2-H and C6-H), 3.79 (b-d, 1, C6-H, J = 16.5 Hz), 5.43 (b-s, 1, C5-H), 7.45 (m, 4, naphthyl), 7.70 (dd, 1, naphthyl, J = 1.6 Hz, J = 7.6 Hz), 7.83 (dd, 1, naphthyl, J = 2.6 Hz, J = 7.4 Hz), 8.03 (d, 1, naphthyl, J = 7.6 Hz). Anal. ($C_{18}H_{21}$N·HCl·$^1/_{10}H_2O$), C, H, N.

1-Methyl-4-(2-naphthylethyl)-1,2,3,6-tetrahydropyridine (8c): yield, 24%. The hydrochloride was crystallized from i-PrOH–Et_2O: mp 188–90 °C; ^{1}H NMR (CD_3OD, hydrochloride) δ 2.33–2.47 (m, 4, C3-H and naphthyl-CH_2CH_2), 2.89 (s, 3, NCH_3), 3.22 (m, 3, C2-H and naphthyl-CH_2CH_2), 3.49 (m, C2-H and C6-H), 3.78 (b-d, 1, C6-H, J = 16.3 Hz), 5.43 (b-s, 1, C5-H), 7.30–8.05 (m, 7, naphthyl). Anal. ($C_{18}H_{21}$N·HCl·$^1/_{10}H_2O$) C, H, N.

1-Methyl-4-[2-(2-thienyl)ethyl]-1,2,3,6-tetrahydropyridine (9c): yield, 8.2%. The hydrochloride was crystallized from i-PrOH–Et_2O: mp 181–183 °C); ^{1}H NMR (CD_3OD) δ 2.41 (m, 4, C3-H and thienyl-CH_2CH_2), 2.90 (s, 3, NCH_3), 3.01 (t, 2, thienyl-CH_2CH_2, J = 7.2 Hz), 3.16 (dt, 1, C2-H, J = 5.2 Hz, J = 10.6 Hz), 3.52 (m, 2, C2-H and C6-H), 3.81 (b-d, 1, C6-H, J = 16.3 Hz), 5.47 (b-s, 1, C5-H), 6.85 (m, 2, thienyl C3-H and C4-H), 7.17 (dd, 1, thienyl C5-H, J = 1.3 Hz, J = 5.2 Hz). Anal. ($C_{12}H_{17}$NS·HCl) C, H, N.

1-Methyl-4-[2-(3-thienyl)ethyl]-1,2,3,6-tetrahydropyridine (10c): yield, 0.21 g (11.9%) of a yellow oil. The hydrochloride was recrystallized from i-PrOH–Et_2O: mp 128–30 °C; ^{1}H NMR (CD_3OD) δ 2.40 (m, 4, C3-H and thienyl-CH_2CH_2), 2.81 (t, 2, thienyl-CH_2CH_2, J = 7.2 Hz), 2.89 (s, 3, NCH_3), 3.18 (dt, 1, C2-H, J = 5.2 Hz, J = 10.7 Hz), 3.50 (m, 2, C2-H and C6-H), 3.80 (b-d, 1, C6-H, J = 16.3 Hz), 5.44 (b-s, 1, C5-H), 6.98 (dd, 1, thienyl C4-H, J = 1.2 Hz, J = 4.9 Hz), 7.06 (dd, 1, thienyl C2-H, J = 0.8 Hz, J = 1.8 Hz), 7.30 (dd, 1, thienyl C6-H, J = 2.9 Hz, J = 4.9 Hz). Anal. ($C_{12}H_{17}$NS·HCl) C, H, N.

***trans*-4-[2-(3-Thienyl)ethenyl]pyridine (20).** 3-Thiophenecarboxaldehyde (2.9 mL, 33 mmol) and 4-picoline (2.9 mL, 30 mmol) were reacted in acetic anhydride as described earlier[10] for other stilbazoles. After concentration of the reaction mixture, the residue was passed through a short column of silica gel (eluting consecutively with CH_2Cl_2 and 35% acetone-hexane). The desired fractions were concentrated to yield 1.53 g (27%) of the product as a brown solid: ^{1}H NMR ($CDCl_3$) δ 6.81 (d, 1, thienyl-CH=CH, J = 16.2 Hz), 7.28 (m, 6, thienyl C2-H, C4-H, and C5-H, thienyl-CH=CH, pyridyl β-H), 8.53 (m, 2, pyridyl α-H); CIMS (NH_3) m/e (intensity) 188.1 ($M^+ + 1$, 100.0).

Procedure C. 4-[2-(1-Naphthyl)ethyl]pyridine (21). The previously described 17[9] (4.29 g, 19 mmol) was hydrogenated over Pd/C (50 mg) in EtOH (100 mL). The crude product upon workup was chromatographed on SiO_2 (hexane–acetone–Et_3N, 80:20:1). The desired fraction was collected and concentrated under reduced pressure to give 3.23 g (74.9%) of a yellow oil: ^{1}H

NMR ($CDCl_3$) δ 3.02 (dd, 2, pyridyl-C*H*=CH, J = 6.9 Hz, J = 8.7 Hz), 3.36 (dd, 2, pyridyl-CH=C*H*, J = 7.3 Hz, J = 9.8 Hz), 7.08 (m, 2, pyridyl β-H), 7.21–8.07 (m, 7, naphthyl), 8.53 (m, 2, pyridyl α-H); CIMS (NH_3) m/e (intensity) 233.9 ($M^+ + 1$, 100.0).

4-[2-(2-Naphthyl)ethyl]pyridine (22). From the previously described 18,[10] compound **22** was obtained in 59% as a yellow oil (procedure C): ^1H NMR ($CDCl_3$) δ 3.03 (m, 4, CH_2CH_2), 7.09 (dd, 2, pyridyl β-H, J = 1.4 Hz, J = 4.5 Hz), 7.30 (dd, 1, naphthyl C3-H, J = 1.7 Hz, J = 8.4 Hz), 7.44 (m, 2, naphthyl C6-H and C7-H), 7.58 (s, 1, naphthyl C1-H), 7.75 (m, 3, naphthyl C4-H, C5-H, and C8-H), 8.48 (dd, 2, pyridyl α-H, J = 1.6 Hz, J = 4.4 Hz).

4-[2-(2-Thienyl)ethyl]pyridine (23). The reaction of 2-thiophenecarboxaldehyde (3.1 mL, 33 mmol) and 4-picoline (2.9 mL, 30 mmol) (procedure B) yielded crude **19**.[24] The latter was hydrogenated in EtOH (100 mL) over Pd/C (100 mg) for 2 days. Chromatography on silica gel (hexane–acetone–Et_3N, 80:20:1) afforded 1.02 g (17.9%) of the yellow solid, **23**: ^1H NMR ($CDCl_3$) δ 2.93 (m, 2, pyridyl-CH_2CH_2), 3.12 (m, 2, pyridyl-CH_2CH_2), 6.71 (m, 1, thienyl C3-H), 6.86 (m, 1, thienyl C4-H), 7.07 (m, 3, thienyl C5-H and pyridyl β-H), 8.48 (m, 2, pyridyl α-H); CIMS (NH_3) m/e (intensity) 189.9 ($M^+ + 1$, 100.0).

4-[2-(3-Thienyl)ethyl]pyridine (24). Crude **20** obtained from the reaction of 3-thiophenecarboxaldehyde (2.9 mL, 33 mmol) and 4-picoline (2.9 mL, 30 mmol) (procedure C) was hydrogenated (procedure D) to yield, after chromatography on silica gel (hexane–acetone–Et_3N 80:20:1), 1.64 g (28.8%) of the yellow solid **24**: ^1H NMR ($CDCl_3$) δ 2.91 (m, 4, CH_2CH_2) 6.88 (m, 2, thienyl C2-H and C4-H), 7.06 (m, 2, pyridyl β-H), 7.24 (m, 1, thienyl C5-H), 8.46 (m, 2, pyridyl α-H); CIMS (NH_3) m/e (intensity 109.2 ($M^+ + 1$, 100.0).

Biological Methods. MAO A from human liver, expressed in yeast, was isolated according to a published method,[11] and MAO B was isolated from beef liver by the procedure of Salach,[12] as modified by Weyler and Salach.[13] All assays were conducted at 30 °C. Initial rates of oxidation of MPTP analogs were measured polarographically in 50 μM sodium phosphate buffer, pH 7.2, containing 0.2% (w/v) Brij-35, in a total volume of 1.9 mL. Following temperature equilibration, a small volume (5–10 μL) of MAO A or B was added to start the reaction. The amount of enzyme used was adjusted for each compound so as to permit accurate rate measurements within 60–120 s after initiation of the assay. Turnover numbers were calculated from V_{max}, on the basis of double-reciprocal plots, divided by the flavin content. Determination of flavin content was accomplished by anaerobic reduction by the standard substrates, benzylamine (MAO B) and kynuramine (MAO A), and measurement of the change in optical density at the extinction coefficient of 11.3 mM. As in previous kinetic experiments, K_m and V_{max} were based on at least two independent determinations. The values of these kinetic constants were obtained from Lineweaver–Burke plots composed of more than six points, and the replicates differed by no more than 5%.

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References

(1) Chiba, K.; Trevor, A.; Castagnoli, N. Metabolism of the neurotoxic amine, MPTP, by brain monoamine oxidase. *Biochem. Biophys. Res. Commun.* **1984**, *120*, 574–578.
(2) Salach, J.; Singer, T. P.; Castagnoli, N.; Trevor, A. Oxidation of the neurotoxic amine 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) by monoamine oxidases A and B and suicide inactivation of the enzymes by MPTP. *Biochem. Biophys. Res. Commun.* **1984**, *125*, 831–835.
(3) Heikkila, R. E.; Manzino, L.; Cabbat, F. S.; Duvoisin, R. C. Protection against the dopaminergic neurotoxicity of 1-methyl-4-phenyl-1,2,5,6-tetrahydropyridine by monoamine oxidase inhibitors. *Nature* **1984**, *311*, 467–469.
(4) Langston, J. W.; Irwin, I.; Langston, E. B.; Forno, L. S. Pargyline prevents MPTP-induced parkinsonism in primates. *Science* **1984**, *225*, 1482–1484.
(5) Heikkila, R. E.; Kindt, M. V.; Sonsalla, P. K.; Giovanni, A.; Youngster, S. K.; McKeown, K. A.; Singer, T. P. Importance of monoamine oxidase A in the bioactivation of neurotoxic analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine. *Proc. Natl. Acad. Sci. U.S.A.* **1988**, *85*, 6172–6176.
(6) Youngster, S. K.; McKeown, K. A.; Jin, Y.-Z.; Ramsay, R. R.; Heikkila, R. E.; Singer, T. P. Oxidation of analogs of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine by monoamine oxidases A and B and the inhibition of monoamine oxidases by the oxidation products. *J. Neurochem.* **1989**, *53*, 1837–1842.
(7) Krueger, M. J.; Efange, S. M. N.; Michelson, R. H.; Singer, T. P. Interaction of flexible analogs of N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine and of N-methyl-4-phenylpyridinium with highly purified monoamine oxidase oxidase A and B. *Biochemistry* **1992**, *31*, 5611–5615.
(8) Efange, S. M. N.; Michelson, R. H.; Remmel, R. P.; Boudreau, R. J.; Dutta, A. K.; Freshler, A. Flexible N-Methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogues: synthesis and monoamine oxidase catalyzed bioactivation. *J. Med. Chem.* **1990**, *33*, 3133–3138.
(9) Smith, J. C.; Cavallito, C. J.; Foldes, F. F. Choline acetyltransferase inhibitors: a group of styrylpyridine analogs. *Biochem. Pharmacol.* **1967**, *16*, 2438–2441.
(10) Baker, B. R.; Gibson, R. E. Irreversible Enzyme Inhibitors. 181. Inhibition of brain choline acetyltransferase by derivatives of 4-stilbazole. *J. Med. Chem.* **1971**, *14*, 315–322.
(11) Tan, A. K.; Weyler, W.; Salach, J. I.; Singer, T. P. Differences in substrate specificities of monoamine oxidase A from human liver and placenta. *Biochem. Biophys. Res. Commun.* **1991**, *181*, 1084–1088.
(12) Salach, J. I. Monoamine oxidase from beef liver mitochondria: simplified isolation procedure, properties and determination of its cysteinyl flavin content. *Arch. Biochem. Biophys.* **1979**, *192*, 128–137.
(13) Weyler, W.; Salach, J. I. Iron content and spectral properties of highly purified bovine liver monoamine oxidase. *Arch. Biochem. Biophys.* **1981**, *212*, 147–153.
(14) Fuller, R. W.; Hemrick-Luecke, S. K. Depletion of heart norepinephrine in mice by some analogs of MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine). *Res. Commun. Chem. Pathol. Pharmacol.* **1987**, *56*, 147–156.
(15) Fuller, R. W.; Robertson, D. W.; Hemrick-Luecke, S. K. Comparison of the effects of two 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine analogs, 1-methyl-4-(2-thienyl)-1,2,3,6-tetrahydropyridine and 1-methyl-4-(3-thienyl)-1,2,3,6-tetrahydropyridine, on monoamine oxidase in vitro and on dopamine in mouse brain. *J. Pharmacol. Expt. Ther.* **1987**, 415–420.
(16) Fuller, R. W.; Hemrick-Luecke, S. K. Persistent depletion of striatal dopamine and its metabolites in mice by TMMP, an analogue of MPTP. *J. Pharm. Pharmacol.* **1987**, *39*, 667–669.
(17) Reynolds, G. P.; Riederer, P.; Rausch, W. D. Dopamine metabolism in human brain: effects of monoamine oxidase inhibition in vitro by (-)-deprenyl and (+)- and (-)-trancycypromine. *J. Neural Transm. (Suppl.)* **1980**, *16*, 173–178.
(18) Riederer, P.; Youdim, M. B. H.; Rausch, W. D.; Birkmayer, W.; Jellinger, K.; Seeman, D. On the mode of action of L-deprenyl in the human central nervous system. *J. Neural Transm.* **1978**, *43*, 217–226.
(19) Glover, V.; Sandler, M.; Owen, F.; Riley, G. J. Dopamine is a monoamine oxidase B substrate in man. *Nature* **1977**, *265*, 80–81.
(20) Fowler, C. J.; Tipton, K. F. On the substrate specificities of the two forms of monoamine oxidase. *J. Pharm. Pharmacol.* **1984**, *36*, 111–115.
(21) Fowler, C. J.; Callingham, B. A.; Mantle, T. J.; and Tipton, K. F. Monoamine oxidase A and B: a useful concept? *Biochem. Pharmacol.* **1978**, *27*, 97–101.
(22) Johnson, J. P. Some observations upon a new inhibitor of monoamine oxidase in brain tissue. *Biochem. Pharmacol.* **1968**, *17*, 1285–1297.
(23) Efange, S. M. N.; Boudreau, R. J. Molecular determinants in the bioactivation of the dopaminergic neurotoxin N-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP). *J. Computer-Aided Molec. Design* **1991**, *5*, 405–417.
(24) Frank, W. C.; Kim, Y. C.; Heck, R. F. Palladium-catalyzed vinylic substitution reactions with heterocyclic bromides. *J. Org. Chem.* **1978**, *43*, 2947–2949.